N° d'ordre :

REPUBLIQUE ALGERIENNE DEMOCRATIQUE & POPULAIRE

MINSTERE DE L'ENSIENMENT SUPERIEUR

& DE LA RECHERCHE SCIENTIFIQUE

UNIVERSITE KASDI MERBAH – OUARGLA

FACULTE MATHEMAIQUE ET SCIENCES DE LA MATIERE

THESE DOCTORAT

CHIMIE

Présenté Par M^elle^ BELBOUKHARI Mebarka

Intitulé

Flavonoides dans deux Asteraceae médicinales du sud – ouest Algerien : *Launeae nudicaulis* et *Warionia saharae* : Quantification, identification et évaluation antimicrobienne et antioxydante

Flavonoids in two medicinal Asteraceae from the southwest of Algeria :*Launaea nudicaulis* and *Warionia saharae*: :Quantification, identification and antimicrobial and antioxidant evaluation

Soutenu le : 26 /11/2015

Devant le jury composé de :

Président : **SAIDI Mokhtar**	***Professeur***	***Univ. K.M. Ouargla***
Examinateur : **DENDOUGUI Hocine**	***Professeur***	***Univ. K.M. Ouargla***
Examinateur : **YOUSFI Mohamed**	***Professeur***	***Univ. A.T. Laghouat***
Examinateur : **BECHKI Lazhar**	***MCA***	***Univ. K.M. Ouargla***
Directeur de thèse : **CHERITI Abdelkrim**	***Professeur***	***Univ. T.M. Béchar***
Co-Directeur de thèse: **BELBOUKHARI Nasser**	***Professeur***	***Univ. T.M. Béchar***

Année universitaire 2015-2016

AKNOWLDGEMENTS

This work was performed at the Phytochemistry and Organic Synthesis Laboratory (POSL) of Bechar University under the direction of **Prof. Cheriti Abdelkrim**. I would like to thank him for welcoming me in his laboratory and for giving me the opportunity to be train me in Phytochemistry. I also thank him for giving me the opportunity to present my results in various international conferences and several scientific publications.

I would also like to warmly thank **Prof. BELBOUKHARI Nasser** from Bechar University for having me in his laboratory and his interest in my work.

My gratitude goes to **Prof. SAIDI.Mokhtar** from K.M. University of Ouargla for his availability, his kindness and for agreeing to chair this thesis committee.

I also express my gratitude to **Prof. DENDOUGUI *Hocine*** from K.M. of Ouargla, University for having agreed to consider this thesis and participate in the jury.

I thank **Prof. YOUSFI Mohammed** from of A.T.Laghouat University for agreeing to be part of my thesis committee. Constructive criticisms have contributed to the operational sequences.

My gratitude also goes to **Dr. BECHKI Lazher**, from K.M.Ouargla, University for having kindly agreed to participate in this jury.

Although the thesis is fundamentally an individual work, it could not have been achieved without a team of colleagues who have contribute at the proper functioning of the laboratory, and ensure a serene atmosphere for all work time . For this I thank all my colleagues in Bechar University in particular members of the POSL laboratory.

Finally, my gratitude goes to my parents for their support throughout my studies and during this thesis.

Abstract

Our work focused on the Quantification, identification and evaluation of antioxidant and antibacterial activity of extracts and pure compounds of *Launeae nudicaulis* and *Warionia saharae* (*Asteraceae*).

The *in vitro* antioxidant activity was investigated with two different methods: thin layer chromatography test with β-carotene and DPPH radical scavenging assay. The preliminary activity screening in TLC allowed us to locate the DPPH scavenging activity in the BuOH, MeOH and EtOAC extracts which showed that these extracts are rich in phenolic compounds.

The quantitative evaluation of DPPH scavenging activity confirmed that BuOH, MeOH and EtOAc are the most active extracts .

MeOH extract of *Launeae nudicaulis and Warionia saharae* showed an important antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* .

Key words: *Launeae nudicaulis* , *Warionia saharae* , *Asteraceae* , antioxidant , DPPH , beta carotene , free radical , antibacterial

Résumé

Notre travail à porter sur la Quantification, identification et évaluation de l'activité antioxydant et antibactérienne des extraits et des composés pures de *Launeae nudicaulis* et *Warionia saharae* (*Asteraceae*).

L'activité antioxydante in vitro a été étudiée avec deux méthodes différentes : test sur CCM à la β-carotène et technique de réduction du radical libre DPPH. Le criblage préliminaire des extraits sur CCM a permis de cibler l'activité antioxydant dans les extrait BuOH, MeOH et ACOET ce qui révèle la richesse des extraits en composés phénolique. de même l'évaluation quantitative du pouvoir piégeur des extraits vis-à-vis du DPPH confirme que les extraits BuOH, MeOH et ACOET sont les plus actifs .

L'extrait MeOH de *Launeae nudicaulis et Warionia saharae* présent une activité antibactérienne importance contre *Staphylococcus aureus, Bacillus subtilis* et *Escherichia coli.*

Mots clés : *Launeae nudicaulis* , *Warionia saharae* , *Asteraceae* , antioxydant , DPPH , béta caroténe , radical libre , antibactérienne

ملخص

ارتكز هذا العمل على التقييم الكمي ، تحديد و تقييم النشاط المضاد للأكسدة و المضاد للبكتيريا للمستخلصات و المركبات النقية لنبات *Launeae nudicaulis* و *Warionia saharae* (*Asteraceae*) .

تمت دراسة النشاطية المخبرية المضادة للأكسدة باستعمال طريقتين : طريقة كروماتوغرافيا الطبقات الرقيقة بإستعمال β-caroténe و تقنية ارجاع الجدر الحر DPPH ، التحليل الأولي للمستخلصات باستعمال تقنية كروماتوغرافيا الطبقات الرقيقة سمحت بتعيين نشاطية مضادة للأكسدة في مستخلصات MeOH , BuOH و AcOEt الذي يثبث أن هذه المستخلصات غنية بالمركبات الفينولية .و هذا ما أكده التقييم الكمي لقدرة هذه المستخلصات على إزاحة الجدر الحر DPPH أن MeOH , BuOH و AcOEt هم الأكثر نشاطا .

أظهر مستخلص MeOH لنبات *Launeae nudicaulis* و *Warionia saharae* نشاط مضاد لبكتيريا : *Bacillus aureus , staphylococcus aureus* و *Esherichia coli* .

الكلمات المفاتيح : *Asteraceae , Warionia saharae , Launeae nudicaulis* ، مضاد الأكسدة ، *DPPH* ، β-caroténe ، جدر حر ، مضاد البكتيريا .

Abbreviation and Symbols

ROS	Reactive Oxygen Species
LDL	Low Density Lipoprotein
SOD	Superoxide Dismutase
Com	Compound
UV	Ultrat violet
GPx	Glutathione Peroxidase
TEAC	Trolox Equivalent antioxidant activity
ABTS	2,2 azinobis , 3 ethyl benzothiazoline , 6 , sulphonic acid
TRAP	Total Radical Trapping Antioxidant Parameter
ORAC	Oxygen Radical Absorbing Capacity
DPPH	2 , 2 Diphenyl -1 - picrylhydrazyl radical
HAT	Hydrogen atom transfer
ET	Electron transfer
FRAP	Ferric Reducing Antioxidant Power
$CHCl_3$	Chloroform
CH_3CO_2H	Acetic acid
MeOH	Methanol
TLC	Thin layer chromatography
EtOAc	Ethyl acetate
Rf	Retention factor
IR	Infrared
LN	*Launeae nudicaulis*
WS	*Warionia saharae*
δ	Chemical shift in ppm

s Singlet

d doublet

m multiplet

t triplet

AA Antioxidant activity

^{1}H NMR proton nuclear magnetic resonance

^{13}C NMR carbon nuclear magnetic resonance

Table List

FIGURES LIST

TABLE OF CONTENTS

CHAPTER III : MATERIALS AND METHODS

CHAPTER IV: RESULTS AND DISCUSSION

Introduction

Introduction

Plants have always been a major source of drugs. Today a majority of the world population, particularly in developing countries, can be treated only with traditional herbal remedies. Aspirin Taxol, the modern pharmaceutical industry itself still relies heavily on the diversity of plant secondary metabolites to find new molecules with novel biological properties. This source seems inexhaustible since only a small portion of the 400,000 known plant species were investigated on phytochemical and pharmacological plans and that each species can be up to several thousand individual components.

As part of the research activities of biological molecules or new plant, so it is best not to base the choice of plants to study on chance alone, but to define it according to various criteria. The most used is that of employment in traditional or folk medicine that enhances the experience accumulated by different generations.

In this work, we initially interested in validating the use of traditional medicine of two medicinal plants from *asteracea* familly: *Launaea nudicaulis* and *Warionea saharae*, which have valuable pharmacological properties, through their use in traditional medicine . These medicinal properties have been explained, at least in part, by the presence of natural bioactive substances in the secondary metabolites. Thus guided fractionation activity of two plants fee allowed the isolation of some compounds as they may be responsible for antioxydantl activity.

A liquid chromatography analysis was used in our laboratory to solve problems in the separation of products that do not show well with chemical indicators or trace in the extracts of two plants.

The choice of these two plants was guided partly by the traditional use indications, and secondly that *Launaea nudicaulis and Warionia saharae* have been the subject of little chemical and biological investigation.

We present our work in two parts composed of four chapters:

Part A: Focus bibliographical on the *Asteracea* family : case of *Launaea* and *Warionia* genus (Chapter I) and the polyphenol compounds : nature and importance (Chapter II).

Part B presents our personal work, devised in two chapters:

First we discuss in Chapter III, materials and methods: Phytochemical screening , chromatographic and extraction conditions.

The second chapter presents the essential core of our work on the result and discussion of antioxidant activities and new biomolecules (flavonoids) responsible of these activities.

Chapter I

Asteraceae family in Saharan flora *:* case of *Launeae* and *Warionia* genus

CHAPTER 1 : ASTERACEAE FAMILY IN SAHARAN FLORA : CASE OF LAUNEAE AND WARIONIA GENUS

Natural products have served as an important source of drugs since ancient times and about half of the useful drugs today are derived from natural sources. Chemo diversity in nature, e.g. in plants, microorganisms and marine organisms, still offers a valuable source for novel lead discovery.

The family *Asteraceae* or Compositae (Asteraceae or Compositae) is an important family of flowering plants. They have the common characteristic of having flowers gathered in heads. The two species selected for this study are widely used by the local population in the treatment of several diseases. They are endemic medicinal plants of the Sahara.

1- Asteraceae

The family of *Asteraceae* or , alternatively , compositae , known as the aster , daisy or sunflower family , is one of the largest angiosperm families of a taxon of dicotyledenous flowering plants . It comprises about 1400 genera and more than 25000 species of herbaceous plants , shurbs , and trees , spread throughout the world , and classified over three subfamilies and 17 tribes **[1-7].**The composite nature of the inflorescences of these plants led early taxonomists to call this family compositae. Though diverse habits and habitats, composites tend to grow in sunlit places, in temperate and subtropical regions . Some *Asteraceae* plants can share these following characters:

- ✓ Various members of the aster family are familiar species in natural habitats , while others are cultivated plants in gardens and some are grown as food (*lactuca sativa*) (lettuce) , and *cichorium* (chicory).
- ✓ Many members of Asteraceae are pollinated by insects , which explain their value in attracting beneficial insects . Many members of Asteraceae are copious nectar producers and are useful for evaluating pollinator populations during their bloom. *centuarea cyanus* , *helianthus annus* and some species of solid ago are major honey plants .
- ✓ Some species in the aster family have anatomical mechanisms of attaching their seeds to the fur of mammals, for the purposes of dispersal.
- ✓ Phytochemically , species of asteraceae biosynthesize many several metabolites such polyfructanes as storage carbohydrates as opposed to polysaccharides , in the

perennial taxa . In some taxa , some segments of the family accumulate polyacetylenes , flavonoids , various alkaloids and terpenoids essential oils , but iridoids have never been found **[6-8]**

- ✓ Plants in *Asteraceae* are medically important; sesquiterpenes compounds obtained from them are responsible for allergic contact dermatitis. More than 4000 structures with around 30 different skeletal types have so far been reported from several tribes of this family.

1.1- Morphological and characteristics of *Asteraceae*

All plants belonging to the *Asteraceae* family can share the following characteristics according to Judd **[9-13]**

- ✓ The inflorescence is an involucrate capitulum , technically called a calathid or calathidium , but generally referred to as flower head , which is contracted raceme composed of numerous individual sessile flowers , called the florets , all sharing the same receptacle .
- ✓ The flowers are of two basic types: tubular actinomorphic corollas and those with strap shaped or radiate zygomorphic corollas, often with the same head. Either type may be bisexual or unisexual.
- ✓ The calyxes (sepals) of the florets are modified to form a pappus, a tuft of hairs, which often appears on the mature fruit.
- ✓ Anthers are syngenesious with the stamens fused to gether .
- ✓ Leaves and stems very often contain secretory canals with resin or latex (particularly common among the *Cichorioideae)*. The leaves can be alternate, opposite, or whorled. They may be simple, but are often deeply lobed or otherwise incised, and conduplicated or revoluted. The margins can be entire or dentate.
- ✓ The fruit of *Asteraceae* is a specialized type of achene sometimes called cypsela. One seed per fruit is formed. Its morphology is often used to help determining plant relationships at the genus and species level. The seeds usually have little or lack endosperm.

1.2- Importance of *Asteraceae* family

The biodiversity of metabolites products isolated from *Asteraceae* makes this family have an important commercial source. Some *Asteraceae* plants are used as herbs and herbal tea such as :

- Chamomile, which comes from two different species, the annual *Matricaria recutila* and the perennial *Chamaemelum nobile*, also called Roman chamomile.
- Calendula (*Calendula arvensis)*, also called the pot marigold, grown commercially for herbal teas and the potpourri industry.
- Echinacea (*Echinacea purpurea*) used as a medicinal tea.

The industrial use of Compositae is also known. Common in all commercial poultry feed, marigold (*Tagete spatula*) is grown primarily in Mexico. Marigold oil, extracted from *Tagetes minuta*is used in the metric ton in the cola and cigarette industry **[14-19].**

Several species of this family are used as natural remedies; such as:

- ✓ *Anthemis arvensis* L. the whole plant is used as anti-inflammatory, emetic, and sedative.
- ✓ *Artemisia arborescens* L. The flower is used as digestive, stimulant, expectorant.
- ✓ *Calendula arvensis* L. The flower and leaf are used as antispasmodic, burns, diuretic, Disinfectant and vulnerary.
- ✓ *Cichoriumin tybus* L. The leaf and root are used in blood purification, as arteriosclerosis, anti-arthritis, anti-spasmodic, digestive, hypotensive, aperitif, and laxative.
- ✓ *Helychrysum microphyllum* Willd. The flower and leaf were used as an expectorant.

1.3-Description of the genus *launeae cass*.

The plant family *Asteraceae*(Compositae) comprises of a large number of specices that have been and are still used as medicinal plants , particularly in folk medicine and used as food.

Launeae Cass.is a small genus of the family *Asteraceae* , the genus consists of 54 species , of nine which are presented in the flora of Algeria and mainly distributed in the South Mediterranean ,Africa and South West Asia **[20]** . They are perennial to annual herbs, small shrubs or sub shrubs. Many of its plants are used in folk medicine as bitter stomachic for treating diarrhea , gastrointestinal tracts , as anti-inflammatory , for skin diseases , treatment of infected wounds , hepatic pains , children fever , as soporific , lactagogue , diuretic and as insecticidal. Additionally , crude extracts of some species have been reported to exhibit antibacterial , anti - parasitic , antioxidant , cytotoxic , neuro pharmacological and insecticidal activities **[21-23].**

According to classification system on flowering plants , the classification hierarchy of the genus *Launeae* can be tracked as follows :

- Kingdom : Plantae
- Division : Angiosperm
- Class : Eudicots
- Subclass :Asterids
- Order : Asterales
- Family : Asteraceae
- Subfamily :Cichorioideae
- Tribe : Lactuceae (Cichorieae)
- Sub-tribe : Sonchinae
- Genus : *Launeae*

The genus *Launeae* Cass. belongs to the tribe *Lactuceae* of the *Asteraceae* family and contains about 54 species , most of which are adapted to dry , saline and sandy habits . Plants of this genus have several rows of stems , hairless leaves incised into lobes that are themselves lined with white teeth , membranous scales on the edges , yellow ligules , and elongated chain , prismatic or slightly flattened.

The genus *Launeae* is represented in the flora of Algeria by nine species including five endemics of North Africa : *L. angustifolia* , *L. quercifolia* and *L. cassiniana* are the endemic plants of the North Africa , with limited distribution , whereas *L. acanthoclada* and *L. arborescens* are two endemic plants of the north –west of Africa . The other four species *L. nudicaulis* and *L. residifolia* sprout in Algeria and Tunisia Mediterranean Sea , whereas *L. glomerata* and *L. mucronata* grow in the Saharan Atlas **[17]** .

Three of this species are used in Algerian Sahara ethnopharmacopea as medicinal plants ,*L. nudicaulis, L. residifolia* and *L. arborescens*, which are endemic to south west Algeria and south east Morocco **[17].**

a- Ethnopharmacology and bioactivity of the genus *Launeae* Cass.

Algeria with its large area and diverse climate has a varied flora, which is a source of rich and abundant medical matter and, in particular, Sahara part constitutes an important reservoir of many plants which have not been investigated until today **[16].**

Among the flora , some *Launeae* plants have been used in the traditional medicine .Species of the genus *Launeae* are widely applied in traditional folk medicine throughout their areas of distribution . Many of them are used in folk medicine as bitter stomachic , anti tumor , insecticides and against skin diseases **[17].**

Launeae nudicaulis (Vernaculer name : Reghama)is a glabrous, perennial herb with cylindrical heads, spaced along the branches , branched stem of 10 – 30cm , with narrow chain .It is used in the traditional medicine to treat gastric burns , pain of stomach , constipation , to relieve fever for children , in the treatment of itches of skin and eczema.

More than 4000 sesquiterpenoids structures with around 30 different skeletal types have so far been reported from several tribes of *Asteraceae* family including the *Cichorieaceae* tribe . These natural compounds are responsible for allergic contact dermatitis and exhibit a wide range of bioactivities which include plant growth regulation and antimicrobial activity. Also they are used as schistosomicial and insect feeding deterrent agents. In addition, they provoke the toxicity for certain cancer cell lines **[17].**

On the other hand , triterpenoids and flavonoids chemio-characteristic of *Asteraceae* family , including the *Launeae* genus , have been reported to have anti-inflammatory activities , anti-hyperlipidemia , hepato -protection , antioxidant , cyto-protective , giving protection against cardiovascular disease , and certain forms of cancer .

Antibacterial, antifungal and allelopathic potential activities have been proven of many species of *Launeae*. in an antibacterial assay against *Bacillus subtilis* the extracts of *Launeae nudicaulis* and *Launeae residifolia* showed 18.5 and 20.5 mm zones of inhibition .

The antibacterial activity of extracts from *Launeae Arborescens* and *Launeae nudicaulis* which are widely distributed in the south west of Algeria .The methanol extract of the aerial part of *Launeae nudicaulis* showed high activities against *Candida albicans , Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* .The highest inhibition observed in *Staphylococcus aureus,* a human pathogen , explains the use of this plant against a number of infections for generations **[2, 16].** .

b- Secondary metabolites isolated from the Saharan *Launeae* genus

The first work in phytochemistry on species of the genus *Launeae* was started by Prabhu and Venkates warlu in1969, when they were isolated from leaves and roots of *Launeae pinnatifida* two compounds Taraxasterol(Com 01)and Taraxerly acetate (Com 02)

Five years after , in 1974 , Bahadur and Sharma reported the presence of palmitic , stearic, oleic and linoliec acids from the roots of *Launeae nudicaulis*.

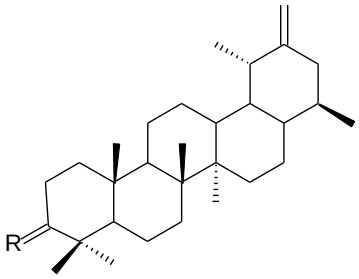
Different secondary metabolites have been identified from the genus *Launeae*. Also, few sesquiterpene lactones have been reported from various species of this genus and the occurrence of flavones glycosides is remarkable **[29]** .

Detailed chemical investigation of *Launeae nudicaulis* yielded some triterpenes such as taraxasterol (Com 01) ,

Two new ursine type triterpenes , nudicauline A (Com 13), and nudicauline B (Com 14) have been isolated from the aerial parts of this species , along with olean - 1 l ,13(18)-diene (Com 15) , 3β - hydroxyl - 13(28) - epoxy - urs - 1 l – ene (Com 16) and 3 - keto - 13(28) - epoxy - urs - 1 l – ene (Com 17),

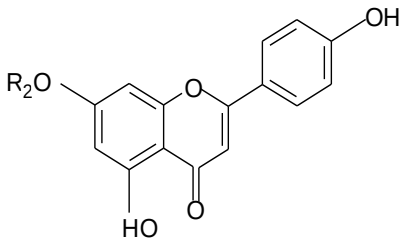
Additionally , flavone glycosides were reported from the 70% EtOH extract of fresh sample of *Launeae nudicaulis* as apigenin - 7 - O – glucoside (Com 03) , luteolin - 7 - O – glucoside (Com 05) , luteolin - 7 -O – rutoniside (Com 07), apigenin - 7 - O – gentiobioside (Com 04) , luteolin - 7 - O – gentiobioside (Com 06) and three glycosides luteolin - 7 , 3' diglucoside (Com 10), luteolin - 7' ,4' – diglucoside (Com 08) and luteolin - 7 - O - gentiobioside - 4' - O – glucoside (Com 09). Moreover , two common coumarins , esculetin (fig 11) and cichoriin (Com 12).

Recently , ethyl acetate soluble fraction of methanolic extract of *Launeae nudicaulis* was subjected to chromatographic purification to get four new compounds including a quinic acid derivative Cholistaquinate (Com 19) , a flavone glycoside Cholistaflaside (Com 18).



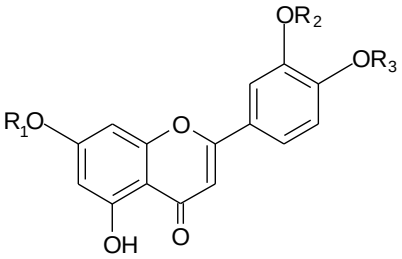
(Com 01) : R = α H , β OH

(Com 02) : R = α H , β OAc



(Com 03) : R_2= Glc

(Com 04) : R_2= Apg



(Com 05) : R_1 = Glc , R_2= H ,R_3=H

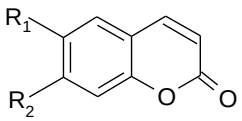
(Com 06) : R_1 = Gen , R_2= H ,R_3=H

(Com 07) : R_1 = Rut , R_2= H ,R_3=H

(Com 08) : R_1 = Glc , R_2= H ,R_3=Glc

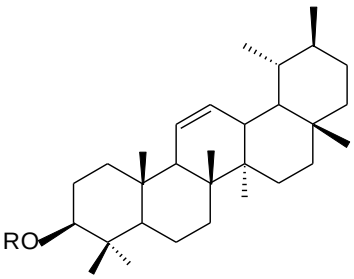
(Com 09) : R_1 = Gen , R_2= H ,R_3=Glc

(Com 10) : R_1 = Glc, R_2= Glc ,R_3=H



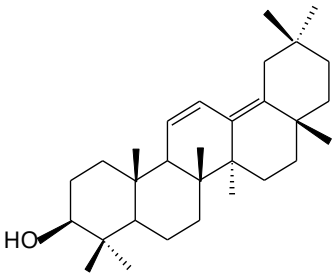
(Com 11): R_1 = OH , R_2= OH

(Com 12) : R_1 = OH , R_2= O-Glc

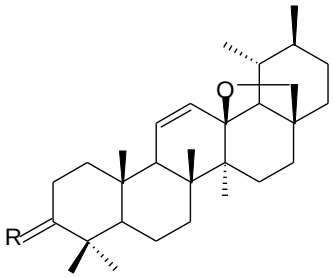


(Com 13): R=OH

(Com14): R = Ac

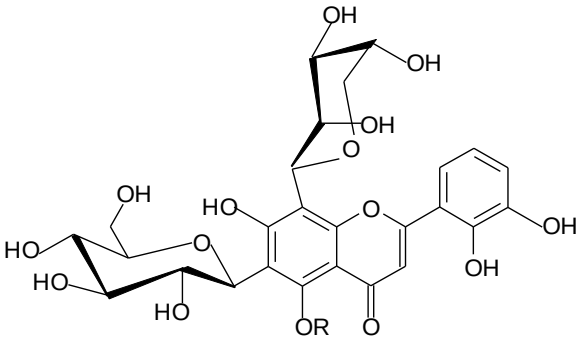


(Com 15)

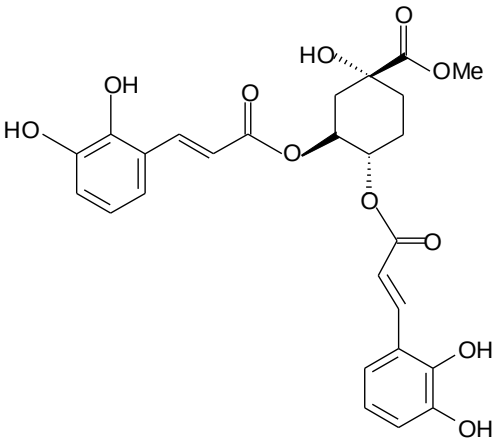


(Com 16): R = αH , βH

(Com 17): R= O



(Com 18)



(Com 19)

Figure I.1: Some secondary metabolites isolated from the genus *Launeae Cass*

1.4-Description of the Genus *Warionia* Benth&Coss.

Warionia Benth. &Coss. is a monotypic genus of *Asteraceae* ,endemic to the northwestern edge of the African Sahara desert. The species *Warionia saharae* Benth.& Coss., known by the vernacular name of "afessas" or "abessas", may be found in several localities on dry shale in northwestern Africa, in Morocco and Algeria. This is a thistle-like aromatic plant, with white latex and pinnately partite, somewhat fleshy leaves. The capitula are homogamous with tubular corollas, the anthers are caudate, and the style branches are dorsally covered by acute collecting hairs extending somewhat below the branches bifurcation. **[30].**

One peculiarity of this plant is the penetrating odor that some people find unpleasant, given by the glandular hairs in the epidermis of the leaves at the slightest contact. **[30].**

According to classification system on flowering plants , the classification hierarchy of the genus *Warionia* can be tracked as follows :

- Kingdom : Plantae
- Division : Angiosperm
- Class : Decotyledon
- Subclass :Asterids
- Order : Asterales
- Family : Asteraceae
- Subfamily :Cichorioideae
- Genus : *Warionia*

Warionia saharae Benth & Coss. (local name Efessas or Kabar Lemíaiz) a genus of the family Asteraceae, is an endemic herbaceous medicinal plant represented by only one species which is widely distributed in the south west of Algeria and south east of Morocco **.[31]**

a- Ethnopharmacology and bioactivity of the genus *Warionia* .

The plant *Warionia saharae* is considered to have medicinal properties mainly by its essential oils. Decoction of dried leaves is used as anti-rheumatic and against epileptic crisis. Crude extracts of the plants showed antibacterial and cytotoxic activities against a cancer cell line **[30].**

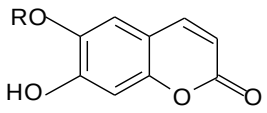
The aerial part of this plant was used in Sahara folk medicine for treating gastrointestinal tracts, icter and as anti-inflammatory **.[31]**

b- Secondary metabolites isolated from the Saharan *Warionia* Genus

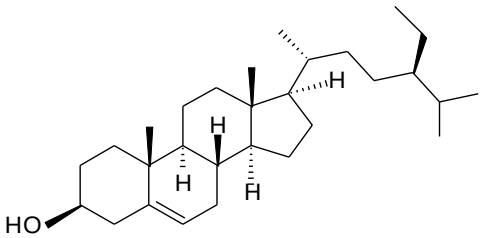
An earlier phytochemical study on *Warionia saharae* focused on the main components of the essential oils and led to the isolation of eudesmol , linalool and nerolidol **[31]** . Other investigations from Morocco of this species, reported the presence of sesquiterpene lactones with guaianolide skeleton type and their cytotoxic and anti-inflammatory activities **[31,32]** .

The phytochemical studies of the chloroform and ethyl acetate soluble parts of the aqueous - EtOH extract of the aerial part of *Warionia saharae* collected during the flowering phase , in the southeast of Algeria give ten compounds including : β - sitosterol as a major component (Com 20) , a mixture of β - sitosterol and stigmasterol (Com 22), scopoletin , esculetin (Com

21) , cirsimaritin ,chrysoeriol , hispidulin, luteolin (Com 24) , taxifolin (Com 23) and quercetin (Com24)

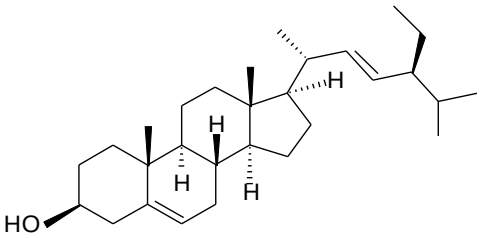


(Com 21)

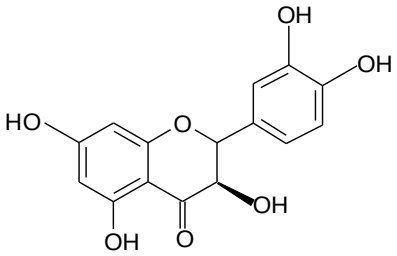


(Com 20)

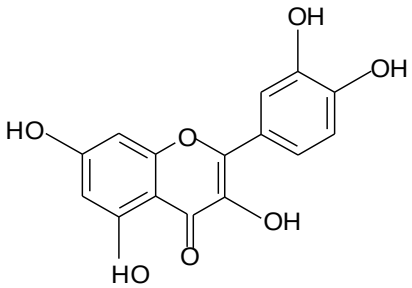
R= H : Esculetin , R= CH_3 : Scopoletin



(Com 22)



(Com 23)



(Com 24)

Figure I.2: Some secondary metabolites isolated from the genus *Warionia*

The family *Asteraceae* has a great importance due to its ethnobotanics, phytochemistry and biological activities, and it is a promising source of various secondary metabolites including terpenoid, saponin and flavonoids. Some of these isolate compounds have been found to exhibit various biological activities. We have attempted to show the high biodiversity of metabolite products generally isolated from this family.

Chapter II

The polyphenols compounds : Nature and Importance

CHAPTER 2 : THE POLYPHENOLS COMPOUNDS : NATURE AND IMPORTANCE

2.1-Polyphenols

Phenolic compounds represent a large group of molecules with a variety of functions in plant growth, development, and defense. Phenolic compounds include signaling molecules, pigments and flavors that can attract or repel, as well as compounds that can protect the plant against insects, fungi, bacteria, and viruses. Most phenolic compounds are present as esters or glycosides rather than as free compounds. Tannins and lignin are phenolic polymers. Tannins are used commercially as dyes and astringents, and lignin accounts for structural rigidity of cells and tissues and is essential to vascular development. From this brief overview it is apparent that phenolic compounds make up a large and fascinating family.

2.2 - Nature of polyphenol

Polyphenols are wide and complex group of secondary plant metabolites **[10-12]** . So far, over 8000 compounds have been identified . Structures of the compounds range from simple molecules such as phenolic acids, to highly polymerized compounds like proanthocyanidins . Polyphenols are essential for the physiology of plants , having functions in growth, structures , pigmentation , pollination , allelopathy and resistance for pathogens , UV radiation and predators **[22, 23]** , in addition , sensory qualities of plant foods and beverages , such as astringency and bitterness are related to their polyphenol content **[22, 24]**

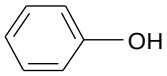
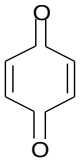
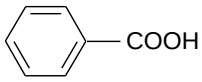
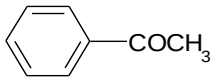
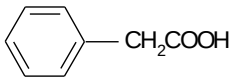
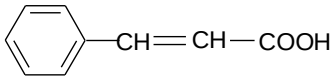
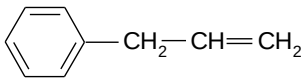
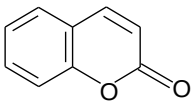
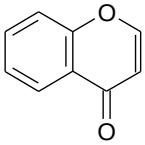
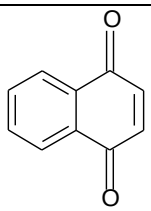
In nature, polyphenols occur primarily in conjugated forms with one or more sugar residues attached to hydroxyl groups **[22, 24, 25]** conjugation increase the polarity of the molecules , which is necessary for storage in plant cell vacuoles . The most common sugar residue is glucose, and residue can be in the form of monosaccharaides or oligosaccharides.

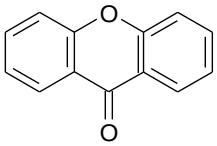
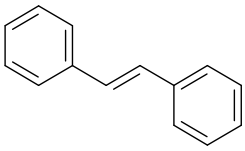
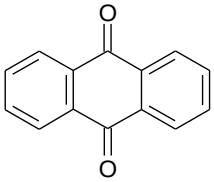
In plant, polyphenols are relatively resistant to heat oxygen, dryness and to some extent also to acidity, but the sensivity to light differs according to their chemical structure **[22, 25 ,26]**

They arise biogenetically from two main synthetic pathways, the shikimate pathway and the acetate pathway **[23,28]**

According to Harborne **[23, 28]** , polyphenols can be divided into at least ten different classes depending on their chemical structure . **Table II.1** illustrates the basic chemical structures of the main polyphenolic compounds.

Table II.1 : The basic chemical structures of the main polyphenolic compounds

Class	Basic skeleton	Basic structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6- C_1	
Acetophenones	C_6- C_2	
Phenylacetic acids	C_6- C_2	
Cinnamic acids	C_6- C_3	
Phenylpropenes	C_6- C_3	
Coumarins , isocoumarins	C_6- C_3	
Chromones	C_6- C_3	
Naftoquinones	C_6- C_4	

Xanthones	C_6- C_1- C_6	
Stilbenes	C_6- C_2- C_6	
Anthraquinones	C_6- C_2- C_6	
Flavonoids	C_6- C_3- C_6	
Lignans , neolignans	$(C_6 C_3)_2$	
Lignins	$(C_6 C_3)_n$	

2.3 -Function and distribution in plants

Phenolics and polyphenolics are essential to the plant's physiology .Several functions of compounds in plants have been proposed or demonstrated. Plants have evolved to produce flavonoids to protect against damage by UV- B light, fungal parasites, herbivores, pathogens and oxidative cell injury. They are also plant hormone secretion controllers and enzyme action inhibitors . In food phenolic may play important roles as natural colorants and in their flavoring of compounds. **[29]**

2.4- Classification of Polyphenols

2.4.1- Phenolic Acid

Phenolic acid are widely distributed in cereals and legumes. They act as free radical terminators .Phenolic antioxidant act to inhibit lipid peroxidation by trapping the peroxyl radical. This radical abstracts a hydrogen atom (or electron after prior less of a proton). From the antioxidant to yield a phenoxyl radical **[33]**

In plant, free phenolic acids occur as substituted benzoic and cinnamic acid derivatives **[33, 34].** Phenolic acids constitute a group characterized by the presence of carboxylic group . Common phenolic acids are gallic acids, vanillic acids, p. coumaric ,ferulic and sinapic acid **[36].**

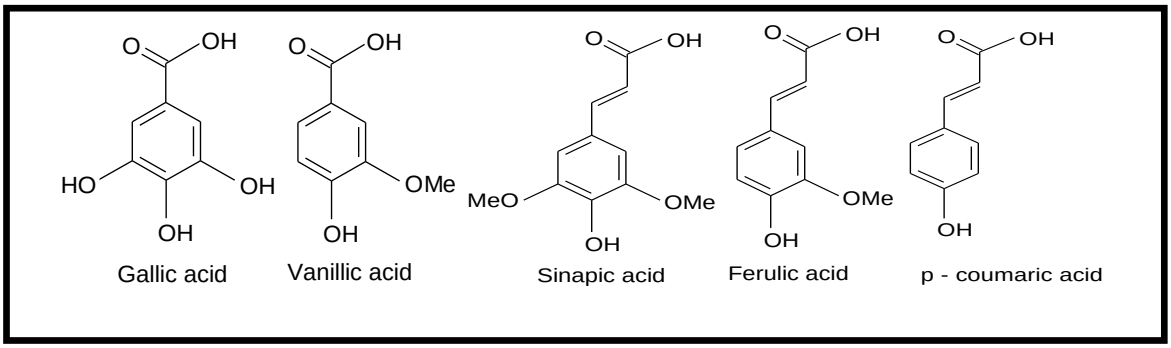


Figure II.1 : Molecule structure of some phenolic acids

2.4.2 – Tannins

Tannins are compounds of intermediate to high molecular weight .Tannins with a molecular mass of up 30,000 DA have been found in carob pods (leguminosae) **[30].**Tannins are highly hydroxylated molecules and can form insoluble complexes with carbohydrates and protein .

The term tannin comes from the tanning capacity of these compounds in transforming animal hide into leather by forming stable tannin protein complexes with skin collagen **[30, 37]**

Plant tannins can be subdivided into two major groups : hydrolysable tannins , condensed tannins . A third group of tannins , the phloro tannins , are found only in marine brown algae and are not commonly consumed by humans **[30,37]**

2.4.3- Flavonoids

Flavonoids represent the most common and widely distributed group of plant phenolics .their common structure is that of diphenylpropane (C_6 C_3- C_6) and consist of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle (**Figure II.2 represent the basic structure and the system of used for carbon numbering of the flavonoid nucleus**) .Biogenetically , the A ring usually comes from a molecules of resorcinol or phloro - glucinol synthesized in the acetate pathway whereas the B ring is divided from the

shikimate pathway **[30, 41]** Flavonoids occasionally occur in plants as aglycones , although they are most commonly found as glycoside derivatives **. [30]**

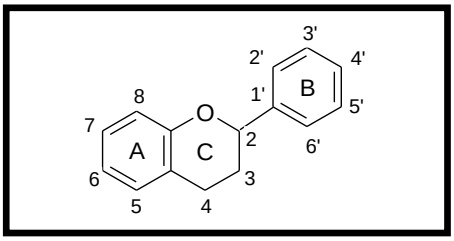


Figure II.2 : Basic structure and numbering system of flavonoid

a- Classification of flavonoids

According to the modifications of the central C ring they can be divided into different structural classes including flavonols , flavones , flavan - 3ols , flavanones , isoflavones and anthocyanidins **.[41]**

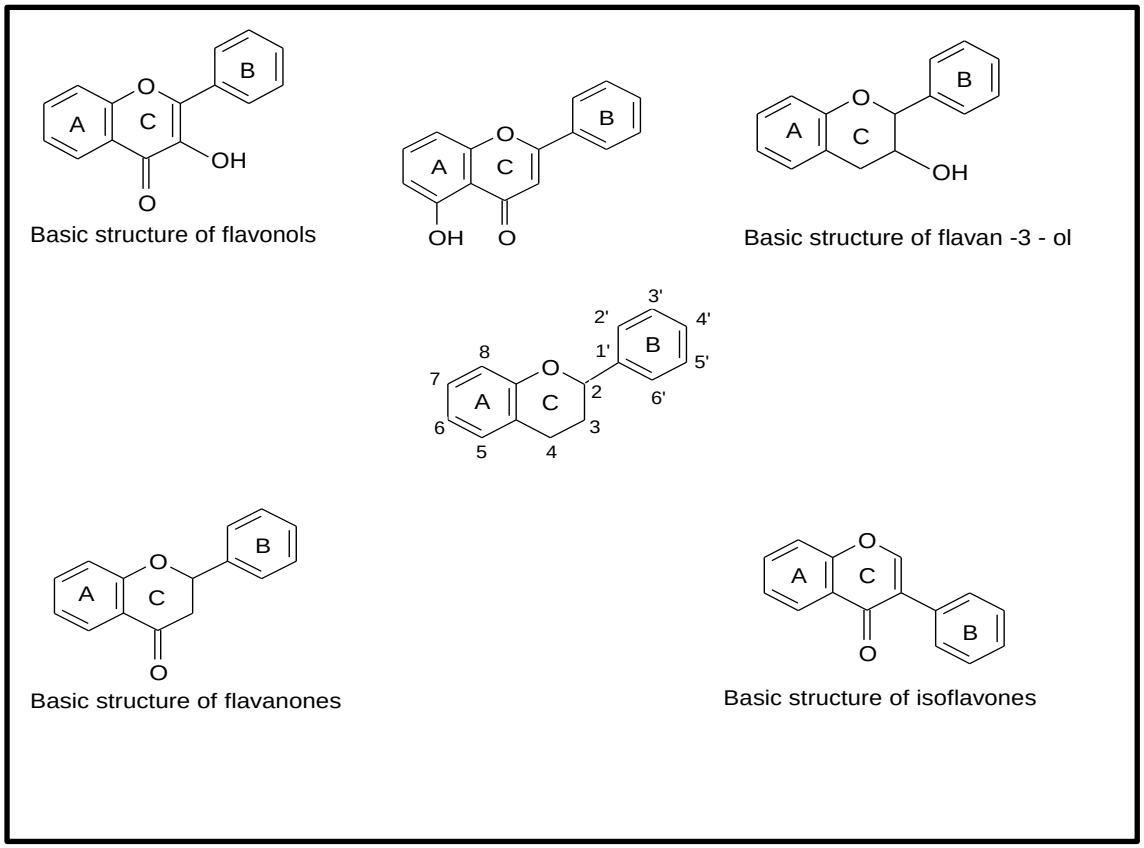


Figure II.3 : Structure of the main flavonoid subgroups

1- Flavonols

The flavonols are the most widespread of the flavonoids in plant food. They vary in color from white to yellow and are closely related in structure to the flavone. They are represented mainly by quercetin ,kaempferol and myricetin . **[37]**

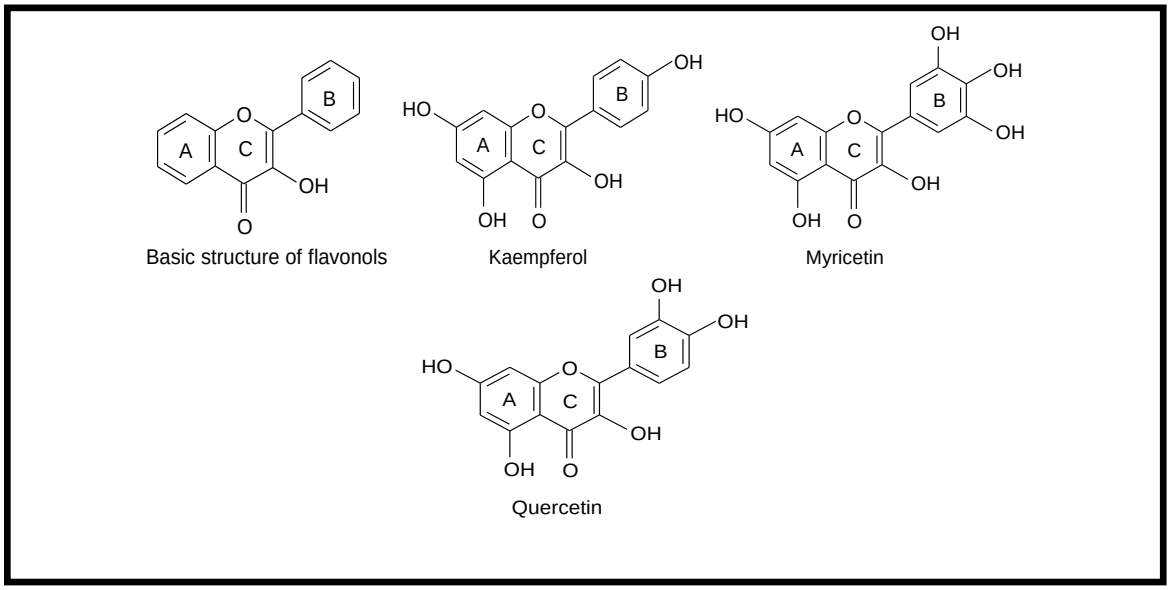


Figure II.4 : Molecule structure of flavonols

2 - Flavones

Flavones are structurally very similar to flavonols and differ only in the absence of hydroxylation at 3 position on the C ring .Flavones are mainly represented in the diet apigenin and lutoelin which are mainly found in vegetables such as celery , sweet red pipper , citrus fruits and herbs . **[37]**

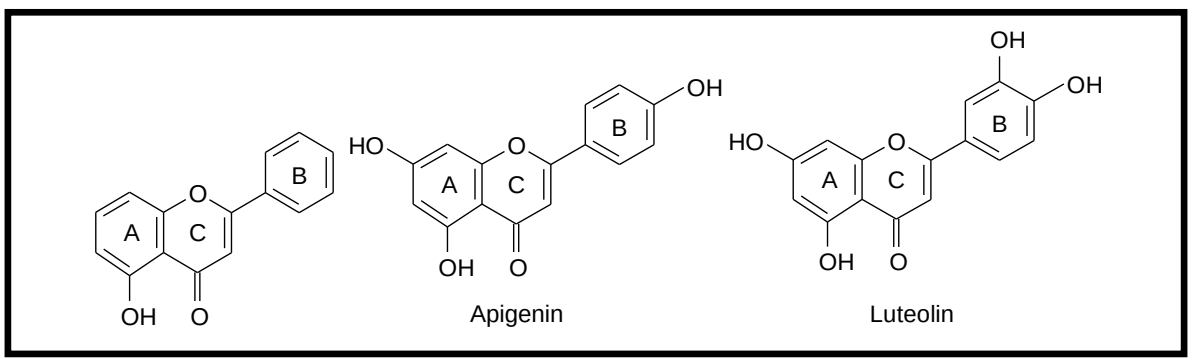


Figure II.5 : Molecule structure of flavones

3 - Flavan- 3-ols

Flavan- 3-ols represent the most common flavonoid , they are structurally the most complex subclass of flavonoids ranging from the simple monomers(+)-catechin and its isomer (-)-epicatechin to the oligomeric and polymeric proanthocyanidins which are also known as condensed tannin **[37,42]**

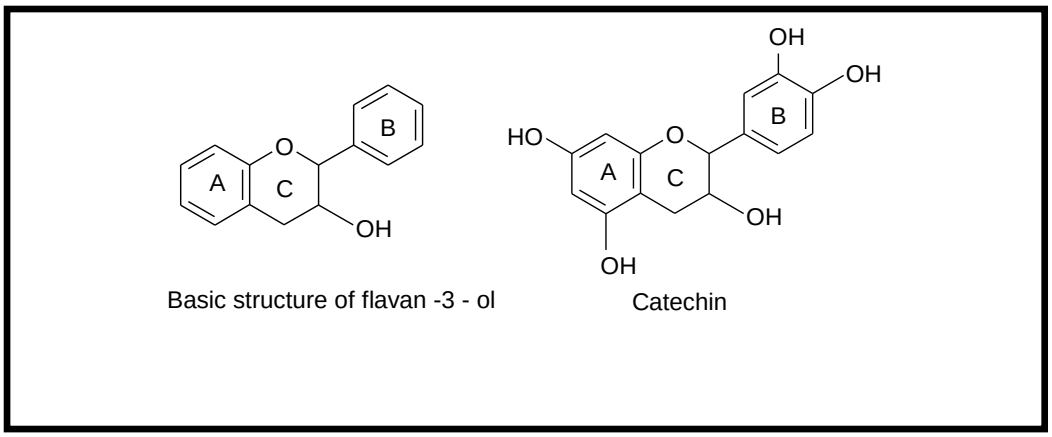


Figure II.6 : Molecule structures of flavan-3-ols

4 - **Flavanones**

Flavanones are characterized by the presence of a keto group at C4 and the absence of 2- 3 double bond .Flavanones have a center of asymmetry at C2 giving two stereoisomers which is of crucial importance of some biological activities . The most common flavanones are naringenin , eriodictyol and hesperitin **[37, 46].**

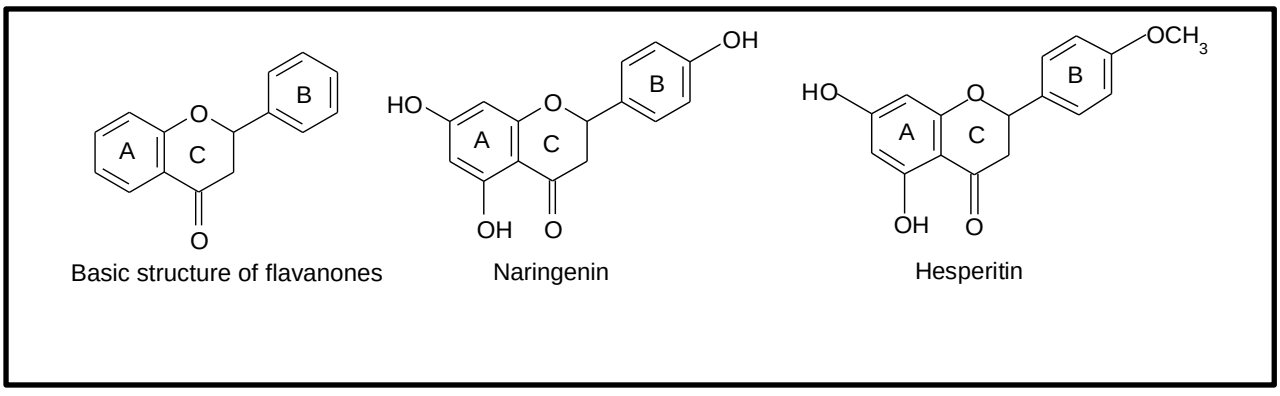


Figure II.7 : Molecule structures of flavanones

5 - Isoflavones

In contrast to most other flavonoids , isoflavones are characterized by having the B ring attached at C3 rather than the C2 position . They have a very limited distribution in the plant kingdom **[37]**. The most common isoflavone are genistien and diadzien **[30]**

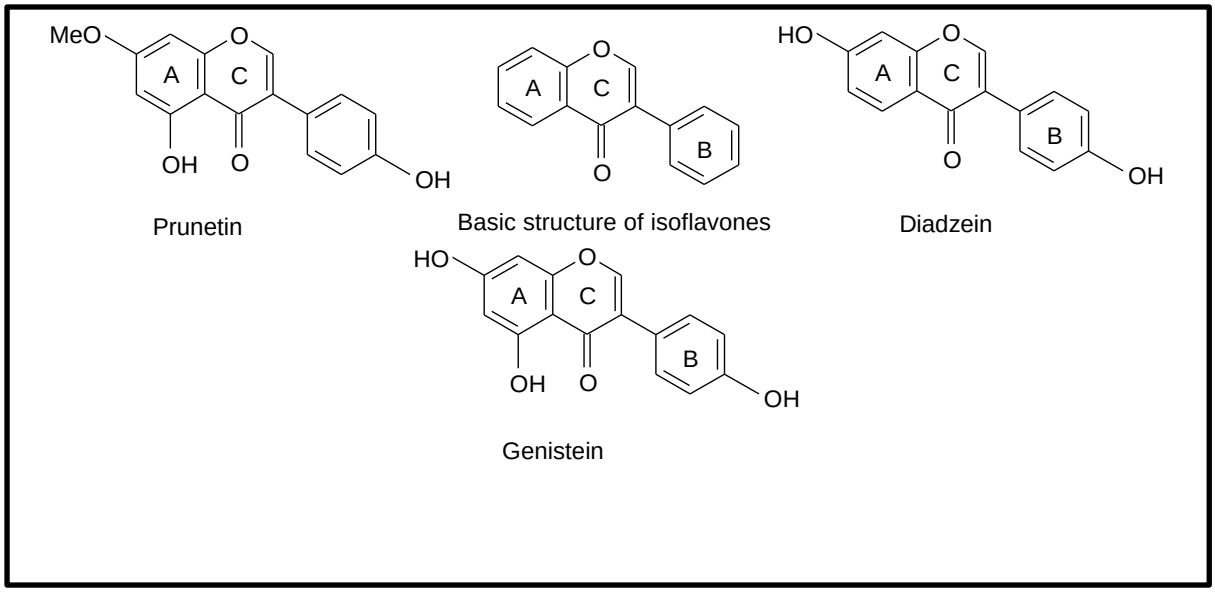


Figure II.8 : Molecule structures of isoflavones

6 - Anthocyanidins

Anthocyanidins are water soluble plant pigments and are particularly evident in fruit and flower tissue where they are responsible for a diverse range of red , blue and purple colors . They occur primarily as glycosides of their respective aglycone. Anthocyanindin chromophores, with the sugar moiety typically attached at the 3 position on the C ring or the 5 position on the A ring **[37]**

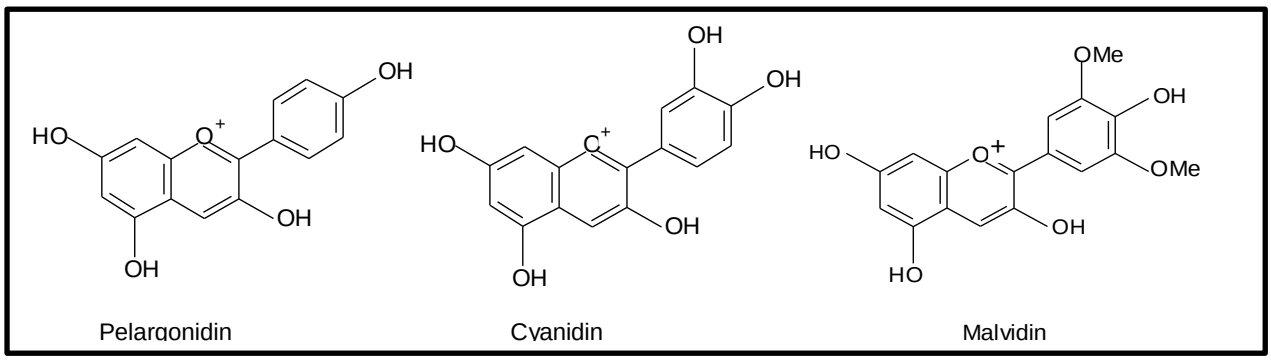


Figure II.9 : Molecule structures of anthocyanidins

b - In vitro antioxidant activity of flavonoids

Flavonoids can prevent injury caused by free radicals by the following mechanisms:

- ✓ Direct scavenging of reactive oxygen species (ROS) ,
- ✓ Activation of antioxidant enzymes ,
- ✓ Metal chelating activity ,
- ✓ Reduction of α – tocopheryl radicals ,
- ✓ Inhibition of oxidases ,
- ✓ Increase in uric acid levels ,
- ✓ Increase in antioxidant properties of low molecular antioxidants .

1- Direct scavenging of (ROS)

Flavonoids are able to scavenge free radicals directly by hydrogen atom donation , the in vitro flavonoid antioxidant activity depends on the arrangement of functional groups on its core structure . Both the conjugation and total number of hydroxyl groups substantially influence the mechanism of antioxidant activity .The B ring hydroxyl configuration is the most significant determinant of ROS scavenging , whereas substitution of the rings A and C has little impact on superoxide anion radical scavenging rate constants.

The main structural features of flavonoids required for efficient radical scavenging could be summarized as follows :

- An ortho – dihydroxy (catechol) structure in the B ring , for electron delocalization **[48]**
- 2,3 –double bond in conjugation with a 4 oxo function in the C ring provides electron delocalization from the B ring **[48]**
- Hydroxyl groups at positions 3 and 5 provide hydrogen bonding to the oxo group **[48]**

2- Ability to activate antioxidant enzymes

Other possible mechanism by which flavonoids act is through interaction with various antioxidant enzymes .Furthermore, some effects may be a reset of a combination of radical scavenging and the interaction with enzyme functions.

Flavonoids are able to induce phase II detoxifying enzymes (e.g. NAD(P)H-quinone oxidoreductase , glutathione S- transferase , and UDP –glucuronosyltransferase), which are the major defense enzymes against electrophilic toxicants and oxidative stress**.[48]**

3- Metal Chelating Activity

Specific flavonoids are known to chelate iron and copper , thereby removing a causal factor for the development of free radicals . Quercetin was able to prevent oxidative injury induced in the erythrocyte membrane by a number of oxidizing agents , which cause release of iron in its free , redox active form . The binding sites for trace metals in the molecule of flavonoids are the catechol moiety in the ring B , the 3-hydroxyl and 4-oxo groups in the heterocyclic ring C , and the 4-oxo and 5-hydroxyl groups between the C and A rings **.[48]**

4- Reduction of α – tocopheryl radicals

The α –tocopheryl represents a major antioxidant in cell membranes and in human low density lipoproteins (LDL) which protects lipoprotein particles against oxidative damage .Flavonoids can act as hydrogen donors to α –tocopheryl radical , which is a potential prooxidant . Furthermore, by interaction with α –tocopheryl radical , they possess a great potential to delay the oxidation of LDL .

5- Ability to inhibit oxidases

Flavonoids inhibit the enzymes responsible for superoxide ($O_2^{\cdot -}$) production , such as xanthine oxidase and protein kinase C . Quercetin and silybin inhibited xantine oxidase activity , thereby resulting in decreased oxidative damage .

6- Increasing in uric acid levels

Interestingly, there are great discrepancies between plasma or serum total antioxidant capacity and plasma concentrations of flavonoids . The large increase in plasma total antioxidant capacity observed after the consumption of flavonoid rich foods is not caused by the flavonoids themselves , but is likely the consequence of increased uric acid levels , which is a major contributor to plasma total antioxidant capacity .

7- Modification of prooxidant properties of low molecular antioxidants

Several authors described prooxidative activity of β-carotene under certain conditions (e.g. UVA irradiation) and suggested that its combination with an antioxidant may have preventive effect. The interaction of flavonoids with naringin ,rutin and quercetin on DNA damage induced by UVA mouse embryo fibroblasts . When each flavonoid was combined with β-carotene during preincubation , UVA induced cellular DNA damage was significantly suppressed .**[48]**

c- Anticancer activity

Flavonoids present in fruits , fruit juices and vegetables have been extensively studied for their potential role as anticancer agents . Cranberry juice, for instance , is largely consumed in North America for its virtues to fight urinary infections , a property due mainly to cranberry proanthocyanidins . Cranberry fruit is rich in phenolic compounds including flavonoids (flavonols ,proanthcyanidins , catechins or flavan-3-ols);a variety of phenolic acids among and triterpenoids . Early investigations have led to the conclusion that cranberry extracts inhibit LDL oxidation **[50],** oxidative damage to neurons and oxidative damage of the vascular endothelium . Therefore, it is believed that these antioxidant activities would make cranberry extracts a potentially good candidate for antitumour activity **.[50]**

Quercetin , one the most studied plant phenols and widely distributed in fruits and vegetables , has shown antiproliferative and antineoplastic activities *in vitro* against numerous cell lines .

d- Antitumor activity

Many researchers have conducted *in vitro* studies on the potential antitumor activity of flavonoids .for example , the antitumor activity on catechin , a flavanol present in green tea , *Areca catechu* , *Cinnamomum cassia* , *Polygonum multiflorum* , was examined using tumor invasion models . this particular flavonoid inhibited such invasiveness and it was suggested that the activity of catechin may be related to its ability to bind tissue type plasminogen activator (t-PA) to laminin , a molecule of extracellular matrix that play an important role during tumor cell adhesion , leading to partial inactivation of t-PA . *In vitro* studies have been made on the antiproliferative effect of *Citrus* flavonoids auercetin ,taxifolin , nobletin and tangeretin on human squamous cell carcinoma. Whereas nobletin and tageretin , which are

both polymethoxylated flavonoids , markedly inhibited carcinoma cell growth at all concentrations tested **.[51]**

e- Anti-inflammatory activity

Anti-inflammatory properties of flavonoids have been studied both *in vivo* and *in vitro* .many groups carried out *in vitro* research and showed that many flavonoids studied were capable of modifying the metabolism of platelet arachidonic acid . Some flavonoids , such as myricetin and quercetin , blocked both the cyclooxygenase and lipooxygenase pathways .

Flavonoids were demonstrated as possessing *in vivo* anti-inflammatory properties .some reports suggested that they had good anti-inflammatory activity without the ulcerogenic side effect of other anti-inflammatory drugs **.[51]**

f- Antiulcer effect

One of the most important side effect of conventional anti-inflammatory drugs is their ulcerogenic activity .Flavonoids are found to be good anti-inflammatory compounds and were also able to protect the gastric mucosa against a variety of ulcerogenic agents . Many studies were performed examining the antiulcerogenic actions of flavonoids using both naturally derived and synthetic compounds. Solon is a synthetic isoprenyl flavonoid derived from sophoradin , itself isolated from the traditional Chinese medicinal plant *Sophorasubprostat*. This compound showed good antiulcerogenic and gastro protective effects .

Other flavonoids that appear to exert antiulcerogenic activity are narnigin and quercetin .in experiment using a rat model of ethanol induced gastric ulcers , both of these flavonoids displayed significant antiulcerogenic effects **.[51]**

g- Antimicrobial activity

One of the undisputed functions of flavonoids is their role in protecting plants against microbial invasion . This not only involves their presence in plants as constitutive agents but also their accumulation as phytoalexins in response to microbial attack **.[54]** because of their widespread ability to inhibit spore germination of plant pathogens , they have been proposed also for use against fungal pathogens of man , there is an ever increasing interest in plant flavonoids for treating human diseases **.[54]**

The isoflavonoid maackiain (3-hydroxy-8,9-methylenedioxypterocarpan) is well known as a constitutive antifangal agent in heartwood of legume trees and as an inducible phytoalexin in herbaceous legumes , such as *Pisum sativum* and *Trifolium* spp .Haware and Stevenson(1999) have claimed it to be both constitutive and inducible in the plant *Cicer bijugum* , a wild relative of the chickpea *C. arietinum* .thus , two strains of *C.bijugum* , resistant to *Botrytis cinerea* infection.

The majority of flavonoids recognised as constitutive antifungal agents in plants are either isoflavonoids ,flavans or flavanones . The recognition that a flavones glycoside , namely luteolin 7-(2"-sulphatoglucoside), is an antifungal constituent of the marine angiosperm *Thalassia testudinum*is noteworthy **[54].**

Several recent papers report the regular presence of antibacterial activity among flavonoids .Thus, the retrochalcone ,licochalcone C (4,4'-dihydroxy-2'-methoxy-3'-prenyl) is active against *Staphylococcus aureus*. Also the compound 5,7-dihydroxy-3,8-dimethoxyflavone has a minimum growth inhibitory concentration of 50 μg ml^{-1} towards *Staphylococcus epidermis* .

Yet one further property of flavonoids that has been researched recently has been antiviral activity , most notably against the human immuno eficiency virus (HIV) , the causative agent of AIDS .some flavonoids are inhibitory to enzymes required for viral replication .the two biflavones robstaflavone and hinokiflavone , are active against HIV-1 reverse transcriptase . also ,quercetin 3-(2"-galloylarabinopyranoside) isolated from *Acer okamatoanum* ,is active against HIV-1integrase**.[54]**

h- Antidiabetic effects

Flavonoids , especially quercetin , has been reported to process antibetic activity . Vessal et al reparted that quercetin brings about regeneration of pancreatic islets and propably increases insulin release in strptozotocin induced diabetic rats **.[55].** also in another study , Hif and Howell reported that quercetin stimulate insulin release and enhanced Ca^{2+} uptake from isolated islets cell which suggest a place for flavonoids in non-insulin dependent diabetes**.[55]**

i- Antithrombogenic effects

Platelet aggregation plays a pivotal role in the physiology of thrombotic diseases .activated platelet adhering to vascular endothelium generate lipid peroxides and oxygen free radicals prostacyclin and nitrous oxide . It shown in the 1960s that tea pigment can reduce blood

coagulability , increase fibrinolysis and prevent platelet adhesion and aggregation . some flavonoids such as quercetin , kaempferol and myricetin were shown to be effective inhibitors of platelet aggregation in dogs and monkeys **[55].**flavonols are particularly antithrombotic because they directly scavenge free radicals , thereby maintaining proper concentration of endothelial prostcyclin and nitric oxide**.[55]**

2.5 - Occurrence of polyphenols in food

Polyphenols are ubiquitous in plant kingdom and practically all plant foods and beverages contain at least some amounts of these compounds **[22, 24, 26].** The richest sources are fruits , berries , vegetables , cereals , legumes , nuts and beverages such as wine , tea , coffee and cocoa . However, the types and amounts of compounds may vary greatly between different foods **[22].**

Out of two groups of phenolic acids , hydroxyl cinnamic acids , the latter is much more common **[22, 25].** The most common hydroxyl cinnamic acids are caffeic and ferilic acid **[22, 30].** Caffeic acid mainly occurs in esterified form with quinic acid forming chlorogenic acid. Caffeic acid and chlorogenic acid are present in very high amounts in coffee **[22, 30].** Ferrilic acid is present in food items rich in cereals **[22, 25, 31]**

Flavonols occur widely in fruits and vegetables as well as in beverages **[22, 25, 32, 33].** Quercetin , which is the most common flavonol , is especially rich in onions and kale **[22, 25].** flavan 3 ol subclass, which consist of both monomeric (catechins) and polymeric (proanthocyanindins)forms are one of the most ubiquitous flavonoids in plant foods **[22, 25].** Rich sources of flavan 3 ols are berries , nuts , dark chocolate , red wine , green and black tea . citrus fruits and citrus juices are the main sources of flavanones **[22, 25].**

Table II.2 : Primary food sources and their polyphenol content

Subclass	Individual compounds	Primary food sources	Polyphenol content
Hydroxybenzoic acids	Gallic , vanillic , syringic , p-hydroxybenzoic acid	Black currant Raspberry Strawberry	4- 13 6- 10 2- 9

Hydroxycinnamic acids	Caffeic , ferulic , p -coumaric , sinapic acid	Blueberry Coffee beverage	100 35- 175
Anthocyanindins	Cyaniding , delphinidin , malvidin , pelargonidin, peonidin , petunidin	Black currant Black grape Blackberries Cherry Rhubarb Strawberry	130- 400 30- 750 25- 500 35- 450 200 15- 75
Flavonols	Quercetin , kaempferol , myricetin , isorhammnetin	Apple Broccoli Cherry tomato Kale Leek Onion	2- 4 4- 10 1,5- 20 30- 60 3- 23 35- 120
Flavones	Luteolin , apigenin	Celery Parsley	2- 19 24- 184
Flavanones	Hesperitin , naringenin , eriodictyol	Grape fruits juice Lemon juice Orange juice	10- 65 5- 30 20- 70
Flavan- 3 ols monomers	Catechin , epicatechin , gallocatechin , epigallocatechin	Apple Apricot Green tea beverage	2 - 12 10 - 25 10 - 80

2.6 - Antioxidant activity of polyphenols

Recent interest in food phenolics has increased owing to their roles as antioxidant ,antimutagens , and scavengers of free radicals and their implication in the prevention of pathologies such as cancer and cardiovascular disease . Epidemiologic studies have shown a correlation between an increased consumption of phenolic antioxidant and a reduced risk of cardiovascular disease **[30, 55 -57].**

Phenolic antioxidants function as terminators of free radicals and chelators of metal ions that are capable of catalyzing lipid peroxidation .phenolics antioxidants interfere with the oxidation of lipids and others molecules by rapid donation of a hydrogen atom to radicals , as illustrated in the following reactions :

$$ROO^{\cdot} + PPH \longrightarrow ROOH + PP^{\cdot}$$

$$RO^{\cdot} + PPH \longrightarrow ROH + PP^{\cdot}$$

Moreover, the phenoxy radical intermediates are relatively stable ; therefore , a new chain reaction is not easily initiated . The phenoxy radical intermediates also act as terminators of the propagation route by reacting with other free radicals**;[30, 58].**

$$ROO^{\cdot} + PP^{\cdot} \longrightarrow ROOPP + PP^{\cdot}$$

$$RO^{\cdot} + PP^{\cdot} \longrightarrow ROPP$$

However, under certain conditions (high concentrations of phenolic antioxidants, high pH , presence of iron) , phenolic antioxidants can initiate an autooxidation process and behave like prooxidants **[30, 58].**

Flavonoids are among the most potent plant antioxidants because they possess one or more of the following structural elements involved in the antiradical activity :

1 – An o – diphenolic group (in ring B)

2 – A 2 – 3 double bond conjugated with the 4 – oxo function

3 – Hydroxyl groups in positions 3 and 5 **[30, 61 -63].**

Quercetin , a flavonol that combines all of these characteristics , is one of the most potent natural antioxidants . Also, the antioxidant efficiency of flavonoids is directly correlated with their degree of hydroxylation and decreases with the presence of a sugar moiety (glycosides are not antioxidants , whereas their corresponding aglycones are antioxidants) **[30, 58].**

Table II.3 : Antioxidant compounds and their antioxidant capacity

Antioxidant	Sources	Antioxidant activity (mM)
Vitamins		
Vitamin C	Fruit and vegetables	1.0 ± 0.02
Vitamin E	Grains , nuts and oils	1.0 ± 0.03
Flavonoids		
Anthocyanidins		
Cyanidin	Grapes , raspberries and strawberries	4.4 ± 0.12
Delphinidin	Aubergine skin	4.4 ± 0.11
Flavon – 3 –ols		
Quercetin	Onion , apple skin , berries , tea	4.7 ± 0.10
kaempferol	Grapefruit and tea	1.3 ± 0.08
Flavones		
Rutin	Onion , apple skin , berries , tea , black grapes	2.4 ± 0.12
Luteolin	Lemon , olive , celery and red pepper	2.1 ± 0.05
Chrysin	Fruit skin	1.4 ± 0.07
Apigenin	Celery and parsley	1.5 ± 0.08
Flavan – 3- ols		
Epicatechin	Black grapes / red wine	2.4 ± 0.02
Epigallocatechin	Teas	3.8 ± 0.06
Epigallocatechingallate	Teas	4.8 ± 0.06
Epicatchingallate	Teas	4.9 ± 0.02
Flavanones		
Taxifolin	Citrus fruit	1.9 ± 0.03
Naringenin	Citrus fruit	1.5 ± 0.05

Hesperidin Hesperetin	Orange juice Orange juice	1.0 ± 0.03 1.4 ± 0.08
Hydroxycinnamates Caffeic acid Ferulic acid P – coumaric acid	White grapes , olive , asparagus Grains , tomato , asparagus White grapes , tomato , asparagus	 1.3 ± 0.01 1.9 ± 0.02 2.2 ± 0.06

2.7 - Polyphenols and cancer

Anticarcinogenic effects of polyphenols are well documented in animals . Polyphenols , when given to rats or mice before and or after the administration of carcinogenic agent or the implantation of a human cancer cell line , are most often protective and induce a reduction of the number of tumors or of their growth **[37, 64].**These effect have been observed at various sites , including mouth , stomach , duodenum , colon , liver , lung , mammary , or skin. Many polyphenols , such as quercetin , catechins , isoflavones , lignans , flavanones , ellagic acid , red wine polyphenols , resveratrol , or curcumin , were tested ; all of them showed protective effects as anticarcinogenic **[37, 65].**

Polyphenols may act as blocking agents at the initiation stage or act as suppressing agents , and inhibit the formation and growth of tumors from initiated cells , they inhibit cell proliferation *in vitro* **[37, 58 , 63 , 56].**

2.7 - Polyphenols and diabetes

Many plants have been traditionally used in the treatment of diabetes. Polyphenols contained in these plants may explain some of their therapeutic activity **[37, 65 , 32].** The acute or chronic administration of polyphenols to experimental animals influences glycemia .Caffiec acid and isoferulic acid , when administred intravenously to rats , reduce the fasting glycemia and attenuate the increase of plasma glucose in an intravenous glucose tolerance test **[37, 50, 57].** These effects were observed in a generic model of insulin dependent diabetes of rats .

2.8- Antioxidants

Oxidative stress , induced by oxygen radicals is believed to be a primary factor in various diseases as well as in the normal process of aging [**32 , 33]** . The reactive oxygen species formed during normal metabolic processes can easily initiate the peroxidation of membrane lipids , leading to the accumulation of lipid peroxides **[48]**

A number of antioxidant defense mechanisms and repair systems exist in the body to protect against the damage and reduce diverse effect of free radicals , including intracellular antioxidant enzymes such as superoxide dismutase , which removes superoxide radicals by accelerating the formation of hydrogen peroxide ; glutathione peroxidase , converts hydrogen peroxide to water and catalase , which breaks down hydrogen peroxide **[62 ,63]** and non-enzymatic antioxidants like vitamin E , vitamin C , β- carotene and phenolic compounds .

2.8.1- Definition

Antioxidant is a chemical that prevents the oxidation of other chemicals , they protect the key cell components by neutralizing the damaging effects of free radicals , which are natural by products of cell metabolism **[41]**

2.8.2- Classification

Three enzymes are mainly responsible for the inhibition of oxidation stress in the organism

a- enzymatic antioxidants

1 – Superoxide dismutase (SOD)

Superoxide dismutase is present in cell cytoplasm (copper - zinc enzyme) and in mithochondria (manganese – enzyme) in order to maintain a low concentration of superoxide anion **[41, 48 , 61].** The determinant role of superoxide dismutase (SOD) in the antioxidant defense systems has been known since 1968 **[46].** It catalyzes the dismutation of superoxide anion into oxygen and hydrogen peroxide according to the following reaction :

$$2\ O_2^{- -} + 2\ H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Different studies have confirmed that the production of H_2O_2 under the action of SOD is the triggering factor in the natural antioxidant defense mechanisms . SOD , therefore , seems to be the key enzyme in the natural defense against free radical **[46]**

2 – Catalase

Catalase is a heme protein that catalyses the transformation of hydrogen peroxide (H_2O_2) into two water molecules and O_2 **[43 , 52, 12 , 53 - 55].**

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

Catalase provides a protective role that is similar to that of glutathione peroxidase because both are important means of removing hydrogen peroxide **[37, 51].**

3 – Glutathione peroxidase (GPx)

Glutathione peroxidase (Gpx) is a cytoplasmic and mitochondrial enzyme that is important for detoxifying peroxides in the cell **[48 , 55].** The GPx enzymes catalyze the reduction of H_2O_2 to water and organic peroxides (ROOH) to the corresponding stable alcohols (ROH) using glutathione (GSH) as a reducing source **[43 , 57].**

$$ROOH + 2\ GSH \longrightarrow ROH + GSSG + H_2O$$

b- Non enzymatic antioxidants

1- Ascorbic acid

Vitamin C or ascorbic acid is wide spread in nature but sparingly associated with fats of oils because of its hydrophilic nature **[36, 54].** Ascorbic acid in the free form , a water soluble molecule fig is present in intra and extracellular fluids but absent in membranes and lipid region . Vitamin C plays a role in free radical scavenging , particularly in lipid peroxidation inhibition **[30, 55].**

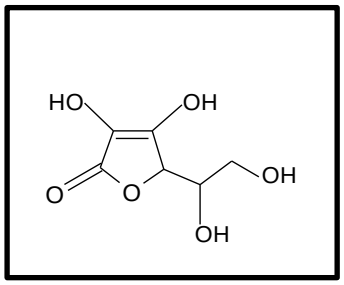


Figure II.10 : Chemical structure of ascorbic acid

In foods , water soluble ascorbic acid acts as a secondary antioxidant and participates in various antioxidative and related functions . Ascorbic acid is capable of quenching various forms of oxygen (singlet oxygen, hydroxyl radicals , and superoxide). In vivo ascorbic acid acts as a primary antioxidant and in tissues it is essential for the prevention of oxidative cellular damage by hydrogen peroxide **[30, 56].**

2- Carotenoids

Carotenoids are ubiquitously found in lipid soluble colored compounds , mainly from green plants , fruits and vegetables . The two main classes of carotenoids , carotene and xanthophylls , are composed of 40 carbon isoprenoid or tetraterpenes with varying structural characteristics . carotenes are polyene hydrocarbons and vary in their degree of unsaturation (e.g B carotene , lycopene).Beta carotene is the most abundantly found provitamin A . Many fats and oils especially those from plant sources , contain b carotene and it contributes to the deep intense orange red color of many oils **[30,57, 58].**

Carotenoids can act as primary antioxidants by trapping free radicals or as secondary antioxidant by quenching singlet oxygen **[30, 57, 58].** Carotenoids may trap free radicals and act as a chain breaking antioxidant **[30, 56, 58]**

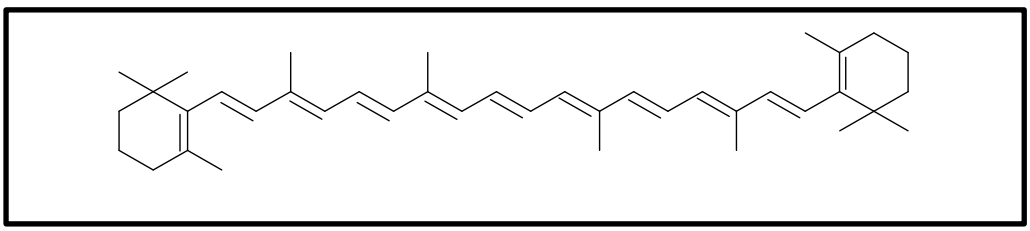


Figure II.11 :Chemical structure of β- carotene

3- Vitamin E

Tocopherols are the natural antioxidative compounds found widely in different tissue , they are found abundantly in vegetable oil derived food . These mono phenolic compounds possess varying antioxidant activities . Tocopherols and tocotrienols comprise the group of chromanol homologs that exert vitamin E activity in diet . These different homologs vary in the extent of methylation of the chromane ring . The α , β , γ and δ-tocopherols contain a saturated phytyl (

trimethyltridecyl) side chain . the antioxidant activity of α , β , γ and δ-tocopherol decreases in order of α > β > γ > δ in vivo **[30, 62, 63].**

Antioxidant activity of tocopherols is mainly by scavenging peroxy radicals thus interrupting chain propagation **[30, 57],** which is based on the tocopherol- tocopherylquinone redox system . The active configuration is the phenolic group in the bezene ring , located at the para position to the oxygen atom bound next to the dihydropyrone cycle . Alpha tocoperol donates a hydrogen atom to a peroxy radical resulting in α-tocopheryl semiquinone radical **[30].**This radical may further donate another hydrogen to produce methyl tocopheryl quinone or react with another tocopheryl semiquinone radical to produce an α-tocopherol dimer **[30, 61].**

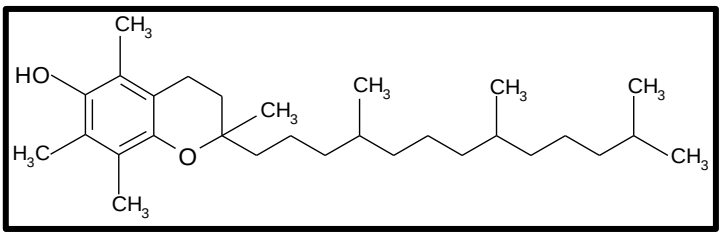


Figure II.12 : Chemical structure of α-tocopherol

4- Polyphenols

Polyphenols are wide and complex group of secondary plant metabolites **[22 , 24 ,26]** . So far , over 8000 compounds have been identified . Structures of the compounds range from simple molecules such as phenolic acids, to highly polymerized compounds like proanthocyanidins .

Polyphenols are essential for the physiology of plants , having functions in growth, structures , pigmentation , pollination , allelopathy and resistance for pathogens , UV radiation and predators **[29, 23]** , in addition , sensory qualities of plant foods and beverages , such as astringency and bitterness are related to their polyphenol content **[10]**

In nature, polyphenols occur primarily in conjugated forms with one or more sugar residues attached to hydroxyl groups **[29, 25, 26]** conjugation increase the polarity of the molecules , which is necessary for storage in plant cell vacuoles . The most common sugar residue is glucose, and residue can be in the form of monosaccharaides or oligosaccharides.

2.8.3- Methods for testing antioxidant activity

A number of techniques have been developed to measure the activity of antioxidant. Some techniques are based on a chemical reaction , while others make use of a biological response .

a- TEAC Trolox equivalent antioxidant activity

The TEAC assay is based on the oxidation induced decolourisation of radical cation ABTS (2,2 azinobis , 3 ethylbenzothiazoline , 6 , sulphonic acid), and determine the ability of hydrophilic H^+ donating antioxidants to scavenge the $ABTS^+$ radical compared with Trolox (a water soluble analogue of vitamin E) **[42, 47]**

The TEAC **[36, 47 , 63]** is equal to the millimolar concentration of a trolox solution having the antioxidant capacity equivalent to a 1 Mm solution of the substance under investigation . as used by Rice Evans and Miller **[36, 47]** , the TEAC reflect the relative ability of hydrogen or electron donating antioxidants to scavenge the ABTS radical cation compared with that of trolox .

The ABTS assay **[37, 48]** has been used to measure the total antioxidant activity in pure substances , in body fluid and in plant material.

b- FRAP assay

The FRAP assay is simple , inexpensive and robust and provides an index of antioxidant activity **[61, 64]** . It describes the ability of a compound to reduce Fe^{3+} to Fe^{2+} , this reduction is accompanied by the formation of a blue colour in the presence of ferric tripyridyltiazine (Fe^{3+} – TPTZ) complex .

Results are obtained as absorbance increases at 593 nm and can be expressed as micromolar Fe^{2+} equivalent or relative to an antioxidant standard **[36]** . The method was originally applied to plasma but has been extended to other biological fluids , foods , plant extracts , juices , etc **[36] .**

c- TRAP Total Radical Trapping Antioxidant Parameter

The total radical trapping antioxidant parameter (TRAP) assay has been widely used to determine total antioxidant activity based on measuring oxygen consumption during controlled lipid oxidation reaction . **[36]** . The TRAP expresses results as the number of µ

moles of peroxyl radicals trapped by 1 l of plasma . The measurement of serum TRAP was based on the determination of the length of time that a subject's serum was able to resist artificially induced oxidation **[36 , 64].**

d – ORAC Oxygen Radical Absorbing Capacity Assay

The ORAC assay is another commonly applied antioxidant assay based on the ability of a test substance to inhibit the oxidation of B‑phycoerythrin by reactive oxygen species , relative to trolox **[30]**

e– β – carotene bleaching assay

This assay developed by Taga and others (1984) , is based on the coupled oxidation of β – carotene and linoleic acid . The method estimates the relative ability of antioxidant compounds to scavenge the radical of linoleic acid peroxide ($LOO^{\cdot}$) that oxidizes β – carotene in the emulsion phase . **[66]**

f- 2 , 2 Diphenyl -1 - picrylhydrazyl radical (DPPH) assay

This method is based on the radical scavenging activity of antioxidants toward the 2 , 2 Diphenyl - 1 - picrylhydrazyl radical ($DPPH^{\cdot}$) . the free radical $DPPH^{\cdot}$ Is reduced to the corresponding hydrazine when it reacts with hydrogen donors .this reaction has been evaluated by the discoloration assay where the decrease in absorbance at 515‑ 528 nm produced by the addition of the antioxidant to $DPPH^{\cdot}$ In methanol or ethanol is measured **[64]**

In plant, polyphenols are relatively resistant to heat oxygen, dryness and to some extent also to acidity, but the sensivity to light differs according to their chemical structure **[22, 26 ,28]**

Table II.4 : List of in vitro antioxidant methods

Antioxidant methods	
Hydrogen atom transfer methods (HAT)	Electron transfer methods (ET)
➢ Oxygen absorbance capacity (ORAC) method ➢ Lipid peroxidation inhibition capacity (LPIC) ➢ Total radical trapping antioxidant	➢ Trolox equivalent antioxidant capacity (TEAC) ➢ Ferric reducing antioxidant power (FRAP) ➢ DPPH free radical scavenginf assay

parameter (TRAP) ➢ Inhibition oxygen uptake (IOC) ➢ Scavenging of H_2O_2 radicals ➢ ABTS radical scavenging method ➢ Scavenging of super oxide radical formation by alkaline (SASA)	➢ Copper (II) reduction capacity ➢ Total phenols by folin Ciocalteu ➢ N,N –dimethyl –p-Phenyllenediamine (DMPD) assay

.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III : MATERIALS AND METHODS

Natural substances from plants have multiple interests put to use in the industry: supply, in cosmetology and dermo pharmacy. Among these compounds are found in a large measure the secondary metabolites which are illustrated especially in therapy. Pharmacy still uses a high proportion of plant-derived medicines and research found in plants of new active molecules, or raw materials for the semi-synthesis. [1]

3.1- Harvesting of the plant

The plants are harvested during winter of the year 2010 of three stations in the wilaya of Bechar (Algeria) **(Fig. III.1).** The chemical study was performed on the aerial part of the plants .The leaves of the plant *Launeae nudicaulis* and *Warionia saharae* were cut and dried in the open air in a dry place and away from direct sunlight, and then finely ground.

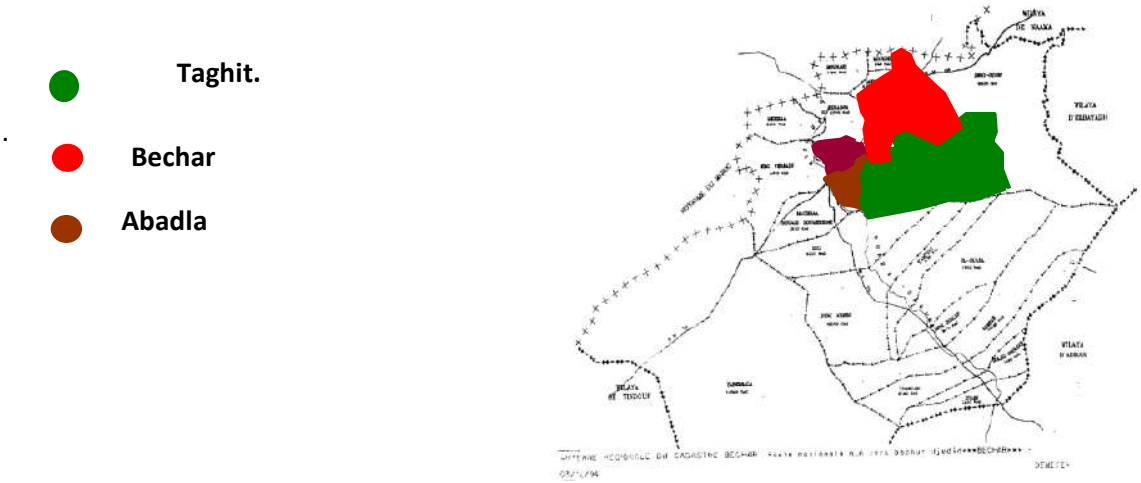


Figure III.1 : Map of the province of Bechar showing the three collection stations. [2]

3.2- Presevation

The plant *Launeae nudicaulis* and *Warionia saharae* freshly harvested, are dried in the shade in a dry and ventilated area for about ten days. Dried parts of the plants are cut then finely grinded and stored in vials in the shelter of the light.

3.3- Geographical situation and crop stations

The plants are harvested at three stations in the province of Bechar (Abadla, Taghit , Boukais and Bechar). Geographic and bioclimatic characteristics of crop areas are summarized in the table below .

Table III.1: Geographic and bioclimatic characteristics of harvesting areas [4].

Region	Altitude	Latitude	Longitude	Bioclimatic characteristics
Bechar	747 m	31° 37' N	2° 13' w	Hot and dry in summer, cold in winter
Taghit	747 m	33° 15' N	1° 47' w	Hot and dry in summer, cold in winter

3.4- Plant material

The aerial part (leaves) of *Launeae nudicaulis* and *Warionia sahara* were harvested in December 2010 from Bechar ,Algeria . Plant samples were dried at room temperature for 03 weeks , than finely ground with an electric grinder .

Figure III.2 : *Launeae nudicaulis* plant

Figure III.3 : *Warionia Saharae* plant

3.5- Phytochemical screening

3.5.1- Plant products exhausted with dilute HCL

3 g of plant material is placed in a beaker, diluted with 50 ml of HCl and the assembly is brought on a hotplate for 15 min, then the mixture is filtered to the following tests:

a-Alkaloids

alkalizing the solution with ammoniac (NH_4OH) until PH= 9-10 , extracting the solution three times with 30ml chloroform ($CHCl_3$) then evaporating of organic phase and add 2-3 drops of Mayer's reagent ; the appearance of white precipitate indicated the existence of alkaloids

3.5.2- Plant products exhausted with distilled water

3g of plant material in the presence of 20 ml of distilled water, bring together on a hot plate for 10 minutes at a temperature below the boiling temperature, filtering the mixture then subjecting the extract to following tests.

a- Saponosids

After cooling the solution , put 10ml of the solution in assay tube, agitate well for one minute and let stand a few seconds and measure the level of the foam.

b- Cardinolids

3g of plant material extract macerated in 20 ml of distilled water for 24 hours. Filter the solution and then extract a 10ml of the filtrate with 10 ml of chloroform / ethanol , evaporate the organic phase and the residue dissolved in 3 ml of glacial acetic acid, add a few drops of $FeCl_3$ and 1 ml of concentrated sulfuric acid. The appearance of the blue green color in the acetic phase indicates the existence of cardinolids.

3.5.3- Product macerated with 70% ethanol

Put 5g of vegetable matter in a bottle with 20 ml of ethanol let macerate for 2 days, then filter the mixture , filtrate was submit the following tests:

- **Steroids**

After evaporation of the solution, dissolve the residue with 15 ml of $CHCl_3$ and filtered several times (3 times) . The filtrate was divided into two parts in two test tubes:

1- First Part

Add 1ml of acetic acid CH_3CO_2H then 1ml of concentrated sulfuric acid H_2SO_4 on the walls of the tube carefully.

The absence of the green color indicates the presence of unsaturated sterols

2- Second Part

Add equal volume of concentrated H_2SO_4 to the walls of the tube carefully the appearance of yellow color which converts red indicates the existence of steroid

3.5.4- Product macerated with diluted HCl

Put in a flask 5 g of plant material with 75ml of dilute hydrochloric acid HCl let macerated for 2 days, filtering the mixture then subjecting the filtrate to the following tests:

-Flavonoids

1- alkalize 10ml filtrate by ammonia NH_4OH the appearance of light yellow color indicates the presence of flavonoids.

2- A 10ml of the filtrate add 5 ml of amyl alcohol, the alcohol phase turns yellow indicates the presence of free flavonoids.

3- Evaporated under vacuum the aqueous phase 1 test dissolve the residue diluted with 3 ml of HCl and heated slightly and then cooled to perform the following tests:

- ✓ Add 2.5 ml of amyl alcohol, the appearance of the yellow color indicates the presence of glycosides flavonoid
- ✓ Add a small amount of Mg in acid extracted the appearance of the red color indicates the presence of flavonoid glycosides.

3.6- Extraction of plant materials

This step consist to extract the maximum of phenolic compounds contained in the leaves of *Launeae nudicaulis* and *Warionia sahara* using organic solvents that accelerate and increase the extraction efficiency .

3.6.1- Reflux extraction

The extraction is performed by successive exhaustion of plant material using different solvents .

***a- Launeae nudicaulis* extraction**

The extraction is performed by using the mixture of solvent (water – MeOH) , 200g of plant material (*Launeae nudicaulis*) were extracted with 350 ml of solvent over 03 hours . the **Figure III.3** illustrates the extraction steps .

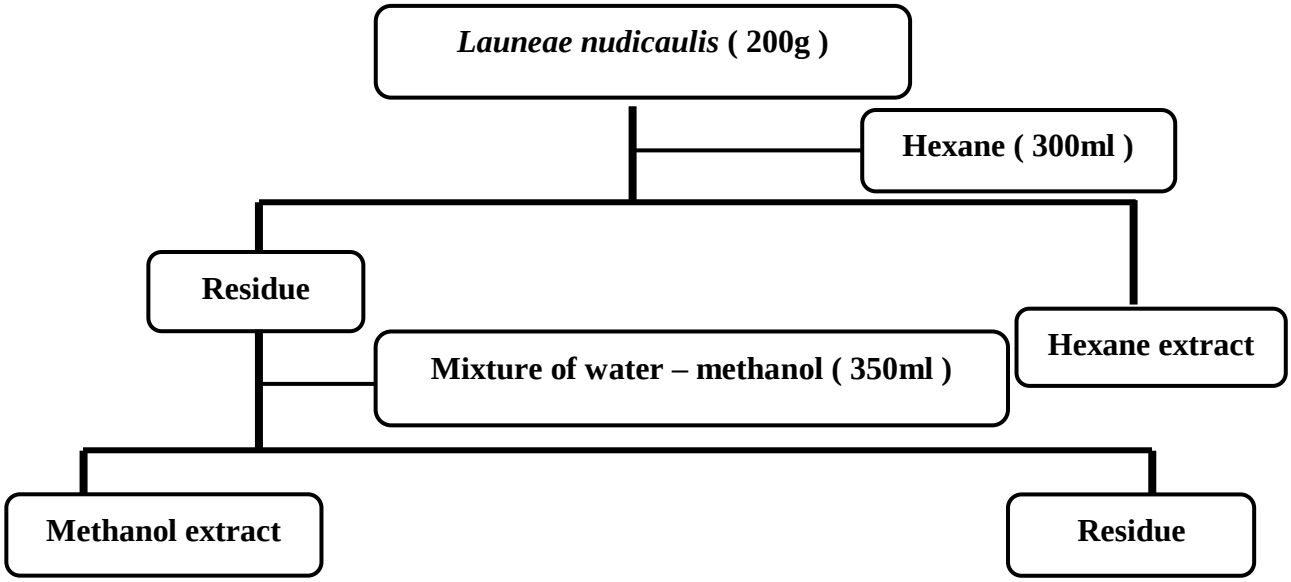


Figure III.3 :*Launeae nudicaulis* Extraction Protocol

b- Warionia saharae extraction

The extraction is performed by successive exhaustion of plant material (reflux extraction) using five solvents with increasing polarity : ether , chloroform , ethyl acetate , butanol and methanol . 50 g of plant material (*Warionia saharae*) was extracted with 300 ml of solvent over 03 hours , after the filtration , the residue is concentrated by rotary evaporation of the solvent in a rotavap .

3.6.2 -Filtration

Filtration is used to separate impurities in solution or suspension through a filter medium , To separate the extract from plants material , filtration was carried out using a filter paper (Whatman).

3.6.3-Rotary evaporation

Separation of solvent from the extract is realized by using the device called rotavapor, in this apparatus the extract is put in around flask and placed in the device. The rotavap spins and heats the flask and reduces the pressure inside by greating a vaccum , the vacuum makes the solvent evaporate and becomes a gas which is collected by cooling it , so it condenses into another flask

3.6.4-Determination of the yield of extraction

The weight of dry extract is determined by the difference between the weight of full bottle (after removing the solvent by rotary evaporation) and the weight of empty bottle.

$$M_{extract} = \text{Mass of full bottle} - \text{Mass of empty}$$

The yield of extraction is the percentage of the mass of dry extract and the mass of plant material.

$$Y = \frac{\text{Mass of dry extract}}{\text{Mass of plant material}} * 100$$

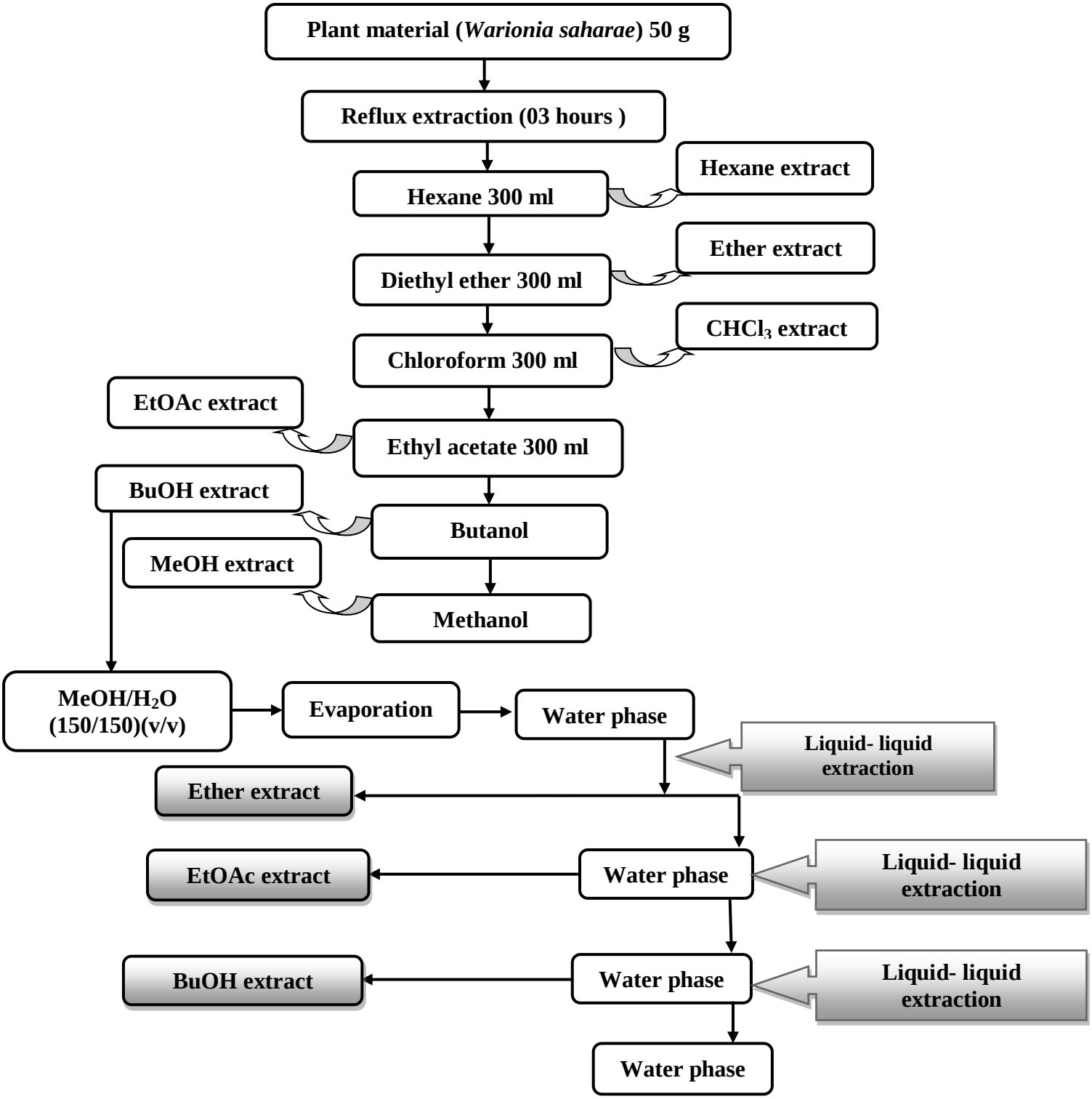


Figure III.5 : *Warionia saharae* Extraction protocol

3.7- Thin layer chromatography analysis of extracts

TLC is an easy , cheap , rapid and basic method for the analysis and isolation of metabolites . it involves the use of particulate absorbent spread on inert sheet of glass , plastic or metal as stationary phase . The mobile phase is allowed to travel up the plate carrying the sample that was initially spotted .

3.7.1-Stationary phase

We used the silica gel (GF254) thin layer chromatography aluminum plate . It is prepared by drawing a line with a pencil 1cm from the edge which is called deposition line , and a line 0,5 cm from the top called stop line .

3.7.2-Mobile phase

The mobile phase is constituted a mixture of organic solvents, for that different solvent systems were tested to choose who given the best separation.

a- Launeae nudicaulis mobile phase

For each extract we use a solvent system as a mobile phase then we choose a solvent which have the best separation, the table shown the solvent system and the solvent system selected for each extact .

Table III.2: Solvent system and the solvent system selected of each extract

Extract	Solvent system	Solvent system selected
Ether	Chloroform／methanol (3／7)	Chloroform／methanol (3／7) Acetone ／hexane (1／1) Hexane ／ethyl acetate (1／1)
	Chloroform／methanol (7／3)	
	Chloroform／methanol (1／1)	
	Methanol	
	Acetone ／hexane (1／1)	
	Chloroform/ ethyl acetate (1/1)	
	Hexane ／ethyl acetate (1／1)	
	Chloroform	

Chloroform	Chloroform／methanol (3／7)	Chloroform／methanol (3／7) Acetone ／hexane (1／1) Acetone／hexane (1／1) Hexane ／ethyl acetate (1／1)
	Chloroform／methanol (3／7)	
	Chloroform／methanol (1／1)	
	Methanol	
	Acetone ／hexane (1／1)	
	Chloroform/ ethyl acetate (1/1)	
	Acetone／hexane (1／1)	
	Hexane ／ethyl acetate (1／1)	
	Dichloromethane	
Ethyl acetate	Chloroform／methanol (3／7)	Chloroform／methanol (3／7) Chloroform／methanol (1／1) Acetone ／hexane (1／1) Hexane ／ethyl acetate (1／1)
	Chloroform／methanol (1／1)	
	Methanol	
	Acetone ／hexane (1／1)	
	Chloroform	
	Hexane ／ethyl acetate (1／1)	
Butanol	Chloroform／methanol (3／7)	Chloroform／methanol (3／7) Chloroform／methanol (1／1) Acetone ／hexane (1／1) Hexane ／ethyl acetate (1／1))
	Chloroform／methanol (1／1)	
	Methanol	
	Acetone ／hexane (1／1)	
	Chloroform	
	Hexane ／ethyl acetate (1／1)	
	Dichloromethane /methanol (1/1)	
	Acetone/water (1／1)	
Methanol	Chloroform／methanol (3／7)	Chloroform／methanol (3／7) Chloroform／methanol (1／1) Acetone ／hexane (1／1) Hexane ／ethyl acetate (1／1)
	Chloroform／methanol (1／1)	
	Methanol	
	Acetone ／hexane (1／1)	
	Chloroform	
	Hexane ／ethyl acetate (5／5)	
	Dichloromethane /methanol (1/1)	

b- ***Warionia sahara*** **mobile phase**

Different solvent system were assayed to give the best separation for each plant extract, the table present the solvent system selected of each extract .

Table III.3: Solvent system and the solvent system selected of each extract

Extract	**Solvent system**	**Solvent system selected**
Ether	Chloroform/methanol (3/7)	Hexane/ ethyl acetate (1⁄1) Chloroform/ ethyl acetate (1⁄1)
	Hexane/ ethyl acetate (1/1)	
	Chloroform / ethyl acetate (1/1)	
	Chloroform// ethyl acetate /acetone/methanol (2/2/1/0.5)	
	Dichloromethane (100%)	
	Chloroforme (100%)	
chloroform	Acetone (100%)	Hexane/ ethyl acetate (1/1) Chloroform/ ethyl acetate /acetone/methanol (2/2/1/0.5)
	Chloroform/methanol (3/7)	
	Hexane/ ethyl acetate (1/1)	
	Chloroform// ethyl acetate (1/1)	
	Chloroform/ethyl acetate /acetone/methanol (2/2/1/0.5)	
	Dichloromethane (100%)	
Ethyl acetate	Chloroform/acEtone (1/1)	Chloroform/acetone (1/1) Hexane/ ethyl acetate (1/1)
	Chloroform/methanol (1/1) et (7/3)	
	Hexane/ /ethyl acetate (1/1)	
	Chloroform/ethyl acetate /acetone/methanol(2/2/1/0.5)	
	Chloroform (100%)	
Butanol	Chloroform/ ethyl acetate /acetone/methanol (2/2/1/0.5)	Chloroforme (100%) Hexane /acétate d'éthyle (1/1)
	Chloroform/methanol (7/3)	

	Hexane // ethyl acetate (1/1)	Chloroforme/methanol (7/3)
	Dichloromethane /methanol (1/1)	
	Chloroforme (100%)	
Méthanol	Chloroforme/ ethyl acetate acetone/methanol (2/2/1/0.5)	Chloroforme/ ethyl acetate /acetone/methanol (2/2/1/0.5)
	Chloroform/methanol (7/3)	Hexane / ethyl acetate (1/1)
	Hexane // ethyl acetate (1/1)	Chloroform/methanol (9/1)
	Dichloromethane /methanol (1/1)	
	Chloroform (100%)	

3.7.3-Deposit

The deposit is made with capillary glass tube for single use, linearly and perpendicular.

3.7.4-Revelation

If the components are colored, they will be directly by visible on the plate, otherwise the revelation can be done with UV lamp at 254 nm .

3.7.5-Retention factor

For each spot was calculated retention factor which is the relationship between the distance traveled by the solvent front and the substances.

$$\mathbf{Rf} = \frac{\textbf{Distance traveled by substance}}{\textbf{Distance traveled by solvent front}}$$

3.8- Isolation of natural compounds of the plant extracts

a- Warionia saharae

A portion of 2 ml of each extract was chromatographed through a silica gel column Sephadex LH 20 Sigma on a mass of 62.3 g, and the elution realized with 300 ml of solvent; the column elution was performed by the best elution of each extract.

Fractions of 3 ml are collected and analyzed on thin layer chromatography plates , these fractions are examined under UV light at 254 and 365 nm.

The fraction or separated product which have the same Rf are collected then calculate its mass and efficiency .

a- Launeae nudicaulis

5g of methanolic extract was chromatographed through a silica gel(50 g) column and elution realized by petrol ether where the polarity was increasing by addition different gradient of solvents (ether , $CHCl_3$, MeOH and EtOAc) .

3.9- Identification of natural compounds of the plant extracts

3.9.1- UV spectroscopy analysis

The UV spectrum of a flavonoid is usually determined in ethanol or methanol and typically consists of two maximum absorptions located in the ranges of 240-285 nm (band II) mainly due to the absorption of the chromophore system of ring B , and 300- 350 nm (band I; due to absorption of the B ring) .

The specific positions and relative intensities of these maximums give precious information concerning the nature of flavonoids and types of hydroxyl or methoxyl substituents for example flavone .

a- Band I

1- Bathochromic effect

Bathochromic effect is also called the red shift; this is a change of the maximum absorption at the longer wave length. It can be produced by a change of the environment or by the presence of an auxochrome.

2- Hypsochromic effect

Hypsochromic effect also called blue change; it is a change towards shorter wavelength.

b- Band II

The effects observed in band II is less intense than in the band I , we can still distinguish the two effects .

1- Bathochromic effect

It can be attributed as the band I , the position and the number of hydroxyl group in the flavone.

2- Hypsochromic effect

While the band II is much more difficult to be changed to the longer wavelength than band I; it has an unexpected property :

When the methoxy group introduced to the 3' and 4' positions of the ring B and the flavone has a hydroxyl group at the 7-position , The band II is changed to the UV - C 237 nm range .

When the methoxy group introduced at three positions of the B ring. Band II may disappearing by a bathochromic effect.

3.9.2- Method of classification and identification of flavonoids

According to the absorption movement concepts of band I and II by the bathochromic and hypsochromic effects and correlation between the spectroscopic data of the literature and the results of UV - VIS spectroscopic analysis of isolated products , the of structures these compounds which belongs to the class of flavonoid can be determined.

Band II give important informations about the classification of flavonoids.

Band I accurate the position and type of substituents in ring A and B.

3.10- IR spectroscopy analysis

Infrared spectroscopy is often used to identify structures because functional groups give rise to characteristic bands both in term of intensity and position .

The samples are examined as a pressed disc , mixing a few milligrams of the substances with a few milligrams of KBr , the infrared radiation are located in the portion of the electromagnetic spectrum between the visible region and that of the microwave

3.11- Measurement of antioxidant activity of plant extracts and their compounds

3.11.1- Beta carotene bleaching assay

1- Extraction of beta carotene

20 g of carrot were mixed with 40 ml of ethanol and potassium hydroxide, which are homogenized for three minutes . The mixture was extracted under reflux for 45 minutes , then cooled at room temperature , the mixture was agitated frequently to avoid any aggregation .

We realized a liquid extraction with 50 ml of hexane , the organic phase was removed with pipette and aqueous phase was extracted with 50 ml of hexane , the extract has been washed with distilled water then filtered through anhydrous sodium sulfate . The residue was removed at reduced pressure at 45 C using rotary evaporation .

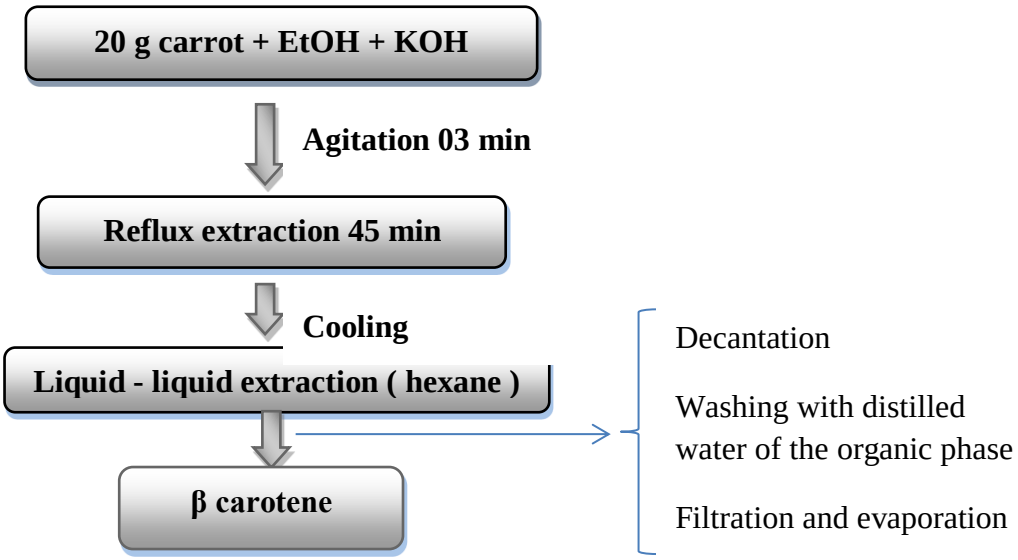


Figure III.8. Protocol of β – carotene **extraction**

2- Beta carotene bleaching assay

The beta carotene is a lipophilic antioxidant that protects fatty acids from the oxidation , the addition of a second antioxidant permits its preservation .

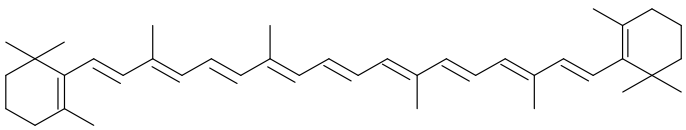


Figure III.9. β-carotene structure

The qualitative assay was realized by the dilution of two milligrams of the dried extract with 1 ml of the appropriate solvent , then a quantity of each dilution of the chloroform, ether, ethyl acetate, methanol and butanol extracts was carefully loaded individually onto the TLC plate and the sample was allowed to dry . the plate was sprayed with chloroformic solution of beta carotene , the plate is exposed under UV lamp at 254 nm until the discoloration , the antioxidant area appears yellow on white .

3.11.2- DPPH radical scavenging method

1- TLC assay

The qualitative assays were performed according to the method of Takao et al (1994). Two milligrams of the dried extract were diluted with 1 ml of the appropriate solvent then, 20(µL) aliquot of each dilution of the chloroform, ether, ethyl acetate, methanol and butanol extracts was carefully loaded individually onto the TLC plate and the sample was allowed to dry. Once dried, the plate was sprayed with 0.2% solution of radical DPPH in methanol. Compound with radical scavenging activity showed a yellow on purple spot due to the discoloration of DPPH.

2- Spectrophotometer

The antioxidant activity of the crude extracts and fractions was assessed by the mean of 2,2-diphenyl -1-picrylhydrazyl (DPPH·**)** colorimetric method as described by Velazquez et al (2003), slightly modified . This method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine and the remaining DPPH·**,** which showed maximum absorption at 517 nm was measured (spectrophotometer). About 2ml of a 20 mg / ml DPPH solution were added to 1Ml of a methanolic solution of each extracts and fractions (1-100µg/ mL). a mixture of 2 ml of DPPH and 1 ml of methanol served as control . the mixture was shaken vigorously , then incubated for 15 min in darkness at room temperature . absorbance was measured at 517 nm , each experiment was performed in triplicate . The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPHradicalscavengingactivity}\ (\%) = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}}$$

A_{sample} : bsorbance of sample

$A_{control}$: absorbance of control

Figure III.4 : Spectrophotmeter

3.12- Antibacterial activity of plant extracts (Disc diffusion method)

The disc diffusion method has been carefully standardised by the Clinical Laboratory Standard Institute (CLSI) methodology (CLSI 2006a, 2006b).

- ✓ A sterile cotton swab was dipped into the suspension and inoculated evenly over the entire surface of the medium by rotating the plate.

- ✓ The discs containing different concentration of extracts and oils were applied (5 and 10 µl) to the plates individually using sterile forceps and then gently pressed down onto the agar. Generally, no more than 4 disks were placed on a 90-mm plate .

- ✓ This prevents overlapping of the zones of inhibition and possible error in measurement. After the disks were placed on the plate, the plate was inverted and incubated at 35°C for 16 to 24 h dependent on strains being tested. After incubation, the diameters of the zones of complete inhibition were measured and recorded in millimetres (mm).

Chapter IV

Results and discussion

1- Phytochemical screening results

a. *Launeae nudicaulis* exhausted with diluted HCl , distilled water , 50% ethanol and $CHCl_3$

The phytochemical screening with exhaustion of *Launeae nudicaulis* indicates the presence of saponin , tannin an unsaturated sterol . Mayer test did not show the existence of alkaloid in *Launeae nudicaulis* .

Table IV.1 : Phytochemical screening with exhaustion of *Launeae nudicaulis*

Compound family	*Launeae nudicaulis*
Alkaloid	-
Saponin	++
Tannin	++
Unsaturated sterol	++

b. *Launeae nudicaulis* macerated in distilled water , diluted HCl and 70% ethanol

Phytochemical survey with maceration of *Launeae nudicaulis* allows the presence of cardenolid , steroid, flavonoids, free flavonoid and glycosylated flavonoid are predominantly in *Launeae nudicaulis* .

Table IV.2 : Phytochemical screening with maceration of *Launeae nudicaulis*

Compound family	*Launeae nudicaulis*
Cardenolid	+
Steroid	+

Flavonoid	+++
Free flavonoid	++
Glycosylated flavonoid	+++

c. *Warionia saharae* exhausted with diluted HCl , distilled water , 50% ethanol and $CHCl_3$

The phytochemical screening with exhaustion of *Warionia saharae* shows the presence of saponin , tannin and unsaturated sterols .However alkaloids did not exist in *Warionia saharae.*

Table IV.3 : Phytochemical screening with exhaustion of *Warionia saharae*

Compound family	***Warionia saharae***
Alkaloid	-
Saponin	++
Tannin	++
Unsaturated sterol	+

d. *Warionia saharae* macerated in distilled water , diluted HCl and 70% ethanol

The phytochemical screening with maceration of *Warionia saharae* shows the presence of cardinolid , steroid , flavonoid , free flavonoid and glycosylated flavonoid .

Table IV.4 : Phytochemical screening with maceration

Compound family	*Warionia saharae*
Cardenolid	+
Steroid	+
Flavonoid	+++
Free flavonoid	++
Glycosylated flavonoid	+++

4.2 Plant material extraction

Extraction of the compounds from dried plant material (*Launeae nudicaulis* and *Warionia saharae*) realized by using different solvents .

4.2.1 *Launeae nudicaulis* extraction

To obtain the methanolic extract of *Launeae nudicaulis* powdered leaves , we realized reflux extraction with the mixture of water- methanol(MeOH) solvent , after the filtration , the residue is concentrated by rotary evaporation . The table IV.5 shown the yield and description of the crude extract obtained from *Launeae nudicaulis* plant .

Table IV.5 : Color and yield of *Launeae nudicaulis* methanolic extract

Extract	Color	Yield (g)	Yield (%)
MeOH	Brown	16,93	8,465

The results indicated that methanolic extract yield of *Launeae nudicaulis* plant was similar to the solvent polarity.

4.2.2 *Warionia Saharae* extraction

To obtain different extracts of the powdered leaves of *Warionia Saharae* plant , we realized solid – liquid extraction (reflux extraction) with different solvents in increasing polarity : Diethyl ether , chloroform ($CHCl_3$) , ethyl acetate (EtOAc) , butanol (BuOH) and methanol (MeOH water) .

Then we realize a liquid – liquid extraction of the water phase of hydro-methanolic extract with Diethyl ether , ethyl acetate (EtOAc) and butanol (BuOH) three times.

1. **Solid – liquid extraction**

The solid - liquid extraction of *Warionia saharae* plant permitted to obtain five crude extracts : Ether extract , chloroform extract, ethyl acetate extract , butanol extract and methanol extract.

The calculation of yield extraction compared to the total weight of *Warionia saharae* powder leaves show that the polar extracts butanol (BuOH) and (MeOH) have given the highest proportions in comparaison with apolar extracts . This can be explained that diethyl ether , chloroform and ethyl acetate are apolar organic solvents used just to degrease the plant .

Table IV.6 :Color and efficiency of the *Warionia saharae* extracts

Extract	**Color**	**Yield (g)**	**Yield (%)**
Diethyl ether	**Green**	**0,625**	**1,25**
Chloroform ($CHCl_3$)	**Green**	**1,31**	**2,62**
Ethyl acetate (EtOAc	**Green**	**1,5**	**3**
Butanol (BuOH)	**Brown**	**2,675**	**5,35**
Methanol (MeOH)	**Green**	**2,31**	**4,62**

The butanol extract present a highest yield extraction (5,35%) , followed by methanol extract (4,62%) and then with ethyl acetate extract (3%) , chloroform extract (2,62%) , the lowest yield extraction is observed in ether extract (1,25%).

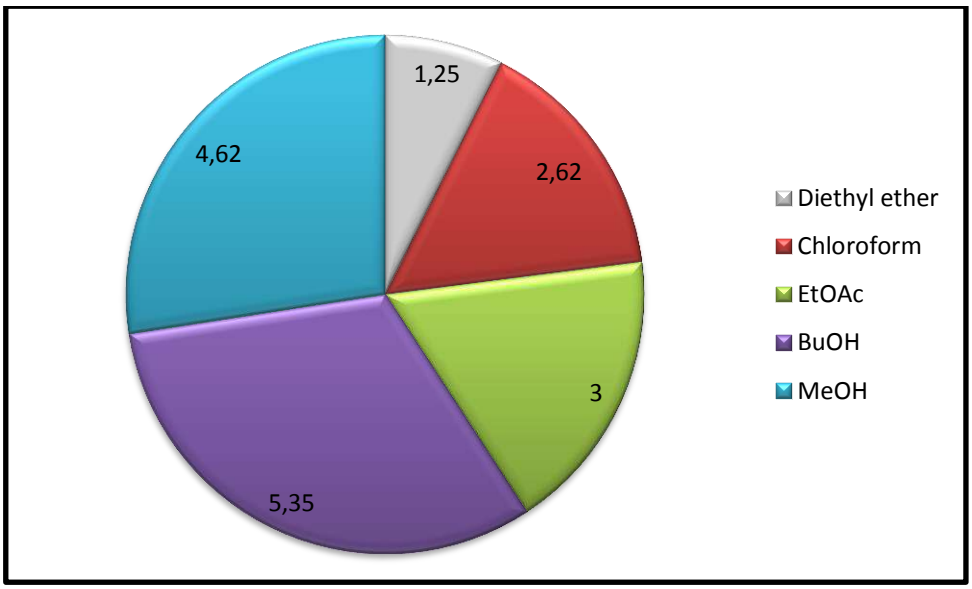


Figure IV.1 Efficiency of the *Warionia saharae* extracts

2. Liquid – liquid extraction

The liquid- liquid extraction of *Warionia saharae* plant provides three crude extracts : diethyl ether extract , ethyl acetate extract and butanol extract .

Table IV.7 : Color and yield of *Warionia saharae* liquid – liquid extraction .

Extract	Color	Yield (g)	Yield (%)
Diethyl ether	**Green**	**1**	**2**
Ethyl acetate (EtOAc)	**Green**	**1,1**	**2,2**
Butanol (BuOH)	**Brown**	**2,5**	**5**

The results indicate that the extraction yield is linked to the polarity of the extraction solvent , where the butanol extract present a high proportion with 5 % followed by ethyl acetate extract with 2,2% and ether extract with 2% .

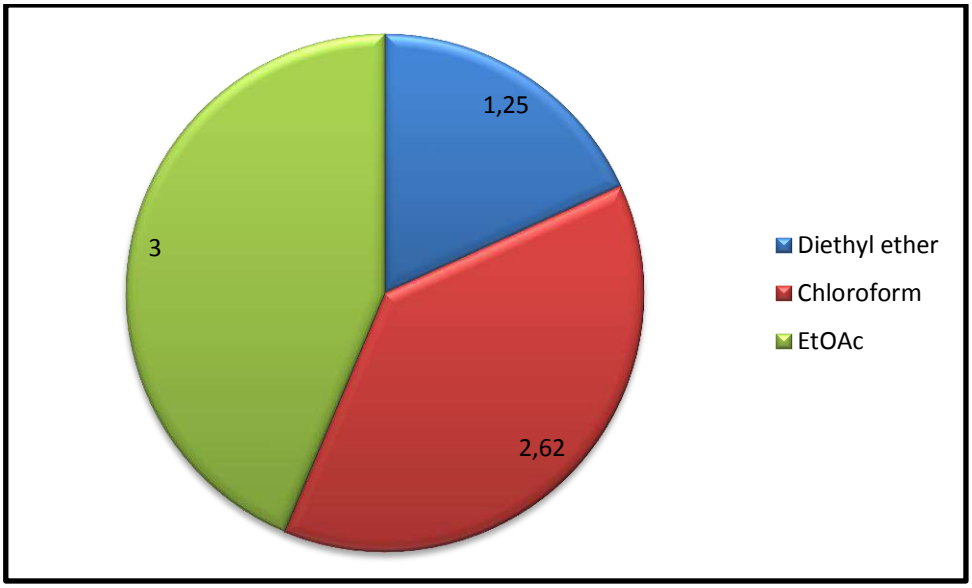


Figure IV.2 Efficiency of the *Warionia saharae* extracts (liquid extraction)

4.3 Thin layer chromatography analysis results

4.3.1 Launeae nudicaulis extraction

TLC analysis of the crude extract of *Launeae nudicaulis* by using MeOH as an eluent present four compounds with Rf equal to 0,63 – 0,72 – 0,87 and 0,90 under light 365 nm and present one spot with Rf equal to 0,80 by using $CHCl_3$/ MeOH as eluent system .

Table IV.8 : Thin layer chromatography analysis of *Launeae nudicaulis* methanolic extract results

Extract	Eluent system selected	Spot number	Rf	Fluorescence
MeOH	MeOH	**04**	**0.63** **0.72** **0.87** **0.90**	Purple Purple Blue Purple
MeOH	**MeOH/$CHCl_3$ (6/3)**	**01**	0.80	Purple

4.3.2 Warionia saharae

1. Solid – liquid extraction

Thin layer chromatography of the crude plant extracts carried out using Silicagel aluminum plate. The results of TLC analysis of all extracts of *Warionia saharae* are summarized in table IV.9 present the information on the retention factor of the chemical constituent and their fluorescence.

Table IV.9 : Thin layer chromatography analysis of *Warionia Saharae* solid – liquid extraction results

Extract	**Eluent system selected**	**Spot number**	**Rf**	**Fluorescence**
Diethyl ether	MeOH	**7**	**0** **0,16** **0,3** **0,46** **0,60** **0,76** **0,90**	Brown / / / / / Brown
$CHCl_3$	MeOH	**4**	0,06 0,2 0,3 0,96	Purple Purple Purple Purple
EtOAc	MeOH	**3**	0,4 0,66 0,83	Brown Brown Brown
BuOH	MeOH/$CHCl_3$	**3**	0,23 0,53 0,76	Brown Brown Purple

MeOH	MeOH/$CHCl_3$ (7/3)	**5**	0.31 0.45 0.59 0.7 0.83	Yellow / Yellow / /

The compound which has the retention factor Rf of 0,3 separated in the both extracts diethyl ether and chloroform with the same eluent system which is MeOH .Whereas the compound which has the Rf of 0,76 is separated with different eluent MeOH / $CHCl_3$(7/3) for the butanol extract and methanol for the ether extract.

2. **liquid – liquid extraction**

According to the results summarized in the table , we observed that the compound which has the retention factor of 0,33 separated in the both extracts diethyl ether extract and ethyl acetate extract with two different eluent MeOH/$CHCl_3$ (7/3) and $CHCl_3$.

Table IV.10 : Thin layer chromatography analysis of *Warionia Saharae* liquid – liquid extraction results

Extract	**Eluent system selected**	**Spot number**	**Rf**	**Fluorescence**
Diethyl ether	$CHCl_3$	**5**	**0** **0,16** **0,2** **0,33** **0,73**	Brown Brown Brown Brown Brown
EtOAc	MeOH/$CHCl_3$ (7/3)	**5**	0,33 0,7 0,76 0,83 0,96	Brown / Brown Brown Brown

BuOH	$CHCl_3$	**3**	0 0,66 0,86	Brown Purple Purple

4.4 Isolation and identification of natural compounds

4.4.1 Launeae nudicaulis methanolic extract

The crude extract is submitted a fractionation by column liquid chromatography by using eluent in increasing polarity , we obtain 230 fractions of 30 ml , the results of this fractionation are shown in table IV.11

Table IV.11 : Fractionation of MeOH extract of *Launeae nudicaulis*

Fraction	Eluent system	TLC observation
1-15	Pure Petrol ether	Nothing
16 – 25	Diethyl ether	Nought
26 – 34	Diethyl ether / Chloroform (90/10)	Trail + 02 spots
35 – 46	Diethyl ether / Chloroform (80/20)	Complex mixture
47 – 58	Diethyl ether / Chloroform (70/30)	Mixture of 03 spots
59 – 71	Diethyl ether / Chloroform (60/40)	Trail + 02 spots
72 – 83	Diethyl ether / Chloroform (30/70)	Trail
84 – 95	Chloroform	Yellow spot
96 – 109	Chloroform / Ethyl acetate (80/20)	02 spots + trail

110 – 123	Chloroform / Ethyl acetate (60/40)	02 spots + trail
124 – 142	Ethyl acetate	02 Spots in majority + trail
143 – 155	Ethyl acetate/ Methanol (95/5)	Trail
156 – 172	Ethyl acetate/ Methanol (90/10)	Mixture of five spots
173 – 180	Ethyl acetate/ Methanol (50/50)	Complex mixture
181 - 200	Ethyl acetate/ Methanol (30/70)	Trail + 03 Spots
201 – 230	Methanol	Trail + 02 spots in majority

The fractions that present similarities are combined to 13 lots , the table IV.12 present 13 lots

Table IV. 12 . Lot number and their TLC observation

N lot	**Fraction**	**Weight**	**TLC observation**
01	1-25	00	Nothing
02	26 - 34	200	Trail + 2 spots
03	35 - 46	150	Complex mixture
04	47 - 71	420	Mixture of 3 spots
05	72 - 83	90	Trail
06	**84 – 95**	**130**	**Yellow spot**
07	96 - 123	230	02 Spots + trail

08	**124 - 142**	**720**	**02 spots in majority + trail**
09	143 - 155	420	Trail
10	**156 - 172**	**660**	**Mixture of 05 spots**
11	173 - 180	300	Complex mixture
12	181 - 200	340	Trail + 03 spots
13	**201 - 230**	**980**	**Trail + 02 spots in majority**

4.4.2 Purification of product

1. Study of lot N 06

Chromatographic analysis under UV light of lot N 06, shows the presence of the single yellow spot, so this product possess the following characteristics:

Table IV .13 : Characteristics of lot N 06

Lot N 06	
Wieght (mg)	**130**
TLC condition	Chloroform / Methanol ($CHCl_3$ / MeOH : 10/1)
Fluorescence	invisible compound under UV light , and provides a pink color after revelation to sulfuric vanillin .
Efficiency	**2,60%**
Code	**LN A**
Physical aspect	**Solid**

2. Study of lot N 08

Chromatography analysis of lot N°08 demonstrate the appearance of two spots in majority , by using eluents in increasing polarity .

Table IV .14 : Fractionation of lot N°08

Fraction	Eluent system	TLC observation
1 – 10	Petrol ether	Nothing
11 – 18	Chloroform	Trail
19 – 28	Chloroform/ ethyl acetate (30/70)	Complex mixture
29 – 40	Chloroform/ ethyl acetate (40/60)	Trail
41 – 52	Chloroform/ ethyl acetate (50/50)	Spot + trail
53 – 68	Chloroform/ ethyl acetate (70/30)	Spot + trail
69 – 85	Chloroform/ ethyl acetate (80/20)	Trail
86 – 100	**Ethyl acetate**	**Spot in majority + trail**

The similar fractions are grouped in 07 sub lots , the table IV.15 shows the sub lots of fraction N°08

Table IV.15 : Sub lots of fraction N°08

Sub lot	Fraction	Weight(mg)	TLC observation
01	1 – 10	00	Nothing
02	11 – 18	60	Trail
03	19 – 28	100	Complex mixture
04	29 – 40	90	Trail

05	41 – 68	110	Spot + trail
06	69 – 85	70	Trail
07	**86 – 100**	**240**	**Spot in majority + trail**

The sub lot N°07 submitted a new liquid chromatography by using the following elution system : diethyl ether , gradient of diethyl ether in ethyl acetate then the pure ethyl acetate , this fractionation permit to isolate compound B which has the following characteristics :

Table IV .16 : Characteristics of lot N 08 , sub lot N 07

Lot N 08	
Sub lot N 07	
Wieght (mg)	**190**
TLC condition	Hexane /Dichloromethane / Methanol (1/ 1/0.2)
Retention factor	0,57
Fluorescence	visible: yellow , 365 nm : red , 254nm : green .
Efficiency	**3,80%**
Code	**LN B**
Physical aspect	**Solid green**

3. Study of lot N 10

The fractionation of lot N°10 by column chromatography realized as the following :

Table IV . 17 : Fractionation of lot N°10

Fraction	System eluent	TLC observation
1 – 12	Petrol ether	Nothing
13 – 18	Ether / Ethyl acetate (90/10)	Small spot
19 – 29	Ether / Ethyl acetate (80/20)	02 spots
30 – 45	Ether / Ethyl acetate (60/40)	02 spots
46 – 58	Ether / Ethyl acetate (20/80)	Trail
59 – 80	Methanol	02 spots in majority

The fractions that present similarities were grouped into 05 sub lots

Table IV .18 : Sub lots of fraction N°10

Sub lot N	Fraction	Weight (mg)	TLC observation
01	1 – 12	00	Nothing
02	13 – 18	65	Small spot
03	19 – 29	80	02 spots
04	30 – 58	130	Trail
05	**59 – 80**	**320**	**02 spots in majority**

The fractionation of sub lot N°05 by column chromatography and using eluents in increasing polarity permit to separate the compound C which has the following characteristics :

Table IV .19 : Characteristics of lot N 10

Lot N 10	
Sub lot N 05	
Wieght (mg)	**180**
TLC condition	Chloroform /Methanol/ Water($CHCl_3$/ MeOH/ Water : 10/1/0.5)
Retention factor	0,78
Fluorescence	visible: green , 365 nm : red , 254nm : brown .
Efficiency	**3,60%**
Code	**LN C**
Physical aspect	**Solid brown**

4. Study of lot N 13

Chromatographic analysis of lot N°13 demonstrate a mixture of two compounds , the separation of this both compounds realized with liquid chromatography and using elution system in increasing polarity , the table show the fraction of lot N°13.

Table IV .20 : Fractionation of lot N°13

Fraction	**System eluent**	**TLC observation**
1 – 07	Petrol ether	Nothing
08 – 19	Ether / Ethyl acetate (80/20)	Trail
20 – 31	Ether / Ethyl acetate (60/40)	Small spot + trail
32 – 40	Ether / Ethyl acetate (50/50)	Small spot + trail
41 – 49	Ether / Ethyl acetate (20/80)	Small spot + trail

50 – 65	Ethyl acetate	One spot
66 – 73	Ethyl acetate/ Methanol (80/20)	Small spot + trail
74 – 88	Ethyl acetate/ Methanol (60/40)	Mixture of 04 spots
89 – 96	Ethyl acetate/ Methanol (50/50)	Mixture of 04 spots
97 – 102	Ethyl acetate/ Methanol (30/70)	Mixture of 04 spots
103 – 110	Ethyl acetate/ Methanol (20/80)	Mixture of 04 spots
111 -125	Methanol	Spot in majority + small brown spot

Systematic exam by thin layer chromatography of fractions permit to grouped in 07 sub lots as shown in the table :

Table IV .21 : Sub lots of fraction N°13

Sub lot	Fraction	Weight (mg)	TLC observation
01	1 – 7	00	Nothing
02	8 – 19	60	Trail
03	20 – 49	70	Small spot + trail
04	**50 – 65**	**150**	**One spot**
05	66 – 73	95	Trail + small spot
06	74 – 110	180	Mixture of 04 spots
07	**111 – 125**	**280**	**Spot in majority + small brown spot**

Thin layer chromatography analysis of sub lot N°04 shows that contains a single spot therefore it is a separated compound which has the following characteristics :

Table IV .22 : Characteristics of lot N 13 , Sub lot N 04

Lot N 13	
Sub lot N04	
Wieght (mg)	**150**
TLC condition	Chloroform /Methanol($CHCl_3$/ MeOH : 10/1)
Retention factor	0,52
Fluorescence	254 nm : yellowish
Efficiency	**3,00%**
Code	**LN D**
Physical aspect	**Solid white**

The sub lot N°07 submitted a liquid chromatography by using different eluent permit to isolate the compound E in majority, which present the following characteristic:

Table IV .23 : Characteristics of lot N 13 , sub lot N 07

Lot N 13	
Sub lot N07	
Wieght (mg)	**200**
TLC condition	Chloroform /Methanol/ Water($CHCl_3$/ MeOH/ Water : 10/1/0.5)
Retention factor	0,52
Fluorescence	254nm : brown .
Efficiency	**3,00%**

Code	**LN E**
Physical aspect	**Solid brown**

4.4.3 Warionia saharae Solid – liquid extraction

According to the results show in table , the isolated products that have Rf equal to **0,65** , **0,85** are found in all extracts with different mass , while the compounds which have Rf equal to **0,91** , **0,82** , **0,88** , **0,83** , **0,94** are separated in butanol extract , ethyl acetate extract and chloroform extract .

Table IV.24 : Results of TLC analysis of separated compounds (solid – liquid extraction) with liquid column chromatography

Extract	Lot	Rf	M(mg)
Diethyl ether extract	**Ws-E -01**	**0,85**	1
	Ws-E -02	0,71	10
	Ws-E -03	**0,65**	50
$CHCl_3$ extract	**Ws-CH -01**	0,97	700
	Ws-CH -02	**0,85**	300
	Ws-CH -03	**0,94**	200
	Ws-CH -04	**0,88**	400
	Ws-CH -05	0,28	700
	Ws-CH -06	0,77	400
	Ws-CH -07	**0,91**	800
	Ws-CH -08	**0,65**	600
	Ws-CH -09	**0,82**	100
	Ws-CH -10	**0,83**	200
EtOAc extract	**Ws-Et -01**	0,74	500
	Ws-Et -02	0,71	300
	Ws-Et -03	0,68	400
	Ws-Et -04	**0,65**	700
	Ws-Et -05	**0,91**	100

	Ws-Et -06	0,62	200
	Ws-Et -07	0,48	400
	Ws-Et -08	0,28	600
	Ws-Et -09	0,61	600
	Ws-Et -10	**0,88**	100
	Ws-Et -11	**0,85**	200
	Ws-Et -12	0,40	600
	Ws-Et -13	0,51	100
	Ws-Et -14	**0,94**	500
	Ws-Et -15	0,97	500
	Ws-Et -16	**0,83**	700
	Ws-Et -17	0,80	400
	Ws-Et -18	**0,82**	100
	Ws-Et -20	0,57	800
BuOH extract	**Ws- Bu -01**	01	200
	Ws- Bu -02	**0,91**	200
	Ws- Bu -03	0,68	400
	Ws- Bu -04	**0,65**	700
	Ws- Bu -05	0,77	100
	Ws- Bu -06	**0,85**	100
	Ws- Bu -07	0,74	100
	Ws- Bu -08	**0,88**	300
	Ws- Bu -09	0,92	100
	Ws- Bu -10	**0,83**	100
	Ws- Bu -11	0,87	200
	Ws- Bu -12	**0,94**	200
	Ws- Bu -13	0,86	200
	Ws- Bu -14	**0,82**	200
	Ws- Bu -15	0,90	160
	Ws- Bu -16	0,89	200

4.4.4 *Warionia saharae* Liquid – liquid extraction

The compounds which have Rf similar to **0,82** , **0,88** , **0,91** and **0,94** are separated with two solvent diethyl ether and ethyl acetate .

Isolated compounds that possess Rf equal to 0,82 , 0,88 , 0,91 , and 0,94 are extracting with all solvent in the case of two extraction method : solid –liquid extraction and liquid –liquid extraction .except in the case of ether in solid– liquid extraction .

The compound which has retention factor equal to 0,85 was found in all extracts of solid – liquid and liquid – liquid extraction .

Ethyl acetate extract don't contain high separated compounds quantities .

Table IV . 25 : Results of TLC analysis of separated compounds (lquid – liquid extraction) with liquid column chromatography

Extract	Lot	Rf	Mass (mg)
Diethyl ether extract	**Ws-E -01(L-L)**	0,68	1
	Ws-E -02(L-L)	0,77	2
	Ws-E -03(L-L)	**0,82**	100
	Ws-E -04(L-L)	**0,88**	10
	Ws-E -05(L-L)	**0,91**	10
	Ws-E -06(L-L)	**0,94**	10
	Ws-E -07(L-L)	0,97	20
	Ws-E -08(L-L)	**0,85**	100
EtOAc extract	**Ws-Et -01(L-L)**	0,71	1
	Ws-Et -02(L-L)	**0,82**	10
	Ws-Et -03(L-L)	0,97	40
	Ws-Et -04(L-L)	**0,94**	10
	Ws-Et -05(L-L)	**0,91**	40
	Ws-Et -06(L-L)	0,74	1
	Ws-Et -07(L-L)	**0,88**	70
	Ws-Et -08(L-L)	0,57	10
	Ws-Et -09(L-L)	0,77	70
	Ws-Et -10(L-L)	0,62	10

	Ws-Et -11(L-L)	0,65	10
	Ws-Et -12(L-L)	0,45	30
	Ws-Et -13(L-L)	0,40	100
	Ws-Et -14(L-L)	**0,85**	30

4.5 Identification and classification of isolated compounds

4.5.1 Launeae nudicaulis methanolic extract

1. UV spectroscopy

- **LN.A**

The compound LN.A is in the form of a yellow solid soluble in methanol, this compound is invisible under UV light and gives a pink color after revelation to vanillin sulfuric.

- **LN.B**

The compound LN.B is isolated as a light green powder, soluble in the methanol and chloroform. The UV spectrum in methanol shows maximum absorption at 270 nm related to band II , and another to 318 nm relative to band I characteristic of a flavonol.

Table IV.26 : UV spectroscopy analysis results of LN.B with different reagents according to Mabry

Reagent	Band I (nm)	Band II (nm)
Eluent	**318**	**256 – 271**
NaOAc	**349**	**253 ep , 266**
MeONa	**311**	**253 ep , 266**
$AlCl_3$	**312 , 373**	**253 ep , 266**
$AlCl_3$ / H^+	**312 , 375**	**253 ep , 266**

The UV spectrum observed in the eluent shows two peaks in the band II , the presence of a system of 3', 4' dioxygen.

The appearance of a second maximum in the band I at 375 nm is observed with $AlCl_3/H^+$ revealing to a bathochromic shift induced with OH in position 5, while the same reagent without acid not make changes to this spectrum.

The addition of the weak base does not cause a bathochromic shift of the band II, which means that the hydroxyl group at position 7 is free. In the same spectrum the bathochromic shift of the band I at 31 nm indicates that the hydroxyl group at 4' position is free.

- **LN.C**

The compound LN.C is a brown solid, soluble in methanol, as well as other polar solvent, and chloroform. This compound showing a red fluorescence under UV light at 365 nm and brown at 254 nm envisage a structure of flavonoid confirmed by both maxima at 258 and 352 nm of UV spectrum characteristic of band I and II.

- **LN.E**

The compound LN .E is a brown solid , soluble in polar solvent , it present in TLC a brown spot under UV light at 254 nm .

The UV spectrum realized in MeOH demonstrate three maximum absorption bands at 271 , 312 and 318 nm characterized of a flavanone .

Table IV . 27 : UV spectroscopy analysis results with different reagents according to Mabry

Reagent	Band I (nm)	Band II (nm)
Eluent	**312 , 318**	**271**
NaOAc	**323 , 368**	**280**
MeONa	**323 , 355**	**231 ep**
$AlCl_3$	**324 , 395 ep**	**246**
$AlCl_3$ / H^+	**324 , 373**	**250**

Add of weak base NaOAc don't induce to a bathochromic shifting of band II , There is not a free hydroxyl group at position 7 .

Appearance of second maximum in band I at 373 nm , observed with $AlCl_3$ / H^+ reagent indicate a bathochromic shift of hydroxyl group in position 5 .

The solution $AlCl_3$ demonstrate with the maximum band I at 395 nm the presence of two group ortho – hydroxyl on cycle B (3' , 4' position).

2. Infrared spectroscopy identification

- **LNA**

Infrared spectrum of LNA compound indicate the presence of hydroxyl group (3442,79 cm^{-1}) , olefin (1656,45 cm^{-1}) and absorption band of aliphatic C-H link at 2918,36 and 2852,81 cm^{-1}

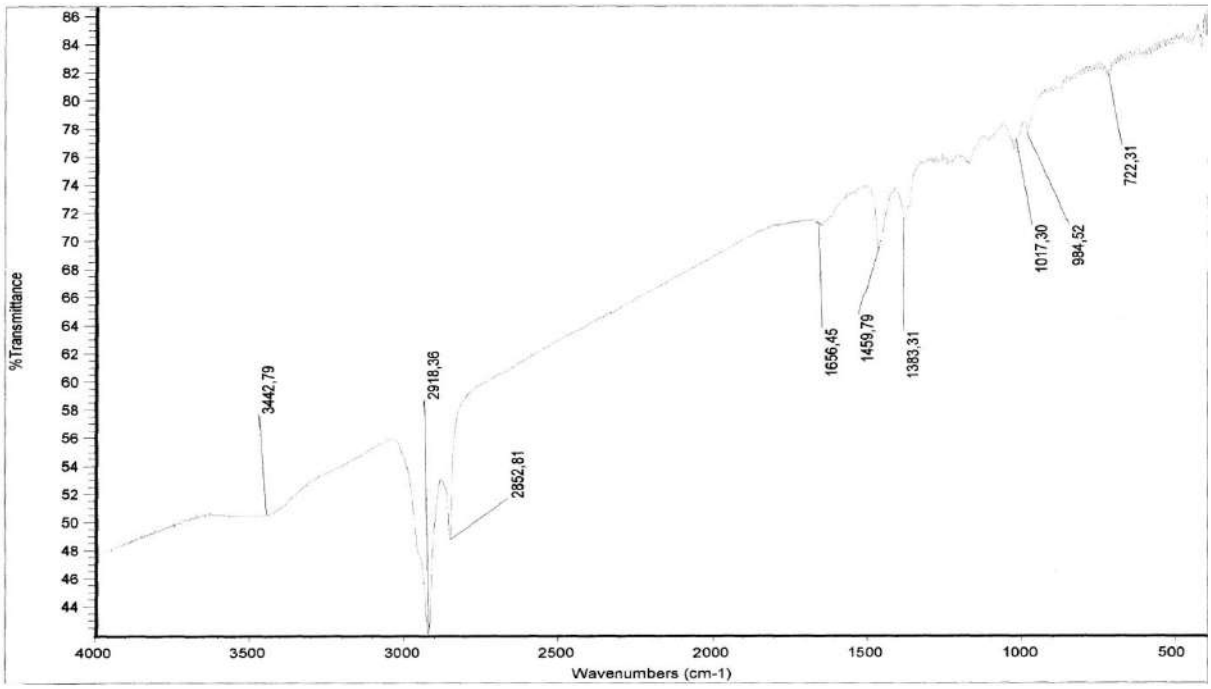


Figure IV.3 IR spectrum of LN.A compound

- **LNB**

Infrared spectrum of LNB compound show the presence of hydroxyl group at 3415,48 cm^{-1} , carbonyl group at 1727, 47 cm^{-1} , olefin at 1607,28 cm^{-1} and absorption bands of aliphatic C-H link at 2918,36 and 2852, 81 cm^{-1}.

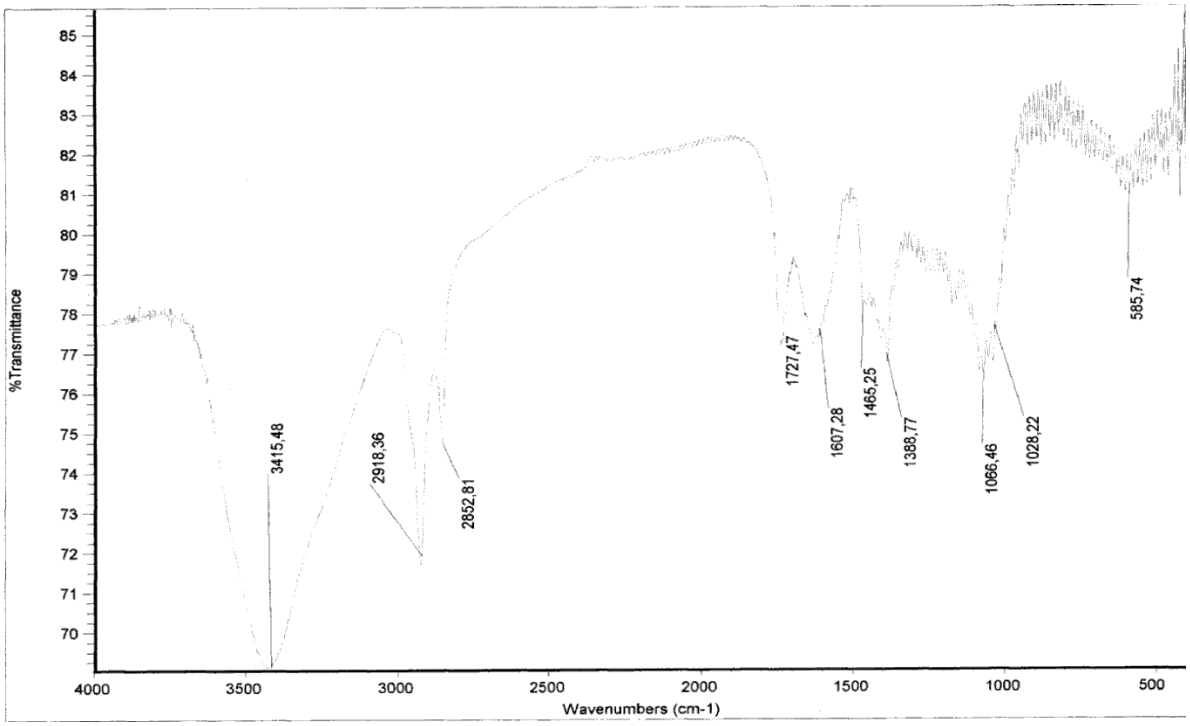


Figure IV.4 IR spectrum of LN.B compound

- **LNC**

Infrared spectrum of LNC compound indicate the presence of hydroxyl group (3442,79 cm^{-1}), carbonyl (1738,39cm^{-1}) and aromatic (1465,29cm^{-1}).

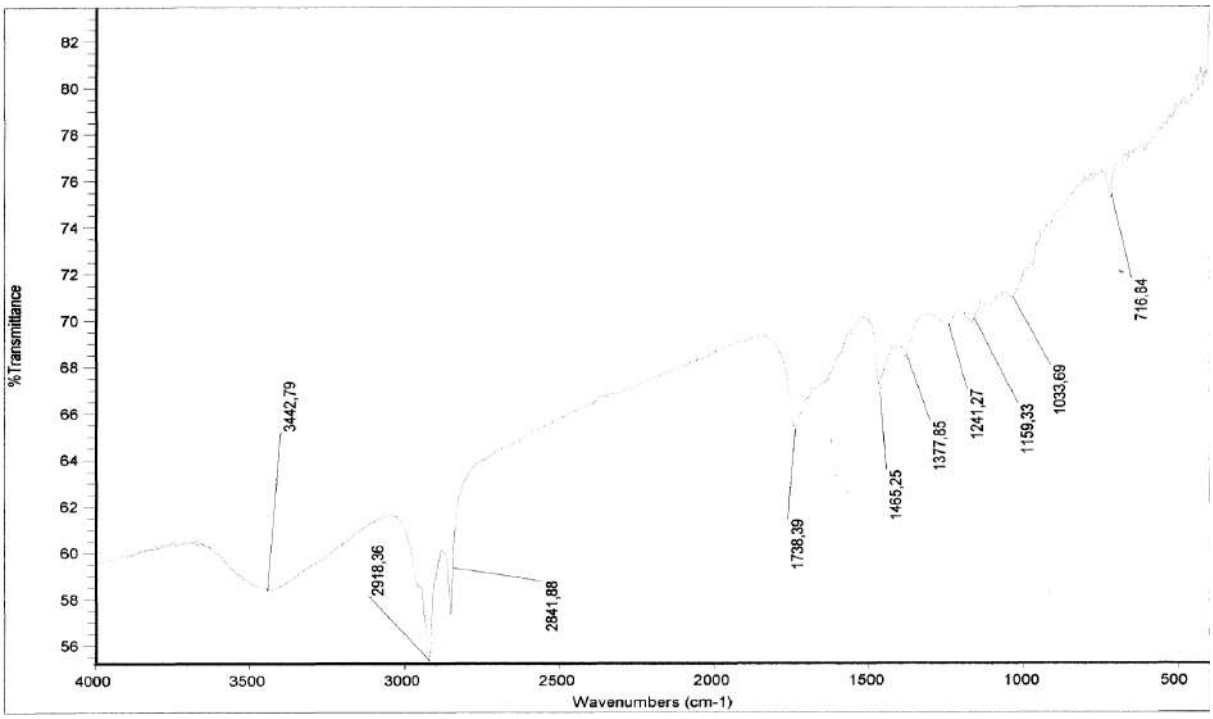


Figure IV.5 IR spectrum of LN.C compound

- **LNE**

Infrared spectrum of LNE compound shows the presence of hydroxyl group (3431,87cm^{-1}) , olefin (1601,82 cm^{-1}) , carbonyl (1738,93 cm^{-1}) and absorption bands of aliphatic C-H link at 2956,60cm^{-1} , 2918,36 cm^{-1} and 2847,34 cm^{-1} .

The characteristic bands observed in the spectrum of the separated compounds located at 3415,48 cm^{-1} – 3459,18 cm^{-1} come from the stretching of O-H bond .

The appearance of intense bands varied between 2841,88 and 2956,60 cm^{-1} corresponding to stretching vibration of CH_2 and CH_3 group .

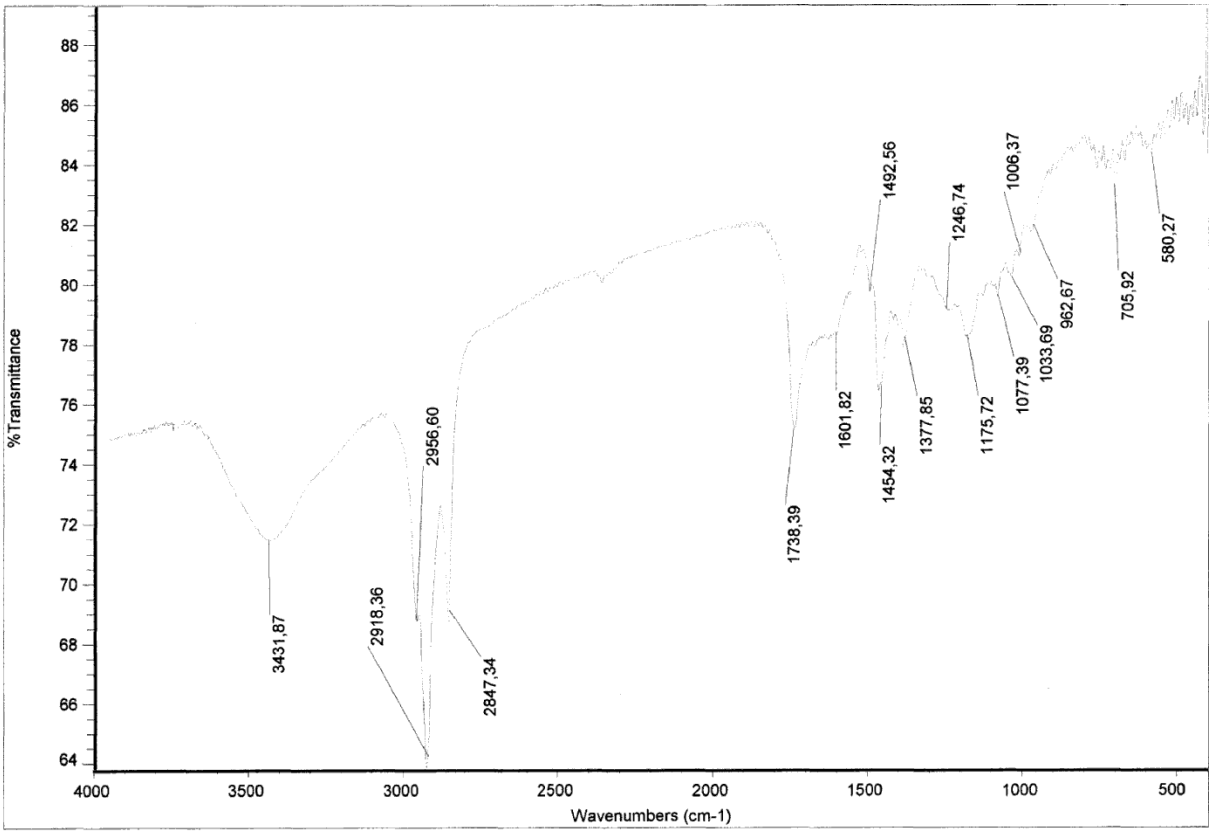
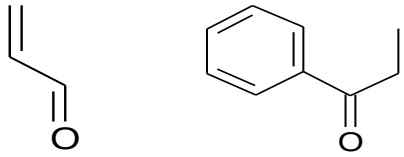


Figure IV.6 IR spectrum of LN.E compound

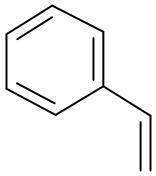
All infrared spectrums of the separated compounds except LNA compound possess absorption peaks around 1700,15 cm^{-1} and 1743 cm^{-1} is due to carbonyl group .

Theoretically, carboxylic acids, ester , ketones , aldehydes and amides show a strong stretching absorption of C=O joint between 1800cm^{-1} – 1650 cm^{-1} .

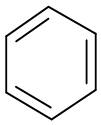
The infrared spectra present bands between 1645,52 cm^{-1} – 1667,37 cm^{-1} correspond to C=O bond of ketone α ,β unsaturated .



Intense peaks of 1601 cm^{-1} and 1607 cm^{-1} characterize ethylene bond .

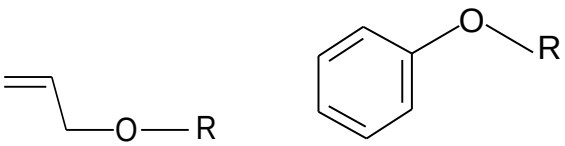


The low frequencies between 1465,52cm^{-1} and 1454,32 cm^{-1} are typically of the aromatic compounds .



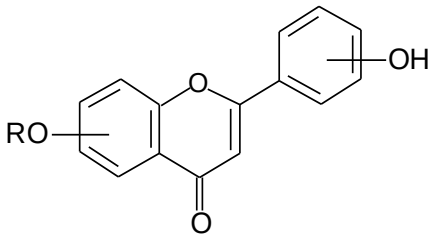
The absorption at 1006,37 cm^{-1} and 1181,18cm^{-1} confirm stretching band of C-O, this involves the presence of an aliphatic ether CH_3-O- .

The band appears at 1241,27 cm^{-1} and 1246,74 cm^{-1} indicates the presence of conjugated ether oxide .

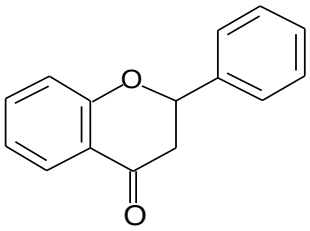


According to the spectral analysis of separated compounds, we suggest the presence of two flavonoids classes and structure of sterol.

A flavone substituted with hydroxyl or methoxy group .



It is a flavanone that is due to by the absence of ethylene function C=C.



Band(cm^{-1}) Separated compounds	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$ ester	$\nu_{C=C}$ Arom	δ_{CH_3}	δ_{O-H}	ν_{C-O} α,β insaturated	ν_{C-O}	$\gamma_{=C-H}$
LN.A	3442,79	2918,36 2852,81	-	1656,45	1456,45 1459,79	1383,31	-	-	984,52 722,31
LN.B	3415,48	2918,36 2852,81	1727,47	1607,28	1455,25	1388,77		1066,46 1028,22	585,84
LN.C	3449,79	2918,36 2841,88	1738,39	1465,25		1377,85	1241,27	1033,69 1159,33	716,84
LN.E	3431,87	2918,36 2847,34 2956,60	1738,39	1601,82	1454,32 1492,56	1377,85	1246,74	1175,72 1077,39 1033,66 1006,37	580,27 705,92 962,67

Table IV.28 : Infrared spectroscopy of *Launeae nudicaulis* compounds

3. NMR spectroscopy

- **LNA Proton NMR spectroscopy**

^{1}H NMR spectrum of the LNA compound present methyl signals towards a sterol structure .

This spectrum shows the following methyl signals :

- ✓ Two singlet signal (δ_H = 0,893 ppm and δ_H = 1,292 ppm) correspond to methyl group CH_3(18) and CH_3(19) respectively .
- ✓ A signal at δ_H = 0,918 ppm as doublet form attributed to methyl group CH_3(21) .
- ✓ A multiplet signal at δ_H = 2,844 ppm corresponds to proton H(3).
- ✓ A signal at δ_H =5,337 ppm as doublet form characterize an olefin proton H(6).

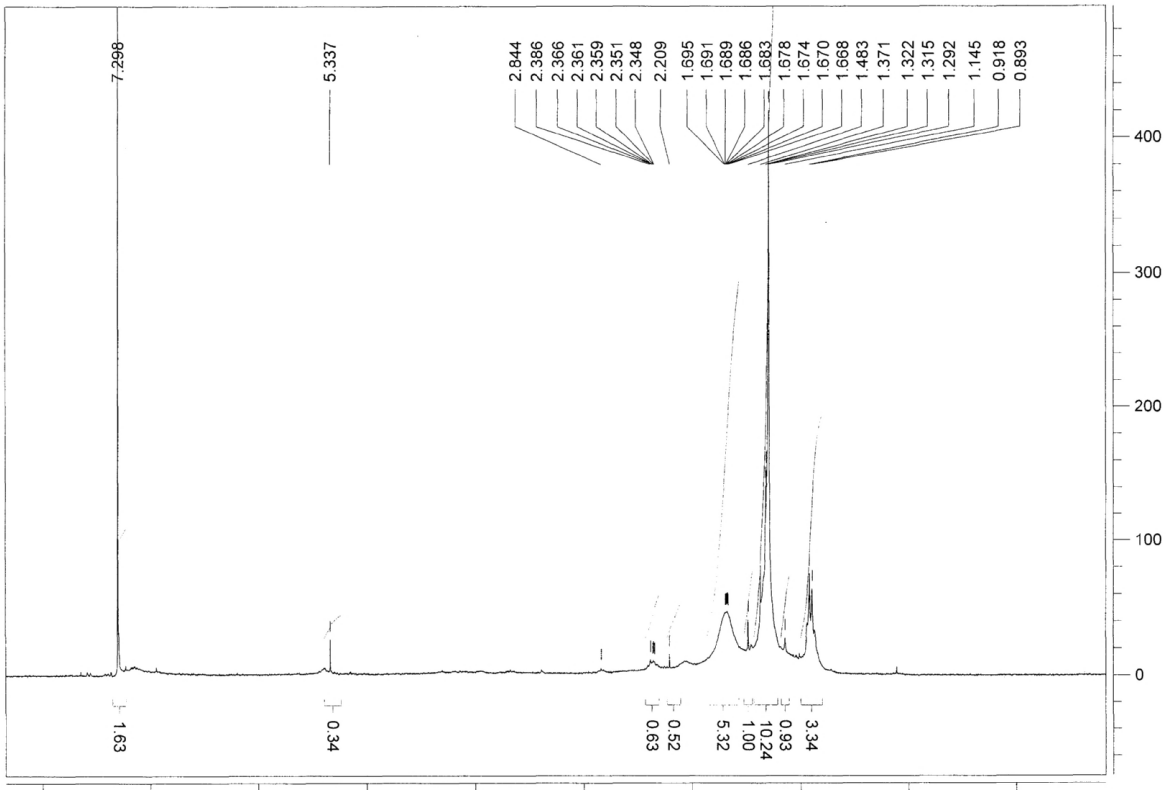


Figure IV.7 ^{1}H NMR spectrum of LN.A compound

- **LNA ^{13}C NMR spectroscopy**

The study of the ^{13}C NMR spectrum of this compound shows the presence of 29 carbon atom.

This last confirm the presence of :

- ✓ Double bond with signals at δ_C = 13O,20 ppm which correspond to quaternary carbon C(5) and δ_C = 115,5ppm relating to ethylic carbon C(6).
- ✓ Six methyl signals varied between 12,5 and 19,5 ppm correspond to six methylic carbons .
- ✓ A signal at δ_C = 72,855 ppm attributed to C(3) carrying an hydroxyl group .

These information are characteristic to a phyto sterol which having an hydroxyl group (OH) on the carbon 3 and a double bond at C_5-C_6 .

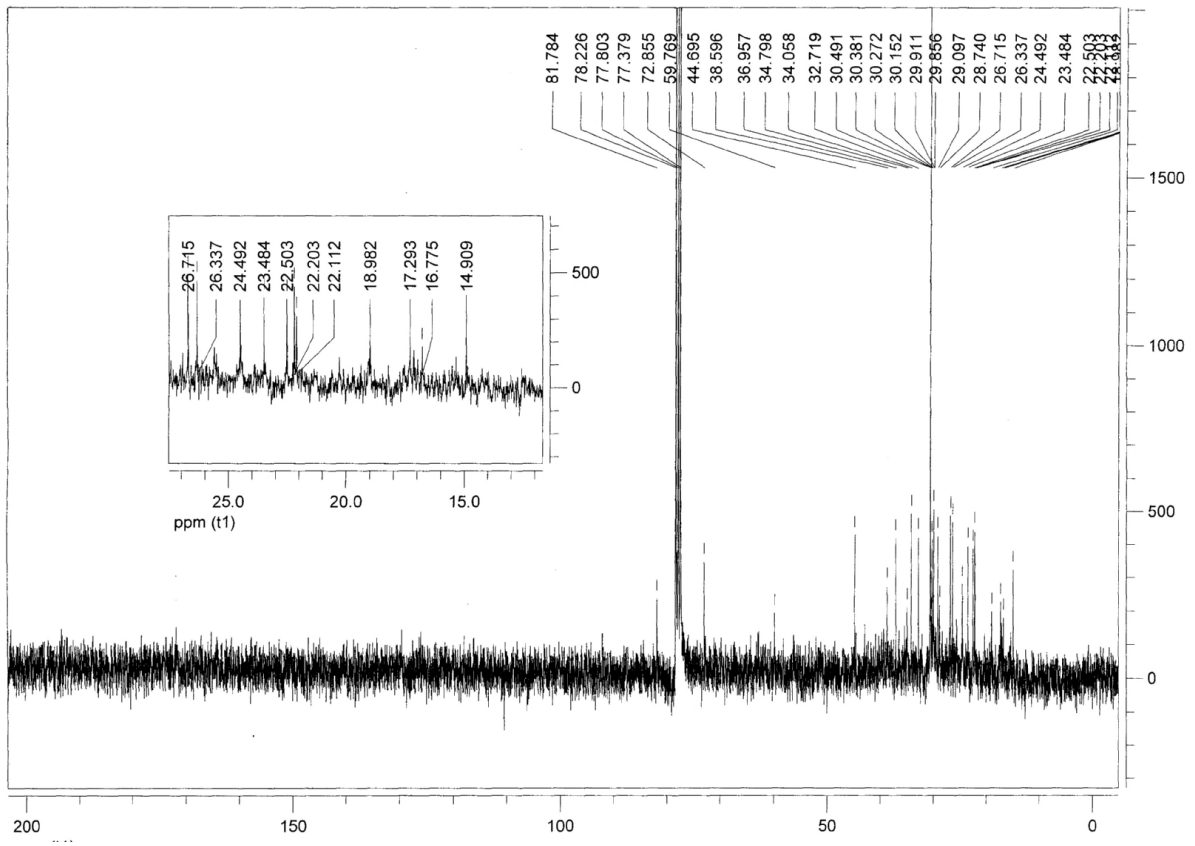


Figure IV.8 ^{13}C NMR spectrum of LN.A compound

Table IV .29 : Proton and carbon NMR spectroscopy of LNA compound

Position	δ_C	δ_H
1	36,957	1,691
2	29,856	1,483
3	72,855	2,844
4	38,596	2,386
5	130,20	-
6	115,5	5,337
7	30,491	2,200
8	30,381	1,371
9	44,695	0,918
10	34,058	-
11	22,112	1,689
12	34,798	1,145
13	42,3	-
14	59,769	1,371
15	24,429	1,668
16	28,740	1,678
17	56,53	1,141
18	14,909	0,893
19	36,997	1,315
20	17,293	0,918
21	32,719	1,683
22	30,272	1,670
23	30,272	1,315
24	44,695	1,670
25	29,097	0,893
26	16,175	0,893
27	19,5	1,322

28	23,484	0,893
29	12,5	

By combination of different spectroscopic informations , we can identified the LNA compound as : 24 – ethyl – cholest – 5 – en – 3ol .

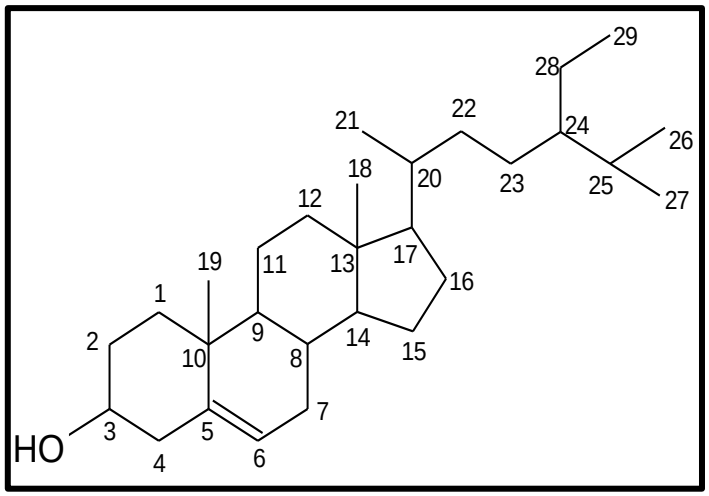


Figure IV.10 : Proposed Structure of LNA compound

- **LN.B Proton NMR spectroscopy**

The majority signals of ^{1}H NMR spectrum of the LNB compound is observed in the region of high field indicating the presence of the aliphatic protons.

- ✓ The absence of signals between 6,5 and 7,9 ppm indicates that all positions of the aromatic ring A and B are fully substituted.
- ✓ Two protons have a chemical shift between δ_H =5,286 and 5,356 are corresponding to H(21) and H(31).

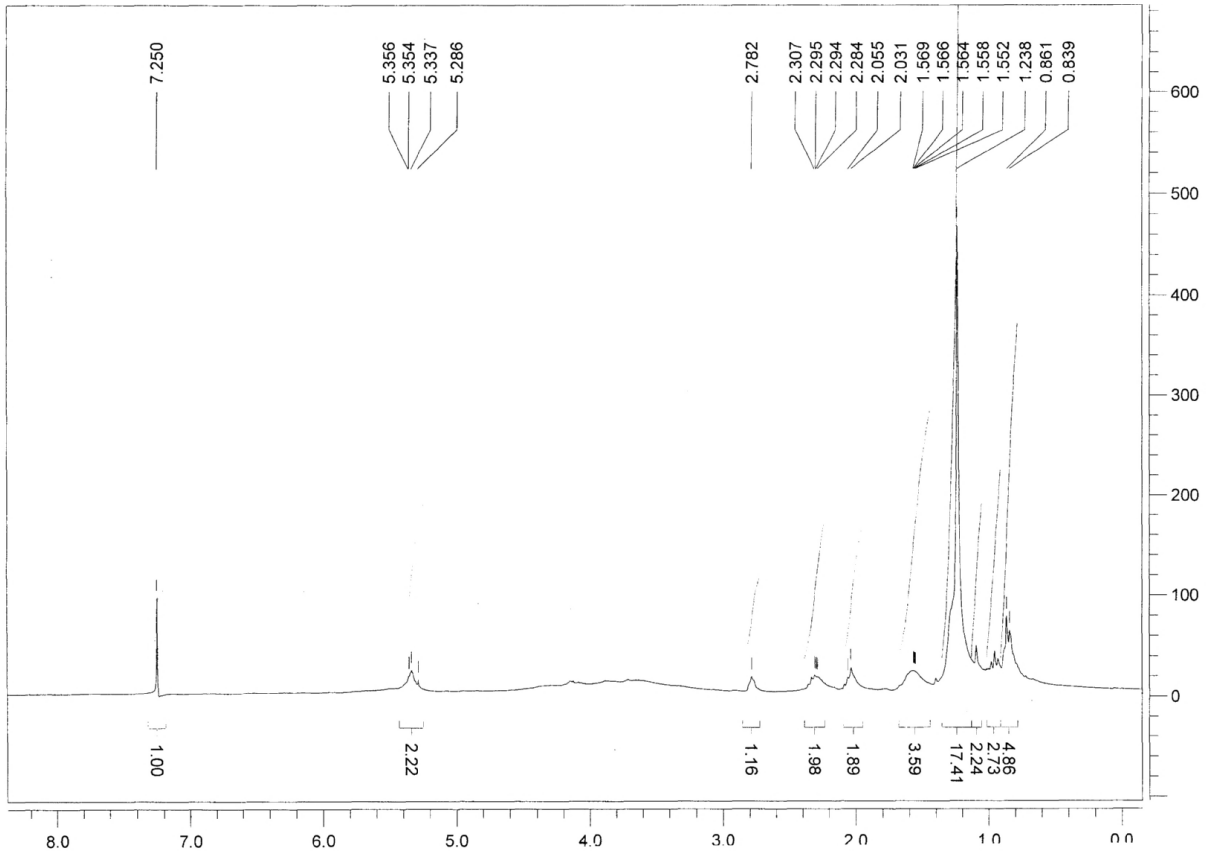


Figure IV.11 ^{1}H NMR spectrum of LN.B compound

- **LN.B ^{13}C NMR spectroscopy**

The study of the ^{13}C NMR spectrum of the compound LNB provides complementary information by displaying 18 signals. We observe several quaternary carbons that we can attributed to the ring A and B.

- ✓ A characteristic signal of carbonyl signal present a chemical shift at δ_C = 174,363 ppm
- ✓ The carbons resonant at δ_C = 162,567 , 150,881 , 150,688 ppm corresponding to : C(5) , C(8a) and C(2) respectively .
- ✓ The other signals that resonate in the region of high fields are consistent with aliphatic groups : CH , CH_2 , CH_3 .

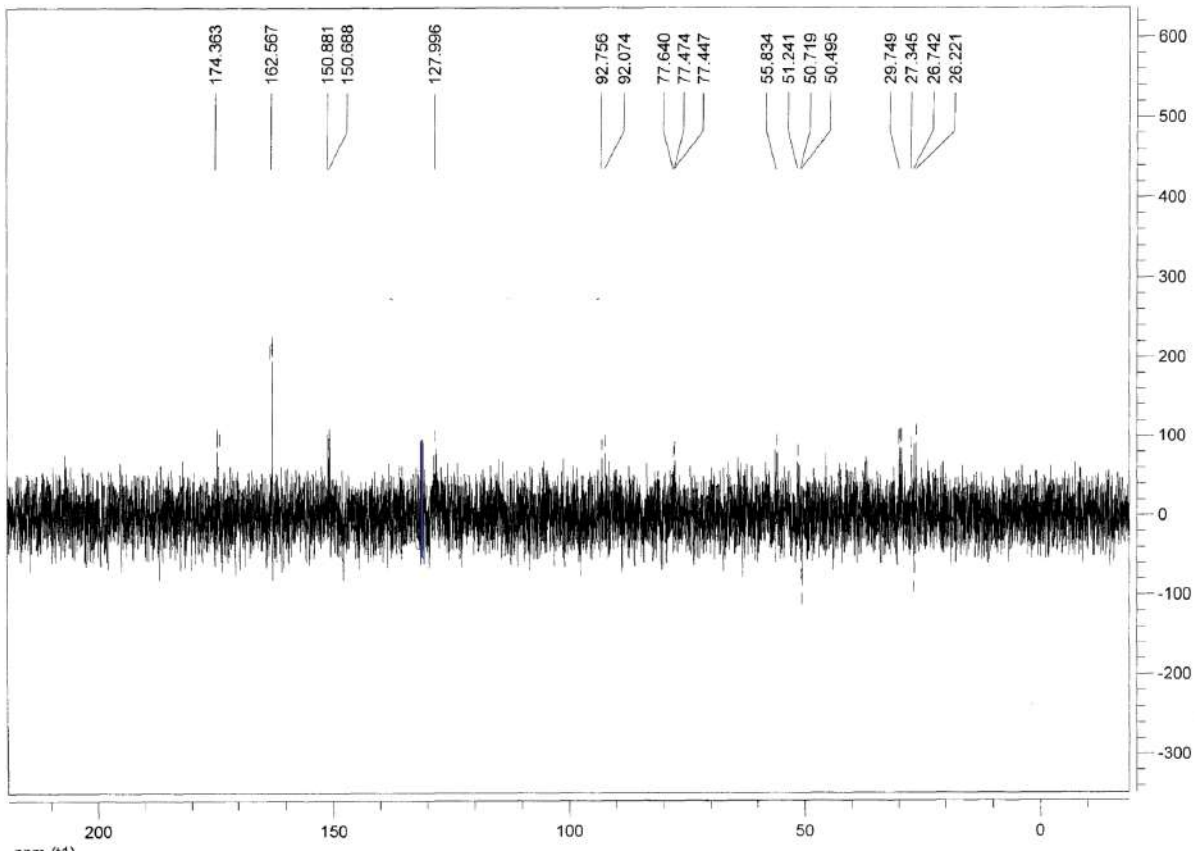


Figure IV.12 ^{13}C NMR spectrum of LN.B compound

Table IV .30 : Proton and carbon NMR spectroscopy of LNB compound

Position	δ_C	δ_H
2	150.688	-
3	135.50	-
4	174/363	-
5	162.567	-
6	92.074	-
7	166.1	-
8	92.756	-
8a	150.881	-
4a	102.00	-
1’	127.996	-
2’	156.3	-

3’	148.5	-
4’	140.00	-
5’	148.20	-
6’	156.00	-
9	30.25	2.28-2.30, t, 2H
10	33.20	1.566, m, 2H
11	29.749	1.566, m, 2H
12	27.347	1.564, m, 2H
13	26.742	1.552, m, 2H
14	27.345	1.564, m, 2H
15	29.747	1.566, m, 2H
16	16.50	0.839, s, 3H
17	16.50	0.861, s, 3H
18	18.20	1.238, d, 3H
19	25.45	2.782, brs, 1H
20	20.35	1.569, d, 3H
21	121.15	5.28-5.35, t, 1H
22	131.50	-
23	20.10	1.569, s, 3H
24	23.50	1.569, s, 3H
4’’	30.23	2.055, t, 2H
5’’	28.10	2.031, t, 2H
6’’	78.35	-
25	27.345	1.564, s, 3H
26	27.350	1.566, s, 3H
27	30.20	2.03-2.05, d, 2H
28	125	5.28-5.35, t, 1H
29	131.5	-
30	23.5	1.569 , s, 3H
31	25.70	1.569 , s, 3H

All these data is used to establish the structure of this compound as the : 5 , 7 , 3', 4' – tetra hydroxyl 8 –(11 , 15 – dimethyl octyl) 6 –(19, 22 – dimethyl but – 21 – enyl) 2'- (29-methyl but -28- enyl) 6'' , 6 '' – dimethyl tetra hydroxyl pyrano [2'', 3'', 5',6'] flavonol

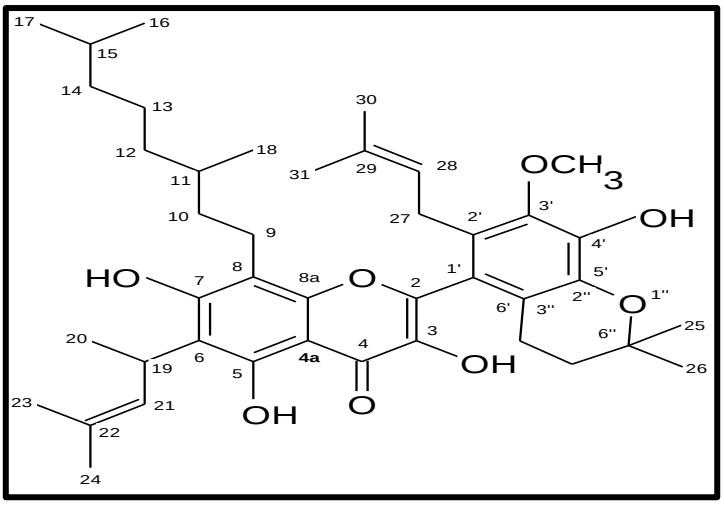


Figure IV. 13 : Proposed structure of LNB

- **LN.C Proton NMR spectroscopy**

^{1}H NMR spectrum of the LN.C compound provides the following results :

- ✓ The protons of ring A of flavonoid usually appear on the ^{1}H NMR spectrum between 6 and 6,5 ppm .
- ✓ On the ^{1}H NMR spectrum of LNC compound, we observe the absence of these aromatic protons, whih indicates that all positions of the A ring are substituted.
- ✓ The protons of B ring of flavonoid are visible on ^{1}H NMR spectrum between 6,5 and 7,9 ppm.
- ✓ On this ^{1}H NMR spectrum, we observe the presence of two aromatic singlet protons at δ_H = 7,335 ppm , These protons are with H(2') and H(6').
- ✓ The signal at δ_H = 5,372 ppm is a signal that corresponds to the proton H(2) .
- ✓ On the same spectrum, we observe a triplet signal resonant between δ_H = 3,692ppm and δ_H = 3,736 ppm is attributed to the proton H(3).
- ✓ The other signals are according to the aliphatic proton CH ,CH_2 , and CH_3 .

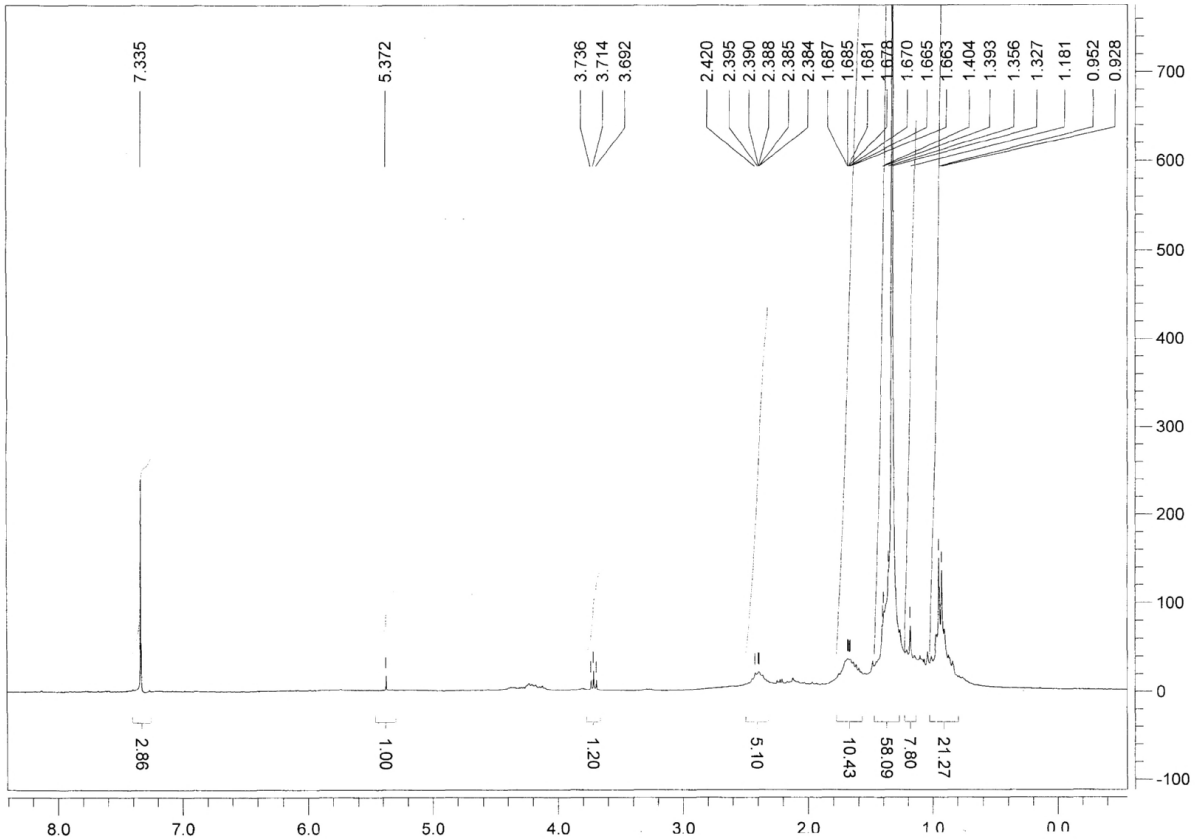


Figure IV.14 ^{1}H NMR spectrum of LN.C compound

- **LNC ^{13}C NMR spectroscopy**

The study of ^{13}C NMR spectrum of the LN.C compound provides additional information , We observe seven quaternary carbons that we can attributed to the ring A and B of a substituted flavonoid at 5, 6, 7 ,8 , 3', 4' and 5' .

- ✓ A characteristic signal of carbonyl (C- 4) with a resonance at δ_C = 192,42 ppm .
- ✓ The carbons with resonance at 153,34 , 148,7 and 141,10 ppm corresponding to C(4') , C(3') ,C(5') and C(7) respectively .
- ✓ The resonance of 128,13 ; 126,6 . 122,6 ppm are attributed to the following carbons : C(1') , C(2') , C(6') and C(6) .
- ✓ A characteristic signal at $^{\delta}C$ = 75,7 ppm concerning to C(2) of flavanone .
- ✓ Another characteristic signal at δ_C =68, 97 ppm which corresponding to C (3) of flavanone .

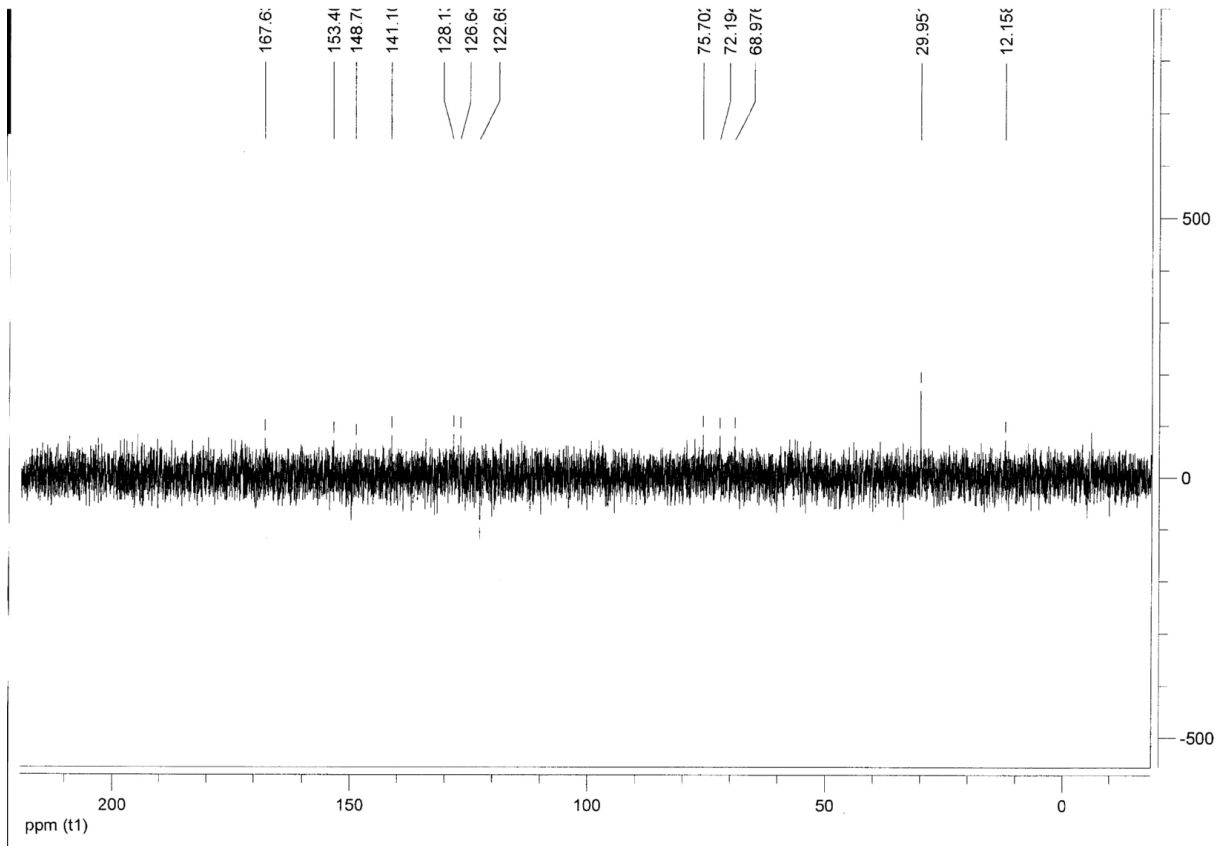


Figure IV.15 ^{13}C NMR spectrum of LN.C compound

Table IV . 31: Proton and carbon NMR spectroscopy of LNC compound

Position	δ_C	δ_H
2	75.702	5.372, d, 1H
3	68.978	3.714, t , 1H
4	195.42	-
5	167.60	-
6	126.62	-
7	141.10	-
8	92.756	-
8a	159.10	-
4a	103.20	-
1’	128.1	-
2’	122.6	7.335, s, 1H
3’	148.7	-

4'	**153.7**	**-**
5'	**148.7**	**-**
6'	**122.6**	**7.335, s, 1H**
9	**31.90**	**2.38-2.42, m**
10	**33.2**	**1.687, m, 2H**
11	**29.90**	**1.681, m, 1H**
12	**27.347**	**1.678, m, 2H**
13	**26.742**	**1.663, m, 2H**
14	**27.345**	**1.670, m, 1H**
15	**25.80**	**1.678, m, 1H**
16	**12.50**	**0.928, d, 3H**
17	**12.55**	**0.952, d, 3H**
18	**13.50**	**1.181, d, 3H**
19	**29.90**	**2.38-2.42, m**
20	**25.60**	**1.356, d, 3H**
21	**25.20**	**1.327, d, 3H**
4''	**32.90**	**2.38-2.42, m**
5''	**72.19**	**-**
22	**25.54**	**1.404, s, 3H**
23	**25.10**	**1.393, s, 3H**

The combination of the different spectroscopic data , we can purpose the following structure of LN.C compound : 5 , 3', 4' , 5' – tetra hydroxyl , 8- (11, 15 – dimethyl octyl) 3 – isopropyl , 5'' , 5'' – dimethyl tetra hydroxyl furano [2'' , 3'', 6 , 7] flavanone .

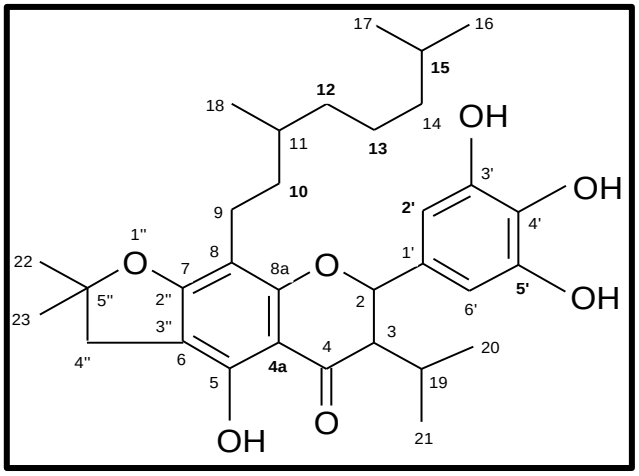


Figure IV.16 : Proposed structure of LNC

- **LN.E Proton NMR spectroscopy**

- ✓ The ^{1}H NMR spectrum of LN.E compound present signals of the aromatic protons of ring B of flavonoid at δ_H = 7,283 ppm and δ_H = 7,231 ppm forming singlets corresponding to protons H(2') and H(6') .
- ✓ Furthermore, signals at δ_H = 2,82 ppm (1H , dd , H-3a) , δ_H = 3,74 ppm (1H, dd , H-3b) and δ_H = 5,382 ppm (1H , dd , H-2) are characteristic signals of flavanone .

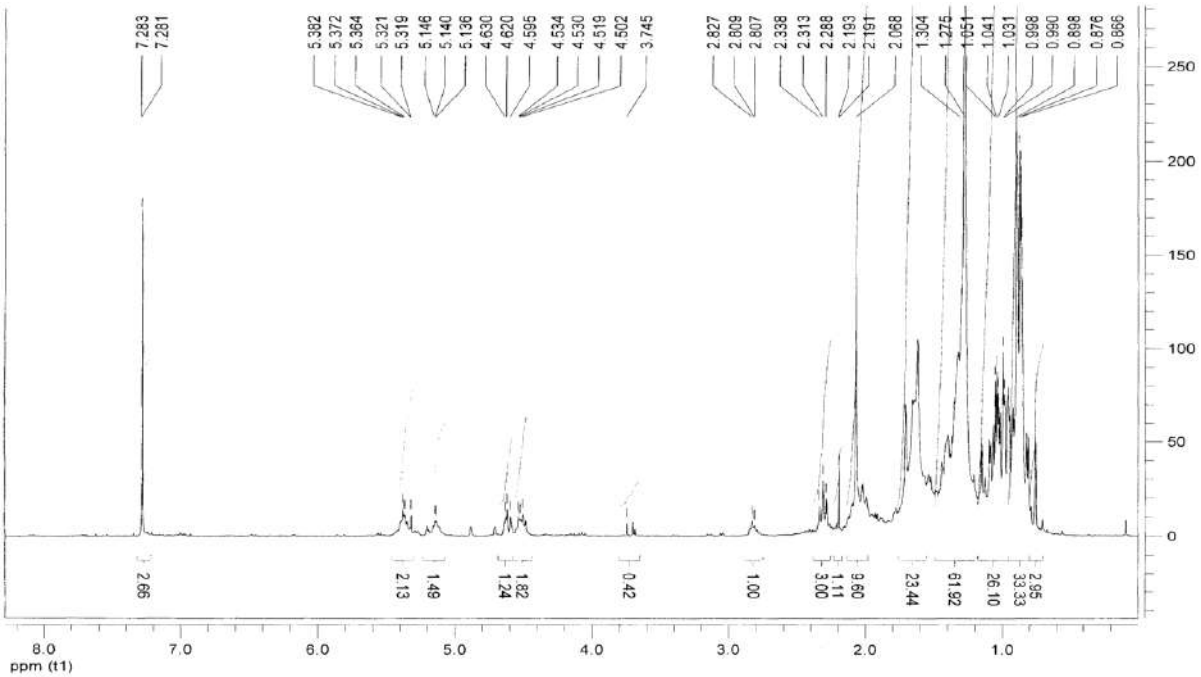


Figure IV.17 ^{1}H NMR spectrum of LN.E compound

- **LNE ^{13}C NMR spectroscopy**

- ✓ Analysis of ^{13}C NMR spectrum provide more information , This spectrum has shown a signal at δ_C = 187,046 ppm concerning of carbonyl group and aliphatic carbon at δ_C = 43,756 ppm . In addition, deshielding signals between δ_C = 162,916 ppm and δ_C = 167,215 ppm are characteristic of quaternary carbon .
- ✓ The information provided by infrared spectrometry, ultraviolet and ^{1}H NMR, ^{13}C NMR , we can say that the LNE compound has the characteristic of a flavonoid , which concern a structure of flavanone .

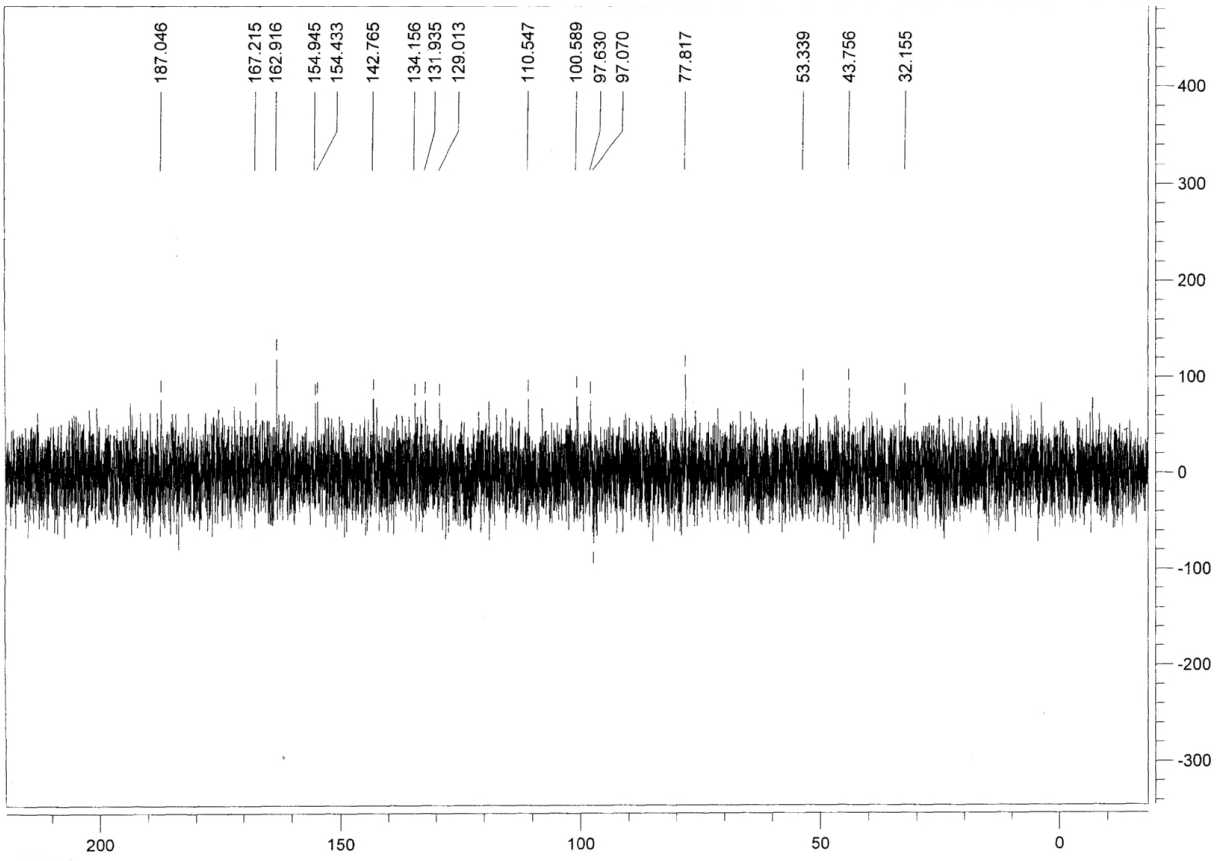


Figure IV.18 ^{13}C NMR spectrum of LN.E compound

Table IV . 32: Proton and carbon NMR spectroscopy of LNE compound

Position	**δ_C**	**δ_H**
2	**77.817**	**5.382, dd, 1H**
3	**43.756**	**2.827, dd, 1Ha** **3.745, dd, 1Hb**
4	**187.046**	**-**

5	**162.916**	**-**
6	**97.63**	**-**
7	**167.215**	**-**
8	**110.547**	**-**
8a	**154.945**	**-**
4a	**100.589**	**-**
1'	**131.935**	**-**
2'	**129.013**	**7.281, s, 1H**
3'	**134.156**	**-**
4'	**154.433**	**-**
5'	**142.765**	**-**
6'	**129.013**	**7.283, s, 1H**
9	**97.070**	**4.620, d, 1H**
10	**122.2**	**5.319, d, 1H**
11	**135.40**	**-**
12	**17.525**	**2.191, s, 3H**
13	**14.605**	**2.068, s, 3H**
4''	**30.20**	**2.338, t, 2H**
5''	**28.10**	**2.288, t, 2H**
6''	**53.339**	**-**
14	**32.155**	**1.057, s, 3H**
15	**23.150**	**1.031, s, 3H**
16	**130.20**	**-**
17	**115.62**	**4.534, d, 1H and 4.502, d, 1H**
18	**18.50**	**1.304, s, 3H**

The suggested structure of the LN.E compound is : 5 , 3' , 4' – trihydroxy , 8-(9 –hydroxy – 11 – methylbut – 10 – enyl) 5'- (16- methylprop -16- enyl) 6" , 6" – dimethyl tetrahydroxy pyrano [2" , 3" , 6 , 7] flavanone .

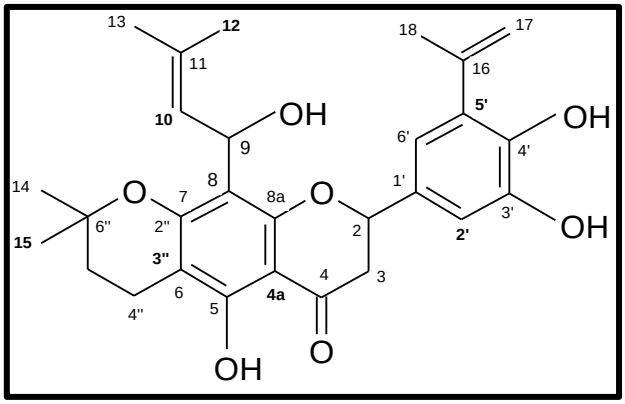


Figure IV. 19 : Proposed structure of LNE

4.5.2 Warionia saharae Solid – liquid extraction

1. UV spectroscopy

a. Butanol fraction

The sixteen isolated compounds belong to flavonoid class , this fraction is rich in hydroxyl and methoxyl flavone (10 flavones , 2 flavanol derived from taxifolin , a flavanone and an isoflavone)

Table IV.33 : Classification and identification of compounds isolated from *Warionia saharae* butanol fraction of the solid – liquid extraction .

Lot	Band I	Band II	Class	identification
Ws- Bu -01	329 338	262,268,272,274, 276,278, 282,291	Flavone	3',4' ,5' Trimethoxy 5,6,7 Trihydroxy flavone
Ws- Bu -02	326,333, 338,343,334, 351,355	264,273,375,281	Flavone	3',4' dimethoxy 5,7,8 Trihydroxy flavone
Ws- Bu -03	325,331	260,269,271 273,275,297	Flavone	3',4' dimethoxy 6-hydroxy flavone
Ws- Bu -04	329,335,342	279,273,275, 280,284	Flavone	3',4',7-Trihydroxy flavone

Ws- Bu -05	334,338	263,270,272, 276,278,281	Flavone	3',4' dimethoxy 5,7 dihydroxy flavone
Ws- Bu -06	324,328,336 337,339, 343,344	262,268, 276,278	Flavone	3',4',5' Trimethoxy 5,6,7 Trihydroxy flavone
Ws- Bu -07	-	265,271,278,283		-
Ws- Bu -08	300,307,313, 316, 320,328,336, 344, 349,355,359, 363,365	262,266,271, 275,283	Flavone	3',4' dimethoxy 5,7 dihydroxy flavone
Ws- Bu -09	329,341	265,273, 281,285,	Flavanol	Taxifolin
Ws- Bu -10	-	272,277,	Nd	nd
Ws- Bu -11	334,343	269,272,279,284	Flavone	3',4' dimethoxy 7,8 – dihydroxy flavone
Ws- Bu -12	335,338 340,350	262,271,276	Flavanol	4',5,7 taxifolin
Ws- Bu -13	329,343,349	266,275,280,	Isoflavone	6- Hydroxy Biochanin A
Ws- Bu -14	326,330,333 337,340,343 348,352,356,360	260,261, 270,277,	Flavanone	Eriodictyol 3' , 4' , 5' trimethoxy
Ws- Bu -15	321,327,336, 341,349,358,360	270,276,283	Flavone-glycosyl	Luteolin-O-glycosyl 4'-methoxy 5,7 dihydroxy
Ws- Bu -16	327,336,342	260,266,269 271,273,278,280	Flavone	3',4' – dimethoxy 5,7 dihydroxy flavone

b. Ethyl acetate fraction

The ethyl acetate fraction contains only three compounds which belongs to flavonoid class , the identification of these compounds shows that we have two derivatives flavones and a flavanol (derivative of taxifolin).

Table IV.34: Classification and identification of compounds isolated from *Warionia saharae* ethyl acetate fraction of the solid – liquid extraction .

Lot	Band I	Band II	Class	Identification
Ws-Et- 01	-	262,268,272,274 ,276 278,282,291	-	-
Ws-Et- 02	-	264,273,275,281 ,	-	-
Ws-Et- 03	-	260,269,271,273 , 275,297	-	-
Ws-Et- 04	-	279,273,275, 280,284	-	-
Ws-Et- 05	-	263,270,272,27, 278,281	-	-
Ws-Et- 06	-	262,268,276,278	-	-
Ws-Et- 07	-	265,271,278,283	-	-
Ws-Et- 08	-	262,266,271, 275,283	-	-
Ws-Et- 09	-	265,273,281,285	-	-

Ws-Et- 10	-	272,277	-	-
Ws-Et- 11	328,334, 341	269,272,279,284	Flavone	3', Methoxy , 4',5,6,7 Tetrahydroxy flavone
Ws-Et- 12	-	262,271,276	-	-
Ws-Et- 13	-	266,275,280	-	-
Ws-Et- 14	-	260,261,270,277	-	-
Ws-Et- 15	-	270,276,283	-	-
Ws-Et- 16	-	260,266,269,271 273,278,280	-	-
Ws-Et- 17	339	260,269,271,273 278,281	Flavone	3',4',5,6,7- pentahydroxy flavone
Ws-Et- 18	338	261,270,272,275 279,282,292	Flavanol	Taxifolin 6- hydroxy
Ws-Et- 19	-	263,269,271,278	-	-
Ws-Et- 20	-	269,270,272,279	-	-

c. Diethyl ether fraction

The compounds isolated from ether fraction don't belong to flavonoid class and in general don't present any band I .

Table IV.35: Classification and identification of compounds isolated from *Warionia saharae* diethyl ether fraction of the solid – liquid extraction .

Lot	Band I	Band II	Class	identification
Ws-E- 01	-	270,276,279,280	-	-
Ws-E- 02	-	270,274,277,279,284	-	-
Ws-E- 03	-	262,266,272,276,284	-	-

d. Chloroform fraction

According to maximal value of band I , the no identified compound belong to flavonoid class is a an isoflavonoid .

Table IV.36 : Classification and identification of compounds isolated from *Warionia saharae* chloroform fraction of the solid – liquid extraction .

Lot	Band I	Band II	Class	identification
Ws-CH- 01	-	**260,263,267,274,279**	-	-
Ws-CH- 02	-	**261,263,268,270,274,277**	-	-
Ws-CH- 03	-	**262,265,268,279,274,278,284**	-	-
Ws-CH- 04	-	**261,263,267,277,282**	-	-
Ws-CH- 05	**305**	**262,268,272,277,281,285**	**isoflavonoid**	**Nd**
Ws-CH- 06	-	**262,269,274,280**	-	-

Ws-CH- 07	-	**263,268,277**	-	
Ws-CH- 08	-	**264,272**	-	-
Ws-CH- 09	-	**262,269,277**	-	-
Ws-CH- 10	-	**262,265,269,274,278**	-	-

4.5.3 Warionia saharae Liquid – liquid extraction

a. Ethyl acetate fraction

The ethyl acetate fraction of liquid – liquid extraction is rich in flavone and isoflavone as: Biochanin A , Genistein and Formononetine .

TableIV.37 : Classification and identification of compounds isolated from *Warionia saharea* ethyl acetate fraction of the liquid – liquid extraction .

Lot	Band I	Band II	Class	Identification
Ws-Et- 01(L-L)	327,337,339 341,344	260,263,267,271,275 278,283,287,291	Flavone	3',4'-dimethoxy 5,7,8-Trihydroxy flavone
Ws-Et- 02(L-L)	301,306 327 ,342	265,271,273,278	Flavone	3',4'-dimethoxy 5,7- dihydroxy flavone
Ws-Et- 03(L-L)	325,330 339,349	265,275,278,283	Isoflavone	6 –Hydroxy Bionchanin A
Ws-Et- 04(L-L)	303,306 326,347	260,262,269,272,274	Flavone	3',4',5'-Trimethoxy 5,6 –Dihydroxy flavone
Ws-Et- 05(L-L)	301,319 328,340	268,271,273,277,279	Isoflavone	8 –Hydroxy 3',5'-Dimethoxy Formononetin

Ws-Et- 06(L-L)	324,327 331	265 ,270,278,281	Isoflavone	8 –Hydroxy 3'-Methoxy Genistein
Ws-Et- 07(L-L)	308,326	266,271,275,278	Flavone	5,7- dihydroxy 4'-Methoxy flavone
Ws-Et- 08	301	265,272,275,279	Isoflavone	Formononetine
Ws-Et- 09	315,317,351	266,271,275,278	Flavone	5,7-dihydroxy 4'-Methoxy flavone
Ws-Et- 10	304,311,326 333,339,341,344	263,270,272 274,277,282	Flavone	5,6,7-Trihydroxy 3' ,4'-Dimethoxy flavone

b. Diethyl ether fraction

Ether fraction is rich in isoflavone and flavone , three kind of isoflavone (Biochanin A , Genistein and Formononetine).

Table IV.38 : Classification and identification of compounds isolated from *Warionia saharae* diethyl ether fraction of the liquid – liquid extraction .

Lot	Band I	Band II	Class	Identification
Ws-E- 01(L-L)	-	261,270,276,279	-	-
Ws-E- 02(L-L)	301,306,327,342	265,271,273,278	Isoflavone	3'-Methoxy ,5-Hydroxy Formononetine
Ws-E- 03(L-L)	325,330,339,349	265?275,278,283	Isoflavone	3',6,8 –Trihydroxy Biochanin
Ws-E- 04(L-L)	303,306,326,347	260,262,269,272,274	Flavone	3',4',5,6,7 Pentahydroxy flavone
Ws-E- 05(L-L)	301,319,328,340	268,271,273,277,279	Isoflavone	Formononetine ,6,8 Dihydroxy

Ws-E- 06(L-L)	324,327,331,344	265,270,278,281	Isoflavone	3',5' –Dimethoxy 6,8-hydroxy Genisteine
Ws-E- 07(L-L)	308,326	264,271,273,276,279	Flavone	4'-Methoxy 5,7dihydroxy flavone
Ws-E- 08(L-L)	301	265,272,275,279	Isoflavone	Formononetine

2. Infra-red spectroscopy analysis

The bands appears between (3377 – 3454) cm^{-1} corresponding to stretching vibration of O-H , the C-H aliphatic bond are present in infrared spectrum with fine and intense bands at 2957 cm^{-1}(asymmetric stretch vibration of CH_3).

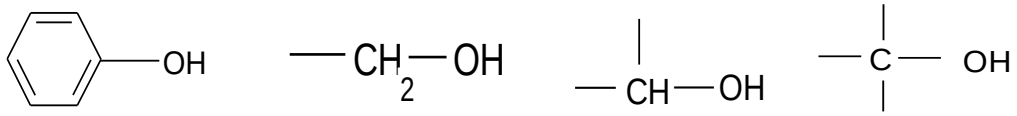
Vibration frequencies located between (2918 – 2929 cm^{-1}) corresponding to asymmetric stretch of CH_2 .Absorption bands at 2847 cm^{-1} associated with symmetric stretching of CH_2 .

Stretching frequencies at 930 cm^{-1} corresponding to deformation vibration out of insaturated hydrocarbon plane .stretching vibration of cyclic ketone with six chain or more , or aliphatic ketone located to 1717 cm^{-1} , the frequencies at 1738 cm^{-1} corresponding to carbonyl function of saturated ester .

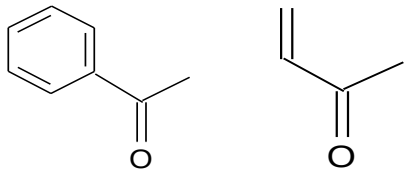
The vibrations at 1766 cm^{-1} and 1771 cm^{-1} for vinyl alcohol ester which have the following fragment : -CO-O-C=C , this group causes an important augmentation of vibration frequencies of carbonyl function (vinyl acetate absorb at 1776 cm^{-1} and phenyl acetate absorb at 1770 cm^{-1}).

The vibration of deformation out of aromatic C-H plane depending to the position of others substituents which have fixing on the benzene ring .

We note the existence of bands between 1150 – 1200 cm^{-1} in all separated compounds , which confirm the presence of aromatic alcohol corresponding to C-O aromatic vibration , and also the existence of bands between 1033- 1170 cm^{-1} which confirms the presence of primary , secondary and tertiary alcohol .



We observe that these separated compounds present intense band between 1600 -1700 cm^{-1}, this band indicates the existence of ketone function which is probably conjugated .



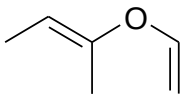
The spectrum of these compounds indicate the presence of substituted aromatic ring confirmed by stretching vibration (C=C) located to 1500 cm^{-1} and 1600 cm^{-1}.

Although , the vibration not appear well on the infrared spectrum due to masking by broadening bands of OH function .

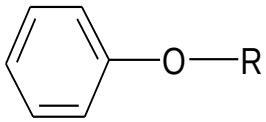
These last bonds are identified by vibration of deformation (=C-H aromatic which absorb around 700cm^{-1} .

Stretching vibration of $–CH_3$, $-CH_2$ appear between 2850 cm^{-1}- 2960 cm^{-1}.

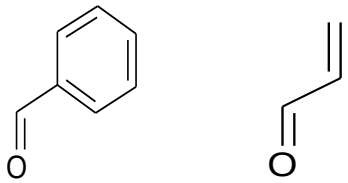
Vibration which exist in absorption domain between 1104 cm^{-1} to 1275 cm^{-1} present in all IR spectrum of the compounds indicate the etheric group .



Absorption bands of 1250cm^{-1} characterize an aliphatic ether oxide aryl .



Absorption band at 924 cm^{-1} characterize a trans ethylic bond and the band at 1601 cm^{-1} can probably attributed to stretching vibration of C=C conjugated group .



According to this interpretation, we conclude that the structure of these compounds is a structure of flavonoid substituted by –OH, -O-CH_3, -O-CO-CH_3.

Table IV .39 : Butanol fraction infrared characteristics

Band(cm^{-1}) Separated compounds	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$ ester	ν_{C-O}	$\nu_{C=C}$ α,β insaturated	$\nu_{C=C}$ Arom	δ_{CH_3}	δ_{O-H}	ν_{C-O} α,β insaturated	ν_{C-O}	$\gamma_{=C-H}$	γ_{CH2}
Ws-Bu-01	3400	2975 2924 2847	1722	1656	1613	1574 1509	1454	1378	1285 1258 1165	1072 1034	875 821 613	711
Ws-Bu-02	3415	2957 2924 2853	1722	/	1607	1509	1460	1378	1285 1159	1072 1034	935 870 821 608	/
Ws-Bu-03	3437	2962 2918 2853	1738	/	1607	/	1465	1378	1263 1165	1083 1028	/	/
Ws-Bu-04	3388	2957 2924 2847	1706	/	1618	1569 1514	1449	1383	1290 1159	1077 1039	935 875 826 695 640 586	/
Ws-Bu-05	3410 3017	2951 2918 2853	1733	1651	1607	1569 1503	1465	1372	1258 1165	1072 1023	908 837 815 608	722

Band(cm^{-1}) Separated compounds	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$ ester	ν_{C-O}	$\nu_{C=C}$ α,β insaturated	$\nu_{C=C}$ Arom	δ_{CH_3}	δ_{O-H}	ν_{C-O} α,β insaturated	ν_{C-O}	$\gamma_{=C-H}$	γ_{CH2}
Ws-Bu-06	3377	2962 2929 2869 2853	1716 1700	1684	/	1503	1443	1378	1285 1165	1072 1034	941 870 821 895 651	/
Ws-Bu-07	3437	2962 2924 2853	1738	1651	1618	/	1465	1378	1269	1165 1116 1072 1034	875 842	717
Ws-Bu-08	3388	2956 2929 2853	1717	1689	1613	1569 1514	1454	1383	1280	1165 1072 1034	941 870 826 700 640 613	/
Ws-Bu-09	3383	2951 2924 2847	1695	/	1618	1564 1503	1460	1383	1285 1203 1159	1034	941 870 821 613	/
Ws-Bu-10	3382	2957 2929 2847	1700	/	1618	1569 1509	1449	1389	1285 1165	1072	935 870 821 777 700 646	/

Ws-Bu-11	3377	2957 2929 2864 2853	1717	/	1613	1564 1509	1443	1400 1378	1285 1165	1072 1039	935 870 821 755 648 635	/
Ws-Bu-12	3343	2951 2924 2847	1733	/	1613	/	1454	1383	/	1094 1023	/	/
Ws-Bu-13	3388	2962 2929 2869	1706	/	1618	1569 1503	1449	1389	1290 1165	1077 1039	940 875 826 755 700 640	/
Ws-Bu-14	3388	2957 2918 3875 2853	1722	/	1613	1564 1509	1443	1389	1285 1159 1121	1077 1034	935 875 821 744 706	/
Ws-Bu-15	3372	2957 2918 2869 2853	1706	/	1613	1569 1503	1449	1400 1372	1284 1285 1258 1165	1072 1034	930 870 821 690 635	/
Ws-Bu-16	3388	2968 2924 2847	1717	/	1618	1569 1514	1454	1400 1378	1290 1159	1077 1039	935 875 826 690 640	/

Table IV.40 : Ethyl acetate fraction infrared spectroscopy characteristics

Band(cm^{-1}) Separated compounds	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$ ester	ν_{C-O}	$\nu_{C=C}$ α,β insaturated	$\nu_{C=C}$ Arom	δ_{CH_3}	δ_{O-H}	ν_{C-O} α,β insaturated	ν_{C-O}	$\gamma_{=C-H}$	γ_{CH2}
Ws-Et-01	**3394**	**2957 2924 2847**	**1727**	**/**	**1624**	**/**	**1465**	**1378**	**1165 1274**	**1066**	**869 789 744 613**	**/**
Ws-Et-02	**3421**	**2962 2924 2853**	**1733**	**/**	**1635**	**/**	**1460**	**1378**	**1285 1165 1127**	**1072 1039**	**608**	**739**
Ws-Et-03	**3426**	**2962 2924 2853**	**1733**	**/**	**1607**	**/**	**1454**	**1383**	**1280 1127**	**1072 1034 1012**	**974**	**733**
Ws-Et-04	**3405**	**2957 2918 2847**	**1733**	**/**	**1629**	**/**	**1460**	**1383**	**1280 1121**	**1066**	**870 788**	**717**
Ws-Et-05	**3399**	**2962 2924 2853**	**1733**	**1662**	**1602**	**/**	**1454 1411**	**1378**	**1263 1225 1170 1132**	**1077 1034**	**908 832 771 673 608**	**728**
Ws-Et-06	**3454**	**2962 2924 2853**	**1733**	**/**	**1618**	**/**	**1460**	**1378**	**1285 1127**	**1072 1034**	**875 793 711**	**/**

Band(cm^{-1}) Separated compounds	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$ ester	ν_{C-O}	$\nu_{C=C}$ α,β insaturated	$\nu_{C=C}$ Arom	δ_{CH_3}	δ_{O-H}	ν_{C-O} α,β insaturated	ν_{C-O}	$\gamma_{=C-H}$	γ_{CH2}
Ws-Et-07	**3454**	**2951 2918 2847**	**1733**	**/**	**1635**	**/**	**1460 1411**	**1378**	**1269 1116**	**1034**	**870 799**	**/**
Ws-Et-08	**3448**	**2957 2924 2853**	**1733**	**/**	**1624**	**/**	**1460**	**1383**	**1269 1127**	**1023**	**881**	**/**
Ws-Et-09	**3415**	**2924 2852**	**1738**	**1673**	**1597**	**/**	**1460 1416**	**1383**	**1121**	**1072 1028**	**864 782 684**	**/**
Ws-Et-10	**3415 3230**	**2957 2924 2847**	**1738**	**/**	**1629**	**/**	**1460**	**1383**	**1154**	**/**	**870 657**	**722**
Ws-Et-11	**3388**	**2957 2918 2847**	**1711**	**1656**	**1607**	**1509**	**1454**	**1378**	**1290 1276 1165**	**1072 1039**	**875 821 777 635**	**/**
Ws-Et-12	**3426**	**2957 2918 2852**	**1733**	**/**	**1607**	**/**	**1460 1422**	**1378**	**1263 1121**	**1028**	**875**	**/**

Band(cm^{-1}) Separated compounds	**ν_{O-H}**	**ν_{C-H}**	**$\nu_{C=O}$** ester	**ν_{C-O}**	**$\nu_{C=C}$** α,β insaturated	**$\nu_{C=C}$** Arom	**δ_{CH_3}**	**δ_{O-H}**	**ν_{C-O}** α,β insaturated	**ν_{C-O}**	**$\gamma_{=C-H}$**	**γ_{CH2}**
Ws-Et-13	**3405 3230**	**2962 2918 2858 2836**	/	/	**1635**	**1542**	**1471 1427**	**1389**	**1219 1148 1121**	**1034**	**870 837 668 608**	/
Ws-Et-14	**3426**	**2962 2924 2874**	**1771 1731**	/	**1607**	/	**1460**	**1378**	**1263 1132**	**1034**	**870 777**	/
Ws-Et-15	**3426**	**2962 2924 2874**	**1771 1733**	**1678**	**1602**	/	**1460 1405**	**1383**	**1263 1225 1181**	**1072 1039 1006**	**914 832 766 700 673 602**	**727**
Ws-Et-16	**3426**	**2957 2918 2847**	/	/	**1624**	/	**1454**	**1383**	**1192 1132**	**1039**	**875 690 613**	/
Ws-Et-17	**3415**	**2957 2924 2858**	**1766 1727**	**1651**	**1607**	**1503**	**1454**	**1378**	**1269 1170 1121**	**1077 1034**	**875 821 771 662**	/

Band(cm^{-1}) Separated compounds	**ν_{O-H}**	**ν_{C-H}**	**$\nu_{C=O}$** ester	**ν_{C-O}**	**$\nu_{C=C}$** α,β insaturated	**$\nu_{C=C}$** Arom	**δ_{CH_3}**	**δ_{O-H}**	**ν_{C-O}** α,β insaturated	**ν_{C-O}**	**$\gamma_{=C-H}$**	**γ_{CH2}**
Ws-Et-18	**3383**	**2957 2918 2853**	**1717**	**1651**	**1602**	**1558 1514**	**1460 1411**	**1383**	**1285 1263 1165**	**1083 1034**	**870 821 766 640**	**/**
Ws-Et-19	**3410**	**2957 2913 2843**	**1733**	**1651**	**1591**	**/**	**1554 1416**	**1378**	**1263 1198 1105**	**1028**	**875 799 619**	**/**
Ws-Et-20	**3410**	**2957 2924 2847**	**1733**	**/**	**1629**	**/**	**1454**	**1383**	**1263 1198 1105**	**1028**	**875 799 619**	**/**

Table IV.41 : Diethyl ether fraction infrared spectroscopy characteristics

Band(cm^{-1}) Separated compounds	**ν_{O-H}**	**ν_{C-H}**	**$\nu_{C=O}$** ester	**ν_{C-O}**	**$\nu_{C=C}$** α,β insaturated	**$\nu_{C=C}$** Arom	**δ_{CH_3}**	**δ_{O-H}**	**ν_{C-O}** α,β insaturated	**ν_{C-O}**	**$\gamma_{=C-H}$**	**γ_{CH2}**
Ws-E-01	3432	2957 2924 2853	1733	/	1618	1541.73	1460	1378	1165 1121	1039	875	/
Ws-E-02	3465	2957 2929 2853	1738	/	1613	/	1465	1372	1252 1165 1121	1028	974	717
Ws-E-03	3437	2951 2924 2853	1782 1738	/	/	/	1465	1383	1247 1170	1072	974	/

Table IV.42 :Chloroform fraction infrared spectroscopy characteristics

Band(cm^{-1}) Separated compounds	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$ ester	ν_{C-O}	$\nu_{C=C}$ α,β insaturated	$\nu_{C=C}$ Arom	δ_{CH_3}	δ_{O-H}	ν_{C-O} α,β insaturated	ν_{C-O}	$\gamma_{=C-H}$	γ_{CH2}
Ws-CH-01	**3426**	**2962 2924 2853**	**1738**	**1662**	**/**	**1498**	**1454**	**1378**	**1230 1176 1116**	**1034**	**744 700 591**	**/**
Ws-CH-02	**3426**	**2995 2962 2929 2847**	**1771 1737**	**1673**	**1607**	**1503**	**1460 1411**	**1383**	**1258 1176 1116**	**1039**	**777 695 619**	**/**
Ws-CH-03	**3465**	**2962 2913 2853**	**1771 1738**	**/**	**1646**	**/**	**1454**	**1383**	**1258 1170**	**1070 1034**	**613**	**/**
Ws-CH-04	**3437**	**2951 2924 2847**	**1771 1738**	**1667**	**1629**	**/**	**1460 1416**	**1378**	**1247 1165**	**1077 1039**	**810 613**	**722**
Ws-CH-05	**3448**	**2962 2924 1858**	**1744**	**1673**	**1613**	**/**	**1465 1422**	**1378**	**1187 1127**	**1034**	**842 788 624**	**/**

Band(cm^{-1}) Separated compounds	**ν_{O-H}**	**ν_{C-H}**	**$\nu_{C=O}$** ester	**ν_{C-O}**	**$\nu_{C=C}$** α,β insaturated	**$\nu_{C=C}$** Arom	**δ_{CH_3}**	**δ_{O-H}**	**ν_{C-O}** α,β insaturated	**ν_{C-O}**	**$\gamma_{=C-H}$**	**γ_{CH2}**
Ws-CH-06	**3443**	**2962 2924 2847**	**1771 1733**	**1662**	**1629**	**/**	**1460 1411**	**1383**	**1235.81 1170.26 1104.70**	**1034**	**761 613**	**/**
Ws-CH-07	**3448**	**2962 2924 2869 2842**	**1722**	**1656**	**1607**	**/**	**1460 1411**	**1383 1312**	**1268.59 1235.85 1153.87 1115.63**	**1077 1039**	**914 886 810 651 602**	**711**
Ws-CH-08	**3448**	**2929 2849**	**1733**	**1662**	**/**	**/**	**1465**	**1372**	**1258 1154**	**1088**	**782 643 602**	**/**
Ws-CH-09	**3443**	**2957 2918 2847**	**1738**	**1662**	**1635 1596**	**1508.95**	**1411**	**1389 1318**	**1263 1176 1121**	**1072 1039**	**837 777 619**	**/**
Ws-CH-10	**3454 3394 3181**	**2951 2918 2853**	**1744**	**/**	**1640 1602**	**/**	**1465**	**1378**	**1263 1170 1116**	**1034**	**903 799 700 619**	**/**

Liquid –liquid extraction

Table IV.43 :Ethyl acetate fraction infrared spectroscopy characteristics

Band(cm^{-1}) Separated compounds	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$ ester	ν_{C-O}	$\nu_{C=C}$ α,β insaturated	$\nu_{C=C}$ Arom	δ_{CH_3}	δ_{O-H}	ν_{C-O} α,β insaturated	ν_{C-O}	$\gamma_{=C-H}$	γ_{CH2}
Ws-Et-02 (L-L)	**3394**	**2929 2853**	**1733**	**/**	**1607**	**1564**	**1460**	**1383**	**1269 1159**	**1077 1028**	**/**	**/**
Ws-Et-03 (L-L)	**3388**	**2957 2924 2853**	**1733**	**/**	**1613**	**1562**	**1460**	**1378**	**1285 1252 1165**	**1094 1039**	**930 821 750 684**	**722**
Ws-Et-04 (L-L)	**3346**	**2957 2924 2853**	**1732**	**/**	**1613**	**1569**	**1465**	**1378**	**1280 1152 1156**	**1094 1023**	**960 821 750 684**	**722**
Ws-Et-05 (L-L)	**3377 3202 3011**	**2957 2924 2853**	**1722**	**1678**	**1613**	**1569 1503**	**1449**	**1378**	**1280 1198 1159**	**1083 1023**	**930 870 821 744 679 657**	**/**
Ws-Et-06 (L-L)	**3343**	**2957 2924 2853**	**1733**	**1684**	**1613**	**1564 1503**	**1465**	**1378**	**1280 1198 1170**	**1083**	**930 875 842 683**	**/**

Band(cm^{-1}) Separated compounds	**ν_{O-H}**	**ν_{C-H}**	**$\nu_{C=O}$** ester	**ν_{C-O}**	**$\nu_{C=C}$** α,β insaturated	**$\nu_{C=C}$** Arom	**δ_{CH_3}**	**δ_{O-H}**	**ν_{C-O}** α,β insaturated	**ν_{C-O}**	**$\gamma_{=C-H}$**	**γ_{CH2}**
Ws-Et-07 (L-L)	**3405**	**2957 2924 2853**	**1717**	**1684 1656**	**1613**	**1564 1503**	**1465**	**1378**	**1280 1263 1170 1116**	**1072 1039**	**930 875 842 815 684**	**722**
Ws-Et-08 (L-L)	**3432**	**2957 2924 2858**	**1733**	**1689**	**1613**	**1558 1509**	**1465**	**1383 1159 1121**	**1274**	**1072**	**/**	**/**
Ws-Et-09 (L-L)	**3422**	**2957**	**1732**	**1684**	**1618**	**1557 1509**	**1457**	**1383**	**1283 1121**	**1075**	**960 817 694**	**722**
Ws-Et-10 (L-L)	**3421**	**2957 2918 2853**	**1700**	**/**	**1624**	**1569 1509**	**1454**	**1378**	**1285 1159**	**1099 1066 1034**	**935 826 651**	**/**

Table IV.44 :Diethyl ether fraction infrared spectroscopy characteristics

Band(cm^{-1}) Separated compounds	ν_{O-H}	ν_{C-H}	$\nu_{C=O}$ ester	ν_{C-O}	$\nu_{C=C}$ α,β insaturated	$\nu_{C=C}$ Arom	δ_{CH_3}	δ_{O-H}	ν_{C-O} α,β insaturated	ν_{C-O}	$\gamma_{=C-H}$	γ_{CH2}
Ws-E-01 (L-L)	3415	2957 2924 2847	1733	1667	1613	/	1465 1416	1372	1121	1034	919 875 793 608	/
Ws-E-02 (L-L)	3437	2957 2918 2853	1744	/	1618	/	1460	1372	1263 1181 1121	1066 1028	870 804 602	711
Ws-E-03 (L-L)	3426	2962 2924 2847	1738	1667	/	1558.12	1471	1383	1263 1159	1077 1028	/	/
Ws-E-04 (L-L)	3634 3470	2962 2918 2847	1869 1733	1640	/	/	1470	1378	1236	1088	951 799	/
Ws-E-05 (L-L)	3437	2951 2951 2853	1733	1629	/	/	1460	1372	1258 1181	1099 1017	799	717

Band(cm^{-1}) Separated compounds	**ν_{O-H}**	**ν_{C-H}**	**$\nu_{C=O}$** ester	**ν_{C-O}**	**$\nu_{C=C}$** α,β insaturated	**$\nu_{C=C}$** Arom	**δ_{CH_3}**	**δ_{O-H}**	**ν_{C-O}** α,β insaturated	**ν_{C-O}**	**$\gamma_{=C-H}$**	**γ_{CH2}**
Ws-E-06 (L-L)	3437	2957 2924 2847	1733	1651	/	/	1465	1372	1258	1099	799	717
Ws-E-07 (L-L)	3415	2962 2924 2847	1738	/	1629	/	1465	1378	1258 1110	1028	/	/
Ws-E-08 (L-L)	3426	2924 2853	1733	/	1629	/	1465	1378	1236 1198	1088	952 804 602	/

Flavone

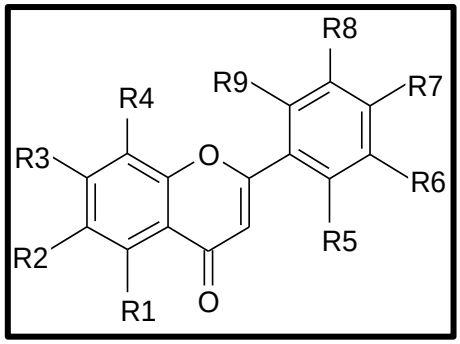


Figure IV. 20 :Flavone structure

Table IV.45 : Identification of flavone separated from different fractions of *Warionia Saharae* extraxts

Fraction	R1	R2	R3	R4	R5	R6	R7	R8	R9
Ws-Bu-01	OH	OH	OH	H	H	OMe	OMe	OMe	H
Ws-Bu-02	OH	H	OH	OH	H	OMe	OMe	H	H
Ws-Bu-03	H	OH	H	H	H	OMe	OMe	H	H
Ws-Bu-04	H	H	OH	H	H	OH	OH	H	H
Ws-Bu-05	OH	H	OH	H	H	OMe	OMe	H	H
Ws-Bu-11	H	H	OH	OH	H	OMe	OMe	H	H
Ws-Et-11	OH	OH	OH	H	H	OMe	OH	H	H
Ws-Et-17	OH	OH	OH	H	H	OH	OH	H	H
Ws-Et-01(L)	OH	H	OH	OH	H	OMe	OMe	H	H
Ws-Et-02(L)	OH	H	OH	H	H	OMe	OMe	H	H

Ws-Et-04(L)	OH	OH	H	H	H	OMe	OMe	OMe	H
Ws-Et-07(L)	OH	OH	OH	H	H	H	H	H	H
Ws-Et-09(L)	OH	H	OH	H	H	H	OMe	H	H
Ws-Et-10(L)	OH	OH	OH	H	H	OMe	OMe	H	H
Ws-E-04(L)	OH	OH	OH	H	H	OH	OH	H	H
Ws-E-07(L)	OH	H	OH	H	H	H	OMe	H	H

Flavone O- glycosyl

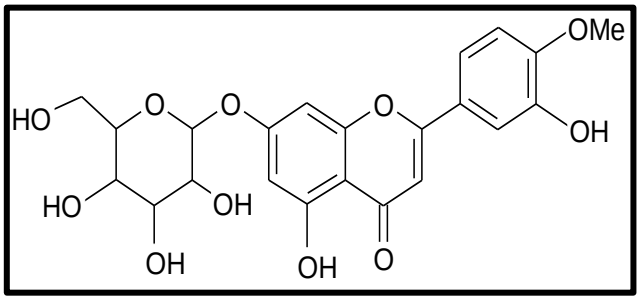


Figure IV. 21 :4'- Methoxy 5,7 –dihydroxy Luteolin – o- glycosyl

Isoflavone

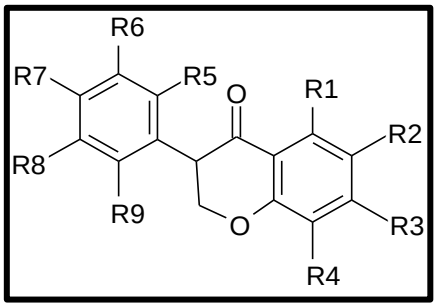


Figure IV. 22 :Isoflavone structure

Table IV. 46: Identification of isoflavone separated from different fractions of *Warionia Saharae* extraxts

Fraction	R1	R2	R3	R4	R5	R6	R7	R8	R9
Ws-Bu-13	OH	OH	OH	H	H	H	OMe	H	H
Ws-Et-05 (L)	H	H	OH	OH	H	OMe	OMe	OMe	H
Ws-Et-06 (L)	OH	H	OH	OH	H	OMe	OH	H	H
Ws-Et-08 (L)	H	H	OH	H	H	H	OMe	H	H
Ws-E-02 (L)	OH	OH	OH	H	H	OMe	OMe	H	H
Ws-E-03 (L)	OH	OH	OH	OH	H	OH	OMe	H	H
Ws-E-06 (L)	OH	H	OH	OH	H	OMe	OH	OMe	H
Ws-E-08 (L)	H		OH	H	H	H	OMe	H	H

Flavanol

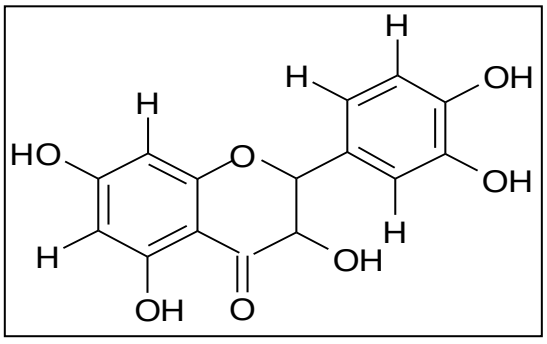


Figure IV. 23 :Flavanol structure

6-Hydroxy Taxifolin

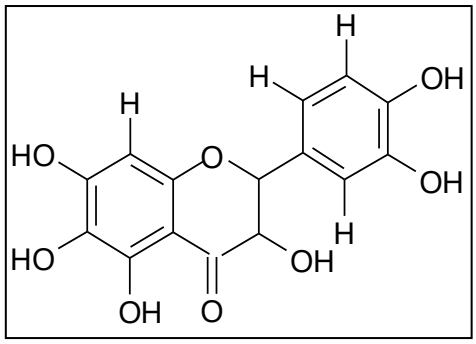


Figure IV. 24 : 6-Hydroxy Taxifolin structure

Flavanone

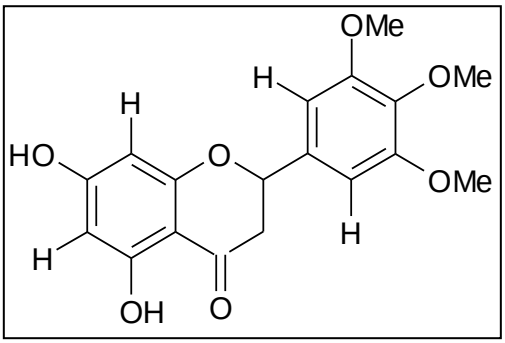


Figure IV. 25 : Flavanone structure

4.6 Evaluation of antioxidant activity

4.6.1 Launeae nudicaulis extracts tested with β carotene

The results show that the oxidation of β-carotene is effectively inhibited by the tested extracts. In fact, the butanol, methanol and ethyl acetate extract of *Launeae nudicaulis* present an antioxidant capacity. The other extracts ether and chloroform did not show any antioxidant activity.

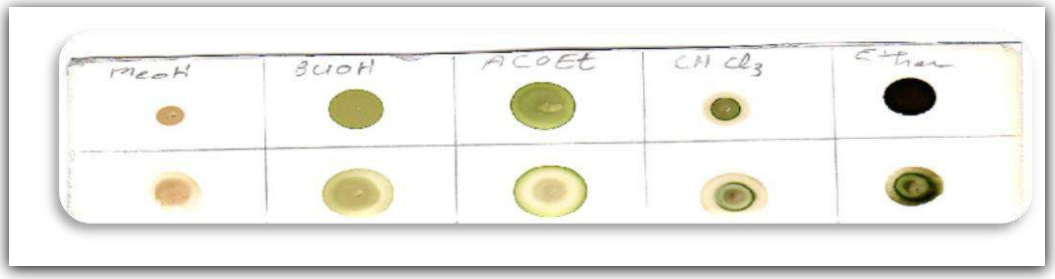


Figure IV.26. Chromatogram of *Launeae nudicaulis* extracts tested with β carotene

The polar extracts butanol, methanol and ethyl acetate are especially rich in soluble chemical substances and their antioxidant activity mainly due to the presence of polyphenolic compounds present in these extracts.

Table IV.47 :. Beta carotene bleaching qualitative assay

Extract	Antioxidant test	
	Positive	Negative
Ether		-
Chloroform		-
Ethyl acetate	+	
Butanol	+	
Methanol	+	

4.6.2 DPPH radical scavenging assay by TLC results - *Launeae nudicaulis*-

a. Launeae nudicaulis extracts

The extract have the antioxidant activity which appear as yellow white spot on purple These extracts are able to reducing the DPPH radical .

- ✓ The DPPH screening method by the mean of thin layer chromatography indicated the presence of antioxidant compounds in methanol (MeOH) , butanol (BuOH) , ethyl acetate (EtOAc) and chloroform ($CHCl_3$) extracts of *Launeae nudicaulis* .

- ✓ The most prominent antioxidant activity was observed particularly in butanol (BuOH) , methanol (MeOH) ,and ethyl acetate (EtOAc) extract respectively , This result demonstrates its richness in soluble chemical substances with high antioxidant activity, this activity may be mainly due to the presence of polyphenolic compounds present in high content in these extracts .

- ✓ In the other hand the ether extract present non antioxidant activity.

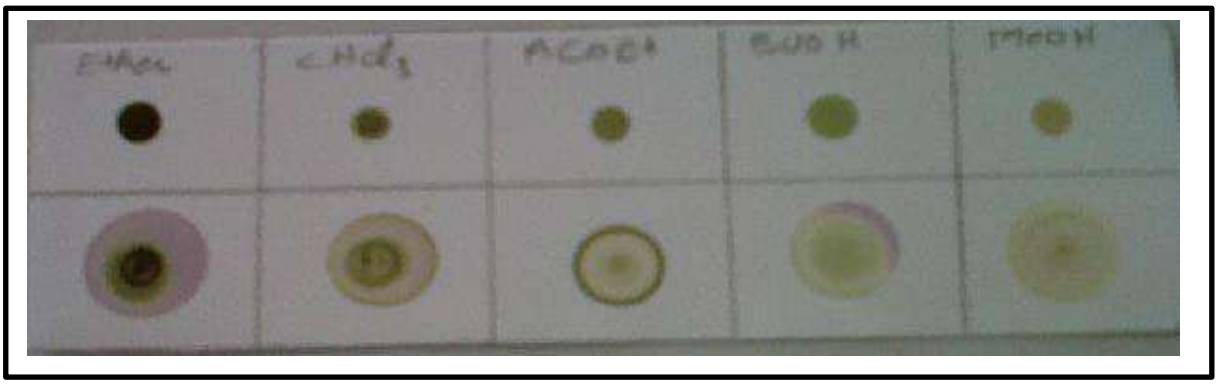


Figure IV.27. Chromatogram of *Launeae nudicaulis* extracts tested with DPPH radical

Table IV.48. DPPH radical scavenging qualitative assay

Extract	Antioxidant test (+/-)	
Ether		-
Chloroform	+	
Ethyl acetate	+	
Butanol	+	
Methanol	+	

***b. Launeae nudicaulis* extracts developed in solvent system**

As shown in **figure IV.28,** the four extracts of *Launeae nuidcaulis* were developed in appropriate mobile phase present a radical scavenging activity except ether extract.

MeOH and BuOH extract of *Launeae nuidicaulis* present the highest anti-radical activity with a massive task extended along the path of the elution. This result demonstrates its richness in soluble chemical compounds with a high radical scavenging activity; this activity may be mainly due to the presence of polyphenolic compounds present in high contents in the both extracts.

The ethyl acetate extract of *Launeae nudicaulis* also shows an interesting radical scavenging activity but less important than BuOH and MeOH extract.

The ether and chloroform extracts are the least active, with spots not very clear, especially for ether extract because of their low contents or absence of polyphenolic compounds.

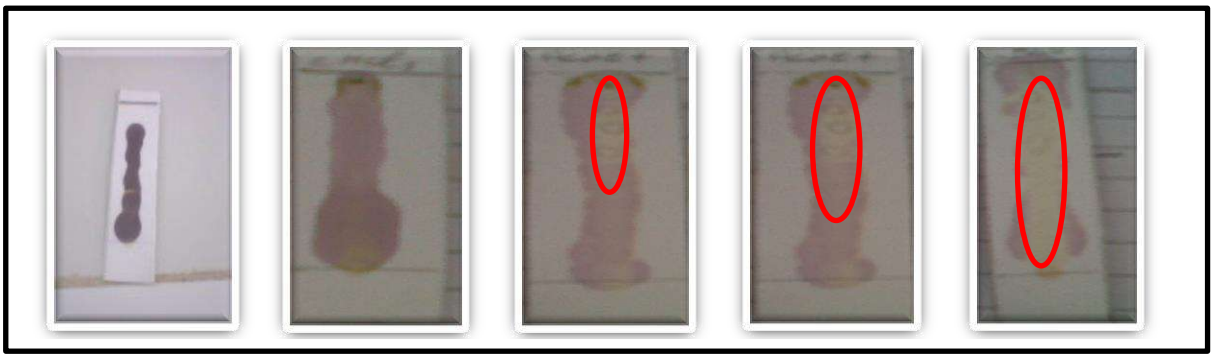


Figure IV.28. Chromatogram of *launeae nudicailis* extract developed in solvent

Table IV.49. Antioxidant activity and Rf of *Launeae nudicaulis* extracts

Extract	Rf	Antioxidant activity
		Positive / Negative
Ether	Rf_1=0.86	+
	Rf_2=0.91	-
	Rf_3=0.95	-
Chloroform	Rf_1=0.78	+
	Rf_2=0.84	-
	Rf_3=0.89	-
	Rf_4=0.93	-
Ethyl acetate	Rf_1=0.85	+
	Rf_2=0.87	+
	Rf_3=0.89	+
	Rf_4=0.91	-
Butanol	Rf_1=0.53	+
	Rf_2=0.62	+
	Rf_3=0.73	+
Methanol	Rf_1=0.62	+
	Rf_2=0.75	+
	Rf_3=0.83	+

- ✓ For the ether extract developed in Ethyl acetate / hexane solvent system, shows a single yellow spot on a purple background with Rf = 0.86 .

- ✓ The Chloroform extract developed in Ethyl acetate / hexane solvent presents a single yellow spot on a purple background with Rf = 0.78 .

- ✓ The ethyl acetate extract developed in Acetone /cyclohexane solvent presents three yellow spots on a purple background with Rf_1=0.85 , Rf_2=0.87 and Rf_3=0.89

- ✓ The three spots present in butanol extract which developed in Chloroform / methanol solvent have a yellow color on purple background with Rf_1=0.53 , Rf_2=0.62 and Rf_3=0.73 , this results show that these three compounds able to scavenge free radical .

- ✓ The methanol extract developed in indicate Chloroform / methanol solvent indicates three spots with yellow color on purple background with Rf_1=0.62 , Rf_2=0.75 and Rf_3=0.83 , these compounds present an antioxidant activity .

4.6.3 *Launeae nudicaulis* Quantitative Analysis of the antioxidant activity

a. Launeae nudicaulis extracts

As shown in **Table IV.50 and Figure IV.29** , butanol extract presents a high antioxidant activity with 91,62% then methanol extract with 91,18 % and ethyl acetate extract with 71,57 % . The other extracts showed lower activity, chloroform with 33.96% and ether with no activity 0%.

The polar extracts are especially rich in soluble chemical substances; the antioxidant activity demonstrated by this method may be mainly due to the presence of phenolic compounds in these extracts.

Table IV.50. Antiradical activity of *Launeae nudicaulis* extracts

Extract	**Antioxidant activity**	**DPPHradical scavengingactivity** (%)
	Positive / Negative	
Ether	-	-
Chloroform	+	**23.45**
Ethyl acetate	+	**71.57**
Butanol	+	**91.62**
Methanol	+	**91,18**

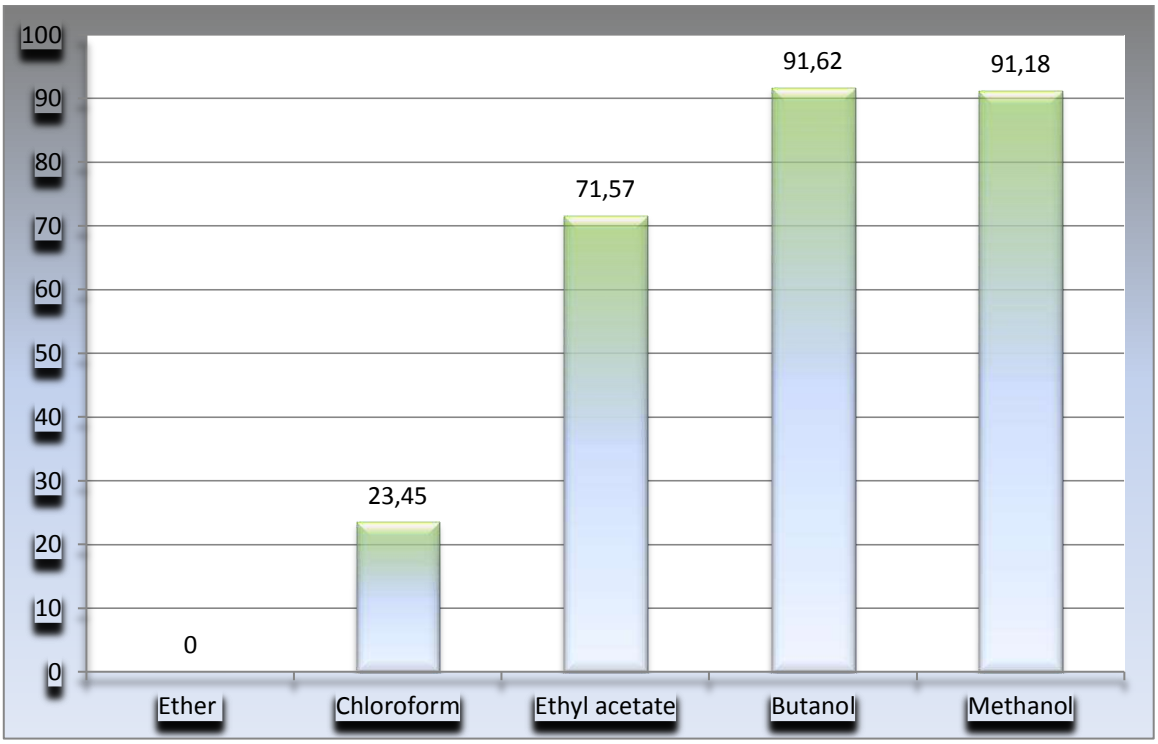


Figure IV.29. Antioxidant activity of *Launeae nudicaulis* extracts

Table IV.51 : Antioxidant capacity Quantification of *Launeae nudicaulis* extracts

Extract	**Antioxidant activity**	**DPPHradical scavengingactivity** (%)
	Positive / Negative	
Ether	-	-
Chloroform	+	**23.45**
Ethyl acetate	+	**71.57**
Butanol	+	**91.62**
Methanol	+	**91,18**

b. **Pure compounds isolated from *Launeae nudicaulis***

The results shown that the compound LNB present an antioxidant activity 82, 05 % belong to flavonol family.

Table IV.52 : Quantitative Analysis of the antioxidant activity of pure compound isolated from *Launeae nudicaulis*

N °	pure compounds	Antioxidant activity		AA %	Class
01	LNA	+		82,94	
02	LNB	+		82,05	Flavonol
03	LNC		-	-	Flavanone
04	LND	+		75,72	
05	LNE		-	-	Flavanone

4.6.4 *Warionia saharae* tested with β- carotene results

a. ***Warionia saharae* extracts**

The results show that the oxidation of β- carotene is effectively inhibited by the tested extracts. In fact , butanol , methanol and ethyl acetate extract of *Warionia saharae* present an antioxidant capacity .The other extracts ether and chloroform did not show any antioxidant activity .

The polar extracts butanol, methanol and ethyl acetate are especially rich in soluble chemical substances and their antioxidant activity mainly due to the presence of polyphenolic compounds present in these extracts.

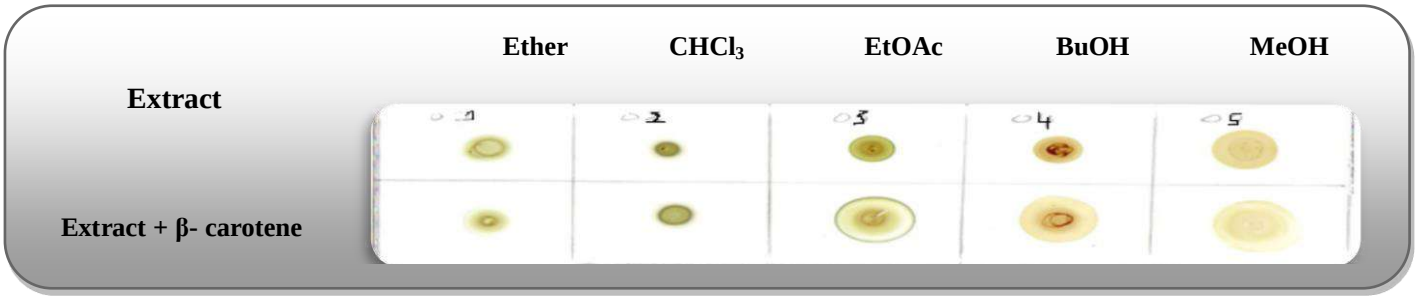


Figure IV.30 : Chromatogram of *Warionia saharae* extracts tested with β- carotene

Table IV.53 : Beta carotene bleaching qualitative assay (*Warionia saharae* extracts)

Extract	Antioxidant activity tested with β- carotene (- / +)	
Ether		-
Chloroform		-
Ethyl acetate	+	
Butanol	+	
Methanol	+	

b. **Pure compounds isolated from *Warionia saharae***

Among the 70 isolated pure compounds were tested, only 19 products that has provided an antioxidant effect, these compounds are butanol and ethyl acetate fraction which belong to : flavone , flavanone , isoflavone , flavanol and glycosylated flavone .

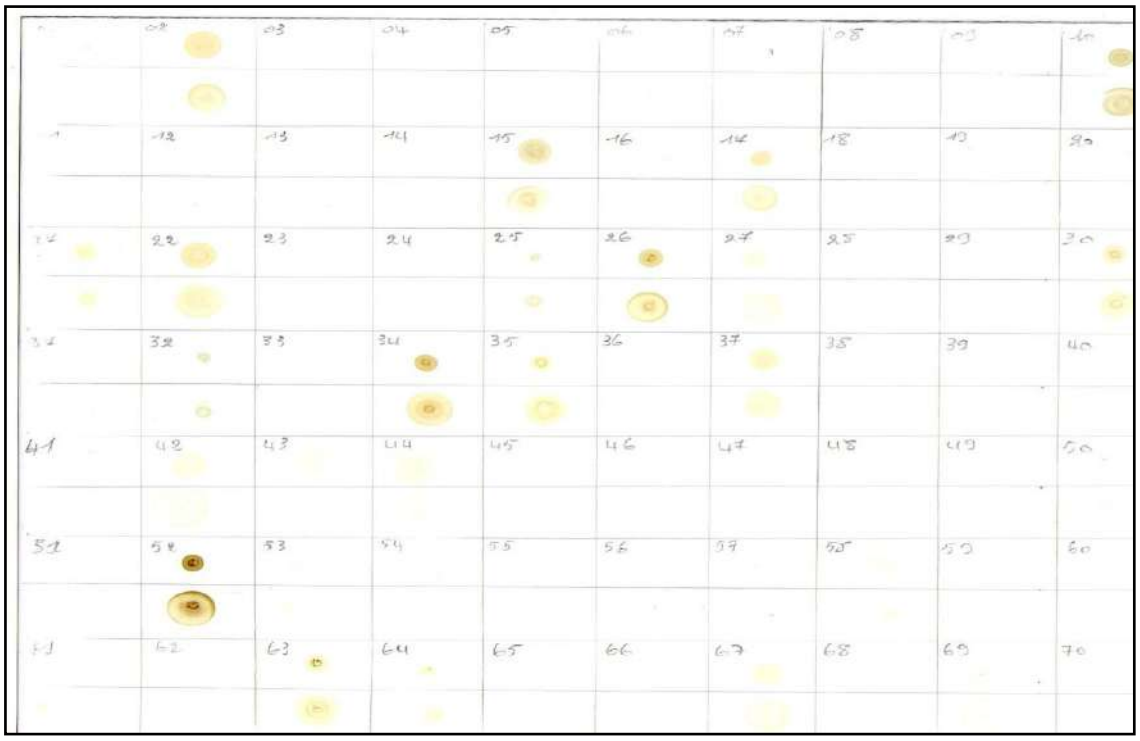


Figure IV.31 : Chromatogram of pure compounds isolated from *Warionia saharae* tested with β- carotene.

Table IV.54: Antioxidant activity of pure compounds isolated from *Warionia saharae* tested with β- carotene.

N°	Pure compounds isolated	Antioxidant activity	
		+	-
01	w.s bu 14	+	
02	w.s Et 05 (L-L)	+	
03	Extraction w.s E 0.85		-
04	w.s CH 02		-
05	w.s Et 02		-
06	w.s Et 01		-
07	w.s Et 12		-
08	w.s E 1		-
09	w.s Et 10		-
10	w.s bu 01	+	
11	w.s Et 06 (L-L)		-
12	w.s Et 08 (L-L)		-
13	w.s E 05 (L-L)		-
14	w.s E 04 (L-L)		-
15	w.s bu 03	+	
16	w.s CH 01		-
17	w.s bu 15	+	
18	w.s Et 20		-
19	w.s Et 07		-
20	w.s Et 07		-
21	w.s Et 17		-
22	w.s bu 11	+	
23	w.s Et 16		-
24	w.s Et 03		-
25	w.s Et 15		-
26	w.s bu 08	+	

27	w.s bu 05	+	
28	w.s E 07 (L-L)		-
29	w.s Et 06		-
30	w.s bu 04	+	
31	w.s E 03 (L-L)		-
32	w.s Et 05		-
33	w.s CH 05		-
34	w.s bu 06	+	
35	w.s Et 03 (L-L)	+	
36	w.s Et 19		-
37	w.s bu Rf : 0.86		
38	w.s E 2		-
39	w.s Et 13		-
40	w.s CH 03		-
41	**w.s E 01** (L-L)		-
42	w.s bu 12	+	
43	w.s E 03		-
44	w.s bu 09	+	
45	w.s Et 09		-
46	w.s CH 08		-
47	w.s Et 10 (L-L)		-
48	w.s E 02 (L-L)		-
49	Extraction (L-L) w.s Et 0.82 02		-
50	w.s CH 04 n° 01		-
51	Extraction (L-L) w.s E 0.94		-
52	w.s bu 02 Rf : 0.914	+	
53	w.s Et 04 0.65		-
54	w.s Et 14 0.94		-
55	Extraction (L-L) w.s Et 0.71 01		-
56	w.s Et 08 0.28		-

57	w.s CH 09 n° 01		-
58	w.s Et 11 0.85	+	
59	Extraction (L-L) w.s Et 0.60 13		-
60	w.s CH 10 n° 01		-
61	Extraction (L-L) w.s Et 0.65 11		-
62	Extraction (L-L) w.s Et 0.42 12		-
63	Extraction (L-L) w.s Et 0.88 07	+	
64	w.s Et 18 0.82	+	
65	w.s CH 06 n° 01		-
66	Extraction (LL) w.s Et 0.77 09		-
67	w.s bu Rf 0.89 16	+	
68	Extraction (LL) w.s Et 0.85 14		-
69	w.s bu 07 Rf : 0.74		-
70	w.s bu Rf : 0.83 10		-

4.6.5 DPPH radical scavenging assay by TLC - *Warionia saharae*-

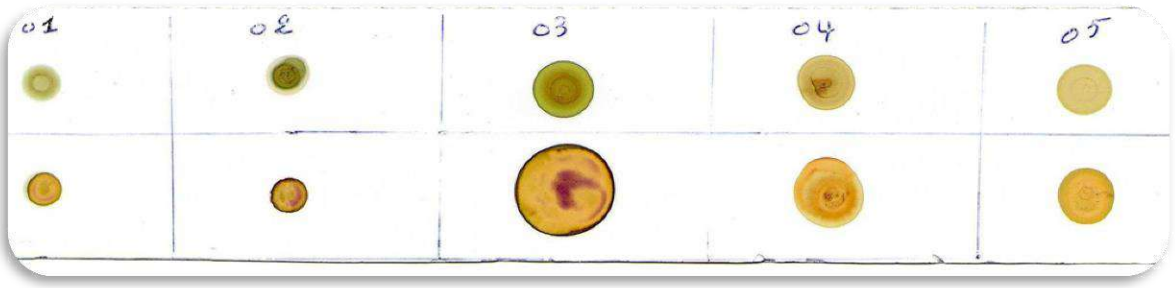
a. *Warionia saharae* extracts

The extract have the antioxidant activity which appear as yellow white spot on purple , these extracts are able to reducing the DPPH radical.

- ✓ DPPH screening method by the mean of thin layer chromatography indicated the presence of antioxidant compounds in methanol (MeOH) , butanol (BuOH) , ethyl acetate (EtOAc) and chloroform ($CHCl_3$) extracts .

- ✓ The most prominent antioxidant activity was observed particularly in butanol (BuOH) , methanol (MeOH) , and ethyl acetate (EtOAc) extract respectively , this result demonstrates its richness in soluble chemical substances with high antioxidant activity , this activity may be mainly due to the presence of polyphenolic compounds present in high content in these extracts .

- ✓ In the other hand the ether extract present no antioxidant activity.



01: Ether , 02 :Chloroform , 03: EtOAc , 04: BuOH , 05:MeOH

Figure IV.32 : Chromatogram of *Warionia saharae* extracts tested with DPPH radical

Table IV.55: DPPH radical scavenging qualitative assay

Extract	**Antioxidant**	
Ether		-
Chloroform	+	
Ethyl acetate	+	
Butanol	+	
Methanol	+	

b. **Pure compounds isolated from *Warionia saharae***

Among the 70 isolated pure compounds were tested, only 19 substances that has given an antioxidant capacity to reduce DPPH radical.

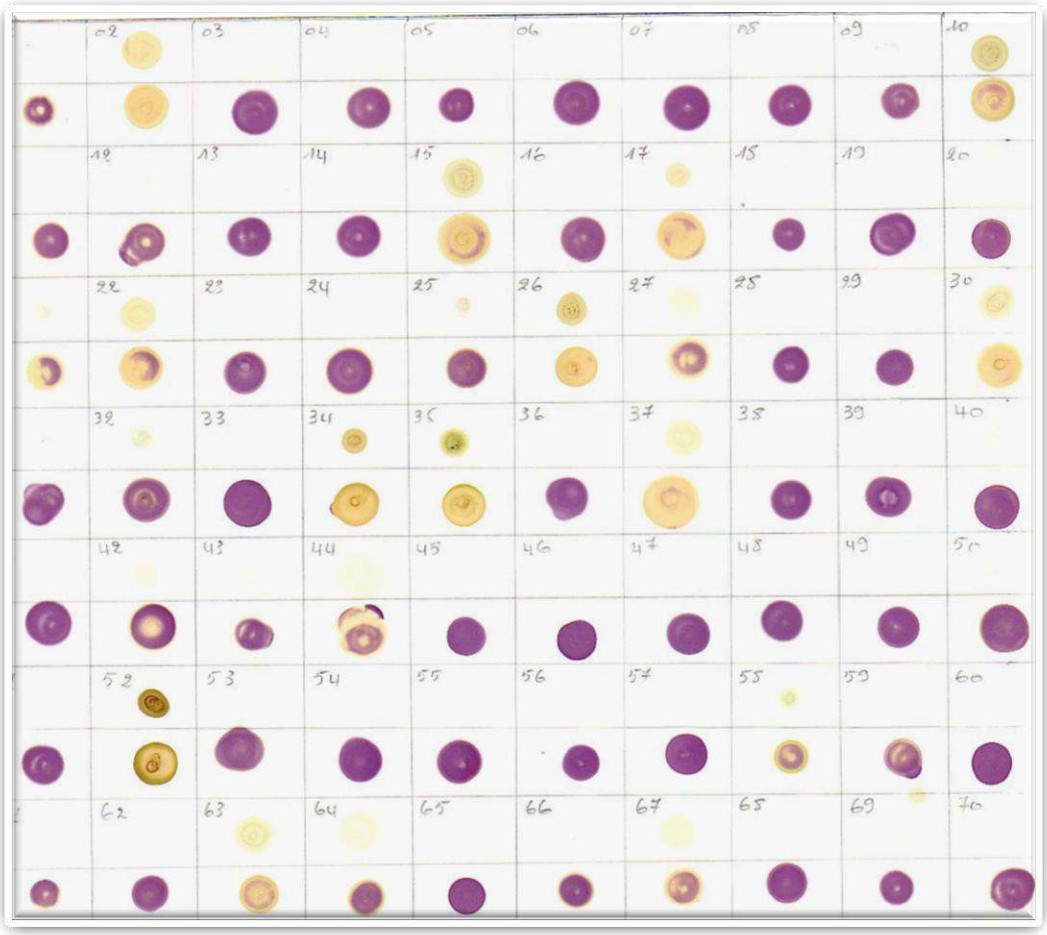


Figure IV.33: Chromatogram of substances isolated from *Warionia saharae* tested by DPPH radical

***c.* *Warionia saharae* extracts developed in appropriate solvent**

- ✓ MeOH and BuOH extracts of *Warionia saharae* presented the best anti-radical activity with a massive task extended along the elution , This result demonstrates its richness in water-soluble substances with a high anti-radical activity , this may be mainly due to the presence of polyphenolic compounds present in high contents in the both extracts .

- ✓ EtOAc extract of *Warionia saharae* also shows an interesting radical scavenging activity but less important than BuOH and MeOH extract .

- ✓ Ether and chloroform extracts are the least active, with spots not very clear, especially for ether extract that because of their low contents or absence of polyphenolic compounds.

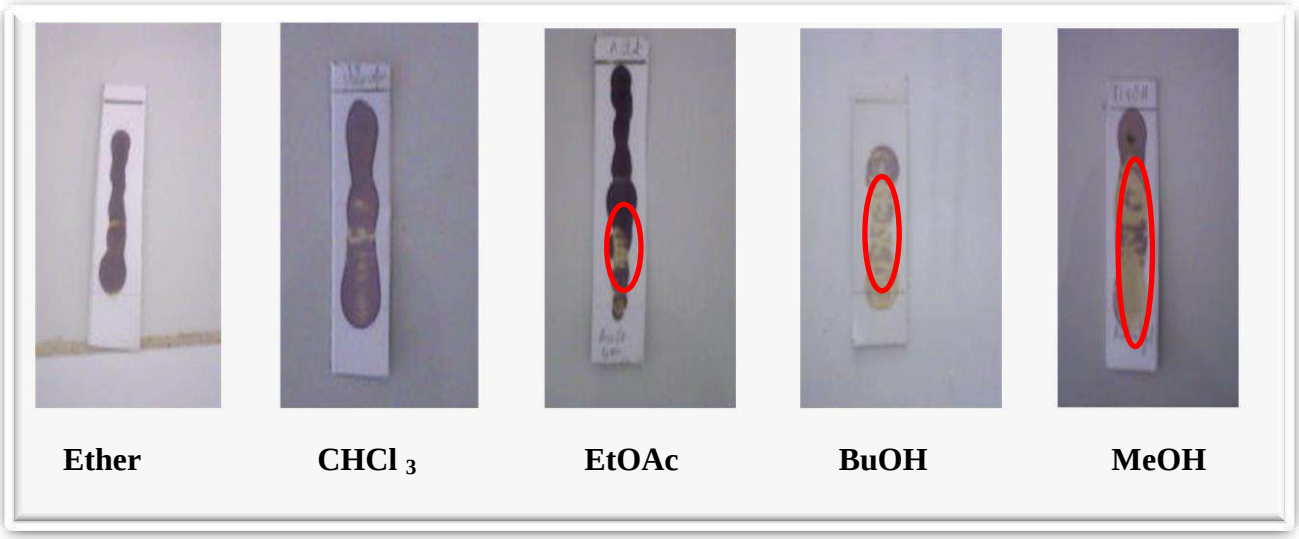


Figure IV.34: Chromatogram of *Warionia saharae* extracts developed in solvent

- ✓ Ether extract developed in acetone / cyclohexane eluent shows a single yellow spot on purple background with an Rf = 0.45.

- ✓ For chloroform extract developed in ethyl acetate / hexane eluent presents a single yellow spot on purple background with an Rf = 0.48.

- ✓ Ethyl acetate extract developed in acetone / hexane eluent illustrates two yellow spots on a purple background with Rf1= 0.54 and Rf2 = 0.68.

- ✓ For butanol extract developed in chloroform eluent shows two yellow spots on a purple background with Rf1= 0.62 and Rf2 = 0.71.

- ✓ For methanol extract developed in acetone / hexane eluent shows two yellow spots on a purple background with Rf1= 0.50 and Rf2 = 0.57.

Table IV.56 : Antioxidant activity and Rf of the *Warionia saharae* extracts

Extract	**Rf**	**Antioxidant activity**	
		+	-
Ether	**Rf=0.45**	+	
	Rf=0.57	-	
	Rf=0.61	-	
	Rf=0.66	-	
	Rf=0.71	-	
	Rf=0.73	-	
	Rf=0.78	-	
Chloroform	**Rf_1=0.48**	+	
	Rf_2=0.58	-	
	Rf_3=0.66	-	
	Rf_4=0.72	-	
	Rf_5=0.77	-	
	Rf_6=0.80	-	
	Rf_7=0.83		
	Rf_8=0.88	-	
Ethyl acetate	**Rf_1=0.54**	+	
	Rf_2=0.62	-	
	Rf_3=0.68	+	
	Rf_4=0.74	-	
	Rf_5=0.8	-	
	Rf_6=0.82	-	
	Rf_7=0.85	-	
Butanol	**Rf_1=0.62**	+	
	Rf_2=0.71	+	
	Rf_3=0.80	-	

Methanol	**Rf_1=0.50**	+
	Rf_2=0.57	+
	Rf_3=0.82	-

4.6.6 Warionia saharae Quantitative analysis of the antioxidant activity

a. Warionia saharae extract

The antiradical activity of Warionia *saharae* extract was evaluated by mean of DPPH radical scavenging method. Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

$$\textbf{DPPH radical scavenging activity}\ (\%) = \frac{\textbf{Acontrol} - \textbf{Asample}}{\textbf{Acontrol}}$$

$\mathbf{A_{sample}}$: absorbance of sample

$\mathbf{A_{control}}$: absorbance of control

The antiradical activity of extracts of the species *Warionia saharae* estimated in percentage is shown in the following table

Table IV.57 : Antioxidant activity of *Warionia saharae* extracts

Extract	**Antioxidant activity**		**AAR%**
	+	-	
Ether		-	
Chloroform	+		33.96
Ethyl acetate	+		70.62
Butanol	+		89.51
Methanol	+		72.16

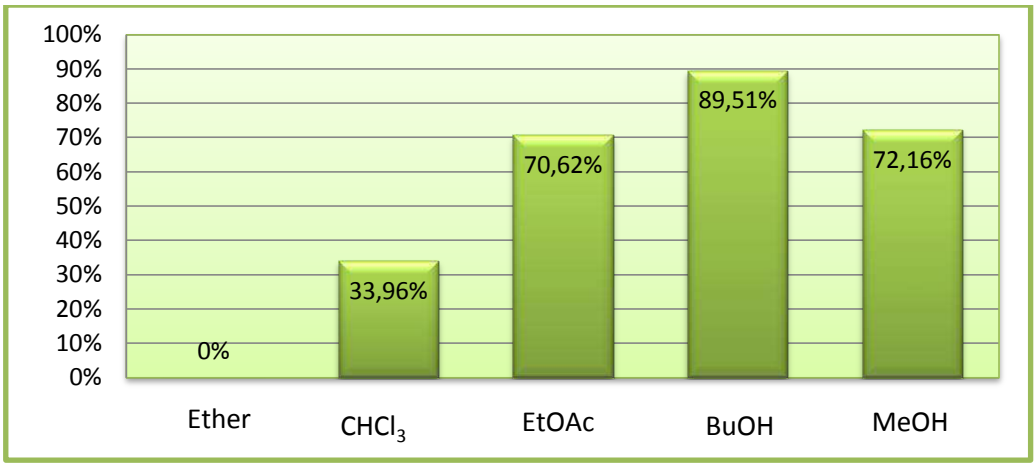


Figure IV.35 : Antioxidant activity of *Warionia saharae* extracts

b. **Pure compounds isolated from *Warionia saharae***

Table IV.58 : Antioxidant activity of the pure compounds of *Warionia saharae*

N°	Fraction	Antioxidant Activity		AAR(%)
		+	-	
01	w.s bu Rf : 0.82 14	+		**78.32**
02	Extraction (L- L) w.s Et 0.91 05	+		**95.30**
03	Extraction L-L w.s E 0.85		-	
04	w.s CH 02 n°01		-	
05	w.s Et 02 0.71		-	
06	w.s Et 01 0.74		-	
07	w.s Et 12 0.40		-	
08	w.s E 1 0.85		-	
09	w.s Et 10 0.58		-	
10	w.s bu 01 Rf 01	+		**83.84**
11	Extraction (L-L) w.s Et 0.74 06		-	
12	Extraction (L-L) w.s Et 0.57 08		-	
13	Extraction (L-L) w.s E 0.91 05		-	
14	Extraction (L-L) w.s E 0.88 04		-	
15	w.s bu 03 Rf : 0.971	+		**75.92**
16	w.s CH 01 n°01		-	
17	w.s bu Rf : 0.90 15	+		**67.56**

18	w.s Et 20 0.57		-	
19	w.s Et 07 0.48		-	
20	w.s Et 07 n° 01		-	
21	w.s Et 17 0.80		-	
22	w.s bu Rf : 0.87 11	+		**69.33**
23	w.s Et 16 0.83		-	
24	w.s Et 03 0.68		-	
25	w.s Et 15 0.97		-	
26	w.s bu 08 Rf : 0.88	+		**76.25**
27	w.s bu 05 Rf : 0.771	+		**95.45**
28	Extraction (L –L) w.s E 0.97 07		-	
29	w.s Et 06 0.62		-	
30	w.s bu 04 Rf 0.8	+		**76.65**
31	Extraction (L-L) w.s E 0.82 03		-	
32	w.s Et 05 0.91		-	
33	w.s CH 05 n° 01		-	
34	w.s bu 06 Rf : 0.85	+		**78.32**
35	Extraction (L –L) w.s Et 0.97 03	+		**86.71**
36	w.s Et 19 0.60		-	
37	w.s bu Rf : 0.86			**69.59**
38	w.s E 2 0.71		-	
39	w.s Et 13 0.51		-	
40	w.s CH 03 n° 01		-	
41	**Extraction (L -L) w.s E 0.685 01**		-	
42	w.s bu Rf : 0.94 12	+		**66.16**
43	w.s E 03 0.65		-	
44	w.s bu Rf : 0.92 09	+		**87.71**
45	w.s Et 09 0.61		-	
46	w.s CH 08 n° 01		-	
47	Extraction (L-L) w.s Et 0.62 10		-	
48	Extraction (L-L) w.s E 0.77 02		-	
49	Extraction (L-L) w.s Et 0.82 02		-	

50	w.s CH 04 n° 01		-	
51	Extraction (L-L) w.s E 0.94		-	
52	w.s bu 02 Rf : 0.914	+		**70.16**
53	w.s Et 04 0.65		-	
54	w.s Et 14 0.94		-	
55	Extraction (L-L) w.s Et 0.71 01		-	
56	w.s Et 08 0.28		-	
57	w.s CH 09 n° 01		-	
58	w.s Et 11 0.85	+		**63.90**
59	Extraction (L-L) w.s Et 0.60 13		-	
60	w.s CH 10 n° 01		-	
61	Extraction (L-L) w.s Et 0.65 11		-	
62	Extraction (L-L) w.s Et 0.42 12		-	
63	Extraction (L-L) w.s Et 0.88 07	+		**81.65**
64	w.s Et 18 0.82	+		**96.40**
65	w.s CH 06 n° 01		-	
66	Extraction (LL) w.s Et 0.77 09		-	
67	w.s bu Rf 0.89 16	+		**73.52**
68	Extraction (LL) w.s Et 0.85 14		-	
69	w.s bu 07 Rf : 0.74		-	
70	w.s bu Rf : 0.83 10		-	

4.6.6 Quantitative analysis of pure compounds isolated from butanol fraction of *Warionia saharae*

Table IV.59 : Quantitative analysis of pure compounds isolated from butanol fraction

N°	Fraction	AAR%	Class
01	w.s bu Rf : 0.82 14	**78.32**	Flavanone
10	w.s bu 01 Rf 01	**83.84**	Flavone
15	w.s bu 03 Rf : 0.971	**75.92**	Flavone
17	w.s bu Rf : 0.90 15	**67.56**	Flavone-glycosyl
22	w.s bu Rf : 0.87 11	**69.33**	Flavone

26	w.s bu 08 Rf : 0.88	**76.25**	Flavone
27	w.s bu 05 Rf : 0.771	**95.45**	Flavone
30	w.s bu 04 Rf : 0.8	**76.65**	Flavone
34	w.s bu 06 Rf : 0.85	**78.32**	Flavone
37	w.s bu 13 Rf : 0.86	**69.59**	Isoflavone
42	w.s bu 12 Rf : 0.94	**66.16**	Flavanol
44	w.s bu 09 Rf : 0.92	**87.71**	Flavanol
52	w.s bu 02 Rf : 0.914	**70.16**	Flavone
67	w.s bu 16 Rf : 0.89	**73.52**	Flavone

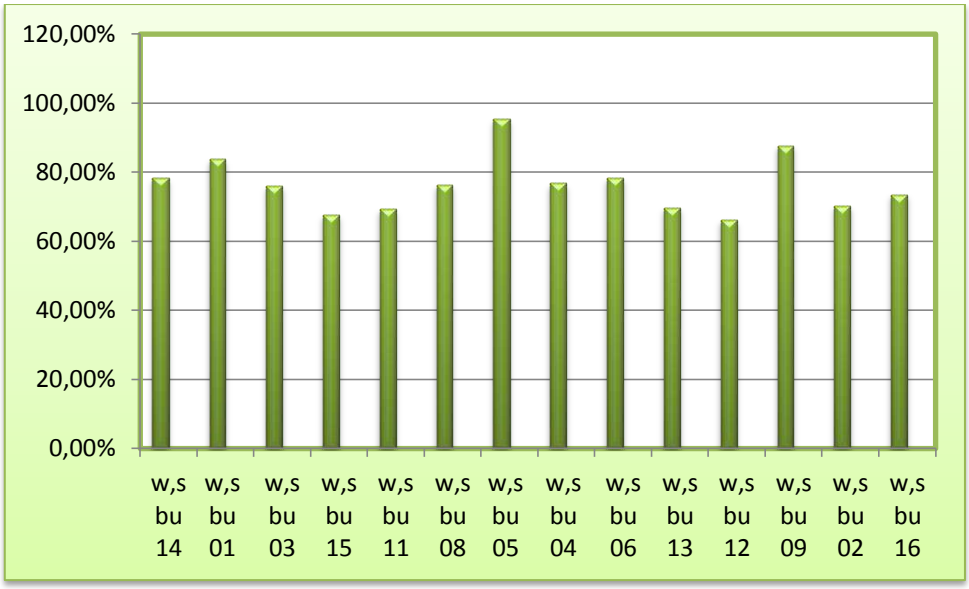


Figure IV.36 : antioxidant activity of pure compounds of *Warionia saharae* butanol fraction

The results present in the table and figure show that pure substances isolated from *Warionia saharae* which have a percentage (%) of higher scavenging activity up to 95% belong to : flavone ; flavanols and flavanones .

4.6.7 Quantitative analysis of pure compounds isolated from ethyl acetate fraction of *Warionia saharae*

Table IV.60 :Quantitative analysis of pure compounds isolated from ethyl acetate fraction

N°	Fraction	AAR%	Class
02	Extraction (L-L) w.s Et 0.91 05	**95.30**	Isoflavone
35	Extraction (L-L) w.s Et 0.97 03	**86.71**	Isoflavone
58	w.s Et 11 0.85	**63.90**	Flavone
63	Extraction (L-L) w.s Et 0.88 07	**81.65**	Flavone
64	w.s Et 18 0.82	**96.40**	Flavanol

Figure IV .37 : Pure compounds isolated from ethyl acetate fraction

According to the above results demonstrating that pure substances isolated from *Warionia saharae* which have a percentage (%) of higher scavenging activity up to 96% belong to: flavanol, isoflavone, flavone.

4.7 Antibacterial activity

The antibacterial activity of the *launeae nudicaulis* and *warionia saharae* extracts was determined by disc diffusion method against the following bacterial strains : *Escherichia coli* , *Bacillus subtilis* and *Staphylococcus aureus* .

Antibacterial activity of *Launeae Nudicaulis*

- ✓ Results show that maximal antibacterial activity , as performed by disc diffusion method , is exhibited by methanolic extract of *Launeae Nudicaulis* especially against *Bacillus subtilis* i.e 18,5 mm zone of inhibition .

Table IV.61 : Antibacterial activity of *Launeae Nudicaulis* extract

Extract	**Concentration**	**Micro organisms**		
		Escherichia coli	*Bacillus subtilis*	*Staphylococcus aureus*
		Inhibition zone in (mm)		
MeOH	Concentrated extract	15	18.5	18
	Diluted extract 10%	0	12	12

- ✓ MeOH extract was tested against *Escherichia coli* and *Staphylococcus aureus* by well method , the maximum zone of inhibition (18 mm) was found against *Staphylococcus aureus.*
- ✓ MeOH extract of *Launeae Nudicaulis* also proved effective against *Escherichia coli* and 15 mm zone of inhibition was seen .

Antibacterial activity of *Warionia Saharae*

Table IV.62 : Antibacterial activity of *Warionia Saharae*

Extract	Concentration	Micro organisms		
		Escherichia coli	*Bacillus subtilis*	*Staphylococcus aureus*
		Inhibition zone in (mm)		
MeOH	Concentrated extract	11,3	11,5	12,5
BuOH	Concentrated extract	10,3	9,5	11,3
EtOAc	Concentrated extract	8,7	8,5	9,5

The antibacterial activity of the various extracts of *Warionia Saharae* were tested in vitro against three microorganisms as seen in the table

- ✓ MeOH extract showed an important antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* with zone of inhibition of 12,5 mm , 11,5 mm and 11,3 mm respectively .

- ✓ BuOH and EtOAc extracts showed moderate antibacterial activity against all microorganisms tested at high concentration.

Relation structure activity

Flavonoid antioxidants function as scavengers of free radicals by rapid donation of a hydrogen atom to radicals, many authors have investigated the antioxidant activity of flavonoids and many attempts have been made to establish the relationship between flavonoid structure and their radical scavenging activity .

In general , the radical scavenging activity of flavonoids depends on the molecular structure and substitution pattern of hydroxyl groups , Previous structure-activity relationship (SAR) studies of flavonoids have pointed to the importance of the number and location of the phenolic OH groups present for the antiradical efficacy. The structural requirement considered to be essential for effective radical scavenging by flavonoids is the presence of a 3',4'-dihydroxy , in the B ring, possessing electron donating properties and being a radical target Also, the 3-OH moiety of the C ring is also beneficial for the antioxidant activity of flavonoids , The C2-C3 double bond conjugated with a 4-keto group, which is responsible for electron delocalization from the B ring, enhances further the radical-scavenging capacity , and saturation of the 2,3-double bond is believed to cause a loss of activity potential , Also, the presence of both 3-OH and 5-OH groups in combination with a 4-carbonyl function and C2-C3 double bond increases the radical scavenging activity , In the absence of the o-dihydroxy structure in the B ring, hydroxyl substituents in a catechol structure on the A-ring were able to compensate and become a larger determinant of flavonoid antiradical activity **[61].**

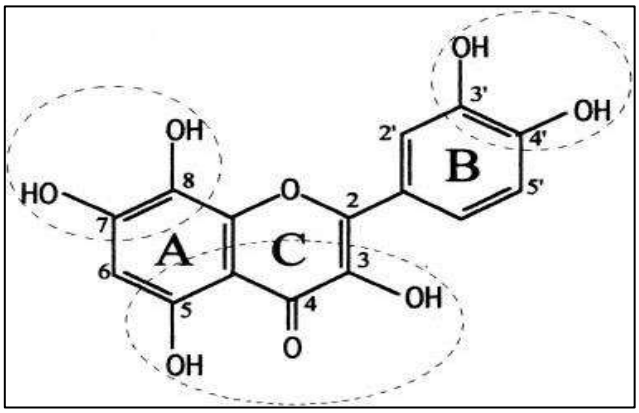
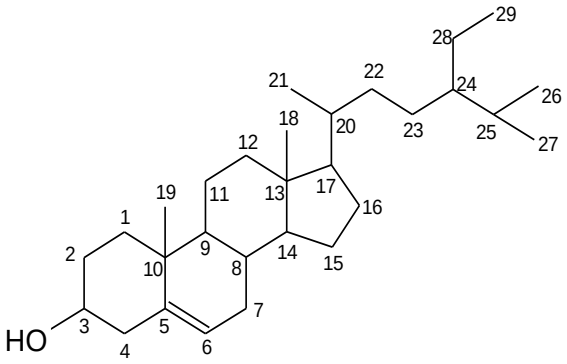
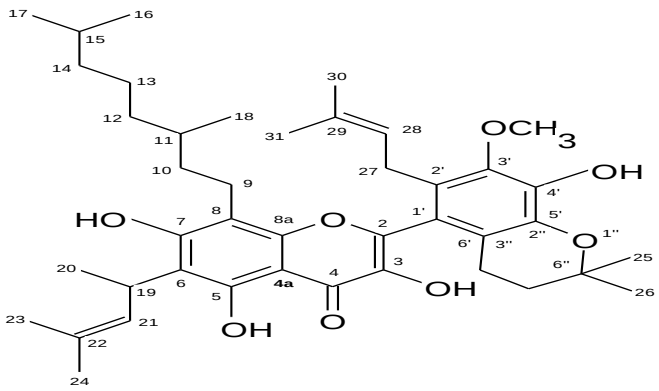


Figure IV .38 : Flavonoid structure

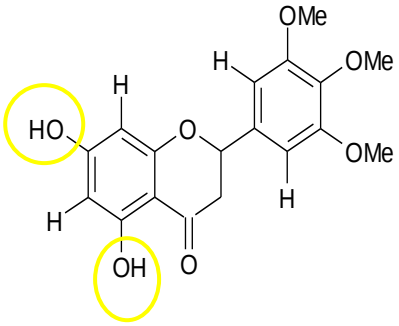
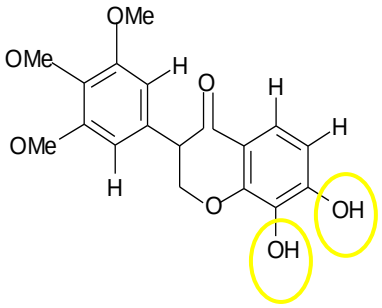
Launeae Nudicaulis

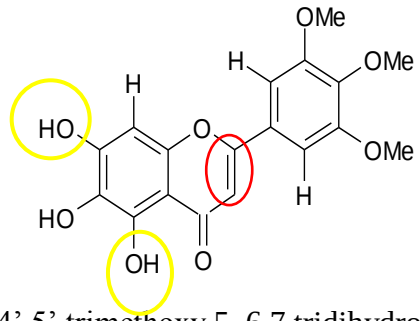
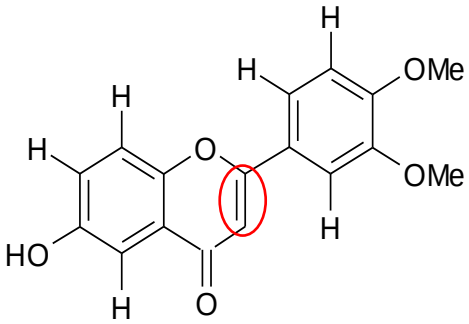
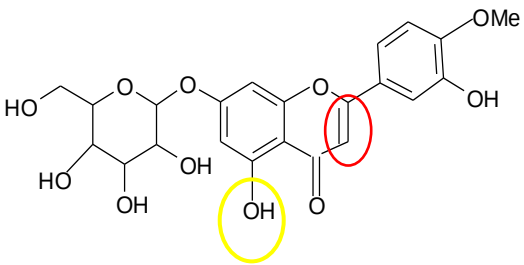
Table IV.63 : Relation structure activity of *launeae nudicaulis*

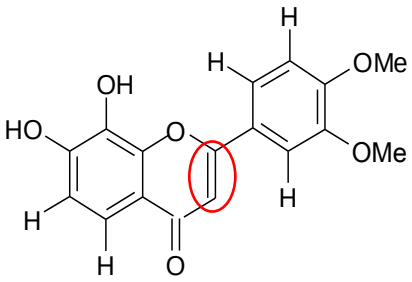
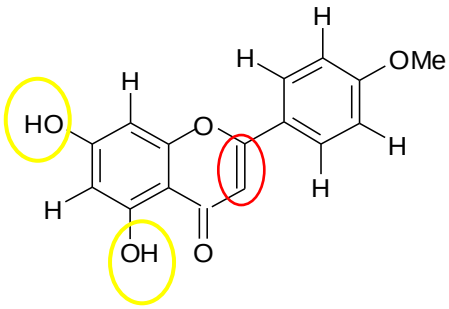
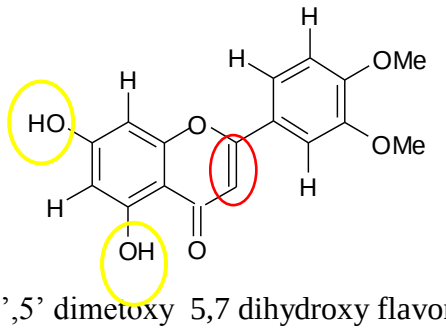
N°	Code	Structure	AA%	Interpretation
01	**LNA**		**82,94**	Presence of hydroxyl group in position 3
02	**LNB**		**83,05**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A.

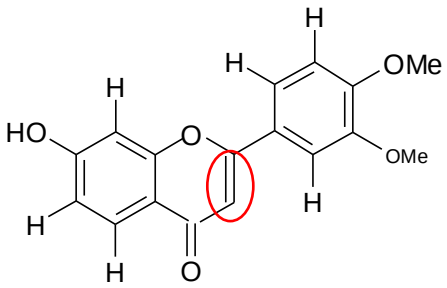
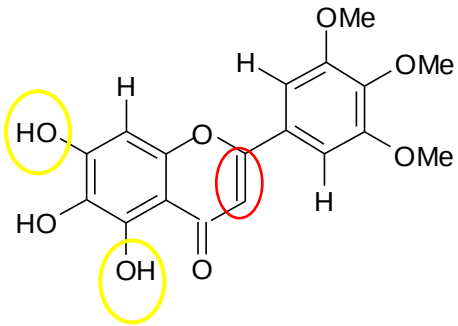
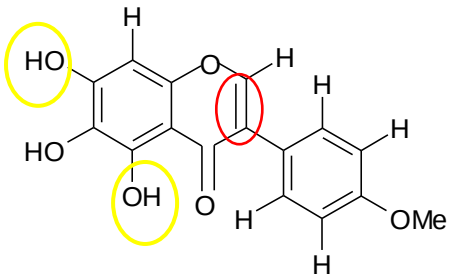
Warionia saharae

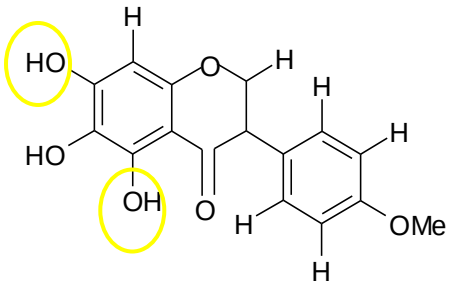
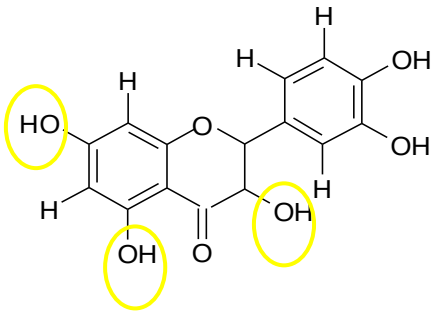
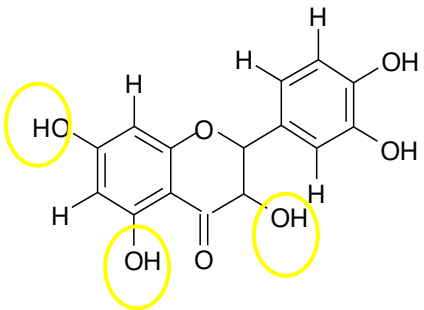
Table IV.64 : Relation structure activity of *Warionia saharae*

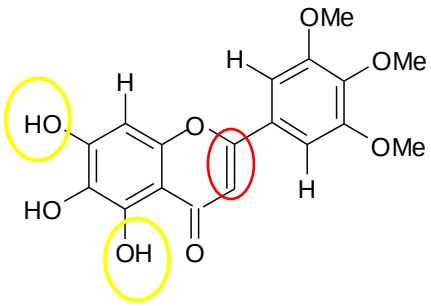
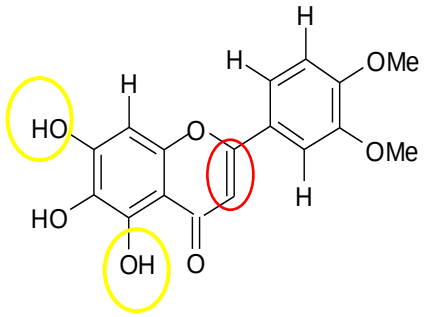
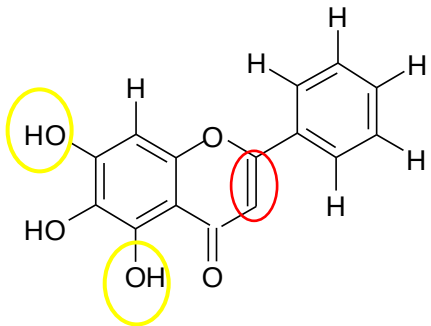
N°	Product	Structure	Antioxidant activity	Interpretation
01	w.s bu 14	 Eriodictyol 3',4',5' trimethoxy (flavanone)	**78.32**	Presence of 5 , 7 dihydroxyl in cycle A
02	w.s Et (L-L) 05	 8-Hydroxy 3',5'-Dimethoxy Formononetin (isoflavone)	**95.30**	Presence of two hydroxyl group

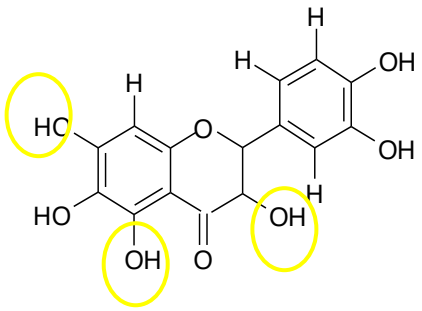
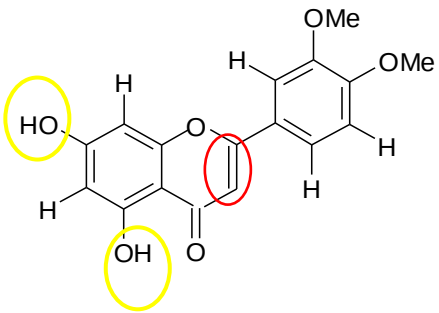
10	w.s bu 01	 3',4',5' trimethoxy 5, 6,7 tridihydroxy flavone (flavone)	**83.84**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A.
15	w.s bu 03	 4',5' dimethoxy 6-hydroxy flavone (flavone)	**75.92**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C.
17	w.s bu 15	 Luteolin 7-o-glycosyl 4'-methoxy 5', 5 dihydroxy (Glycosylate flavone)	**67.56**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of hydroxyl group in 5 position in cycle A .

22	w.s bu 11	 4',5' dimethoxy 7,8-dihydroxy flavone (flavone)	**69.33**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C.
28	w.s bu 08	 4' methoxy 5,7 dihydroxy flavone (flavone)	**76.25**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A.
27	w.s bu 05	 4',5' dimetoxy 5,7 dihydroxy flavone (flavone)	**95.45**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A.

30	w.s bu 04	 4',5' - dimethoxy ,7-hydroxy flavone (flavone)	**76.65**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C.
34	w.s bu 06	 3',4',5' trimethoxy 5,6,7 tridihydroxy flavone (flavone)	**76.32**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A.
35	w.s Et(L-L) 03	 6-Hydroxy Biochanin A (isoflavone)	**86.71**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A.

37	w.s bu 13	 6-Hydroxy Biochanin A (isoflavone)	**69.59**	Presence of 5 ,7 dihydroxy groups in cycle A.
42	w.s bu 12	 4'- methoxy taxifolin (flavanol)	**66.16**	Presence of 5 ,7 dihydroxy groups in cycle A. Presence of hydroxyl group in 3 position in cycle C.
44	w.s bu 09	 Taxifolin (flavanol)	**87.71**	Presence of 5 ,7 dihydroxy groups in cycle A. Presence of hydroxyl group in 3 position in cycle C.

52	w.s bu 02	 3',4' , 5' trimethoxy 5,6,7 Trihydroxy flavone (flavone)	**70.16**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A.
58	w.s Et 11	 3',4' - dimethoxy, 5,6,7 trihydroxy flavone (flavone)	**63.90**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A
63	w.s Et(L-L) 07	 5,6,7 trihydroxy flavone (flavone)	**81.65**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A

64	w.s Et 18	 Taxifolin 6-hydroxy (flavanol)	**96. 40**	Presence of hydroxyl groups in 5 , 7 , and 6 position .
67	w.s bu 16	 3',4'-diMethoxy 5,7 di hydroxy (flavone)	**73.52**	Presence of 2-3 double bond in conjugation with 4 oxo function in cycle C. Presence of 5 ,7 dihydroxy groups in cycle A

Conclusion

Conclusion

Using the technique of activity-guided chromatographic isolation, it is possible to generate many structurally novel bioactive plant secondary metabolites, and examples have been provided in this work of plant secondary metabolites with potential antioxydant or potential cancer chemo preventive activity, comprised by compounds representative of the flavanone, flavone, flavonol, isoflavone, and steroidal alkaloid classes. Several of these novel plant-derived bioactive compounds were isolated along with closely related analogs of previously known structure, and accordingly we have been able to conduct preliminary structure–activity relationship studies with reference to the particular in vitro bioassays in which activity was observed.

The antioxidant activity of the extracts of two medicinal plants : *Launeae nudicaulis* and *Warionea Saharae* evaluated by the mean of two different methods : beta carotene bleaching assay and DPPH radical scavenging shows that the butanol extract present a highest antioxidant activity with 91,62 % .

The results of this study provide evidence that flavonoids have radical scavenging activity or antioxidant activity due to presence of substitution patterns on the B ring appear to be the most important contributor to the antioxidant activity , hydroxyl groups boost the antioxidant activity , whereas methoxy and glycosyl groups reduce the antioxidant activity . presence of unsaturation between C2-C3 in conjugation with 4 oxo function enhances the antioxidant capacity . A hydroxyl group at the C3 position is also beneficial to the ability of flavonoids to scavenge free radical.

The MeOH extract of *Launeae Nudicaulis* was tested against *Bacillus subtilis* and *Staphylococcus aureus* , the maximum zone of inhibition (18 mm) was found against *Staphylococcus aureus.*

The MeOH extract of *Launeae Nudicaulis* proved effective against *Escherichia coli* and 15 mm zone of inhibition was seen .

The antibacterial activity of the various extracts of *Warionia Saharae* were tested in vitro against three microorganisms showed an important antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* with zone of inhibition of 12,5 mm , 11,5 mm and 11,3 mm respectively .

It is hoped that in future research one or more of the compounds described in this work will be subjected to further development or, alternatively, will serve as a lead compound for synthetic optimization.

References

References

1. Belboukhari .M ., Cheriti . A., Belboukhari. N., (2011). **Total phenolic content and *in vitro* antioxidant activity of extracts from endemic medicinal plant *Warionia Saharae* .** NPAIJ . 7(3) : 147-150
2. Belboukhari. N., Cheriti . A.,(2006) .**Antibacterial And Antifungal Activities of Crude extracts from *Launeae Arborescens* .** Pakistan Journal of Biologial Sciences .9(1) : 1-2 .
3. Belboukhari. N., (2002) . Communication 6^{éme} Congrés de la SAChem , Université Ferhat Abbass , Setif.
4. Belboukhari. N., Cheriti . A.,(2008). **Ethnomedical and antimicrobial studies of *Launeae Nudicaulis*** . EJEAFChe ; 7(14) : 2749-2753.
5. Benlabbes , A. (2007) . **Etude phytochimique du *Launeae Nudicaulis* .** université de Bechar .
6. Benguerba , A., (2008) . **Etude phytochimique et de la phase butanolique de l'éspéce *Inula Crithmoides L*** . Costantine .91p.
7. Bitam , F., (2010). **Etude phytochimique de *Launeae Arborescens et Halophilla Stipilacea*** .Batna .242p.
8. Boutti , A ., (2006) . **Etude phytochimique de l'éspéce *Globularia alypum L.*** Algerie .102p .
9. Boudjerda , A., (2007) . **Recherche et détermination structurale des métabolites secondaires de *Achillea ligustica* (*Anthemideae*) , et *Ranunculus Cortusifolius* (*Ranunculaceae*).**Costantine.331p.
10. Boddu , J., Svabek, C., Ibraheem , F., Jones, D., and Chopra , S., 2005, **Characterization of a deletion allele of a sorghum Myb gene and yellow seed showing loss of 3- deoxy flavonoids .** Plant Sci.169: 542-552.
11. Boerjan, W., Ralph, J.,and Baucher , M., 2003, **Lignin biosynthesis .**Ann .Rev.Plant Biol . 54 : 519-546;
12. Brunow , G., Lundquist , K., and Gellsteldt , G.,1999 , **Analytical methods in wood chemistry , pulping and paper making ,** Springer – Verlag , Germany , pp;77-124
13. BRUNETON, J. (1993). **Pharmacognosie, phytochimie, Plantes médicinales**. 2^{e} edition. Lavoisier Technique & Documentation. Paris, France.1119p.

14. CHERITI, A., SAAD, A., BELBOUKHARI, N., GHEZALI, S. (2006). **Chemical composition of the essential oil of *Launaea Arborescens* from Algerian Sahara**. Chemistry of Natural Compounds, Vol. 42. N° 3.

15. CHERITI, A., BELBOUKHARI, N., BOMBARDA, I., GAYDOU, E. (2010). **Antimicrobial Activity and Chemical Composition of the Essential Oil from the Sahara Endemic Medecinal Plant *Warionia Saharae* (Asteraceae)**. Revue des Régions Arides. N° 23 (1-Spécial).

16. CHERITI, A., BELBOUKHARI, N., HACINI, S. (2004). **Ethnopharmacological survey and phytochemical screening of somme medecinal plants of Algerian Sahara**. Ir. J. Pharm. Res. 3(2) :51.

17. CHERITI, A., BELBOUKHARI, M., BELBOUKHARI, N., (2012). **Phytochemical and biological studies on *Launeae* Cass . genus 'Asteraceae) from Algerian sahara** . Current topics in photochemistry , vol 11 .

18. DJELLOULI, M. (2007). **Huiles essentielles, évaluation antimicrobiennes et quantification des flavonoïdes dans trois plantes endémiques du sud-ouest Algérien : *Zilla Macroptera, Warionia Saharae* et *Launaea Nudicaulis*.** Bechar. 93p.

19. ESSAQUI, A., ELAMRANI, A., CAYUELA, J. A., BENAISSA, M. (2007). **Chemical Composition of the Essential Oil of *Warionia Saharae* from Morocco**. Jeobp.10 (3): 241 -246.

20. ESSAQUI, A., ELAMRANI, A., BENAISSA, M., RODRIGUES, A. I., YOONGHO, L. (2004). **Chemical Composition of the leaves extract of *Warionia Saharae* of Morocco**. Jeobp.7 (3): 250 -254.

21. FADOL, A., BOUZIANE, F. (2006). **Etude phytochimique de *Launeae Nudicaulis* (Extraction et isolement des flavonoïdes)**. Bechar. 117p.

22. FERRARI, J. (2002). **Contribution à la connaissance du métabolisme secondaire des thymelaeaceae et investigation phytochimique de l'une d'elles : *Gnidia Involucrata* steud. ex A. Rich**. Lausanne. Suisse. 228p.

23. Gallagher, S. R., 1992, GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, San Diego.

24. Halpin, C., Knight, M. E., Foxon, G. A., Campbell, M. M., Boudet, A. M., Boon, J. J., Chabbert, B., Tollier, M.-T., and Schuch, W., 1994, Manipulation of lignin quality by downregulation of cinnamyl alcohol dehydrogenase, Plant J. 6: 339-350.

25. Haslam, E., and Cai, Y., 1994, Plant polyphenols (vegetable tannins) : gallic acid metabolism, Nat. Prod. Rep. 11: 41-65.

26. Harborne, J. B. and Simmonds, N. W., 1964, Biochemistry of Phenolic Compounds, Academic Press, London, pp. 101.

27. Hatfield, R. and Vermerris, W., 2001, Lignin formation in plants: the dilemma of linkage specificity, Plant Physiol. 126: 1351-1357.

28. Hilmi , F., Sticher , S;, Heilmann, J, J . Nat . Prod., 65, 523-526(2002).

29. HOSTETTMANN, K. (1997). **Tout Savoir sur le Pouvoir des Plantes, Sources de Médicaments.** Favre SA, Lausanne. Suisse. 240p.

30. Landucci, L. L., Deka, G. C. and Roy, D. N., 1992, A 13C NMR study of milled wood lignins from hybrid Salix clones, Holzforsch. 46: 505-511.

31. Lapierre, C., Tollier, M. T., and Monties, B., 1988, Mise en évidence d'un nouveau type d'unité constitutive dans les lignines d'un mutant de maïs bm3, C. R. Acad. Sci. Paris, Ser. III 307: 723-728

32. LEBRUN, J. P. (1979). **Eléments pour un Atlas des plantes vasculaires de l'Afrique sèche**. J.E.M.V.P.T., Bot 6. 2: 11-12.

33. Lu, F., and Ralph, J., 1999, Detection and determination of p-coumaroylated units in lignin. J. Agric. Food Chem. 47: 1988-1992.

34. Hironobu Takahashi, Sachiyo Hirata, Hiroyuki Minami, Yoshiyasu Fukuyama "Triterpene and flavanone glycoside from Rhododendron Simsii" phytochemistry 56 (2001) 875-879.

35. Marita, J., Vermerris, W., Ralph, J., and Hatfield, R. D., 2003, Variation in the cell wall composition of maize brown midrib mutants, J. Agric. Food 1Chem. 51: 1313-1321.

36. Maria Teresa Escribano-Bailon and Celestino Santos-Buelga "Polyphenol Extraction from Foods" Chapter I.

37. MILAN, P., HAYASHI, A. H., APPEZZATO-DA-GLÓRIA B. (2006). **Comparative Leaf Morphology and Anatomy of Three Asteraceae Species.** J BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY. Vol. 49, N°. 1 : 135-144

38. Matvienko, M., Torres, M. J., and Yoder, J. I., 2001, Transcriptional responses in the hemiparasitic plant Triphysaria versicolor to host plant signals, Plant Physiol. 127: 272-282.

39. Manfred Hesse, Herbert Meier et Bernd Zeeh "Méthodes spectroscopiques pour la chimie organique" MASSON, Paris, 1997

40. MOUFFOK, S. (2011). **Etude des métabolites secondaires de *Centaurea Pubescens* ssp. *Omphalotricha* (Asteraceae)**. Batna. 178p.

41. MOREIRA-MUÑOZ, A., MUÑOZ-SCHICK, M. (2007). **Classification, diversity, and distribution of Chilean Asteraceae: implications for biogeography and conservation.** Diversity and Distributions.13 : 818–828.

42. Nelson, J. A., and Falk, R. E., 1993, The efficacy of phloridzin and phloretin on tumor cell growth, Anticancer Res. 13: 2287-2292.

43. Niemetz, R., and Gross, G. G., 2005, Enzymology of gallotannin and ellagitannin biosynthesis, Phytochem. 66: 2001-2011.

44. OZENDA, P. **(**2004). **Flore et Végétation du Sahara**. 3e édition. CNRS, Paris. 662p.

45. OZENDA, P. (1983). **Flor du Sahara**. 2e édition. CNRS. Paris. 307p.

46. PAULIAN, P. (1967), **Guide pour l'Etude de quelque plantes Tropicales**. Gauthier-Villards. Paris. 129p.

47. Pillonel, C., Mulder, M. M., Boon, J. J., Forster, B., and Binder, A., 1991, Involvement of cinnamyl-alcohol dehydrogenase in the control of lignin formation in Sorghum bicolor L. Moench, Planta 185: 538-544.

48. Prithiviraj, B., Singh, U. P., Manickam, M., Srivastava, J. S., and Ray A. B., 1997, Antifungal activity of bergenin, a constituent of Flueggea microcarpa, Plant Path. 46: 224-228.

49. Quideau, S., Jourdes, M., Saucier, C., Glories, Y., Pardon, P., Baudry, C., 2003, DNA Topoisomerase inhibitor acutissimin A and other flavano-ellagitannins in red wine, Angew. Chem. 115: 6092-6099.

50. QUEZEL, F., SANTA, S. (1962). **Nouvelle flore de l'Algérie et des régions désertiques méridionales**. Vol. 2. CNRS, Paris. France. 1170p.

51. Ribéreau-Gayon, P., 1972, Plant Phenolics, Oliver and Boyd, Edinburgh, pp. 254.

52. Ralph, J., 1996, An unusual lignin from Kenaf, J. Nat. Prod. 59: 341-342.

53. RACHID, S., ASHRAF, M., BIBI, S., ANJUM, R. (2000). **Antibacterial and antifungal activities of *Launeae Nudicaulis* and *Launeae Resedofolia***. Pakistan Journal of Sciences. 3(4): 630-632.

54. RAMAUT J. L., HOFINGER M., DIMBI R., CORVISIER M. (1985). **Main Constituents of the Essential Oil of Warionia Saharae Benth and Coss**. Chromatographia. Vol. 20, N° 3.

55. Ralph, J., Hatfield, R. D., Piquemal, J., Yahiaoui, N., Pean, M., Lapierre, C., Boudet, A. M., 1998, NMR characterization of altered lignins extracted from tobacco plants

down-regulated for lignification enzymes cinnamyl alcohol dehydrogenase and cinnamyl-CoA reductase, Proc. Natl. Acad. Sci. USA 95: 12803-12808.

56. Ralph, J., Lapierre, C., Marita, J. M., Kim, H., Lu, F., Hatfield, R. D., Ralph, S., Chapple, C., Franke, R., Hemm, M. R., Van Doorsselaere, J., Sederoff, R. R., O'Malley, D. M., Scott, J. T., Mackay, J. J., Yahiaoui, N., Boudet, A. M., Pean, M., Pilate, G., Jouanin, L., and Boerjan, W., 2001, Elucidation of new structures in lignins of CAD- and COMT-deficient plants by NMR. Phytochem. 57: 993-1003

57. Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P. F., Marita, J. M., Hatfield, R. D., Ralph, S. A., Christensen, J. H., Boerjan, W., 2004, Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids, Phytochem. Revs. 3: 29-60.

58. Ralph, J., MacKay, J. J., Hatfield, R. D., O'Malley, D. M., Whetten, R. W., and Sederoff, R. R., 1997, Abnormal lignin in a loblolly pine mutant, Science 277: 235-238.

59. Ruegger, M., and Chapple, C., 2001, Mutations that reduce sinapoylmalate accumulation in Arabidopsis thaliana define loci with diverse roles in phenylpropanoid metabolism, Genetics 159: 1741-1749.

60. R. R., Mackay, J. J., Ralph, J., Sederoff and Hatfield, R. D., 1999, Unexpected variation in lignin, Curr. Opin. Plant Biol. 2: 145-152.

61. RICE –Evans , C.A., Miller, N.J , Free Radical Res ., 22,375-383(1995).

62. Styles, E. D., and Ceska, O., 1989, Pericarp flavonoids in genetic strains of

63. Swain, T. and Bate-Smith, E. C., 1962, Flavonoid compounds, in: Comparative Biochemistry Vol. III., M. Florkin and H. S. Mason, eds., Academic Press, New York, NY, pp. 755-809.

64. Tanaka, T., Nonaka, G., Ishimatsu, M., Nishioka, I., and Kouno, I., 2001, Revised structure of cercidinin A, a novel ellagitannin having (R)-hexahydroxydiphenoyl esters at the 3,4-positions of glucopyranose, Chem. Pharm. Bull. 49: 486-487.

65. Winkel-Shirley, B., 2001, Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology, Plant Physiol. 126: 485-493.

Appendix

ISSN : 0974 - 7508 Volume 7 Issue 3

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 7(3), 2011 [147-150]

Total phenolic content and in vitro antioxydant activity of extracts from the endemic medicinal plant warionia saharae

Mebarka Belboukhari, Abdelkrim Cheriti*, Nasser Belboukhari

Phytochemistry and Organic Synthesis Laboratory, University of Bechar, Bechar, 08000, {ALGERIA}

E-mail : karimcheriti@yahoo.com

Received: 20th June, 2011 ; Accepted: 20th July, 2011

ABSTRACT

The AcOEt fractions prepared from 80% EtOH extract of the endemic Saharan medicinal plant *Warionia saharae* exhibited the most potent antioxidant capacity (IC_{50} = 3.08 ± 0.40 µg/mL for DPPH and 8.95 ± 0.23 µg/mL for superoxide anion radical), compared to the *n*-BuOH and $CHCl_3$ fractions. Thus, this extract fraction exhibited a strong antioxidant activity and had the most potent scavenging abilities which may be caused by the presence of Phenolic compounds.

KEYWORDS

Antioxydant;
Total Phenolic;
Warionia saharae;
Endemic;
Sahara;
Algeria.

INTRODUCTION

Oxidative damage is caused by free radicals and reactive oxygen species, mostly generated endogeneously, they are recognized to be involved in the pathogenesis of various diseases such as atherosclerosis, cancer, diabetes mellitus and reperfusion disorder[1]. Recently some synthetic antioxidants such as BHT (butylated hydroxyl toluens) and BHA (butylated hydroxyl anisole) have been suspected to dangerous to human health. Therefore, there is great interest in finding antioxidants from natural sources, which could be used in medicine and additive to nutraceuticals to prevent such deleterious effect and to minimize oxidative damage to cells[2-5].

A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants and phenolic compounds, naturally distributed in plants, are effective to counteract deleterious action of ROS[6,7]. It is noted that, plant extracts sometimes have better antioxidant activities than those of pure molecules and there is a growing interest for the use of plant extracts as bioactive agent[8].

As a part of our investigation into medicinal plants growing in Algerian Sahara[9-14]; In this study we investigate the antioxydant activity and compare phenolic and flavonoids contents of extracts from the aerial part of the *Warionia saharae* an endemic Saharan specie.

Warionia saharae Benth & Coss. (local name " Efessas or Kabar Lem'aiz") a genus of the family Asteraceae, is an endemic herbaceous medicinal plant represented by only one species which is widely distributed in the south west of Algeria and south east of Morocco[15]. The aerial part of this plant was used in Sahara folk medicine for treating gastrointestinal tracts, icter and as anti-inflammatory[16-20].

EXPERIMENTAL

Plant material

The leaves of *Warionia saharae* were collected from Bechar (south west Algeria) in May 2008. The plant was identified by Pr A. Marouf (Department of

Full Paper

Biology, University Es Senia, Oran – Algeria) and a voucher specimen is kept in the Herbarium of POSL Laboratory, Faculty of Sciences (University of Bechar, Algeria) under N° CA 02/07.

Preparation of the extracts

Dried and powdered leaves (500 g) of *Warionia saharae* were exhaustively extracted with 80% EtOH solution in Soxhlet apparatus for 6 h. The obtained hydro-alcoholic extract was concentrated by a rotary evaporator and diluted with water (200 mL). The resulting solution was extracted successively by liquid/ liquid partition with solvents of increasing polarity: $CHCl_3$, EtOAc and *n*-BuOH. The organic layers were dried with Na_2SO_4, giving after removal of solvents under reduced pressure $CHCl_3$ (2.30 g), EtOAc (3.10 g) and *n*-BuOH (5.91 g) extracts.

Antioxidant activity

DPPH radical scavenging activity

The stable radical DPPH° (1,1-diphenyl-2-picryl hydrazyl) was used for determination of free radicals cavenging activity of the extracts obtained from leaves of *Warionia saharae* based on the described methods[21-23], with minor modifications. A solution of 0.2 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of extract in methanol (with different concentrations 5 to 200 µg/ml). The reaction mixture was shaken vigorously and allowed to stand in the dark for 30 min at room temperature. The control contained all reagents without the sample was used as blank. The DPPH radical scavenging activity was determined by measuring spectrophotometrically the absorbance at 517 nm with a Unicam UV 300 spectrophotometer, using a 10 mm quartz cuvette. All measurements were made in triplicate and ascorbic acid and quercetin were used as references for comparison. The DPPH radical scavenging activity I (%) of the sample was calculated using the following equation:

$$\mathbf{I\ (\%) = [1 - Ab_s / Ab_c] \times 100}$$

Where Ab_s is the absorbance of the plant extract containing DPPH, Ab_c is the absorbance of blank solution of DPPH without the sample.

The IC_{50} value which was defined as the concentration (in µg/mL) of the extract necessary to decrease the absorbance of DPPH by 50% was calculated from the data obtained by sigmoid non-linear regression model using plots.

Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity was determined by NBT (nitro blue tetrazolium) reduction method as described early[23-25] with minor modifications. The superoxide anion radical reduces the yellow dye NBT^{2+} to produce the blue formazan, whose absorbance was measured at 560 nm. The antioxidants compounds are able to inhibit the formation of purple NBT. The extract of *Warionia saharae* at 50, 100 or 150 µg/mL was mixed with 5 mL of 0.05 M of sodium carbonate buffer solution (pH 10.2) containing 1.3 µM riboflavin, 0.02 M methionine and 5.6 µM NBT. After 30 min at light the absorbance was then measured at 560 nm. The superoxide anion radical scavenging activity (%) was calculated according to the equation:

$$\mathbf{\%\ Inhibition = [1 - Ab_s / Ab_c] \times 100}$$

Where Ab_s and Ab_c are the absorbance of sample and blank control (mixture without any sample) respectively.

Determination of total phenolic content (TPC)

The total phenolic contents (TPC) were determined according to the early described procedures [26] with the slight modification of using a Folin and Ciocalteu's phenolic reagent. Briefly, 1 ml of extract solution was mixed with 2 ml of Folin and Ciocalteu's reagent and allowed to react for 3 min. Then, 2 ml of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 ml with distilled water. The reaction mixture was stand for 1 h before the absorbance was read at 760 nm (spectrophotometer UV-Unicam 300). Gallic acid was used as a standard phenolic compound and the results were expressed as mg of gallic acid equivalents/g of extract (mg GAE/g extract).

Determination of total flavonoids content (TFC)

The total flavonoid contents (TFC) in the extracts were estimated spectrophoto-metrically according to the literature[27], with minor modifications. 1 ml of the extract solution added to a test tube which contained 4 ml of distilled water, and then added 0.4 ml of 5% sodium nitrite solution and allowed to stand. After 5 min, 0.8 ml of 10% aluminium chloride was added and allowed to react for 5 min, then 2 ml of sodium

hydroxide solution (15%) was added and the mixture was diluted with another 2 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately. Rutin was used for constructing the standard curve and flavonoids content was expressed as mg of rutin equivalents/g of extract (mg RE/g extract)

TABLE 1 : Radical scavenging activities (DPPH and superoxide) of fractions from EtOH extract of *Warionia saharae*

Fraction (From 80% EtOH extract)	IC_{50}*	
	DPPH	Superoxide
$CHCl_3$	53.50 ± 9.05	25.2 ± 1.1
EtOAc	3.08 ± 0.40	8.95 ± 0.23
n-BuOH	4.40 ± 0.43	10.25 ± 0.63

*** IC_{50} expressed as μg/mL**

RESULTS AND DISCUSSION

In this work, we investigated for the first time the antioxidant activity of organic extracts from *Warionia saharae* Benth & Coss. (Asteraceae), a Saharan endemic medicinal specie. The relatively stable organic radical, DPPH and superoxide anion radical have been widely used in the determination of antioxidant activity of single compounds, as well as of different plant extracts[28,29]. The IC_{50} values (μg/mL) for radical scavenging activities tests of liquid-liquid fractionalisation of the crude 80% EtOH extract along with $CHCl_3$, EtOAc and *n*-BuOH extracts from leaves of *W. saharae* are summarized in TABLE 1.

According to the IC_{50} values presented in TABLE 1, the AcOEt extract from leaves of *W. saharae* exhibited the most potent antioxidant capacity, compared to the $CHCl_3$ and *n*-BuOH extracts. Thus, this extract has an important role in scavenging abilities of various radicals and IC_{50} values of antioxidant activities followed 3.08 ± 0.40 μg/mL for DPPH and 8.95 ± 0.23 μg/mL for superoxide anion radical.

Data expressed in Mean ± SD (standard deviation) from triplicate experiments.

These results using the DPPH and superoxide anion radicals, suggesting that the EtOAc and *n*-BuOH fractions were more enriched in antiradical compounds, which suggests that phenolic derivatives present in these extracts are responsible for the scavenging activity of *W. saharae* (TABLE 2). Literature surveys indicated that plant phenolic derivatives are one of the major groups of compounds having multiple biological effects and acting as antioxidants[30,31]. Thus, a highly positive relationship between total phenols and antioxidant activity was found in many plant species[32].

TABLE 2 : TCP and TFC of fractions from EtOH extract of Warionia saharae

Fraction (From 80% EtOH extract)	TCP (GAE, mg/g)	TFC (RE, mg/g)
$CHCl_3$	6.2 ± 0.5	ND*
EtOAc	143 ± 1.2	11.6 ± 0.5
n-BuOH	63.25 ± 1.1	5.7 ± 0.14

GAE: Gallic Acid Equivalents; RE: Rutin Equivalents

*** ND: Not Determined, Data from triplicate experiments.**

The total phenolic contents of the liquid-liquid fractionalisation of the crude 80% EtOH extract from leaves of *W. saharae*, as determined by the Folin and Ciocalteu method, are : 6.2 ± 0.5, 143 ± 1.2 and 63.25 ± 1.1 mg GAE/g extract, respectively for $CHCl_3$, EtOAc and *n*-BuOH fractions. The maximum PTC (143 ± 1.2 mg GAE/g extract) and FTC (11.6 ± 0.5 mg RE/g extract) in EtOAc fraction suggesting that this fraction extract was more enriched in phenolic derivatives that are responsible for the high scavenging activity. As can be seen in TABLE 1 and 2, the current results indicate that there is a positive correlation between total antioxidant activity and the total phenolic and flavonoids contents.

It was reported that the antioxydant activity could be due to the action of a free hydroxyl group of phenolic compounds. Specially, flavonoids with polyhydroxylated substitution on ring A or B and a free 3-hydroxyl substitution and the lower strength of the O–H bond present in these compounds corresponds to a higher scavenging activity[33,34]. However, these compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates[35]. These results show that in general, the rich-flavonoids plants could be a good source of antioxidants that would help to increase the antioxidant capacity of an organism, protect it against lipid peroxidation and could have a direct action on different diseases in relation with ROS[36].

Full Paper

CONCLUSION

The results obtained in this study clearly showed that both EtOAc and *n*-BuOH extracts fractions from the 80% EtOH extract of leaves of the Saharan endemic medicinal specie *Warionia saharae*, possess antioxidant activity. The EtOAc extract exhibited a strong antioxidant activity and had the most potent scavenging abilities of various radicals which may be caused by the presence phenolic compounds. The antioxidant activity of the *W. saharae* suggests that the extracts obtained by polar solvents from the leaves could be used as an effective natural source of antioxidant. Further studies should be carried out for the isolation and identification of phenolic derivatives, and antioxidant studies are also needed for an understanding of their mechanisms of action.

ACKNOWLEDGEMENTS

We are thankful to MESRS and DGRST for the financial support given through this project and to Pr. A. Marouf, University of Oran for helping.

REFERENCES

[1] N.A.Leong Cheng, M.Tako, I.Hanashiro, H.Tamaki; Food Chemistry, **109**, 415-420, **(2008)**.
[2] I.Bravo; Nutrition Review, **56**, 317-333, **(1998)**.
[3] B.H.Havsteen, Pharm.Therap., **96(2-3)**, 67-202 **(2002)**.
[4] H.N.Shivaprasad, M.S.Gupta, M.D.Kharya, A.C. Rana, S.Mohan; Nat.Prod., Indian J.; **1(1-2)**, 28-30 **(2005)**.
[5] S.K.Rath, J.K.Patra, M.P.Kanji, H.N.Thatoi, S.K. Dutta; Nat.Prod., Indian J.; **6(2)**, **(2010)**.
[6] I.C.Ferreira, P.Baptista, M.Vilas-Boas, L.Barros; Food Chemistry, **100**, 1511-1516 **(2007)**.
[7] J.A.Pereira, P.G..Pereira, I.C.Ferreira, P.Valentao, P.B.Andrade, R.Seabra; J.Agricu.Food Chem., **54**, 8425-843 **(2006)**.
[8] C.A.Calliste, P.Trouillas, D.P. Allais, J.L.Duroux; Agricu.Food Chem.,**53**, 282-288 **(2005)**.
[9] A.Cheriti, A.Saad, N.Belboukhari, S.Ghezali; Chem. Nat.Comp. **42(3)**, 360-361 **(2006)**.
[10] A.Cheriti, A.Saad, N.Belboukhari, S.Ghezali; Flavour Fragr.J., **22**, 286-288 **(2007)**.
[11] N.Belboukhari, A.Cheriti; Asian J.Plants Sc., **4(5)**, 465-467 **(2005)**.
[12] N.Belboukhari, A.Cheriti; Pak.J.Bio.Sc., **9(1)**,1-2, **(2006)**.
[13] N.Belboukhari, A.Cheriti, E.Bombarda, E.Gaydou; Rev.Reg.Arides, NS, 103-107, **(2010)**.
[14] A.Cheriti, M.F.Talhi, N.Belboukhari, Y. Belhadjadji, S.Ghezali; Chem.Techno., Indian J.; **6(1)**, 13-17 **(2011)**.
[15] P.Quezel, S.Santa; « Nouvelle Flore de l'Algerie et des Regions Desertiques et Meridionales », Ed. CNRS, Paris, France, **(1963)**.
[16] J.Belakhdar; « La pharmacopée marocaine traditionnelle ; Médicine arabe ancienne et savoirs populaires». Ibis Press, Paris, France, **(1997)**.
[17] F.Hilmi, O.Sticher, J.Heilmann; J.Nat.Prod., **65**, 523-526 **(2002)**.
[18] F.Hilmi, O.Sticher, J.Heilmann; Planta Med., **69(5)**, 462-464 **(2003)**.
[19] A.Cheriti, N.Belboukhari, S.Hacini; Ir.J.Pharm. Res., **3(2)**, 51 **(2004)**.
[20] A.Cheriti, N.Belboukhari, S.Hacini; Ann. Univ. Bechar, **1**, 4-7 **(2005)**.
[21] M.S. Blois; Nature, **181**, 1199-1200 **(1958)**.
[22] K.P.Suja, A.Jayalekshmy, C.Arumughan; Food Chemistry, **91**, 213-219 **(2005)**.
[23] F.Sharififar, G..Dehghn-Nudeh, M.Mirtajaldini; Food Chemistry,**112**, 885-888 **(2009)**.
[24] C.Beauchamp, I.Fridovich; Analytical Biochemistry, **44**, 276-277 **(1971)**.
[25] K.N.Prasad, B.Yang, X.Dong, G.Jiang, H.Zhang, H.Xie, Y.Jiang; Innov.Food Sc.Emer.Techno., **10**, 627-632 **(2009)**.
[26] V.L.Singleton, J.A.Rossi; Amer.J.Enology and Viticu., **16**, 144-158 **(1965)**.
[27] M.G.Hertog, P.C.Hollman, M.B.Katan, J.Agric. Food Chem., **40**, 2379-2383 **(1992)**.
[28] I.Parejo, F.Viladomat, J.Bastida, A.Rosas-Romero, N.Flerlage, J.Burillo, C.Codina; J.Agric.Food Chem., **50**, 6882-6890 **(2002)**.
[29] V.Katalinic, M.Milos, M.Jukic; Food Chemistry, **94(4)**, 550-557 **(2006)**.
[30] C.A.Rice-Evans, N.J.Miller, P.G.Bolwell; Free Radical Res., **22**, 375-383 **(1995)**.
[31] P.G..Pietta; J.Nat.Prod., **63**,1035-1042 **(2000)**.
[32] İ. Gulcin; Int.J.Food Sci.Nut., **56**, 491-499 **(2005)**.
[33] P.Siddhuraju, P.S.Mohan, K.Becker; Food Chemistry, **79**, 61-67 **(2002)**.
[34] N.K.Prasad, S.Divakar, G..R.Shivamurthy, S.M. Aradhya; J.Sc.Food and Agric., **85**, 1461-1468 **(2005)**.
[35] M.E.Cuvelier, H.Richard, C.Berset; Biosci. Biotech.Biochem., **56**, 324-325 **(1992)**.
[36] H.Matsuda, T.Wang, H.Managi, M.Yoschikawa; Bioorg.Med.Chem., **11**, 5317-5323 **(2003)**.

Current Topics in Phytochemistry
Vol. 11, 2012

Review

Phytochemical and biological studies on *Launaea* Cass. genus (Asteraceae) from Algerian Sahara

Abdelkrim Cheriti[1,*], Mebarka Belboukhari[1], Nasser Belboukhari[2] and Houria Djeradi[1]

[1]Phytochemistry and Organic Synthesis Laboratory, [2]Bioactive Molecules and Chiral Separation Laboratory, University of Bechar, Bechar, 08000, Algeria

ABSTRACT

Traditional remedies have been employed for the treatment and management of various ailments since the beginning of human civilization. *Launaea* Cass. is a small genus of the family Asteraceae (tribe Lactuceae, subtribe Sonchinae), consisting of 54 species, of which 9 are presented in the flora of Algeria and is mainly distributed in the South Mediterranean, Africa and SW Asia. Plants in the *Launaea* genus have been used ethnobotanically as bitter stomachic, for treating diarrhea, gastrointestinal tracts, as anti-inflammatory, for skin diseases, treatment of infected wounds, hepatic pains, children fever, as soporific, lactagogue, diuretic and as insecticidal. The aim of this review is to present as much information as was established from the available scientific literature. The review covers the ethnopharmacological, biological activity related and phytochemical information on the species from genus *Launaea*, especially those growing in Algerian Sahara and used as medicinal plants.

KEYWORDS: Asteraceae, *Launaea* Cass., ethnopharmacological, biological activities, phytochemical, Sahara

INTRODUCTION

The World Health Organization (WHO) has recognized the potential utility of traditional remedies and strives to preserve the primary health care involving medicinal plants. Thus, there is ample archaeological evidence indicating that medicinal plants were regularly employed by people in prehistoric times. In several ancient cultures botanical products were ingested for biomedically curative and psychotherapeutic purposes [1]. Plants have formed the basis of Traditional Medicine (TM) systems that have been in existence for thousands of years and continue to provide mankind with new remedies, such as, the oldest known medicinal systems of the world: Ayurveda, Arabian medicine, Chinese and Kempo medicine. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and thousands of years [2]. One of the most efficient ways of finding new bioactive compounds is collecting data on the use of medicinal plants in traditional pharmacopeia. Nearly 50,000 species of higher plants have been used for medicinal purposes. They are also used in food, cleaning, personal care and perfumery. In systems of traditional healing, major pharmaceutical drugs have been either derived from or patterned after compounds from biological diversity [3]. Natural products have made enormous contributions to human health through compounds such as quinine, morphine, aspirin (a natural product analog), digitoxin and many others. Thus natural products are very important to conduct research on and they can be a source of new compounds [4]. A trend in phytomedicine is the use of new plant origin bioactive compounds with the potential for chemical modification, which will broaden phytomedical

*karimcheriti@yahoo.com

importance. Molecular biology is also being used in this process and the pharmacological profiles of these compounds are screened using new research equipment and new technology [5-8]. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world and in which higher plants contribute to no less than 25% [2].

A dozen potent drugs have been derived from plants including: derived diosgenin; reserpine and pilocarpine. Other natural products are metabolites from fungi, bacteria, algae, and marine organisms. So, the diversity of structures obtained and the different therapeutic activities shown by the natural products make the isolation, identification, synthesis and biosynthesis of new natural compounds a field of enormous interest. Only a small part of the 400,000 vegetable species known have so far been investigated for their phytochemical and pharmacological aspects, and each species could contain up to several thousands of different components [9].

The plant family Asteraceae (Compositae) comprises of a large number of species that have been and are still used as medicinal plants, particularly in folk medicine and used as a food.

Launaea Cass. is a small genus of the family Asteraceae (tribe Lactuceae, subtribe Sonchinae). The genus consists of 54 species, of which 9 are presented in the flora of Algeria and is mainly distributed in the South Mediterranean, Africa and SW Asia. They are perennial to annual herbs, small shrubs or sub shrubs. Many of its plants are used in folk medicine as bitter stomachic, for treating diarrhea, gastrointestinal tracts, as anti-inflammatory, for skin diseases, treatment of infected wounds, hepatic pains, children fever, as soporific, lactagogue, diuretic and as insecticidal. Additionally, crude extracts of some species have been reported to exhibit antibacterial, antiparasitic, antioxidant, cytotoxic, neuropharmacological and insecticidal activities. From a chemical point of view, only ten species of the genus *Launaea* Cass. have been subjected to previous phytochemical investigation, namely, *Launaea acanthoclada, L. arborescens, L. asplenifolia, L. capitata, L. cassiniana, L. mucronata, L. nudicaulis, L. pinnatifida, L. resedifolia* and *L. tenuiloba*. Different secondary metabolites including terpenoids, steroids, triterpenoid saponin, sesquiterpene lactones, coumarins, flavonoids, flavone glycosides and phenolic compounds have been reported.

We attempt to present a review on the ethnopharmacological and phytochemical studies and biological activities of plants from the genus *Launaea* Cass., especially those growing in Algerian Sahara and used as medicinal plants.

Botanical taxonomy of the genus *Launaea* Cass.

Asteraceae family (Compositae), known as the aster, daisy or sunflower family, is one of the largest angiosperm families of dicotyledenous flowering plants. It comprises about 1400 genera and more than 25000 species of herbaceous plants, shrubs, and trees, spread throughout the world, and classified over three subfamilies and 17 tribes [10]. Asteraceae plants tend to grow in sunlit places, in temperate and subtropical regions and can share these following characters [11]:

Various members of the aster family are familiar species in natural habitats, while others are cultivated plants in gardens and some are grown as food (*Lactuca sativa*). Many members of Asteraceae are pollinated by insects, which explain their value in attracting beneficial insects and are major honey plants.

The flowers of this family are of two basic types: tubular actinomorphic corollas and those with strap shaped or radiate zygomorphic corollas, often with the same head. Either type may be bisexual or unisexual.

Leaves and stems very often contain secretory canals with resin or latex (particularly common among the Cichorioideae). The leaves can be alternate, opposite, or whorled. They may be simple, but are often deeply lobed or otherwise incised, and conduplicated or revoluted. The margins can be entire or dentate.

The fruit of Asteraceae is a specialized type of achene sometimes called cypsela. One seed per fruit is formed.

Due to their chemo-diversity, the sesquiterpene lactones are the most suitable class of naturals

products for chemo-systematic studies within the family [12, 13].

The tribe *Lactuceae* Cass. The tribe Lactuceae (Cichorieae, Asteraceae family) comprises 98 genera and more than 1550 species. The milky latex and the floral structure make the tribe easily distinguishable from all other Asteraceae. The flowering heads are composed of wholly ligulate florets that are usually 5-lobed [10].

According to classification system on flowering plants [14], the classification hierarchy of the genus *Launaea* can be tracked as follows:

Kingdom : Plantae
Division : Angiosperms
Class : Eudicots
Subclass : Asterids
Order : Asterales
Family : Asteraceae
Subfamily : Cichorioideae
Tribe : Lactuceae (Cichorieae)
Sub-tribe : Sonchinae
Genus : *Launaea*

The genus *Launaea* Cass. belongs to the tribe Lactuceae of the Asteraceae family and contains about 54 species, most of which are adapted to dry, saline and sandy habits [15]. Plants of this genus have several rows of stems, hairless leaves incised into lobes that are themselves lined with white teeth, membranous scales on the edges, yellow ligules, and elongated chain, prismatic or slightly flattened.

The genus *Launaea* is represented in the flora of Algeria by nine species including five endemics of North Africa: *L. angustifolia*, *L. quercifolia*, and *L. cassiniana* are the endemic plants of the North Africa, with limited distribution [15, 16], whereas *L. acanthoclada* and *L. arborescens* are two endemic plants of the north-west of Africa. The other four species *L. nudicaulis* and *L. residifolia* sprout in Algeria and Tunisia Mediterranean Sea, whereas *L. glomerata* and *L. mucronata* grow in the Saharan Atlas [16].

Three of this species are used in Algerian Sahara ethnopharmacopea as medicinal plants, *L. nudicaulis, L. residifolia and L. arborescens*,

Figure 1. *Launaea arborescens* (Batt.) and flower– south west Algeria.

which is endemic to south west Algeria and south east Morocco [17].

Launaea arborescens (Batt.) Murb, (syn. *Zollikoferia spinosa* DC) is an almost leafless, xerophilous, perennial spiny shrub, 40-120 cm. high, with typical zig-zag shaped stems (Figure 1). The young stems are green, glabrous and erect. The older ones become tough spines. The leaves are narrow and dissected in small lobes, evergreen at the base but shed after flowering from the stems. The flowers are yellow, and abundant flowering occurs from March to June, but flowers and achenes are produced throughout the year. The roots are very deep, the leaves and stems have white latex which is similar in appearance to milk (thus the local name “Oum loubina”) [15-18].

Ethnopharmacolgy and bioactivity of the genus *Launaea* Cass.

It is well known that species from Asteraceae family are used as natural remedies such as: *Anthemis arvensis* L. (anti-inflammatory, emetic, sedative), *Artemisia arborescens* L. (digestive, stimulant, expectorant), *Calendula arvensis* (antispasmodic, burns, diuretic, disinfectant and vulnerary), *Cichorium intybus* L. (blood purification, arteriosclerosis, anti-arthritis, anti-spasmodic, digestive, hypotensive, aperitif and laxative) and *Helychrysum microphyllum* Willd. (expectorant).

Algeria with its large area and diversified climate has a varied flora, which is a source of rich and abundant medical matter and, in particular, Sahara part constitutes an important reservoir of many plants which have not been investigated until today. Among this flora, some *Launaea* plants have been used in the traditional medicine [17-19]. Species of the genus *Launaea* are widely applied in traditional folk medicine throughout their areas of distribution. Many of them are used in folk medicine as bitter stomachic, anti-tumour, insecticides and against skin diseases.

Launaea residifolia (Vernacular name: Lemkar) is a medicinal plant used in folkloric medicine mainly for the treatment of hepatic pains.

Launaea nudicaulis (Vernacular name: Reghama) is used in the traditional medicine to treat gastric burns, pain of stomach, constipation, to relieve fever in children, in the treatment of itches of skin and eczema.

Launaea arborescens (Batt) (Vernacular name: Oum Lbina) commonly used in popular medicine as an antidiarrhoic and antispasmodic, to relieve fever, and as a vermifuge in children. The latex is applied locally to cure sore throats and in the treatment of furuncles. The powdered root mixed with *Artemisia herba-alba* is taken for diabetes. The plant is appreciated by livestock, mainly by camel [17-21].

Many phytochemicals are potent effectors of biologic processes and have the capacity to influence disease risk via several complementary and overlapping mechanisms [22].

More than 4000 sesquiterpenoids structures with around 30 different skeletal types have so far been reported from several tribes of Asteraceae family including the Cichorieae tribe. These natural compounds are responsible for allergic contact dermatitis and exhibit a wide range of bioactivities which include plant growth regulation and antimicrobial activity. Also they are used as schistosomicidal and insect feeding-deterrent agents. In addition, they provoke the toxicity for certain cancer cell lines by inhibition of nuclear DNA synthesis, especially the enzymatic activity in tumour cells of DNA polymerase and thymidylate synthetase [12, 23, 24].

On the other hand, triterpenoids and flavonoids chemio-characteristic of Asteraceae family, including the *Launaea* genus, have been reported to have anti-inflammatory activities, anti-hyperlipidemia, hepatoprotection, antioxidant, cytoprotective, giving protection against cardiovascular disease, and certain forms of cancer [25, 26]. Antibacterial, antifungal and allelopathic potential activities have been proven for many species of *Launaea*. In an antibacterial assay against *Bacillus subtilis* the extracts of *L. nudicaulis* and *L. residifolia* showed 18.5 and 20.5 mm zones of inhibition, respectively, as determined by the disc diffusion method. The antifungal activity against *Aspergillus* spp. was determined by measuring the linear growth in slants on 4^{th} day of incubation. Methanol extracts of *L. nudicaulis* and *L. residifolia* were active at 0.209 mg/ml levels exhibiting 45 ± 6 mm and 37 ± 6 mm linear growth which decreased to 22 ± 5 mm and 28 ± 4 mm, respectively, at 0.838 mg/ml concentration [27].

As a part of our works on medicinal plants of Algerian Sahara, recently we have reported the antibacterial activity of extracts from *Launaea Arborescens* and *L. nudicaulis* which are widely distributed in the south west of Algeria. The methanol extract of the aerial part of *L. nudicaulis* showed high activities against *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The highest inhibition observed in *S. aureus*, a human pathogen, explains the use of this plant against a number of infections for generations. Very interesting antifungal activity against *Candida albicans* and *Saccharomyces cerevisae* and antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella entrecocus* have been reported for the methanol extract of *Launaea Arborescens* [18, 28].

Hydroalcoholic extract from aerial parts of *Launaea arborescens* was evaluated for acute and subacute toxicity in *Swiss* mice after ingestions of the extract. The LD50 of the extract is higher than 2.75 g/kg and the subacute treatment did not shows any change in corporal weight and haematological parameters, which suggest that the plant seems to be destituted of toxic effects in mice [29].

Aerial part and roots of *Launeae arborescens* were used to evaluate their extracts for antifungal activity against Fusarium oxysporum f. sp. albedinis Foa. The antifungal test was conducted using disc diffusion technique and relative virulence (RV) test (on potato tuber tissue). For both tests, four extract quantities were used (200, 400, 800 and 1,600 µg). The relative virulence was presented as necrotic tissue weight (mg) of potato tuber tissue. Among all solvents, methanol had the best extraction yield (mean: 6.35%, minimum: 2.27%, maximum: 9.80%) [30].

Coumarins isolated from *L. resedifolia* showed high antibacterial activity against some Gram-positive bacteria such as *Bacillus cereus* and *Staphyllococcus aureus* in minimum inhibitory concentrations of 200 and 400 µg/mL. However, they showed no effect on tested Gram-negative bacteria such as *Serratia* Sp., *Pseudomonas* Sp. and *Escherichia coli* [31]. The ethanol extract of *L. resedifolia* showed neuropharmacological properties in animal models. The extract exhibited an inhibitory effect on the locomotor activity of mice in the open field test, an anti-nociceptive effect by increasing the hot plate reaction time in the hot plate test, and an anti-inflammatory activity in the carrageen-induced paw oedema. This finding has demonstrated that the extract of *L. resedifolia* possesses sedative, analgesic and anti-inflammatory properties, and some effect on body weight. The anti-infl ammatory effect of the plant was found to be as active as the prototype non-steroidal anti-inflammatory drug (NSAID) aspirin [32].

Allelopathic potential effect of aqueous extract of *Launaea procumbens* was observed in the soil application by a significant retarding effect on wheat growth while shoot spray or root dip treatment had no such effect and methanol and chloroform fraction from this specie exhibited efficient antioxidant scavenging activities, attributed to the phenolic and polyphenolic compounds such as myricetin, catechin, vitexin, orientin, hyperoside and rutin, revealed in HPLC [33].

Other research has shown that extracts from *Launaea procumbens* provide effective protection for kidneys against the CCl_4-induced oxidative damage in rats, through antioxidant and free radical scavenging effects of flavonoids and saponins present in this plant, which might be responsible for the elimination of various kidneys insults [34].

Phytochemistry of the genus *Launaea* Cass.

a. Secondary metabolites from the 2ed group of Lactuceae tribe

The biodiversity of metabolite products isolated from Asteraceae makes this family an important phytochemical and commercial source. Several phytochemical studies of some genera of *Lactuceae* tribe (Cichorieae) revealed to be rich in secondary metabolites, specifically sesquiterpene lactones exhibiting the eudesmane, germacrane and guaiane carbon framework. A total of 360 sesquiterpene lactones and related compounds have been isolated from 139 taxa belonging to 31 different genera of the *Lactuceae*. Studies realized for these genera revealed that most sesquiterpenoids within the Cichorieae belong to the guaianolide class, particularly: 92 representatives of costus lactone type, 75 compounds of lactucin type, and 29 representatives of hieracin type [35, 36].

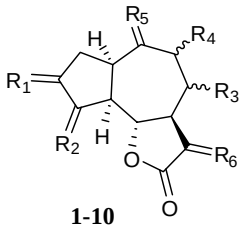
Some phenolic compounds, such as flavonoids and coumarins were also isolated [37-42]. In addition, triterpenes have also been detected [43, 44]. Recently, Sareedenchai and Zidorn indicated that a total of 135 flavonoids have been isolated from 299 species of the Cichorieae (Lactuceae) tribe. The reported compounds encompass flavanones, flavanonols, flavones, flavonols, anthocyanidins, isoflavonoids, chalcones and aurone [45].

Based on the similarity of their sesquiterpenes profiles, Zidorn grouped the 31 genera of the *Lactuceae* into seven main clusters and classified Launaea with the 2ed group characterized by the prevalence of guaianolides, formed by 11 genera, sub-divided into four sub-groups: a) *Scorzoneroides*; b) *Notoseris, Lactuca,* and *Cichorium*; c) *Launaea, Crepidiastrum, Reichardia,* and *Cicerbita* d) *Taraxacum, Helminthotheca,* and *Hypochaeris* [35].

Phytochemical investigation of 2ed group of the Cichorieae tribe resulted in the identification and isolation of differents metabolites including:

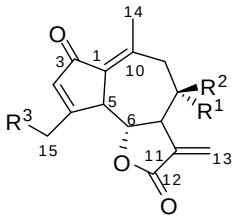
Sesquiterpenoids

Costus lactone type guaianolides such as dehydrocostruslactone **1**, ixerisoside B **2**, C **3** and D **4**, scorzoside **5**, zaluzanin C **6**, glucozaluzanin C **7,** 11 β,13-dihydrozaluzanin C **8**, 8β-hydroxy-4 β,15-dihydrozaluzanin C **9** and prenantheside C **10** [38, 39]. Lactucin type guaianolides, Lactucin **11**, 8-O-acetate Lactucin **12**, Crepidiaside A **13**, 11β, 13dihydrolactucin **14**, 8-O-acetate, 11β, 13 dihydrolactucin **15** and 8-Deoxylactucin **16**. The eudesmane derivatives santamarin **17**, ixerisoside E **18,** lactuside D **19**, sonchuside C **20** and artesin **21** [39, 46], costinolide type germacranolides such as picriside B **22**, C **23**, sonchuside A **24**, B **25** and cichoerioside C **26**, [41, 47, 48], and melampolides type, lactulide A **27**, lactuside A **28** and B **29** [38, 39, 49, 50] and in some case sesquiterpenoid sulphate, 8-deoxy-15-(3'-hydroxy-2'-methylpropanoyl) lactucin-3'sulfate **30** [51].

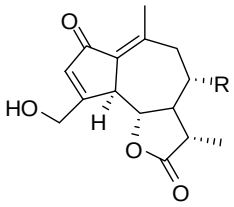


1-10

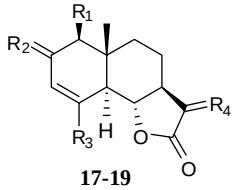
	R_1	R_2	R_3	R_4	R_5	R_6
1	H,H	CH_2	H	H	CH_2	CH_2
2	α H,β OGlc	α CH_3 ,βH	β OH	H	CH_2	CH_2
3	α H,β OGlc	CH_2	H	H	CH_2	α CH_3 ,βH
4	H,H	CH_2	H	α OGlc	CH_2	CH_2
5	H,H	CH_2	H	α OGlc	CH_2	α CH_3 ,β
6	α H,β OH	CH_2	H	H	CH_2	CH_2
7	α H,β OGlc	CH_2	H	H	CH_2	CH_2
8	α H,β OH	CH_2	H	H	CH_2	α CH_3 ,βH
9	α H,β OH	α CH_3 ,βH	β OH	H	CH_2	CH_2
10	α H,β OGlc	CH_2	α OH	H	CH_2	α CH_3 ,Bh



11 : R_1 = R_3=OH , R_2=H
12 : R_1 = OAc , R_2=H , R_3=OH
13 : R_1 =R_2=H , R_3=O-glc

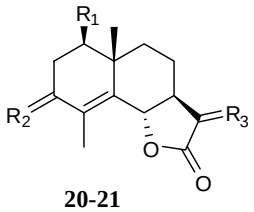


14 : R=OH
15 : R = OAc
16 : R=H



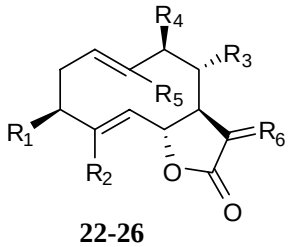
17-19

	R_1	R_2	R_3	R_4
17	OH	H ,H	CH_3	CH_2
18	OH	H ,H	CH_2OGlc	CH_2
19	O-PPA	H ,H	CH_2OGlc	CH_2



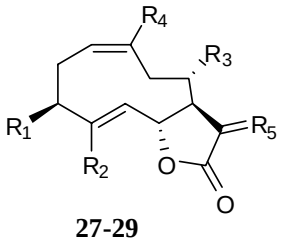
20-21

	R_1	R_2	R_3
20	H	α H, β OGlc	α CH_3, β H
21	OH	H, H	α CH_3, β H



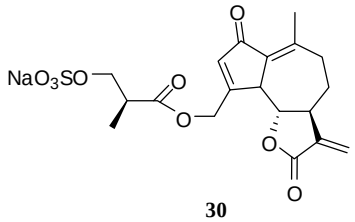
22-26

	R_1	R_2	R_3	R_4	R_5	R_6
22	H	CH_2OGlc	H	H	CH_3	CH_2
23	OGlc	CH_3	H	H	CH_3	CH_2
24	O-Glc	CH_3	H	H	CH_3	α CH_3, β H
25	OGlc	CH_3	H	O-PMP	CH_3	CH_2
26	OGlc	CH_3	OH	H	CH_3	α CH_3, β H



27-29

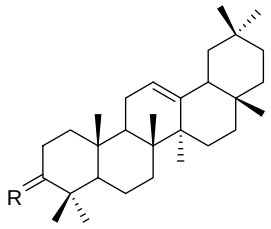
	R_1	R_2	R_3	R_4	R_5
27	OH	CH_3	H	CHO	α CH_3 ,βH
28	OGlc	CH_3	H	CHO	α CH_3 ,βH
29	OGlc	CH_3	H	CH_2OH	α CH_3 ,βH



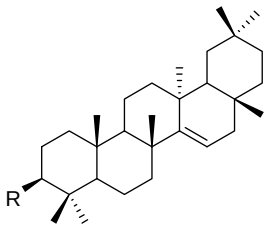
30

Terpenoids

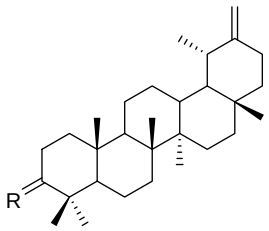
The majority of these triterpenes are pentacyclic and belong to lupane, oleanane, gammacerane and ursane groups, with some tetracyclic compounds such as β-amyrin **31**, β-amyrin acetate **32**, taraxerol **33**, taraxeryl acetate **34**, taraxasterol **35**, taraxasterone **36**, taraxasteryl acetate **37**, ψ-taraxasteryl derivatives **38**, **39**, α-amyrin derivatives **40**, **41**, lupeol **42**, lupenone **43**, and lupenyl acetate **44** [39, 44].



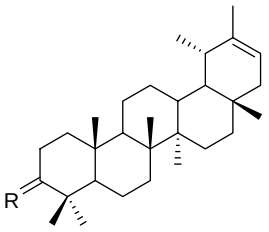
31 R= α H, βOH
32 R= α H, βOAc



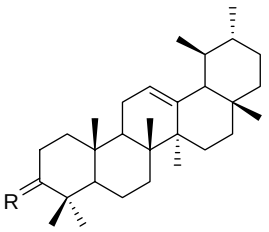
33 R= OH
34 R= OAc



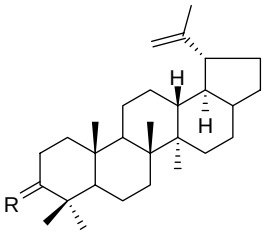
35 R= α H, βOH
36 R= O
37 R= α H, βOAc



38 R= α H, βOH
39 R= α H, βOAc



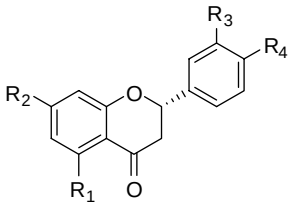
40 R= α H, βOH
41 R= α H, βOAc



42 R=α H, βOH
43 R= O
44 R= α H, βOAc

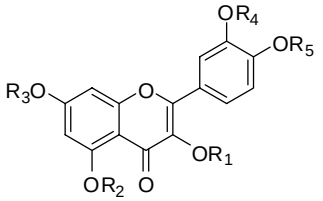
Phenolic compounds

Several phenolic compounds were identified in the aerial parts and roots of some species of the 2ed group of Lactuceae tribe such as small phenolic compounds: *p*-hydroxybenzoic acids, 4- caffeoylquinic, chlorogenic, *trans*-caffeate, methyl and ethyl *p*-hydroxyphenylacetate, and *p*- coumaric**,** affeic acids as well as their glycoside derivatives, dihydroconiferin, syringin and dihydrosyringin [38, 39]. In addition this group of Lactuceae tribe contains various flavonoids and flavonoid glycosides such as flavanone type: 7-hydroxyflavanone **45**, 7-methoxyflavanone **46**, naringenin **47**, naringenin 7-methyl ether **48**, miscanthoside **49**, hesperitin **50,** quercetin derivatives: Isorhamnetin **51**, quercetin 7-*O*-glucoside **52**, quercetin 7-*O*-gentiobioside **53**, hyperin **54**, quercetin 3-*O*-glucuronide **55**, quercetin 3-*O*-rhamnoside **56**, quercetin 3-*O*-rutinoside **57**, isorhamnetin 3-*O*-glucoside **58** and isorhamnetin 3-*O*-glucuronide **59.** Various apigenin, luteolin and isoetin groups were founds in the tribe such as: Apigenin 4'-methyl ether **60**, apigenin 4'-*O*-glucoside **61**, apigenin 7-*O*-glucoside **62**, scutellarin A **63**, apigenin 7-*O*-gentiobioside **64**, linarin **65,** luteolin **66**, luteolin 4'-*O*-glucoside **67**, luteolin 7-*O*-galactoside **68**, luteolin 7-*O*-glucoside **69**, luteolin 7-*O*-rhamnoside **70**, luteolin 7-*O*-gentiobioside **71**, luteolin 7-*O*-rutinoside **72**, luteolin 7,4'-*O*-diglucoside **73**, luteolin 7-*O*-gentiobioside-4'-*O*-glucoside **74**, luteolin 7,3'-*O*-diglucoside **75** and isoetin glycosides, 7-O-glucoside **76**, 7-O-glucoside-2'-O-arabinoside **77**, 7-O-glucoside-2'-O-xyloside **78**, 7-O-glucoside-2'-O-(4-O-acetyl)-xyloside **79.** It is well noted that flavonoids are considered as chemosystematic markers in the tribe Cichorieae of the Asteraceae family. Furthermore, usually coumarin compounds are found in the 2ed group of the Cichorieae tribe such as, umbelliferone **80**, scopoletin **81**, esculetin **82** and cichoriin **83** [41, 45, 52, 53].



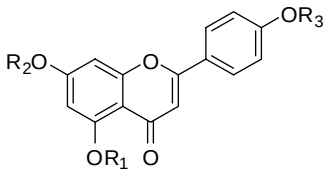
45-50

	R_1	R_2	R_3	R_4
45	H	OH	H	H
46	H	OCH_3	H	H
47	OH	OH	H	OH
48	OH	OCH_3	H	OH
49	OH	O-Glc	OH	OH
50	OH	OH	OH	OCH_3



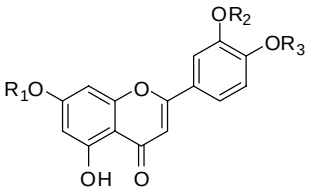
51-59

	R_1	R_2	R_3	R_4	R_5
51	H	H	H	CH_3	H
52	H	H	Glc	H	H
53	H	H	Gen	H	H
54	Gal	H	H	H	H
55	Glu	H	H	H	H
56	Rha	H	H	H	H
57	Rut	H	H	H	H
58	Glc	H	H	CH_3	H
59	Glu	H	H	CH_3	H



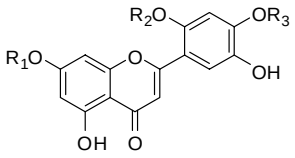
60-65

	R_1	R_2	R_3
60	H	H	CH_3
61	H	H	Glc
62	H	Glc	H
63	H	Glu	H
64	H	Gen	H
65	H	Rut	CH_3



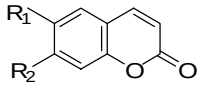
66-75

	R_1	R_2	R_3
66	H	H	H
67	H	H	Glc
68	Gal	H	H
69	Glc	H	H
70	Rha	H	H
71	Gen	H	H
72	Rut	H	H
73	Glc	H	Glc
74	Gen	H	Glc
75	Glc	Glc	H



76-79

	R_1	R_2	R_3
76	Glc	H	H
77	Glc	Ara	H
78	Glc	Xyl	H
79	Glc	4-*O*-acetyl Xyl	H



80-83

	R_1	R_2
80	H	OH
81	OMe	OH
82	OH	OH
83	OH	O-Glc

b. Secondary metabolites isolated from the Saharan *Launaea* genus

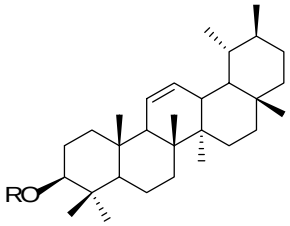
Different secondary metabolites have been identified from the genus *Launaea*. In addition, few sesquiterpene lactones have been reported from various species of this genus and the occurrence of flavones glycosides is remarkable. The first works in phytochemistry on species of the genus *Launaea* was started in 1969 by Prabhu and Venkateswarlu [54], when they isolated from leaves and roots of launaea pinnatifida two compounds Taraxasterol **35** and Taraxerly acetate **37**. Five year after, in 1974, Bahadur and Sharma [55] reported the presence of palmitic, stearic, oleic and linoleic acids from the roots of *Launaea nudicaulis*. Twenty year ago, in 1989, Gupta *et al.* [56] investigated *Launaea asplenifolia* and isolated nine compounds namely, taraxasterol, taraxasterone, taraxasteryl acetate and the common compounds stigmasterol, ethypalmitate, ethylstearate, hexacosanol, octacosanol and octacosanoic acid.

Launaea nudicaulis

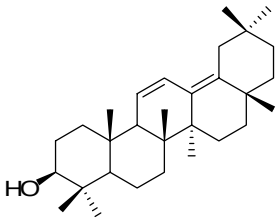
The light petroleum extract of *Launaea nudicaulis* leads to the characterization of some Δ^7 and Δ^5 phytosterols: β-sitosterol, brassicasterol, campesterol, stigmasterol, fucosterol, 24β-Δ^7-ergosten-3β-ol and stigmasta-7,24(28)-dien-3-ol [57]. Detailed chemical investigation of *Launaea nudicaulis* yielded some

triterpenes such as taraxasterol 35, ψ- taraxasterol 38, β-amyrin 34, 3β- taraxerol 33, α- amyrin 39, and lupeol 41 [58].

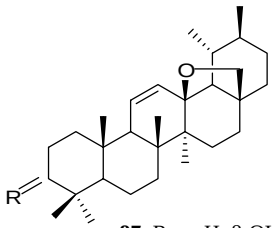
Two new ursene type triterpenes, nudicauline A **84**, and nudicauline B **85** have been isolated from the aerial parts of this species, along with olean-11,13(18)-diene **86**, 3β-hydroxy-13(28)-epoxy-urs-11-ene **87** and 3-keto-13(28)-epoxy-urs-11-ene **88** [59]. Additionally, flavone glycosides were reported from the 70% EtOH extract of fresh sample of *Launaea nudicaulis* and identified as apigenin-7-*O*-glucoside **62**, luteolin-7-*O*-glucoside **69**, luteolin-7-*O*-rutinoside **72**, apigenin-7-*O*-gentiobioside **64**, luteolin-7-*O*-gentiobioside **71**, and three glycosides luteolin-7,3'-diglucoside **75**, luteolin-7',4'-diglucoside **73** and luteolin-7-*O*-gentiobioside-4'-*O*-glucoside **74** [60], which are common metabolites within the 2ed group of Lactuceae tribe as indicated above. Moreover, two common coumarins, esculetin **82**, and cichoriin **83**, were also described [61, 62].



84 R= OH
85 R = Ac

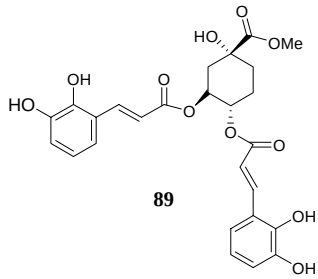


86

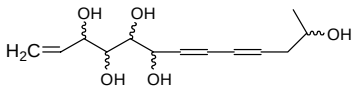


87 R= α H, β OH
88 R = O

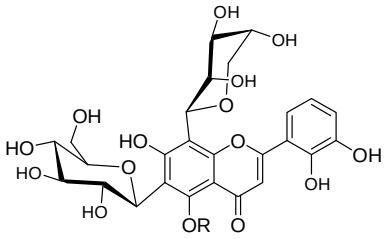
Recently, ethyl acetate soluble fraction of methanolic extract of *Launaea nudicaulis* was subjected to chromatographic purification to get four new compounds including a quinic acid derivative Cholistaquinate **89**, a pentahydroxy acetylene analog: trideca-12-ene-4,6-diyne-2, 8, 9, 10, 11-pentaol **90**, a flavone glycoside Cholistaflaside **91** and a sesquiterpene lactone nudicholoid **92**. Cholistaquinate **89** exhibited significant activity in DPPH free radical scavenging assay with an IC50 value of 60.7 mM, whereas, nudicholoid **92** exhibited a moderate inhibitory activity against the enzyme butyrylcholinesterase with an IC50 value of 88.3 mM [63].



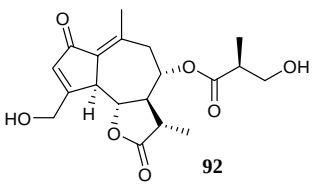
89



90



91



92

***Launaea residifolia* (L.)**

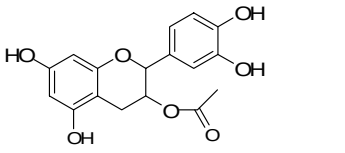
Chemical studie of the plant led to the isolation of triterpenes α-amyrin **40**, lupeol **42**, lupeol acetate **44** and their epimer moretenol together with the Δ^7-stigmasterol. From the aerials parts of *Launaea residifolia* growin in Algeria, four coumarin compounds, cichoriin **83,** esculetin **82**, scopoletin **81** and its isomere isoscopoletin, were isolated [64].

On the other hand, the chemical composition of essential oils from this species (0.9%) has been identified using the ordinary GC-MS technique. Nineteen compounds of essential oil of *L. residifolia* L. were identified representing 86.68% of the total oil. The compounds were identified by spectral comparison to be mainly esters, alcohols, ketones, and terpenes. The principal constituents are dioctyl phthalate (39.84%), Decanoic acid, decyl ester (12.09%), 11-Octadecenal (11.24%), and Eucalyptol (07.31%) [65].

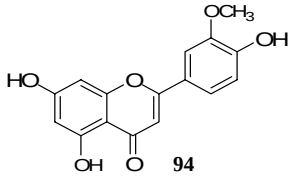
Launaea arborescens

Chemical data on this species are scarce in literature and few published papers describe phenolic components of the plant. In their studies on *Launaea* genus from Spain including *L. arborescens*, Giner *et al.* [66] isolated common phenolic compounds namely, luteolin **66**, luteolin-7-*O*- glucoside **69**, luteolin-7-*O*-rhamnoside **70**, esculetin **82** and its glycoside cichoriin **83**, and simple compounds, ethyl-caffeoate and ferulic acid. The authors remarked that cichoriin **83** was the most abundant compound in all studied species.

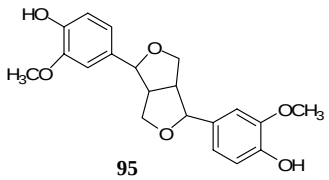
We are the first initiators on the phytochemical study of the Algerian sample of *L. arborescens* collected from the Sahara [67]. From the methanol extract of the aerial parts of this species, we have described the isolation of four compounds, two flavonoids, 3- acetyl-5-methoxy-7,3',4'-trihydroxyflavan-3-ol-8-*O*-glycoside **93,** 5,7,4'-trihydroxy-3'- methoxyflavone (chrysoeriol) **94**, one lignan, 4,4'-dihydroxy-3,3'-dimethoxy-7,9':7,9'-diepoxylignan **95**, and a diterpene, methyl-15,16-epoxy-12-oxo-8(17), 13(16), 14-ent-labdatrien-19-oate **96**.



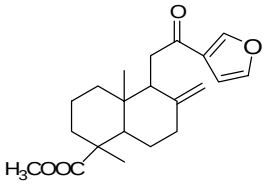
93



94



95



96

A diversity structure of triterpenes oleanane (3β-hydroxy-11α-ethoxy-olean-12-ene) and sesquiterpenes type guaianolides (9α- hydroxy-11β,13-dihydro-3-epi-zaluzanin C, 9α-hydroxy-4α,15-dihydro-zaluzanin C) and costinolide (3β,14-dihydroxycostunolide-3-O-β-Gluc.,3β, 14-dihydroxycostunolide-3-O-β-Gluc.-14-O-p-hydroxyphenylacetate) together with the lactucin-sulfate **30** were chemically characterised from both the aerial parts and roots of *L. arborescens* [68]. The hydrodistillation of the aeriel part of *Launaea arborescens* gave a green yellowish oil in an yield of 0.07% from dried material. Seventeen compounds were identified, representing 84.96% of the total oil. The essential oil of *L. arborescens* was a mixture of different substances, including oxygen-containing monoterpenes, alcohols, aldehydes, and esters. Esters were the dominant group in the oil (58.24%) with dioctyl phthalate (38.6%) and

decanoic acid, decyl ester (12.07%) as the main constituents. Alkenes and ketones were the minor constituents of the oil. The terpenoid portion consisted of two oxygenated monoterpenes accounting for 7.24% of the oil. We also found aldehydes in considerable amounts (16.09%) [69].

In a recent study, we were interested in the chiral separation and determination of the diastereomerisation barriers of two flavanone glycosides hesperidin and naringin isolated from the aerial part of *Launeae arborescens*. The chiral separation HPLC screening of diastereomers of hesperidin and naringin by HPLC methods was accomplished in the normal-phase mode using 11 chiral stationary phases and various n-hexane/alcohol mobile phases. The rate constants and activation energy of diastereomerisation (DG#) of flavanones, naringin and hesperidin were determined, respectively, on Chiralpak IC and Chiralpak IA. Separation of (2R/2S)-flavanone glycosides using the Chiralcel OD-H as CSP indicat that Epimer selectivity values (R) ranged from 1.81 for naringin to 1.16 for hesperidin, Chiralpak IA ranged in different conditions from 1.25 to 1.13 for naringin and hesperidin and Chiralpak AD-H presented a good chiral separation of naringin and hesperidin with a selectivity factor towards 1.28. The ChiralpakAD phase presented only the epimer separation of hesperidin with a selectivity factor towards 1.21. Analogously, the resolution factor (Rs) ranged from 2.27 for naringin to 0.97 for hesperidin. The values of R and Rs obtained for naringin were much better than those obtained using another polysaccharide-derived CSP (Chiralpak AD) and a very similar mobile phase (1.51 and 0.7, respectively) [70].

CONCLUSION

The genus *Launaea* has great importance due to its ethnobotanics, phytochemistry and biological activity, and it is a promising source of various secondary metabolites including sequiterpenoids, terpenoids and flavonoids. Some of these isolates compounds have been found to exhibit various biological activities. We have attempted to show the high biodiversity of metabolite products isolated from of the *Launaea* genus as well as their biological significance. This review presents information on the importance of the ethnobotany, phytochemistry and biological activities of the members of this genus, especially the species growing in Algerian Sahara. The given information can be the base for undertaking future research. It is necessary to carry out more studies and to propagate utilization of medicinal plants as a way to diminish the costs of public health programs.

REFERENCES

1. Halberstein R. 2005, Ann. Epidemiol., 15, 686.
2. Gurib-Fakim A. 2006, Traditions of yesterday and drugs of tomorrow, Molecular Aspects of Medicine, Special Edition, Elsevier Publications, UK.
3. Bisset N. 1994, Herbal drugs and phytopharmaceuticals, A handbook for practice on a scientific basis, Ed. Medpharm. Sc. Publishers, Sttutgarts-CRC Press, Boca Raton.
4. Newman, D. J. and Cragg, G. M. 2007, J. of Nat. Prod., 70, 461.
5. Cordell, G. 2000, Phytochemistry, 55, 463.
6. Cordell, G. and Colvard, M. 2005, J. Ethnopharmacol., 100, 5.
7. Yaniv, Z. and Bachrach, U. 2005, Handbook of Medicinal Plants, Harworth Press, Inc. New York, London.
8. Pieters, L. and Vlietinck, A. 2005, J. Ethnopharmacol., 100, 57.
9. Hostettmann, K., Potterat, O. and Wolfender, J.-L. 1998, Chimia, 52, 10.
10. Bremer. K. 1994, Asteraceae: Cladistics and Classification, Timber Press, Portland.
11. Judd, W. S., Campbell, C. S., Kellog, E. A. and Stevens, P. F. 1999, Plant Systematics: A Phylogenetic Approach, Sinauer Associates, Sunderland, MA.
12. Seaman, F. C. 1982, Bot. rev., 48, 123.
13. Zdero, C. and Bohlmann, F. 1990, Plant Syst. Evol., 171, 1.
14. Funk, V. A., Susanna, A., Stuessy, T. F. and Bayer, R. J. 2009, Systematics, Evolution and Biogeography of Compositae. International Association for Plant Taxonomy, Vienna, Austria.
15. Ozenda, P. 2004, Flore et Végétation du Sahara. CNRS, Paris.

16. Quezel, P. and Santa, S. 1963, In: Nouvelle Flore d'Algérie et des Régions Désertiques Méridionales, Vol. 1-2, CNRS, Paris.
17. Cheriti, A., Belboukhari, N., Sekkoum, K. and Hacini, S. 2006, J. Algerien reg. Arides, 5, 7.
18. a) Cheriti, A., Belboukhari, N. and Hacini, S. 2001, Annales Univ. de Bechar, 1, 4. b) Belboukhari, N., Cheriti, A. and Roussel, C. 2007, eCAM, 4(S1), 55.
19. Belboukhari, N. and Cheriti A. 2008, Elec. J. Environ. Agron. Food Chem., 7(14), 2749.
20. a) Chehma, A. 2006, Catalogue des plantes spontanées du Sahara septentrional Algérien, Ed. Dar ElHouda, Ain Mila, Algeria. b) Cheriti, A. 2000, Plantes Médicinales de la Région de Bechar, Sud Ouest Algérie: Etude Ethnopharmacologique, Rapport CRSTRA', Algeria.
21. Bellakhdar, J. 1997, La pharmacopée marocaine traditionnelle, Médecine arabe ancienne et savoirs populaires, IBIS Press.
22. Lampe, J. W. and Chang, J-L. 2007, Interindividual differences in phytochemical metabolism and disposition, Seminars in cancer biology, 17(5), 347.
23. Kupchan, S. M., Giacobbe, T. J., Krull, I. S., Thomas, A. M., Eakin, M. A. and Fessier, D. C. 1970, J. Org. Chem., 35, 3539.
24. Picman, A. K. 1986, Biochem. Syst. Ecol., 14, 255.
25. Lie, J. 1995, J. Ethnopharmacol., 49, 57.
26. Cheriti, A., Babadjamian, A. and Balansard, G. 1994, J. Nat. Prod., 57, 1160.
27. Rashid, S., Ashraf, M., Bibi, S. and Anjum, R. 2000, Pak. J. Bio. Sci., 3, 630.
28. Belboukhari, N. and Cheriti, A. 2006, Pak. J. Biolog. Sci., 9(1), 1.
29. Salah Ramadan, B., Cheriti, A., Belboukhari, N. and Zaouani, M. 2008, Annales Univ. Bechar, 4, 48.
30. Boulenouar, N., Marouf, A., Cheriti, A. and Belboukhari, N. 2012, J. Agr. Sci. Tech., 14, 659.
31. El-Bassuony1, A. and Kabbash, A. M. 2008, Phcog Mag., 4(16), 249.
32. Auzi, A. A., Hawisa, N. T., Sherif, F. M. and Sarker, S. D. 2007, Rev. bras. farmacogn., 17, 160.
33. a) Shaukat, S. S., Tajuddin, Z. and Siddiqui, I. A. 2003, Pak. J. Bio. Sci., 6, 225. b) Rahmat, A. K., Muhammad, R. K., Sumaira, S. and Mushtaq, A. 2012, Chem. Central J., 6, 4.
34. Rahmat, A. K., Muhammad, R. K. and Sumaira, S. 2010, J. Ethnopharm., 128, 452.
35. a) Zidorn, C. 2006, Biochem. System. Ecol., 34, 144. b) Zidorn, C. 2008, Phytochemistry, 69, 2270.
36. Zidorn, C., Ellmerer, E.-P., Heller, W., Jöhrer, K., Frommberger, M., Greil, R., Guggenberger, M., Ongania, K.-H. and Stuppner, H. 2007. Z. Naturforsch., 62b, 132.
37. Miyase, T. and Fukushima, S. 1987, Chem. Pharm. Bull., 35, 2869.
38. Kisiel, W. and Barszcz, B. 2000, Fitoterapia, 71, 269.
39. Kisiel, W. and Michalska, K. 2006, Fitoterapia, 77, 354.
40. Kisiel, W. and Zielinska, K. 2003, Z. Naturforsch., 58c, 789.
41. Michalska, K. and Kisiel, W. 2007, Biochem. System. Ecol., 35, 714.
42. Mulinacci, N., Innocenti, M., Romani, A., LA Marca, G. and Vincieri, F. F. 2001, Chromatographica, 54, 455.
43. Schutz, K., Carle, R. and Schieber, A. 2006, J. Ethnopharmacol., 107, 313.
44. Takasaki, M., Konoshima, T., Tokuda, H., Masuda, K., Arai, Y., Shiojima, K. and Ageta Hiroyuki. 1999, Biol. Pharm. Bull., 22, 606.
45. Sareedenchai, V. and Zidorn, C. 2010, Biochem. System. Ecol., 38, 935.
46. EL-Masry, S., Ghazy, N. M., Zdero, K. and Bohlmann, F. 1984, Phytochemistry, 23, 183.
47. Takeda, Y., Musuda, T., Morikawa, H., Ayabe, H., Hirata, E., Shinzato, T., Aramato, M. and Otsuka, H. 2005, Phytochemistry, 66, 727.
48. Khalil, A. T., El-Fattah, H. A. and Mansour, E. S. 1991, Planta Med., 57, 190.
49. Nishimura, K., Miyase, T., Ueno, A., Noro, T., Kuroyanagi, M. and Fukushima, S. 1986, Phytochemistry, 25, 2375.
50. Wang, X.-X., Lin, C.-J. and Jia, Z.-J. 2006, Planta Med., 72, 764.
51. Sessa, R. A., Bennett, M. A., Lewis, M. J., Mansfield, J. W. and Beale, M. H. 2000, J. Bio. Chem., 275, 26877.

52. a) Aboul-Ela, M. A., Abdul-Ghani, M. M., El-Fiky, F. K., El-Lakany, A. M., Mekky, H. M. and Ghazy, N. M., 2002, Alexandria. J. Pharm. Sci., 16, 152. b) Dem'yanenko, V. G. and Dranik, L. I. 1973, Khim. Prir. Soedin., 9, 119.
53. Clifford, M. N., Shutler, S., Thomas, G. A. and Ohiokpehai, O. 1987, Food Chem. 24, 99.
54. Prabhu, K. R. and Venkateswarlu, V. 1969, J. Indian Chem. Soc., 46, 176.
55. Bahadur, K. and Sharma, S. 1974, Proc. Natl. Acad. Sc., India, Sect. A, 44, 264. (Chem. Abst. 84: 19232h)
56. Gupta, M. M., Verma, R. K. and Singh, S. C. 1989, Fitoterapia, 60, 476.
57. Behari, M. and Gupta, R. 1980, Indian J. Chem. Section B:, 19B(10), 926.
58. a) Majumder, P. L. and Laha, S. 1982, J. Indian. Chem. Soc., 59, 881. b) Hook, F., Behari, M., Gupta, R. and Mutsumoto, T. 1984, Indian Drugs, 21, 366.
59. Zaheer, A., Dildar, A. and Abdul, M. 2006, Magn. Reson. Chem., 44, 717.
60. Ragaa, M. A., Mansour, A., Ahmed, A. A. and Nabiel, A. M. S. 1983, Phytochemistry, 22, 2630.
61. Sarg, T. M., Omar, A. A., Ateya, A. M. and Hafiz, S. S. 1986, Egypt. J. Pharm. Sci., 25, 35.
62. a) Mansour, R. M. A., Ahmed, A. A. and Saleh, N. A. M. 1983, Phytochemistry, 22, 2630. b) Mansour, R. M. A., Saleh, N. A. M. and Boulos, L. 1983, Phytochemistry, 22, 489.
63. Saleem, M., Parveen, S., Riaz, N., Nawaz, Tahir, M., Ashraf, M., Afzal, I., Shaiq Ali, M., Malik, A. and Jabbar, A. 2012, Phytochemistry Let., under press.
64. a) Abdel-Fattah, H., Zaghloul, A. M., Halim, A. F. and Waight, E. S. 1990, Egypt. J. Pharm. Sci., 31, 81. b) Gherraf, N., El-Bassuony, A. A., Zellagui, A., Rhouati, S., Ahmed, A. A. and Ouahrani, M. R. 2006, Asian. J. Chem., 18, 2348.
65. Zellagui, A., Gherraf, N., Ladjel, S. and Hameurlaine S. 2012, Org. Med. Chem. Let., 2, 2.
66. Giner, R. M., Diaz, J., Manez, S., Recio, M. C., Soriano, C. and Rios, J. L. 1992, Biochem. System. Ecol., 20, 187.
67. Belboukhari, N. and Cheriti, A. 2006, Pak. J. Biol. Sci., 9, 2930.
68. Bitam, F., Ciavatta, M. L., Manzo, E., Dibi, A. and Gavagnin, M. 2008, Phytochemistry, 69, 2984.
69. Cheriti, A., Saad, A., Belboukhari, N. and Ghezali, S. 2006, Chem. Nat. Prod., 42(3), 360.
70. Belboukhari, N., Cheriti, A., Roussel, C. and Vanthuyne, N. 2010, Nat. Prod. Res., 24(7), 669.

Asian Journal of Chemistry; Vol. 25, No. 9 (2013), 4723-4725

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14050

Structure-Antioxidant Activity Relationship of Some Flavonoids Isolated from *Warionia saharae* (*Asteraceae*)

MEBARKA BELBOUKHARI[1,2,2], ABDELKRIM CHERITI[1] and NASSER BELBOUKHARI[2,*]

[1]Phytochemistry and Organic Synthesis Laboratory, University of Bechar, Bechar, 08000, Algeria
[2]Bioactive Molecule and Chiral Separation Laboratory, University of Bechar, Bechar, 08000, Algeria

*Corresponding author: E-mail: nasro14@maktoob.com; belboukhari.nasser@yahoo.com

(*Received*: 11 May 2012; *Accepted*: 4 March 2013) AJC-13057

The antioxidant behaviour of flavonoids including flavone, flavanol and isoflavone isolated from leaves of *Warionia saharae* and the related activity-structure relationships were investigated by measuring their abilty to scavenge the free radical 2,2-diphenylpicrylhydrazyl show that the antioxidant activity depends both on substitution of hydroxyl groups of the flavonoids skeleton and the presence of an unsaturation at the C2 -C3 bond in conjugation with 4 oxo function.

Key Words: Flavonoid, *Warionia saharae*, Antioxidant, Free radical, Scavenge.

INTRODUCTION

Reactive oxygen species readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA. This oxidative damage is a crucial etiological factor implicated in several chronic human diseases namely cardiovascular diseases, rheumatism, diabetes mellitus and cancer[1,2].

The human body possesses many defense mechanisms against oxidative stress, including antioxidant enzyme and non-enzymatic compounds[3], antioxidants are chemical substances that reduce or prevent oxidation, they have the ability to counteract the damaging effects of free radicals in tissues[1,4].

Many studies have shown that phenolic compounds display antioxidant activity as a result of their capacity to scavenge free radicals. Flavonoids belong to a group of phenolic compound with a number of biological activities such as antibacterial,antimutagenic, cytotoxic, anticarcinogenic and antioxidant activity[5].

The antioxidant property of flavonoids was the first mechanisms of action studied in particular with regard to their protective effect against cardiovascular diseases, flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals[6,7].

The objective of this study was to elucidate the antioxidant activity of flavonoids isolated from leaves of *Warionia saharae* and determine their activity-structure relationships as antioxidant by using the DPPH radical scavenging.

EXPERIMENTAL

DPPH radical scavenging method: The antioxidant activity of the flavonoids isolated from leaves of *Warionia saharae* was assesses by the mean of 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) colorimetric method[1]. This method depends on the reduction of purple DPPH to a yellow coloured diphenylpicrylhydrazine and the remaining DPPH$^{\cdot}$, which showed maximum absorption at 517 nm was measured (spectrophotometer). About 2 mL of a 20 mg/mL DPPH solution were added to 1 mL of a methanolic solution of each fractions (1-100 µg/mL). A mixture of 2 mL of DPPH and 1 mL of methanol served as control. The mixture was shaken vigorously then incubated for 15 min in darkness at room temperature. Absorbance was measured at 517 nm, each experiment was performed in triplicate. The DPPH radical scavenging activity was calculated according to the following equation:

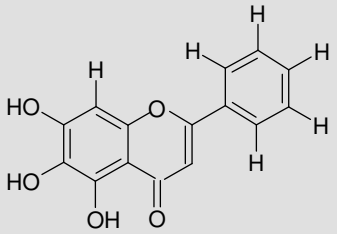
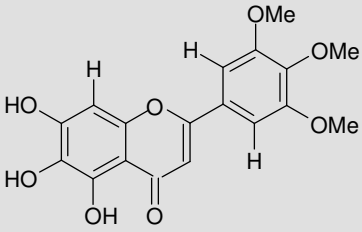
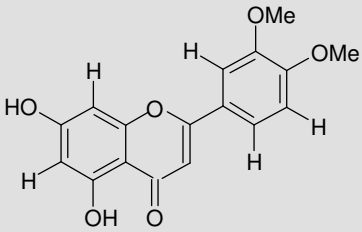
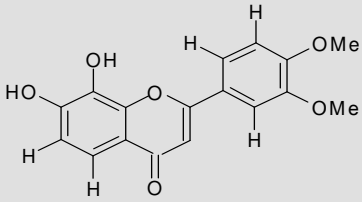
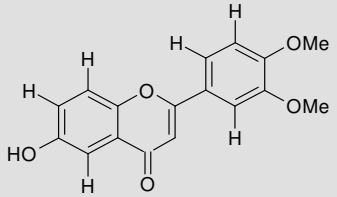
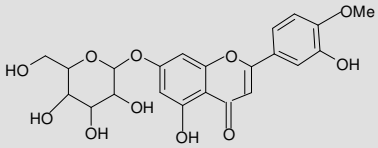
$$\text{DPPH radical scavenging activity (\%)} \quad \frac{A_{control} - A_{sample}}{A_{control}}$$

where A_{sample}: absorbance of sample; $A_{control}$: absorbance of control.

RESULTS AND DISCUSSION

Ten different flavonoids including flavones, flavanol and isoflavone isolated from leaves of *Warionia saharae* were measured for antioxidant properties in this study by using DPPH radical scavenging.

TABLE-1
STRUCTURE AND ANTIOXIDANT ACTIVITY OF TESTED FLAVONE

N	Name	Code	Structure	AA %
1	5,6,7-Trihydroxy flavone	w.sAD(L-L) 07		81.65
2	3',4'-Dimethoxy-5,7,8-trihydroxy flavones	w.sbu 02		70.16
3	3',4'-Dimethoxy-5,7-flavone	w.s bu 16		73.52
4	3',4'-Dimethoxy-7,8-dihydroxy flavones	w.sbu 11		69.33
5	3',4'-Dimethoxy-6-hydroxy flavone (flavone)	w.sbu 03		75.92
6	Luteolin-O-glycosyl-4'-methoxy-5-hydroxy (flavone-glycosylé)	w.sbu 15		67.56

As shown in Table-1, the 5,7 hydroxyl group in A ring the C2-C3 double bond in conjugation with 4 oxo[8-10] function present in compound **1** (5,6,7-trihydroxy flavones), is known to improve antioxidant efficiency and this may be the reason why this flavone has a high potent antioxidant activity (86.65 %).

As present in Table-1, it is observed that compounds **2**, **3**, **4** having a lower antioxidant activity than the activity of compound **1** due to presence of methoxylation group in 3', 4' and 5' position in A ring[9,10].

The presence of glycosylation group in 7 position for compound **6** reduce the antioxidant activity[10].

In the Table-2, the antioxidant activity of taxifolin 6-hydroxy is higher 96.40 % due to existence of hydroxyl group in 5, 7 position in A ring and 3 position in C ring[8-10].

The taxifolin 6-hydroxy, 4' methoxy has a low antioxidant activity percentage than taxifolin 6-hydroxy due to presence of methoxylation group in 5' position in A ring[10].

As present in Table-3, the compound **1** has a high antioxidant activity 86.71 % due to presence of hydroxyl group in 5, 6, 7 position in A ring and the unsaturation between C2-C3 in conjugation with 4-oxo function in C ring[8-12].

The absence of unsaturation between C2-C3 in C ring reduce the antioxidant activity for compound **2**[9,11-13].

Conclusion

The results of this study provide evidence that flavonoids have radical scavenging activity or antioxidant activity due to presence of substitution patterns on the B ring appear to be the most important contributor to the antioxidant activity,

TABLE-2
STRUCTURE AND ANTIOXIDANT ACTIVITY OF TESTED FLAVANOLS

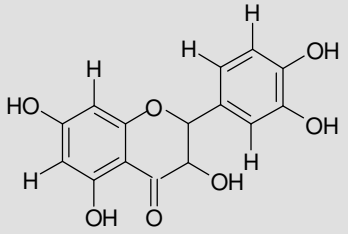
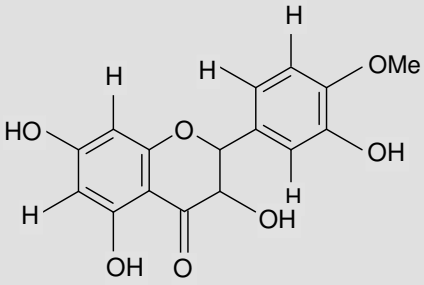
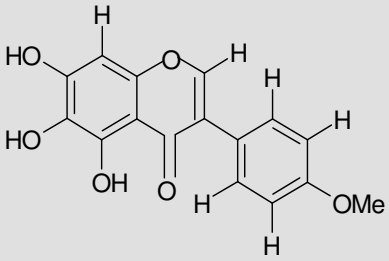
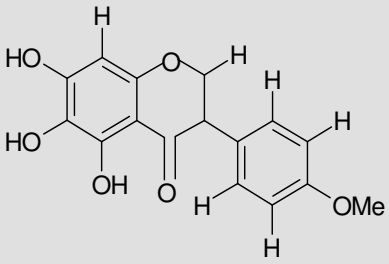
N	Name	Code	Structure	AA %
1	Taxifolin 6-hydroxy (flavanol)	w.s AD 18		96.40
2	4',5,7-Taxifolin (flavanol)	w.sbu 12		66.16

TABLE-3
STRUCTURE AND ANTIOXIDANT ACTIVITY OF TESTED ISOFLAVONE

N	Name	Code	Structure	AA %
1	6-Hydroxy biochanin A (isoflavone)	w.s AD(L-L) 03		86.71
2	6-Hydroxy biochanin A (isoflavone)	w.sbu 13		69.59

hydroxyl groups boost the antioxidant activity, whereas methoxy and glycosyl groups reduce the antioxidant activity. Presence of unsaturation between C2-C3 in conjugation with 4 oxo function enhances the antioxidant capacity. A hydroxyl group at the C3 position is also beneficial to the ability of flavonoids to scavenge free radical.

REFERENCES

1. R.B. Ammar, W. Bhouri, M.B. Sghaier, J. Boubaker, I. Skandrani, A. Neffati, I. Bouhlel, S. Kilani, A.-M. Mariotte, L. Chekir-Ghedira, M.-G. Dijoux-Franca and K. Ghedira, *Food Chem.*, **116**, 258 (2009).
2. K. Pong, *Expert Opinion Biol. Ther.*, **3**, 127 (2003).
3. E.S. Abdel-Hameed, *Food Chem.*, **114**, 1271 (2009).
4. M. Bandyopadhya, R. Chakraborty and U. Raychaudhui, *LWT-Food Sci. Technol.*, **40**, 842 (2007).
5. B. Modak, M.L. Contreras, F. González-Nilo and R. Torres, *Bioorg. Med. Chem. Lett.*, **15**, 309 (2005).
6. P. Montoro, A. Braca, C. Pizza and N. De Tommasi, *Food Chem.*, **92**, 349 (2005).
7. L. Bravo, *Nutr. Rev.*, **56**, 317 (1998).
8. M. Belboukhari, A. Cheriti and N. Belboukhari, *Nat. Prod.*, **7**, 150 (2011).
9. C.A. Rice-Evans, N. Miller and G. Paganga, *Trends Plant Sci.*, **2**, 152 (1997).
10. C.A. Rice-Evans, N.J. Miller and G. Paganga, *Free Radic. Biol. Med.*, **20**, 933 (1996).
11. K.E. Heim, A.R. Tagliaferro and D.J. Bobilya, *J. Nutr. Biochem.*, **13**, 572 (2002).
12. A. Arora, M.G. Nair and G.M. Strasburg, *Free Radic Biol. Med.*, **24**, 1355 (1998).
13. G. Cao, E. Sofic and R.L. Prior, *Free Radic Biol. Med.*, **22**, 749 (1997).