PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

KASDI MERBAH UNIVERSITY OUARGLA

FACULTY OFMATHEMATICS AND MATERIAL SCIENES

DEPARTMENT OF CHEMISTRY

Dissertation

ACADEMIC MASTER

Specialty: Natural Products Chemistry

Title:

Characterization and Modeling of compounds separated from the butanol extract of a plant of the Chenopodiaceae family: analysis of NMR, UV-Vis and MS spectra

Presented by:

BELMABEDI Oumelkhir

on: 13/06/2022

Before the jury composed of:

Pr. BOUZIANE Mebarka	**President**	**UKMO**
Dr. MEKHLEFI Tarak	**Examiner**	**UKMO**
Pr. RAHIM Oumelkheir	**Supervisor**	**UKMO**
Dr. ALLAOUI Messaouda	**Assistant Supervisor**	**UKMO**

University Year: 2021/2022

DEDICATION

قال تعالى :{ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ}

(صدق الله العظيم)

We are for her and if she does, even though she comes to her 💛 God, it is not in my effort and diligence, but with your success and generosity and thanks to me, May God be easy and easy for me beyond and open to me from the doors of your knowledge, God Amen ❤️ Praise be to Allah, the Lord of the Worlds" ❤️

I dedicate my graduation and joy

To all those who are blessed by God with prestige and reverence..

To those who taught me to give without waiting.. To whom I proudly carry his name (My dear father ❤️)

And my angel in life.. To the meaning of love and the meaning of tenderness and dedication.. In his name life and the secret of existence, to those who called it the secret of my success to the most precious loved ones (my dear mother ❤️)

To those close to the heart, supporters in through thick and thin, my brothers and sisters. Thank you.

To my friends and companions on the path to those who are difficult for us. Thank you from heart to heart.

To the guys of the first and last step to those who have been in lean years with a rainy cloud I am very grateful 💙

To those who taught me and have the greatest credit after God (my best teachers at all stages of school life)

ACKNOWLEDGMENTS

First of all we thank God for the most mercy for enabling us to present

this project in the best form that we wanted to be,

we would like to thank my supervisor of this project,

Pr. (RAHIM Oumelkheir) and (Dr. ALLAOUI Messaouda) for their valuable

help and advice to come out with this project.

I thank my faculty and doctors that provided me with all the knowledge.

Most of all I am grateful to my family for

their endless love, assistance, support and encouragement. And for my friends

for their understanding

and support for us to complete this project.

BELMABEDI Oumelkhir

Table of contents

List of figures

CHAPTER I:Botanical aspects and previous research……

CHAPTER II: Materials and Method……..……..…

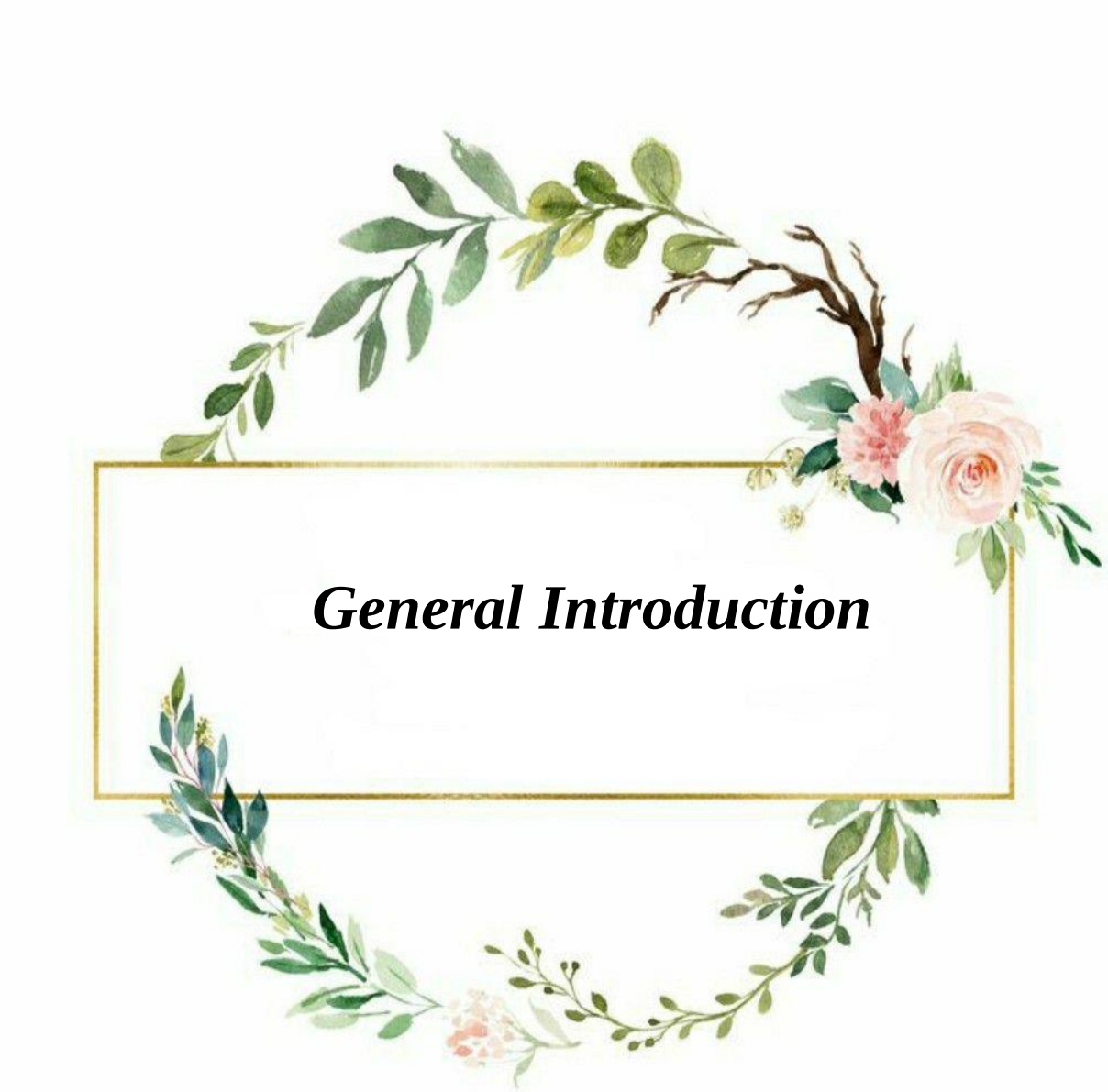
Chapter III : Results and discussion……..……...…

List of tables

List of Abbreviations

Abbreviations	*Definition*
AcOH	Acetic acid
Ac	Acetone
aq	Aqueous
APT	Attached proton test
AUC	Area Under the Curve
TLC	Thin Film Chromatography
IC_{50}	50% inhibition concentration
NMR	Nuclear Magnetic Resonance
DMSO	Dimethylsulfoxide
QSAR	Quantitative structure activity relation ship
Log P	partition coefficient
π	Hansch constant (lipoid substitute constant)
f	hydrophobic fragment constant
σ	Hammett constant
F, R	field and resonance parameters
Es	Taft constant (steric aspect)
rv	Mean radius (Van-der-Waals radii of symmetric substituents)
1D NMR	one dimensional
2D NMR	two dimensional
CAN	Acetonitrile
BuOH	n-Butanol
calcd	calculated
CC	Silica gel Column Chromatography
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
COSY	Correlation Spectroscopy
CPC	Centrifugal Partition Chromatography
d	doublet
dd	doublet of doublets
D_2O	Deuterium Oxide
DEPT	Distortionless Enhancement by Polarisation Transfer
^{13}C ***NMR***	Carbon-13 Nuclear Magnetic Resonance

DNA	Deoxyribonucleic acid
eq	Equation
EtOAc	Ethyl Acetate
EtOH	Ethanol
F.C	Flash Chromatography
HILIC	Hydrophilic Interaction Liquid Chromatography
HMBC	Hetero-nuclear Multiple Bond Correlation Spectroscopy
HPLC	High Performance Liquid Chromatography
HPLC-DAD-MS	High Performance Liquid Chromatography coupled to a Diode Array Detector and a Mass Spectrometer
HPTLC-UV	High Performance Thin Layer Chromatography coupled to a spectro-photometer
MS-ESI	Mass Spectrum Electrospray Ionization
HMQC	Hetero-nuclear Multiple Quantum Correlation spectroscopy
IR	Infrared Spectrophotometry
m	multiplet
Me	Methyl
MeOH	Methanol (***CH_3OH***)
m.p	melting point
MS	Mass Spectrum
NMR	Nuclear Magnetic Resonance
PE	Petroleum Ether
ppm	parts per million (chemical shift value)
Prep. HPLC	Preparative High Performance Liquid Chromatography
Prep. TLC	Preparative Thin-Layer Chromatography
s	singlet
t	triplet
TLC	Thin Layer Chromatography
Tol	Toluene
UV	Ultraviolet
^1H NMR	Proton Nuclear Magnetic Resonance



General Introduction

General Introduction

Saharian plants are known by their resistance to several stress factors. Under extreme climatic conditions, these plants could constitute a reservoir of new natural, safe and effective biomolecules [1]. The Sahara covers 84% of the area of Algeria. The Saharan flora is very diverse if we compare the number of species with the enormity of its surface, but very varied in its systematic composition. The Algerian Sahara has 650 species with 162 endemic species, endemism accounts for about 25% [2].The study of medicinal plant is one of the methods of examining the interaction and relationships between biological and cultural components of the environment [3].Ethnobotanical studies today are recognized as the most viable method of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents [4]. only few studies have been conducted in the assessment; chemical constituents of Saharian medicinal plants specifically in the identification of the structure of bioactive constituents of traditional medicinal plants in the country wise [5]. The medicinal value of these plants backs to their active chemical components which found in their secondary phytochemical metabolites, such as phenolics, alkaloids, flavonoids and terpenoids that have been shown to have significant antimicrobial activities in several studies[6].

Traganum nudatum "Delile" is a plant belonging to Chenopodiaceae family. which is a native halophytic shrub in arid zones in Algerian. It grows in high temperatures and can tolerate increased salinity and aridity [7]. it is used in traditional medicine for the treatment of several diseases such as used to treat wounds, rheumatism, diarrhea, dermatoses [8], treat diabetes and various pains like low back pain [9], also as a remedy for gastric problems, pruritus and pimples [10] and usefulness as protective agents against oxidative DNA damage [11]. The separation of certain compounds from the butanol extract resulted in the isolation of several types of products, the most common of which are flavonoids that are the subject of phytochemical and pharmacological studies. This work allowed several spectroscopic analyses to be carried out to determine and suggest the structure of the resulting pure materials. So, the main objective of this project is to analyses the different spectral arrays to prove and predict the structure and modeling of the molecules and define their QSAR descriptors.

The main parts of this work constitute three chapters, are treated as follows:

The first chapter is devoted to bibliographical and botanical research species studied as well as previous work.

the second part are devoted to the Materials and Working Method realized in this brief in which we have worked

In the third chapter we describe the identification Is reserved for the structural elucidation of compounds isolated from the butanolic phase of T. nudatum, as well as the discussion of the results obtained.

This work will be closed with a general conclusion.

References

[1] ***Bouaziz M, Dhouib A, Loukil S, Boukhris M, Sayadi S. Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia. Afric. J. Biotechn. 2009; 8:(24):7017-7027.***

[2] P. Ozenda, Flore du Sahara : septentrional et central. 1958, Paris Centre National de la Recherche Scientifique.

[3]. ***Bye RA. Medicinal plant of Sierra Madre: Comparative study of Tarahumara and Mexican market plants. Econ. Bot. 1986; 40(1):103-124.***

[4]. ***Ogol C, Ogola P, Odede W, Khayota B. Indigenous knowledge of medicinal and utilitarian plants of Mfangano Island, Lake Victoria, Kenya, East African Journal of Science. 2002; 4:11-28.***

[5]. ***Habtamu A, Mulatu O, Tsdeke L. Traditional medicinal plants utilization, management and threats in Hadiya Zone, Ethiopia. Journal of Medicinal Plants Studies. 2014; 2(2): 94-108..***

[6] Ahmed, F.A., et al., ***Phytochemical Investigation, HPLC Analysis and Antimicrobial Activity of Some Plants from Chenopodiaceae Family.*** **Egyptian Academic Journal of Biological Sciences, H. Botany, 2022. 13(1): p. 13-24.**

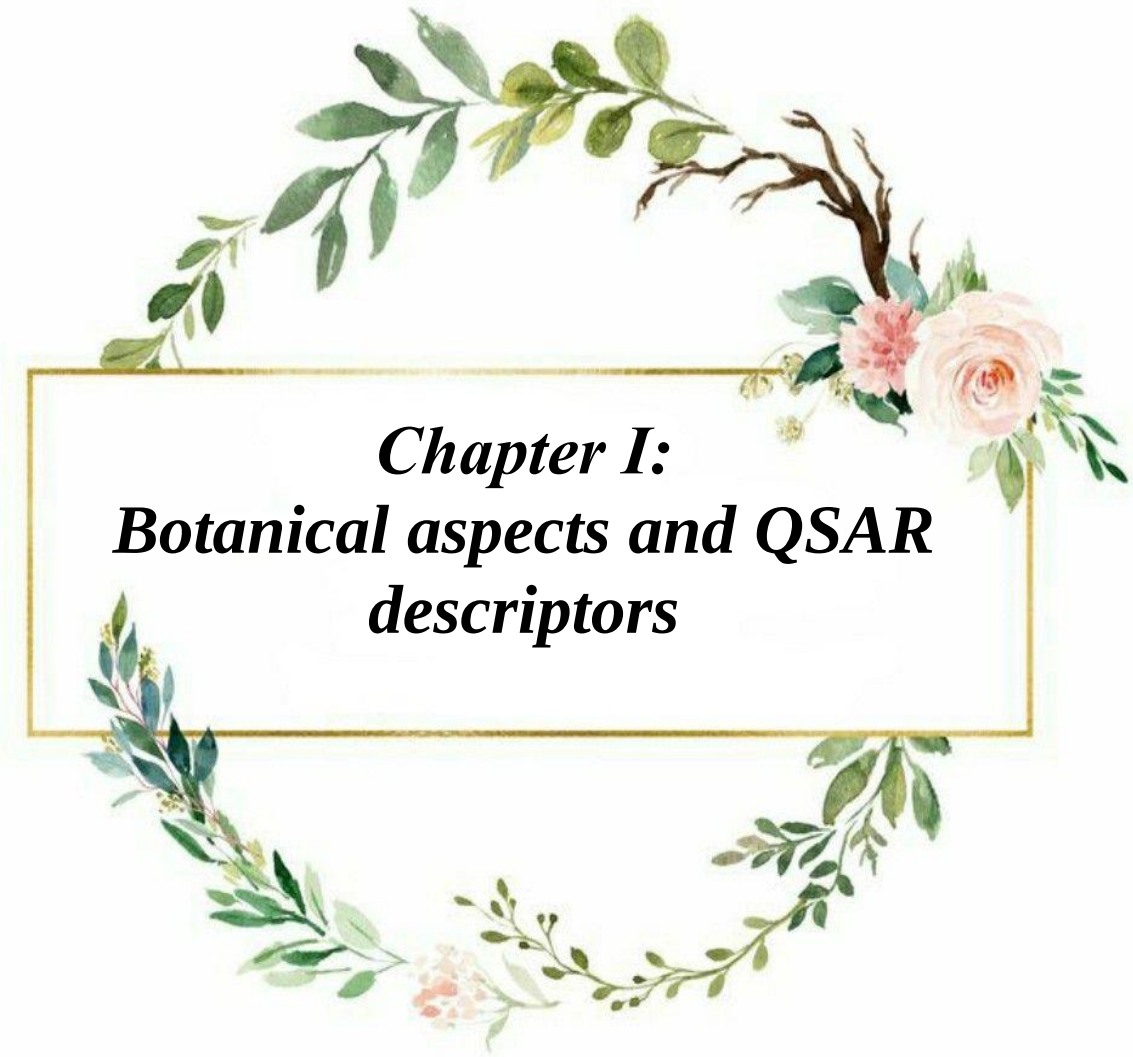
[7] Tan K., Miller A.G. and Cope T.A. Flora of the Arabian Peninsula and Socotra Volume I. ***NordicJournal of Botany,*** **17, 144–144 (1997).**

[8] Ould Elhadj M.D., Hadj-Mahammed M. and Zabeirou H. Place des Plantes Spontanées dans la médicine traditionnelle de la région d'Ouargla (Sahara Septentrional Est). ***Courrier du Savoir,*** **3, 47-51(2003).**

[9] Boualala M., Bradai L. and Abid M. Diversité et utilisation des plantes spontanées du Sahara septentrional algérien dans la pharmacopée saharienne. Cas de la région du Souf. ***Revue ElWahat pour les Recherches et les Etudes,*** **7, 18-26 (2014).**

[10] Bellakhdar J. La pharmacopée marocaine traditionnelle, Ibis Press, Paris, France. (1997).

[11] Daradka H.M., Aljohani H.A., Alotaib M.K., Khabour O.F., et al. Protective effects of ***Traganum nudatum*** **against oxidative DNA damage in human lymphocytes.** ***International medic al journal,*** **25(2), 913-920 (2020).**



Chapter I: Botanical aspects and QSAR descriptors

I-1 Chenopodiaceae family:

I.1.1. Background:

The family Chenopodiaceae is relatively large and worldwide it numbers about 1600 species belonging to more than 100 genera, spread more widely in the moderate and subtropical regions [1].

Plants in this family are mainly salt-tolerant annual or perennial herbs with vesicular (glandular) hairs on the leaves that store salt as sodium or potassium chloride.

Betalain pigments are also present. Tiny, wind-pollinated flowers form in dense clusters. The usually bisexual flower has 5 joined sepals or none, 5 stamens, and a single pistil. In the superior ovary is one chamber (**locale**) containing one ovule that develops into a nutlet fruit[2].

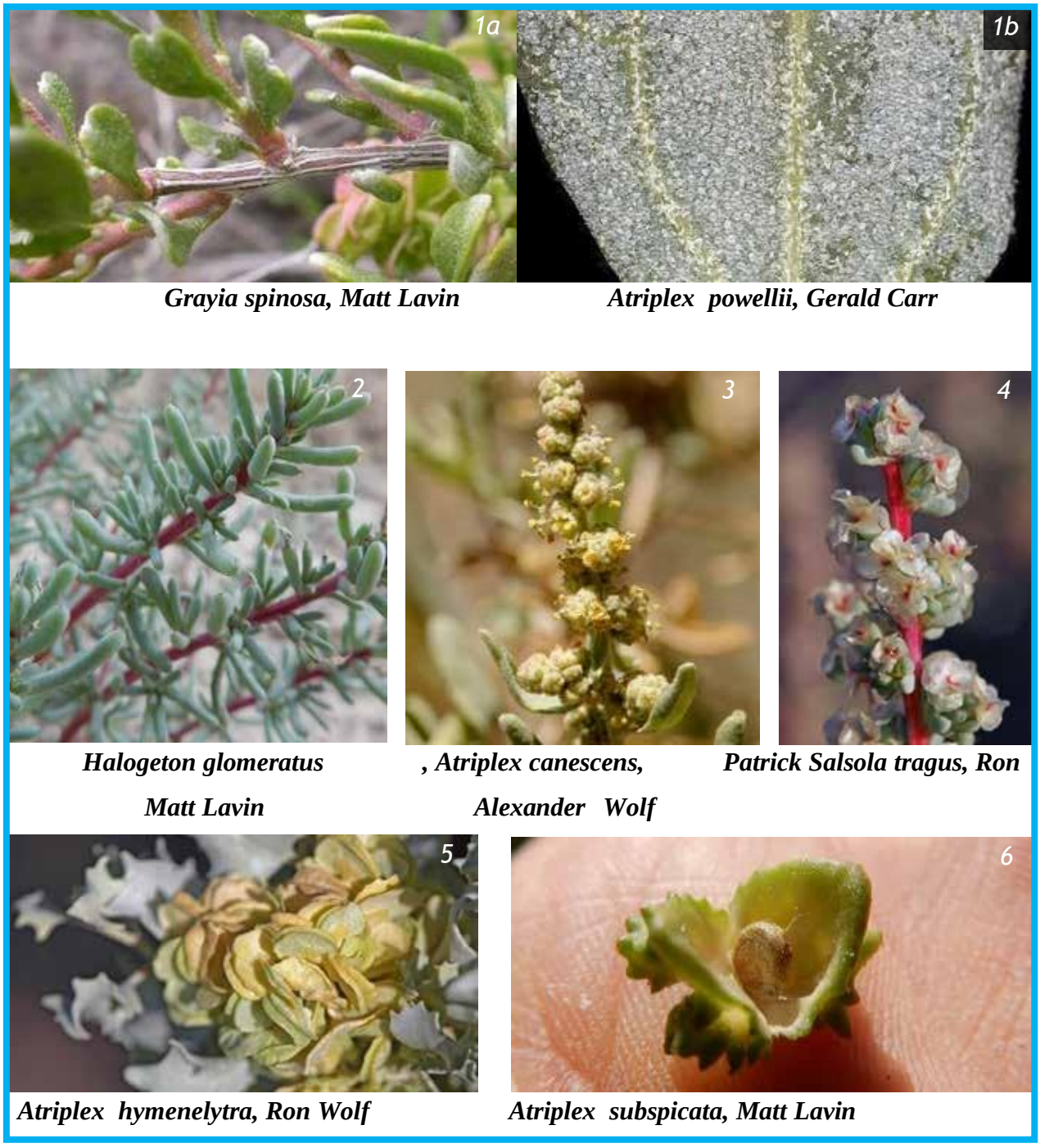
The flowers are tiny and inconspicuous, but some species bear showy masses of fruits. Chenopods are common in deserts and especially in saline or alkaline soils [3].

It contains numerous species which vary greatly in their chemical constituents and uses such as alkaloids, flavonoids, saponins and tannins[4].

I -1-2Identifying Characteristics:

1.Leaves: (a) generally alternate, without stipules; (b) leaf surfaces with simple, stellate or glandular hairs— scurfy leaf surface (covered with scale-like particles)

2. Stems: occasionally fleshy
3. Flowers: unisexual or bisexual, tiny, inconspicuous
4. Perianth: petals absent, (3) 5 free or basally fused sepals, often scarious; Stamens 1-5
5. Ovary: superior
6. Fruit: a chene or utricle, enclosed by persistent sepals or bracts (figure I-1)



Grayia spinosa, Matt Lavin ***Atriplex powellii, Gerald Carr***

Halogeton glomeratus Matt Lavin ***, Atriplex canescens, Alexander Wolf*** ***Patrick Salsola tragus, Ron***

Atriplex hymenelytra, Ron Wolf ***Atriplex subspicata, Matt Lavin***

Figure I-1: Identifying Characteristics of *Chenopodiaceae* family [5]

Table I-1 :Plants from *Amaranthaceae* family used in the treatment of diabetes in Algeria

Family	Plant(s) names	Algerian local name (s)	Algerian study area	Part used	Traditional preparation method	Algerian traditional use(s)	Number of citations Informant(s) frequency	References
Amaranthaceae	Aerva javanica (Burm.f.) Juss. ex Schult.	Makhmila	CS	Aerial part	Decoction	Diabetes,cough, headaches, kidney diseases, lithiasis, urinary decrease, abdominal pains, jaundice	1 (0.58%)	[6] [7]
	Arthrophytum scoparium(Pomel)Iljin Syn. Haloxylon scoparium Pomel	Remt	W	Stems	Infusion	Diabetes	1 (0.58%)	
	Hammada scoparia (Pomel) Iljin (Syn. Haloxylon scoparium Pomel)	Ramth	NE	Flowers, leaves, boughs	Decoction	Diabetes	1 (0.58%)	[8] [9]
	Anabasis articulata (Forssk.) Moq. Atriplex halimus L.	Baguel El gtaf	SE NW, SW	Aerial part Leaves	Decoction Decoction	Diabetes Lowering	4 (2.33%)	[10]
		Guettaf, El-Gtaf	A	Not reported	Not reported			
		G't f Gtaf	***SE E***	reported Leaves Leaves	***Decoction , Infusion Infusion***	hyperglycemia, anemia Diabetes Diabetes, treatment of ovarian cysts, rheumatism, goiter, cholesterol		***[9] [11]***
	Haloxylon scoparium Pomel	***Remth***	***SE***	Aerial part	***Decoction , infusion***	Diabetes	***1 (0.58%)***	***[9]***
	Traganum nudatum Delile	***Domran***	***SE***	Aerial part	***Decoction , powder***	Diabetes	***1 (0.58%)***	***[9]***

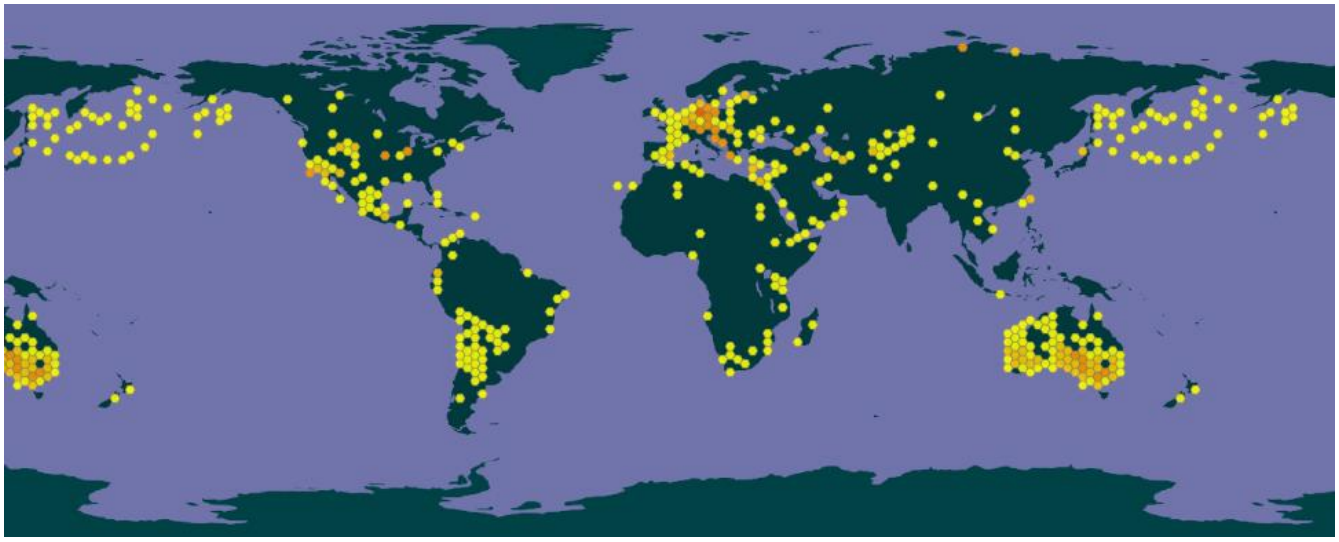


Figure I-2 : Geographical distribution map of the *Chenopodiaceae family*[12]

I-1-3Systematic position of the family *Chenopodiaceae*[12]:

- **Kingdom: Plantae**
- **Subkingdom: Viridiplantae**
- **Infrakingdom: Streptophyta**
- **Superphylum: Embryophyta**
- **Phylum: Tracheophyta**
- **Subphylum: Spermatophytina**
- **Class: Magnoliopsida**
- **Superorder: Caryophyllanae**
- **Order: Caryophyllales**
- **Accepted name Family "*Amaranthaceae*" Synonym = *Chenopodiaceae***

I-2 Description of species studied:

Our work is devoted to the species answered in the South Algerian, this species is *Traganum nudatum* Delile is a plant belonging to Chenopodiaceae family. In Algerian Sahara, it is used in traditional medicine for the treatment of several diseases such as diabetes, rheumatism, diarrhea, otitis, and to treat wounds, skin diseases and low back pain[13].

I-2-1 Classification [12]:

- **Kingdom: Plantae**
- **Phylum: Tracheophyta**
- **Class: Magnoliopsida**
- **Order: Caryophyllales**
- **Family: Amaranthaceae (Chenopodiaceae)**
- **Genus: Traganum**
- **Scientific name: *Traganum nudatum* (Delile)**

Figure I-3 : drawing of Traganum nudatum

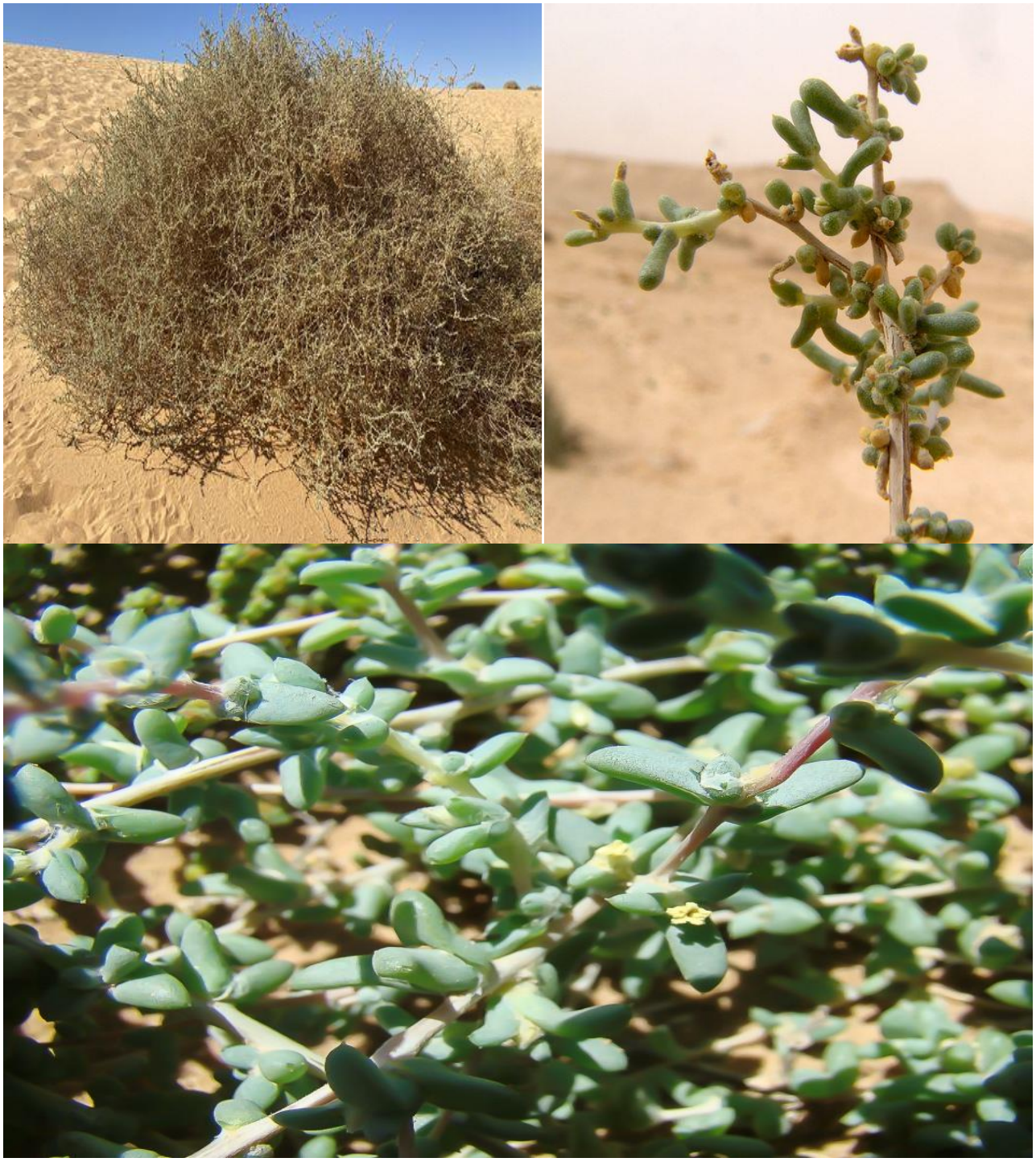


Figure I-4 :photograph of *T. nudatum*[13]

Figure I-5 : Geographical distribution map of the *T. nudatum* [6]

I-2-2Phytochemical Screening:

Samples of *Traganum nudatum* roots, stems and leaves were screened phyto-chemically for the presence of secondary metabolites using the standard methods of Harborne,[14] and Trease and Evans [15]. The secondary metabolites screened for are alkaloids, tannins, Saponins, anther aquinones, flavonoids, Sterols and triterpenes, Steroid derivatives, and cardenolides

Qualitative phytochemical screening of the extracts of *Traganum nudatum* demonstrated the presence of Alkaloids, tannins, Saponins and flavonoids while anther a quinones, cardinolides, Sterols and triterpenes were absent. The results of the antibacterial screening of the plant (TableI-3) revealed that the three extracts showed varying degrees of inhibition on the tested microorganisms at a concentration of 5 mgmL-1. The Roots extract showed a great activity against *Escherichia coli, Staphylococcus aureus* and *Streptococcus Sp* (17 -24mm) but no activity against *Pseudomonas aeruginosa* and *Salmonella typhis*. The stems extract exhibited higher activity against *Escherichia coli* and *Streptococcus Sp* (11–12.5mm) whereas there is no activity against *Staphylococcus aureus, Salmonella typhis* and *Pseudomonas aeruginosa.* The Leaves extract had an important activity against *Escherichia coli, Staphylococcus aureus* and *Streptococcus Sp* (16 -19mm) but no activity against *Pseudomonas aeruginosa* and *Salmonella typhis*. The minimum inhibit or concentration of the different extracts ranged between 0.0625 and 0.125 mg mL-1 (Table 4). The lowest MICs for extracts were those for *Streptococcus Sp Staphylococcus aureus, and Escherichia coli* (0.0625 mg mL-1).

Table I- 2: Phytochemical components of the water extracts of *T. Nudatum*

Phytochemical components	roots	stems	leaves	References
Alkaloids	+	+	+	[15]
Tannins	+	+	+	[15]
Saponins	+	+	+	[15]
Anthraquinones	-	-	-	[15]
flavonoids	+	+	+	[15]
Sterols and triterpenes	-	-	-	[15]
Steroid derivatives	-	-	-	[15]
cardenolides	-	-	-	[15]

I-2-3 Antimicrobial activity:

The antimicrobial activity was determined by the well diffusion method [16]. The diameter of the inhibition zones were measured for each plate and the average reading of the three replicates for each antibacterial species are shown in (Table 3).

Table I-3: Inhibition zone diameter (mm) of different extracts at 5mg/ mL

organisms	Roots extract	Stems extract	Leaves extract	References
Escherichia coli	24	11	16	**[16]**
Pseudomonas aeruginosa	0	0	0	**[16]**
Salmonella typhis	0	0	0	**[16]**
Staphylococeus aureus	23	0	19	**[16]**
Streptococcus Sp	17	12.5	16	**[16]**

The antibacterial activity of the water extracts of *Traganum nudatum* can be attributed to the action of the phytochemical compounds it contains. Polyphenolic compounds are known to inhibit a wide range of organisms [17]. The antimicrobial activity of the extracts could beex plained by the presence of tannins. The mechanism of action of tannins is based on their ability to bind proteins thereby inhibiting cell protein synthesis [18]. There was a significant difference in the antimicrobial activity of the extracts on Gram-negative and Gram-positive bacteria since there was no biological effect against *Pseudomonas aeruginosa* and *Salmone llatyphis* with the prescribed concentration. The roots have been found to be more effective against s*taphylococcus aureus* and *Escherichia coli* than stems and leaves. The three extracts did not show any action against *Pseudomonas aeruginosa* and *Salmonella typhis.* This means that higher doses of the antimicrobial agent will be needed in the treatment of infections caused by *Pseudomonas aeruginosa* and *Salmonella typhis* provided they are not toxic to the tissues[CH].

Table I-4: Minimun inhibition concentration (MIC) of different extracts of *T. Nudatum*

organisms	Roots extract	Stems extract	Leafs extract
Escherichia coli	0.0625	0.125	0.0625
Streptococcus Sp	0.0625	0.125	0.10
Staphylococcus aureus	0.0625	-	0.10
Salmonella typhis	-	-	-
Pseudomonas aeruginasa	-	-	-

I-2-4 Antioxidant activities

DPPH radical scavenging activity The model of scavenging DPPH free radical has been widely used for the determination of antioxidant activity of different plants, vegetables, and fruit extracts. In this method, phenols and flavonoids have the ability to donate electron/hydrogen that results in converting highly reactive free radical DPPH to non-reactive stable diamagnetic molecule DPPH-H [19].

DPPH radical scavenging activities of extracts were proportional to their concentrations (Figure I-6). In fact, the results pointed out that EAFM showed the strongest antiradical capacity with an IC50 about 20 ± 0.11 µg/mL. The IC 50 values of scavenging DPPH radical were in the following order: EAFM < BFM < EAFI < CEM < BFI < CEI (Fig. 3).

However, the ascorbic acid concentration 1 ± 0.14 µg/mL required to reduce 50% of DPPH radical was lower than the extracts. These results sustain those of Kang et al. (2017) who found that the maximum antiradical activity

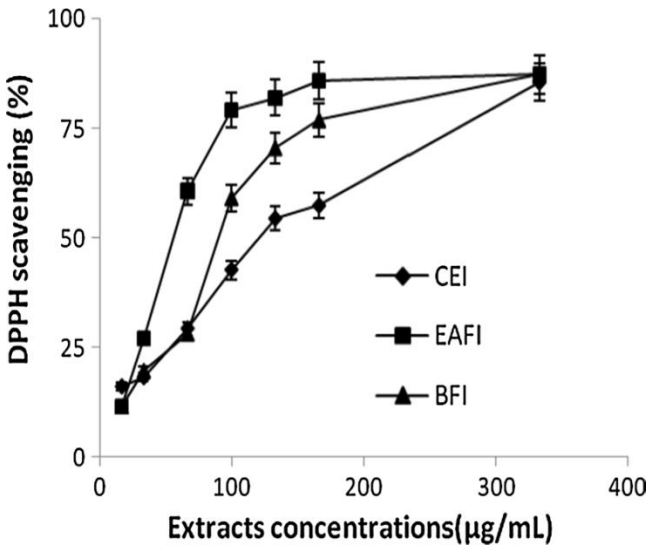


Figure I-6:DPPH radical scavenging activities of crude extractb ethyl acetate and n-butanol

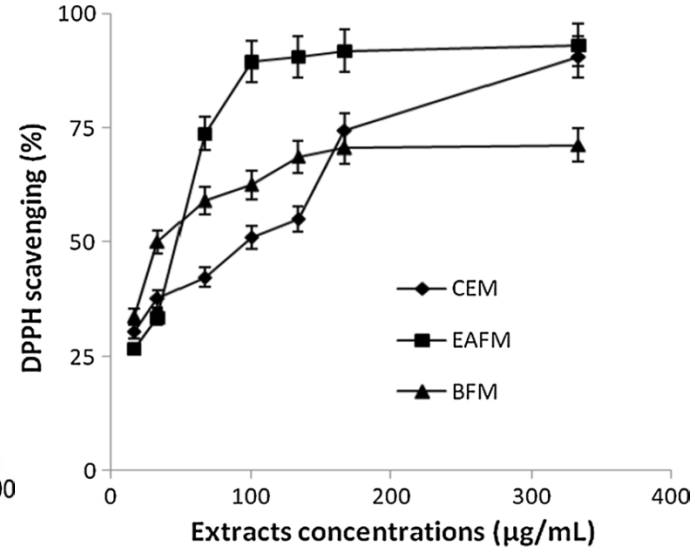


Figure I-7: DPPH radical scavenging activities of crude extract, ethyl acetate and n-butanol

I-2-5 Some results of chromatogram separation (GC-MS) of amyotrophic plant (CH_2Cl_2) extract :

Table I-5: results of chromatogram separation (GC-MS) of amyotrophic plant (CH_2Cl_2) extract

Time Detention (min)	Percentage	Scientific names of vehicles	References
4.876	0.275	(E)-3-hexene	[20]
7.295	0.137	M-hydroxy phenethanol	[20]
9.162	0.221	Trimethyl silyl octadecanoic acid	[20]
16,33	0.806	Tetradecanoic acid	[20]
17,81	0.734	1,10-decandiol	[20]
17,96	0.816	Acetyl choline	[20]
18.647	0.373	Octadecanal	[20]
20,24	8.748	Hexadecanoic acid	[20]
22,74	0.365	1-Heptadecanamine	[20]
23.057	0.536	1-(ethenyloxy)-octadecane	[20]
23,47	2.551	Cyclodecanol	[20]
23,58	5.742	9-Octadecenal	[20]
23,9	0.869	Octadecanoic acid	[20]

I-3-1 QSAR:

Although the study of structure-activity relationships began at the end of the 19th century, this only in the early 1960s did the work of Corwin Hansch propose a mathematical model to correlate biological activity and chemical structure.Over the past 40 years, this field has been extensively studied and the data Bibliographics available on this approach are now important [21].

The QSAR method includes all statistical methods by which activities biological (most often expressed as logarithms of molar activities equipotentials) are related to the structural elements (Free Wilson analysis), the physical-chemical properties (Hansch analysis) or different parameters related to the concept of a field helping to describe the structure (3D QSAR).

Information extracted from QSAR study results can be used to obtain a better knowledge of molecular structures and probably the mode of action at the molecular level. This information can then be used to predict the physicochemical properties and biological activities of new and to design new structures [21].

I-3-2 Principle:

The QSAR/QSPR study consists of defining a mathematical relationship between an activity biological, or a property measured for a series of similar compounds in the same experimental conditions, with molecular descriptors using methods statistics. The objective of these studies is to analyze structural data in order to detected termining factors for the activity or property studied. To do this, different types of statistical methods can be used. The resulting mathematical expression is used as a predictive means of the activity/property studied for new molecules or molecules for which experimental data are not available. [22]

I-3-3 The overall strategy:

To develop a model, it is necessary to start with the search for the maximum possible reliable experimental data. Next, the development of a set of descriptors that characterize the molecular structures of the database compounds for link to the experimental activity/property studied. Once developed, the model must be validated in terms of correlation. The influence of the training set compounds on the model is estimated by methods of internal validation. To estimate the predictive power of the model, it is necessary additional experimental data is external validation. [23]

I-3-4 QSAR/QSPR Models:

In past years, the Quantitative Relations Structure Activity, Quantitative Property Structure (QSAR/QSPR) have become a powerful theoretical tool, to the place of quantum mechanics, for the description and prediction of the properties of complex molecular systems under different circumstances. The QSAR approach assumes a dependent relationship between any

what physical property, chemical affinity, or biological activity of a chemical compound, and its molecular structure. This univocal correspondence can be represented by the chemical composition, the connectivity of atoms, the potential energy surface, and the electron wave function of a compound.

Different physico-chemical molecular descriptors representing the structure can be determined empirically or using theoretical and of different levels of complexity. It was necessary to highlight that the knowledge of exact chemical constitution and/or molecular structure three-dimensional chemical compounds studied is an essential condition for the application of the QSAR approach.

The implementation of the QSAR approach depends on the appropriate use of molecular descriptors[24].

I-3-5 The descriptors:

A molecular descriptor is a parameter (numeric value) specific to a given chemical structure.

These values can be obtained experimentally or calculated from the structure of the molecule. The calculated descriptors allow to make predictions without have to synthesize molecules, which is one of the objectives of molecular modelling. Molecular descriptors play a fundamental role in relationship studies quantitative structure activity/ownership. They are used as independent variables to predict a variable dependent (activity or property).The use of molecular descriptors in model development

QSAR/QSPR is not an easy task. First, a very large number of descriptors of different complexities and designs was introduced in the course of years. Meanwhile, no strict rules have been established for the selection of suitable descriptors from the large number of available descriptors. This choice has often been based on the chemical intuition of researchers, or by bending to tradition [25].

- Constitutional descriptors.
- Topological descriptors.
- Quantum descriptors.
- Reactivity descriptors from the conceptual DFT.
- Geometric descriptors.

- Geometric descriptors: These are descriptors evaluated from the relative positions of the atoms of a molecule in space, as well as atomic rays and masses. They therefore require know the 3D structure of the molecule. This can be obtained experimentally well but most often by molecular, empirical or as initio modelling. therefore, unlike the previous descriptors, they require a certain amount of time to calculus. Among these descriptors are the volume and molecular surface, the moment of inertia or of specific distances, angles or angles between atoms in the molecule. In particular, such a geometric parameter could show its central role for characterize the activity of iron pyridine (II) bis (arylimino) complexes for oligomerization of ethylene. It has been shown that the activity of these complexes is related to a dihedral angle particular φ which governs the accessibility to the metal reactive core of the precursor complex catalytic by its activator.

I-3-6 QSAR Tools and techniques:

I-3-6-1 Biological Parameters:

Biological data are usually expressed on a logarithmic scale in reason for the linear relationship between response and dose logarithm in the region log dose-response curve. Activity logarithms (log 1/C)are also used to obtain higher mathematical values when the structures are biologically highly effective. Examples of biochemical or used in the QSAR analysis are described in (TableI-6) [26].

Table I-6: Types of biological data used in the QSAR analysis

Activity Source	Biological Parameters
1. Isolated Receivers	
Velocity constant	
Michaelis-Menten constant	Log k
Inhibition constant	Log 1/Km
2. Cellular Systems	Log 1/Ki
Inhibition constant	Log 1/IC50
Cross-resistance	Log CR
In vitro biological data	Log 1/C
Gene mutation	Log TA98
3. In vivo systems	BCF log
Bioconcentration factor	Log I (induction)
Velocities of reaction in vivo	Log T (total clearance)
Pharmacodynamic speeds	

I-3-6-2 Molecular properties and their parameters:

Some descriptive parameters of molecules are listed in (Table I-7) [27].

Table I-7: Molecular Properties and Their Parameters [27]

Molecular Property	Corresponding Interaction	Parameters
Lipophilicity	hydrophobic interactions	log P, π , f, R_M, χ
Polarizability	van-der-Waals interactions	MR, parachor, MV
Electron density	ionic bonds, dipol-dipol	σ, *R*, *F*, κ, quantum
	interactions, hydrogen	chemical indices
	bonds, charge transfer	
	interactions	
Topology	steric hindrance geometric fit	E_S, r_V, L, B, distances, volumes

I-3-6-3 Molecular descriptors:

A molecular descriptor can be considered as a consequence of a process logic and mathematics, applied to chemical information codified through the representation of a molecule [28] .

The coded information of a molecular descriptor depends on the type of representation used and the algorithm defined for its calculation. There are:

- Simple molecular descriptors derived from the number of standard atoms or structural fragments of the molecule.
- algorithms applied to a topological representation (graphic molecular) and usually called topological or 2D descriptors.
- molecular descriptors derived from a geometric representation, which are called geometric or 3D descriptors.

I-3-6-4 QSAR Techniques:

Some research projects involve biological and physico-chemical data appropriate. These data can be represented and analyzed in a variety of ways. The grouping and classification of compounds, based on their properties, are the key elements in molecular similarity studies. Regression or correlation between biological and chemical data are generally useful for rationalize structure-activity relationships. Two main types of studies can be defined:

(1) by correlation between biological and physicochemical data using regression techniques,

(2) by classification of compounds or model identification methods

Both types of studies are referred to as multivariate statistical data analysis, or QSAR study [28].

I-3-7 General Scheme of a QSAR Study

The chemo-informatics methods used in building QSAR models can be divided into three groups:

•Extracting descriptors from molecular structure,

•Choosing those informative in the context of analyzed activity,

•Finally using the values of the descriptors as independent variables to define a mapping that correlates them with the activity in question [27].

I-3-8 QSAR Equation:

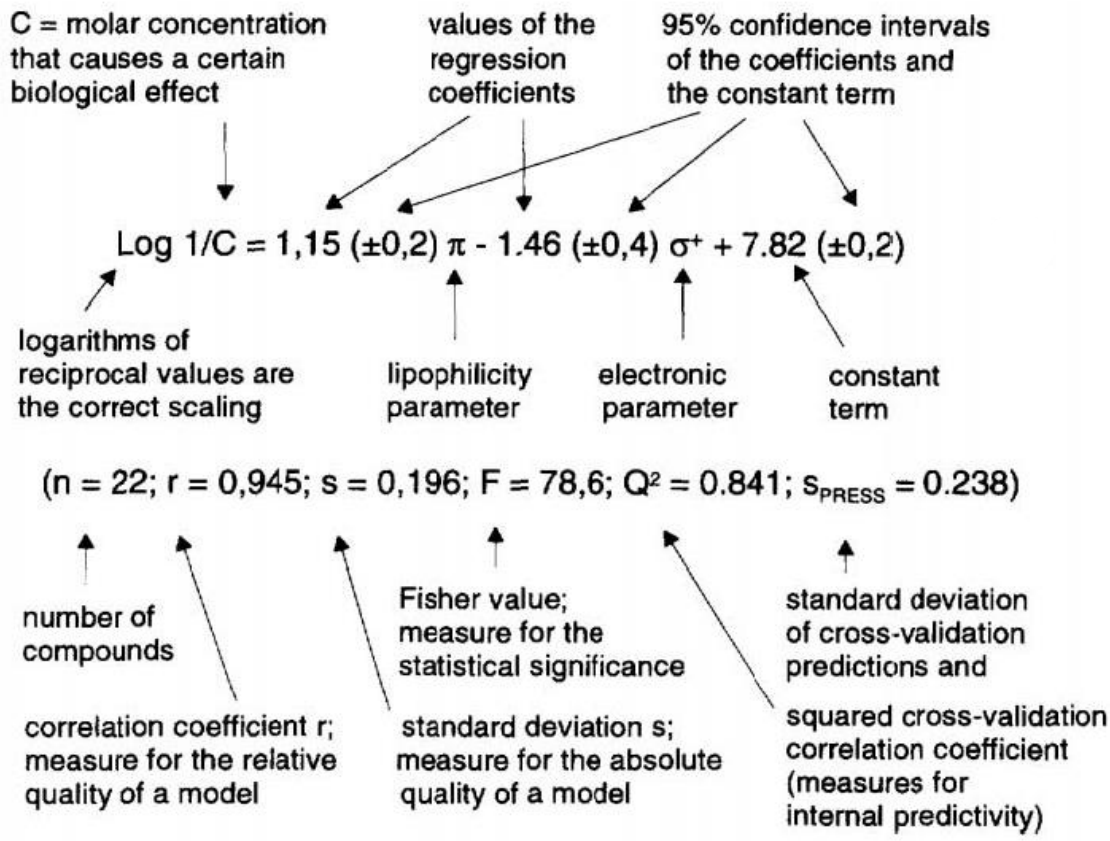


Figure I-8 :QSAR Equation [27]

I-3-9 Relationship Structure Quantitative Activity:

QSAR equations take the following general form: Biological activity = function{parameter(s)}

In the equation, biological activity is normally expressed as log[1/(symbol concentration)], where C is generally the minimum concentration required to cause a defined biological response [29].

The mathematical relationship between a parameter or several physico-chemical parameters and the biological activity of a compound can be expressed through the Hansch equation:

$$Log\frac{1}{C} = -K1(logP)2 + K2logP + K3 + K4Es + K5$$

This equation is obtained by regression analysis. The value of the coefficient obtained after this analysis gives information on the concordance of parameters that are used to form the Hansch equation. If the result obtained for r is! 0,9, this means that the parameters used are valid and that the equation can be used for the rest of the compounds in a 'training set' (or set of molecules).

The Hansch equation is used to predict the biological activity of compounds whose structure is similar to those used to form this equation. However, in the case where the expected value is different from that determined by the experiment, this may mean that the actual biological activity of the compounds is not taken into account by the parameters involved in the equation construction process. The Hansch equation can also be used to show the degree of importance of different parameters on biological activity [30].In the case of drug design, it may be desirable to use parabolic functions in place of linear functions. The descriptor for an ideal drug candidate often has an optimum value. Drug activity will decrease when the value is either larger or smaller than optimum. This functional form is described by a parabola, not a linear relationship. The advantage of using QSAR over other modeling techniques is that it takes into account the full complexity of the biological system without requiring any information about the binding site. The disadvantage is that the method will not distinguish between the contribution of binding and transport properties in determining drug activity. QSAR is very useful for determining general criteria for activity, but it does not readily yield detailed structural predictions [30].

I-3-10Methods for Computing Properties:

The reliability and accuracy of property results vary greatly. There is no generalization that says any given method will compute every property best. However, there are some generalizations to be made. One of these generalizations is that a given type of algorithm will tend to have certain strengths and weaknesses in spite of the type of property being computed. Below are the most common techniques [31].

I-3-10-1From the Energy:

Some of the most important information about chemistry is the energy or relative energetics associated with various species or processes. A few of these are mentioned specifically in this chapter. The accuracy of computed energies is mentioned many other places in this book. Energy is an integral part of most computational techniques. However, some energies are easier to compute than others [31].

I-3-10- 2 From Molecular Geometry:

Some properties, such as the molecular size, can be computed directly from the molecular geometry. This is particularly important, because these properties are accessible from molecular mechanics calculations. Many descriptors for quantitative structure activity or property relationship calculations can be computed from the geometry only [31].

I-3-10-3 QSAR or QSPR:

Quantitative structure property relationships (QSPR) and, when applied to biological activity, quantitative structure activity relationships (QSAR) are methods for determining properties due to very sophisticated mechanisms purely by a curve fit of that property to aspects of the molecular structure. This allows a property to be predicted independent of having a complete knowledge of its origin. For example, drug activity can be predicted without knowing the nature of the binding site for that drug [32].

I-3-10-4 Biological activity:

There is great commercial interest in predicting the activity of a compound in a biological system. This includes both desired properties, such as drug activity, and undesired properties, such as toxicity. Such a prediction poses some very problems due to the complexity of biological systems. No method in existence is capable of automatically computing all the interactions between a given molecule and every molecule found in a single cell, let alone an entire organism. Such an attempt is completely beyond the capabilities of any computer hardware available today by many orders of magnitude. Molecular simulation techniques can be used to predict how a compound will interact with a particular active site of a biological molecule. This is still not trivial because the molecular orientation must be considered along with whether the active site shifts geometry as it approaches.

One very popular technique is to use QSAR. It is, in essence, a curve-fitting technique for creating an equation that predicts biological activity from the properties of the individual molecule only. Once this equation has been created using many compounds of known activity, it can be used to predict the activity of new compounds. Another technique is to use pattern recognition routines.

Whereas QSAR relates activity to properties such as the dipole moment, pattern recognition examines only the molecular structure. It thus attempts to find correlations between the functional groups and combinations of functional groups and the biological activity. Expert systems have also been devised for predicting biological activity [32].

I-3-10-5 Boiling Point and Melting Point:

Several methods have been successfully used to predict the normal boiling point of liquids. Group additivity methods give an approximate estimate. Some group additivity methods gain accuracy at the expense of being applicable to a narrow range of chemical systems. Techniques that use a database to parameterize a group additivity method are significantly more accurate.

QSPR methods have yielded the most accurate results. Most often, they uselarge expansions of parameters obtainable from semiempirical calculations along with other less computationally intensive properties. This is often the method of choice for small molecules. Molecular dynamics and Monte Carlo simulations can be used, but these methods involve very complex calculations. They are generally only done when more information than just the boiling point is desired and they are not calculations for a novice. Melting points are much moreto predict accurately. This is because of their dependence on crystal structure. Seemingly similar compounds can have significantly dicerent melting points due to one geometry being able to pack into a crystal with stronger intermolecular interactions. Some group additivity methods have been designed to give a rough estimate of the melting point[32].

I-3-10-6 Surface Tension:

Surface tension is usually predicted using group additivity methods for neat liquids. It is much more cult to predict the surface tension of a mixture, especially when surfactants are involved. Very large molecular dynamics or Monte Carlo simulations can also be used. Often, it is easier to measure surface tension in the laboratory than to compute it [33].

I-3-10-7 Vapor Pressure:

Dicerent compounds can display a very large dicerence in vapor pressure, depending on what type of intermolecular forces is present. Because of this, dicerent prediction schemes are used, depending on whether the molecule is nonpolar, polar, or hydrogen-bonding. These methods are usually derived from thermodynamics with an empirical correction factor incorporated. The correction factors usually depend on the type of compound, that is, alcohol, keytone, and, and so forth. These methods are applicable to a wide range of temperatures so long as they are not too close to the temperature at which a phase change occurs. Constants for Henry's law are computed from vapor pressure, log P, and group additivity methods [33].

I-3-10-8 Solubility:

A significant amount of research has focused on deriving methods for predicting log P, where P is the octanol–water partition . Other solubility and adsorption properties are generally computed from the log P value. There are some group additivity methods for predicting log P, some of which have extremely complex rules. QSPR techniques are reliably applicable to the widest range of compounds. Neural network based methods are very accurate so long as the unknown can be considered an interpolation between compounds in the training set. Database techniques are very accurate for organic compounds. The solvation methods discussed in chapter 24 can also be used [34]

.I-3-10-9 Diffusivity:

The rate of chemical dicusion in a non-flowing medium can be predicted. This is usually done with an equation, derived from the dicsion equation, that incorporates an empirical correction parameter. These correction factors are often based on molar volume. Molecular dynamics simulations can also be used. Dicusion in flowing fluids can be orders of magnitude faster than in non-flowing fluids. This is generally estimated from continuum fluid dynamics simulations [35].

I-3-10-10 Visualization:

Data visualization is the process of displaying information in any sort of pictorial or graphic representation. A number of computer programs are available to apply a colorization scheme to data or to work with three-dimensional representations. In recent years, this functionality has been incorporated in many [36].

References

[1] Trease, G.E. and W.C Evans, Pharmacognosy , English Language Book Society, Bailliere Tindall, Britain, 1989, 13th edn, 378-480.

[2] OULD ELHADJ M. DIDI, HADJ-MAHAMMED. M., ZABEIROU. H., *Courrier du Savoir* , 2003, 03, 47-51

[3] Cheesbrough M., Tropical Health Technology. Cambridge University Press, U.K. 1984

[4] Harbone. J. B., Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapman and Hall, London, 1984, 2nd edn, 1-168

[5] El Ghazali, G.E.B., *Pollen morphological studies in Amaranthaceae s.lat. (incl. Chenopodiaceae) and their taxonomic significance: A review.* Grana, 2022. 61(1): p. 1-7.

[6] Nasar-Abbas S. M., Kadir Halkman A. K., *International Journal of Food Microbiology*, 2004, 97, 63-69.

[7] National Committee for Clinical Laboratory Standards (NCCLS), *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, NCCLS, Pennsylvania, USA, 2000, N7-A5.

[8] Stern J. L., Hagerman A. E., Steinberg P. D. and Mason P. K., *J. Chem. Ecol*., 1996, 22, 1887-1899.

[9] Labed, B., et al., *The antibacterial activity of water extracts of Traganum nudatum Del (Chenopodiaceae) growing in Algeria.* Der Pharmacia Lettre, 2010. 2(6): p. 142-145.

[10] Telli A, Esnault MA, El Hadj Ould, Khelil A, *An ethnopharmacological survey of plants use in traditional diabetes treatment in south-eastern Algeria,* 2016, Ouargla province. J Arid Environ 127:82–92

[11] Telli A, Esnault MA, El Hadj Ould, Khelil A, *An ethnopharmacological survey of plants use in traditional diabetes treatment in south-eastern Algeria,* 2016, Ouargla province. J Arid Environ

[12] Traganum nudatum – synthese – eFlore – Tela Botanica (tela-botanica.org)

[13] ALLAOUI Messaouda. *Doctoral dissertation*. University kasdi Merbah Ouargla (*2015).*

[14] Ben Menni, D., et al., *Identification of Sterols from Anabasis articulata (Forssk.) Moq.(Chenopodiaceae) Growing in Algeria and Study of Their Potential Bioactivity.* Waste and Biomass Valorization, 2022: p. 1-13.

[15] El Bassossy, T., *Chemical Constituents and Biological Efficacy Evaluation of Traganum Nudatum Aerial Parts.* Egyptian Journal of Chemistry, 2021. 65: p. 521-530.

[16] Senhaji, S., F. Lamchouri, and H. Toufik, ***Phytochemical Content, Antibacterial and Antioxidant Potential of Endemic Plant Anabasis aretioïdes Coss. & Moq. (Chenopodiaceae).*** **BioMed Research International, 2020. 2020: p. 6152932.**

[17] Benhassine, I., et al., ***Anti-inflammatory, analgesic activities and phytochemical study of Traganum nudatum Delile.*** **Iranian Journal of Pharmaceutical Sciences, 2021. 17(3): p. 1-22.**

[18] Senhaji, S., F. Lamchouri, and H. Toufik, ***Phytochemical content, antibacterial and antioxidant potential of endemic plant anabasis aretioïdes coss. & moq.(Chenopodiaceae).*** **BioMed Research International, 2020. 2020.**

[19] O Barnawi, I., ***Traganum nudatum Possesses Anticancer Efficacy against Breast Cancer MDA-MB-231 Cells.*** **2021.**

[20] Messaouda, A., et al., ***COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITY AND PHENOLS AND FLAVONOIDS CONTENTS OF THE ETHYL ACETATE EXTRACTS FROM TWO SAHARAN CHENOPODACEA.*** **Algerian Journal of Arid Environment "AJAE", 2016. 6(1).**

[21] Hugo Kubinyi., QSAR Parameters, Germany. http://www.kubinyi.de/

[22] S Chtita, Modeling biologically heterocyclic organic molecules active by QSAR/QSPR methods. Search for new drugs, 2017

[23] Guillaume F, Development of QSPR models for the prediction of benefits explosibility of nitroaromatic compounds, 2010

[24] David C. Young, ***COMPUTATIONAL CHEMISTRYA Practical Guide for Applying Techniques to Real-World Problems2001, New York.*** **A JOHN WILEY & SONS, INC., PUBLICATION**

[25] H. Waterbeemd, S. Rose, "Quantitative approaches to structure-activity relationships", in ***Book*** **"Quantitative approaches to structure-activity relationships". 2003, Elsevier. 351-367.**

[26] C. D. Selassie, "History of quantitative Structure-Activity relationships", in ***Book*** **"History of quantitative Structure-Activity relationships", D. J. Abraham, Editor. 2003, John Wiley & Sons, Inc.: California.**

[27] H. Gonzalez-Diaz, E. Olazabal, L. Santana, E. Uriarte, Y. Gonzalez-Diaz, N. Castanedo, "QSAR study of anticoccidial activity for diverse chemical compounds: Prediction and experimental assay of trans-2-(2-nitrovinyl)furan." ***Bioorg. Med. Chem.,*** **2007. 15: 962-968..**

[28] G. Thomas, "Fundamentales of Medicinal Chemistry". "The SAR and QSAR approaches to drug design". 2003: John Wiley & Sons, Ltd.

[29] H-J. Bohm, G. Schneider, "Virtual Screening for Bioactive Molecules." 2000, Germany: Wiley-VCH.

[30] O Barnawi, I., *Traganum nudatum Possesses Anticancer Efficacy against Breast Cancer MDA-MB-231 Cells.* 2021.

[31] Ahmed, F.A., et al., *Phytochemical Investigation, HPLC Analysis and Antimicrobial Activity of Some Plants from Chenopodiaceae Family.* Egyptian Academic Journal of Biological Sciences, H. Botany, 2022. 13(1): p. 13-24.

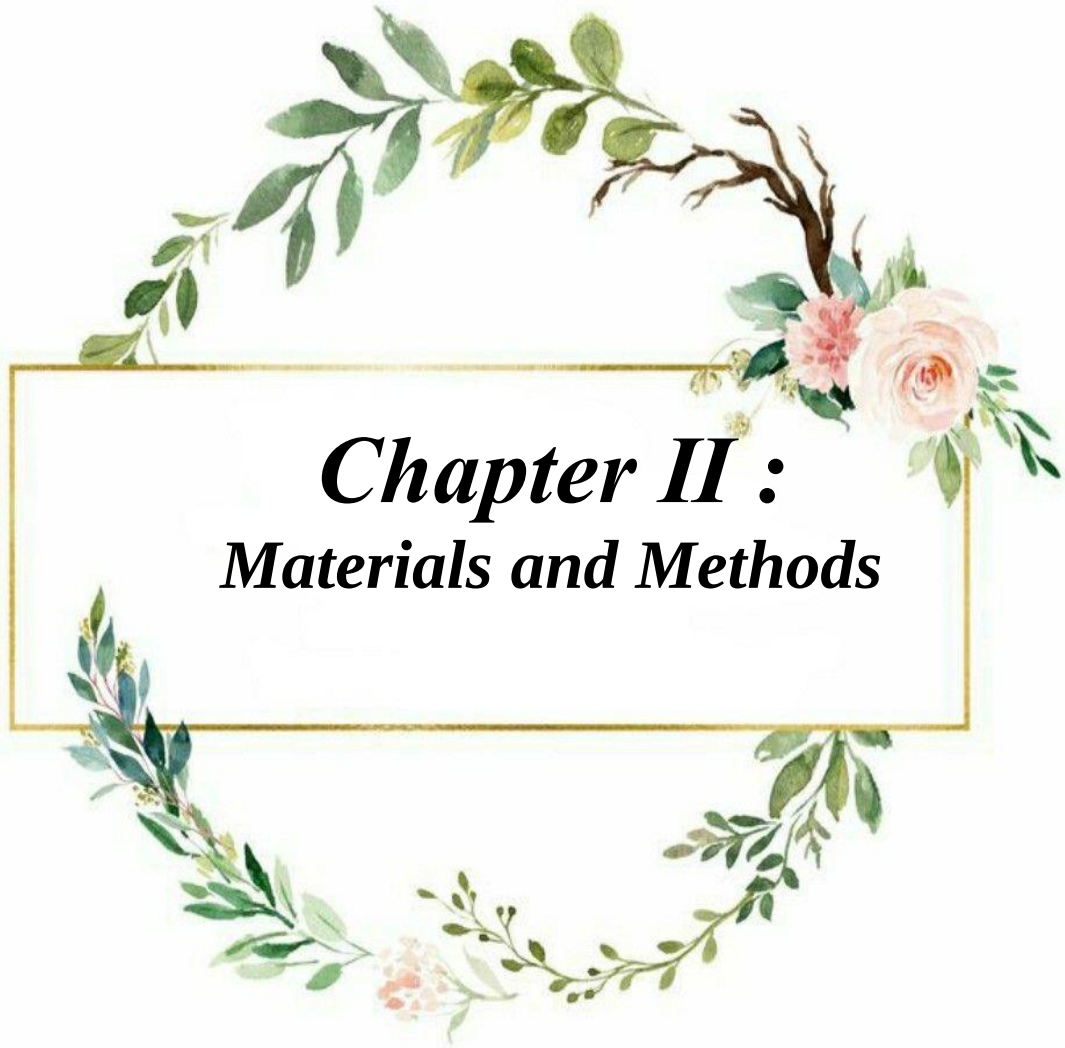
[32] Allaoui, M., et al., *Comparative study of the antioxidant activity and phenols and flavonoids contents of the ethyl acetate extracts from two Saharan chenopodacea: Haloxylon scoparium and Traganum nudatum.*Algerian Journal of Arid And Environment, 2016: p. 71-79.

[33] *Etude des relations Quantitatives Structure-Activité (QSAR)de plusieurs familles de composés sur des données biologiques (1).pdf* .

[34] Chen, B., et al., *Development of quantitative structure activity relationship (QSAR) model for disinfection byproduct (DBP) research: A review of methods and resources.* Journal of hazardous materials, 2015. 299: p. 260-279.

[35] Bouarab-Chibane, L., et al., *Antibacterial properties of polyphenols: characterization and QSAR (Quantitative structure–activity relationship) models.* Frontiers in microbiology, 2019. 10: p. 829.

[36] Muratov, E.N., et al., *QSAR without borders.* Chemical Society Reviews, 2020. 49(11): p. 3525-3564.



Chapter II :

Materials and Methods

II-1 Introduction:

Plants are able to produce different compounds that be used to protect themselves against different types of pathogens [1]. Medicinal plants are important elements of indigenous medical systems in Algeria as well as in other developing countries.

Medicinal plants constitute an unfailing source of compounds (natural products –NPs) utilised in medicine for the prevention and treatment of various deceases. The introduction of new technologies and methods in the field of natural products chemistry enabled the development of high throughput methodologies for the chemical composition determination of plant extracts, evaluation of their properties and the exploration of their potentials.

II-2 Plant material:

Traganum nudatum Del (Chenopodiaceae) is a native halophytic shrub in arid zones of the Mediterranean basin, To our knowledge, there are no published studies focusing on the chemical composition of *T. nudatum*, despite its forage value and importance in North African steppes. Therefore, the purpose of the present study was to characterization and identification chemical element contents in *T. nudatum*[2, 3].

II-3 Phytochemical examination of a plant [4]:

II-3-1 Reaping and drying the plant: The dehydration plant was harvested from the ***Touggourt*** area, located 160Km north of Ouargla, at the time of its flowering in the month of April. After the genie process comes the process of purification and drying which takes place through the following stages:

- purification of the plant from impurities and dead organs.
- Fragmentation of each bush to small parts to facilitate the drying process .
- Brush the collected parts over a clean lid leaving distances for ventilation between the procedures. The drying process is done in the shade with stirring from time to time for ten days.
- The investigation process in the oven is completed for a week.

II-3-2 Grinding and storing plant [4]:

After full drying of the plant crushes its different parts by crushing machine.

- The leaves and their carriers are crushed in a sieve with 2mm pores.

The branches are crushed in a sieve with membranes 1mm.

- Flowers and fruits, owing to their scarcity, are smashed with mortars.

After the crushing process, dry powder is placed inside floating glass receptacles. Sealed and stored pending use.

II-4 Extraction [4]:

II-4-1 Extraction by ethanol and water (EtOH/H2O):

2Kg of vegetable powder soaked in 70% concentrated ethanol for 24 then, Nominates and repeats the process three times; Organic phases combine and concentrate under pressure and then, Dilute with distilled water and leave a full night and then leach, the resulting leach extracts Solvents

The following membership:

1. Petroleum ether ml 400 three times.
2. Methane dichloride ml 400 three times.
3. Ethyl acetate ml 500 once.
4. n-butanol ml 500 three times.

The resulting organic phases every time they dry, leach and then evaporate under pressure and melt the deposit into methanol.

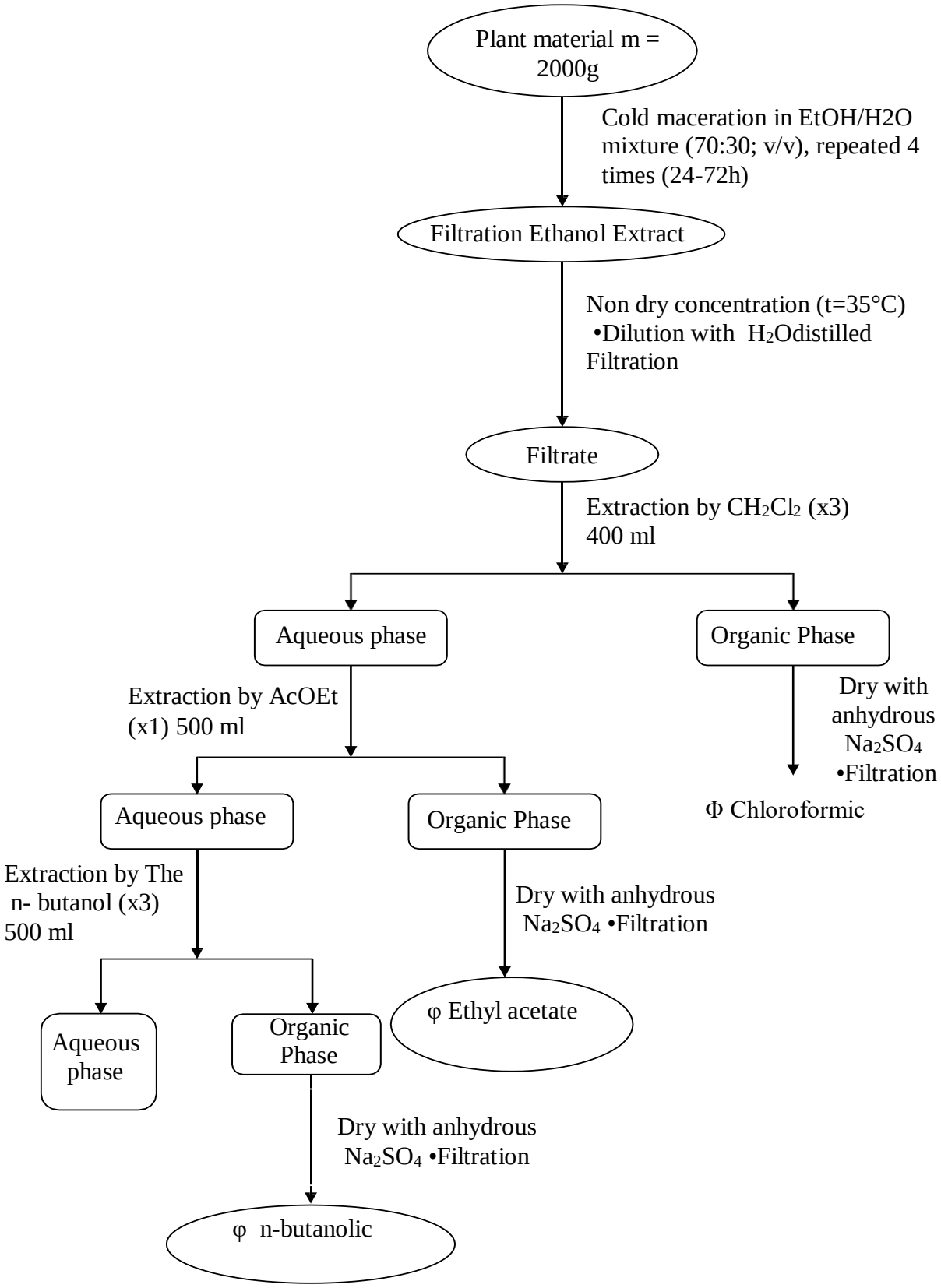


Figure II-1: Extraction Protocol of *T. nudatum*[4]

II-5 Physicochemical methods:

II-5-1 Mass spectrometry:

Also called mass spectroscopy, analytic technique by which chemical substances are identified by the sorting of gaseous ions in electric and magnetic fields according to their mass-to- charge ratios. Method : ESI positive (+) and negative (-) / Operator : FTMS User / Instrument : apex –IV [4]**.**

II-5-2 Magnetic resonance spectrometry:

NMR: Each purified compound was analyzed in NMR in order to establish its structure. For this, we used :The NMR (^{1}H and ^{13}C) spectra were carried out at the University of Jordan, Department of Science and Chemistry Instrument Model : Bruker 500 MHz-Avance III. Solvent DMSO [4].

MHz for the ^{13}C Brüker 500 MHz-Avance III. Solvent DMSO NMR equipped with a cry probe. The data are processed and exploited by Top Spin 2.1 or 3.2 software.

The samples are prepared in analytical tubes of 5 mm diameter in Deuterated solvents DMSO-d6 depending on the solubility of the analyzed compound [4].

Chemical δ displacements are given in ppm with respect to Tetra Methyl Silane(TMS) and coupling constants J in Hz. The standard pulse sequence programs provided by Brüker made it possible to carry out, in addition to the ^{1}H and ^{13}C mono-dimensional experiments, different2D experiences:

Homo-nuclear correlations: COSY (Spectroscopy correlation) allows to observe the scalar couplings2J/3J between geminate and vicious protons

the observation of correlations between nearby protons in the same space if they are not directly related.

Heteronuclear correlations:

- **HMQC** (Heteronuclear Multiple Quantum Coherence) allows you to evidence of 1JH-C couplings and connect each carbon to the protons it carries
- **HMBC** (Hetero-nuclear Multiple Bond Spectroscopy) highlights long-range proton-carbon couplings in 2J and 3J.

II-6Flavonoidsconjugates:

Flavonoids and their conjugates form a very large group of natural products. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs. The chemical structures of this class of compounds are based on a C6-C3-C6 skeleton. They differ in the saturation of the heteroatomic ring C, in the placement of the aromatic ring B at the positions C-2 or C-3 of ring C, and in the overall hydroxylation patterns (Figure 2.1). The flavonoids may be modified by hydroxylation, methoxylation, or *O*-glycosylation of hydroxyl groups as well as *C*-glycosylation directly to carbon atom of the flavonoid skeleton. In addition, alkyl groups (often prenyls) may be covalently attached to the flavonoid moieties, and sometimes additional rings are condensed to the basic skeleton of the flavonoid core. The last modification takes place most often in the case of isoflavonoids, where the B ring is condensed to the C-3 carbon atom of the skeleton. Flavonoid glycosides are frequently acylated with aliphatic or aromatic acid molecules [5].

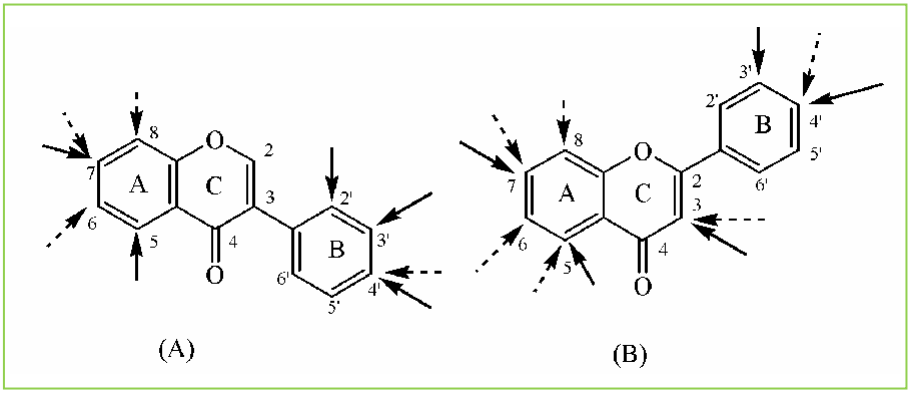


Figure II-2: Flavonoid structures, ring labeling, and carbon atom numbering. (A) Isoflavones. (B) Flavones and flavonols. Full arrows indicate most frequent hydroxylation sites and dashed arrows indicate most frequent C- and/or O- glycosylation sites.

II-7 Structural Characterization and Identification of Flavonoid and their Conjugates :

All physicochemical methods applied in the field of organic chemistry are useful for structural characterization or identification of individual flavonoids and their conjugates. The separation approaches mentioned above may be considered in different ways. The first one is directed toward the analysis of single compounds obtained after exhaustive isolation and purification procedures. The method of choice in this approach is NMR of ^{1}H hydrogen and/or ^{13}C carbon isotopes, dependent on the intensity of the interactions between different atoms within a molecule placed in a high-intensity magnetic field. Different NMR experiments have been developed to achieve information concerning chemical structure of the studied molecule on this basis [6].

Particularly useful are methods enabling recording of two-dimensional spectra showing homo-nuclear interactions [**co**rrelation spectrosco**py** (COSY) and **n**uclear **o**verha user **e**ffect **s**pectrosco**y** (NOESY)] as well as hetero-nuclear [**h**etero-nuclear **s**ingle **q**uantum **c**orrelation (HSQC) and **h**etero-nuclear **m**ultiple **b**ond **c**orrelation (HMBC)] to facilitate the acquisition of all the structural information about an aglycone and the corresponding sugar substitution. In the case of diglycosides, information on the placement of the inter-glycosidic bonds and the possible acyl group substitutions on the sugar rings, and the position of anomeric proton(s) also can be obtained. The limitation of NMR methods is the lower sensitivity in comparison with other instrumental methods. For obtaining good quality spectra containing all the necessary structural information, relatively high amounts of purified compound (more than 1 mg) are necessary, especially when magnets of medium frequency (500 MHz) are used in the NMR spectrometer. The application of ultraviolet and infrared spectrophotometers may give valuable information about specific compounds [6].

Analysis of natural products is possible with different types of MS available on the market. The instruments are equipped with various sample introduction systems and ionization methods, as well as diverse physical phenomena are used for separation of the created ions in MS analyzers. Positive and negative ions are analyzed in MS; the choice of the ionization mode (negative or positive) is sometimes a very important feature. The ionization methods may be divided into two groups differing with respect to the amount of energy transfer red to the molecule during the ionization process. Electron ionization (EI) belongs to the first group. The transfer of energy occurs during the interaction of electrons with the molecule in the vapor state; it may cause the cleavage of chemical bonds and fragmentation of the molecule, which is characteristic for the analyzed compound. Other ionization

methods deliver lower energy to the studied molecules during the protonation (positive ion mode) or deprotonation (negative ion mode) processes [7].

II-7-1 UV absorbance detection :

The positions, types and number of substituents in the conjugated systems could be speculated via means of UV spectrum. Most of the flavonoids in methanol possess two main absorption bands. Band I is at 300–400 nm, which is caused by electron transition of cinnamoyl group. Band II is at 240–280 nm, which is caused by electron transition of benzoyl group, as shown in Figure 2. The structure types and oxygen-bearing substituent types of flavonoids could be determined by the peak locations, shapes and strengths of band I and II, as shown in(TableII-1) [8].

The locations and shapes of Band I and II will be affected by the substituents attached to rings A and B. Normally, red shift of band I increases accordingly when the number of hydroxyl groups located at ring B increases. Similarly, red shift of band II increases accordingly when the number of hydroxyl groups located at ring A increases, but it has trifling impact to band I, with the exception of 5-OH. The corresponding bands will be violet shifted 5–15 nm if the particular hydroxyl is glycosided. Furthermore, the influence of the hydroxyl groups will almost disappear if they are acetylated [8].

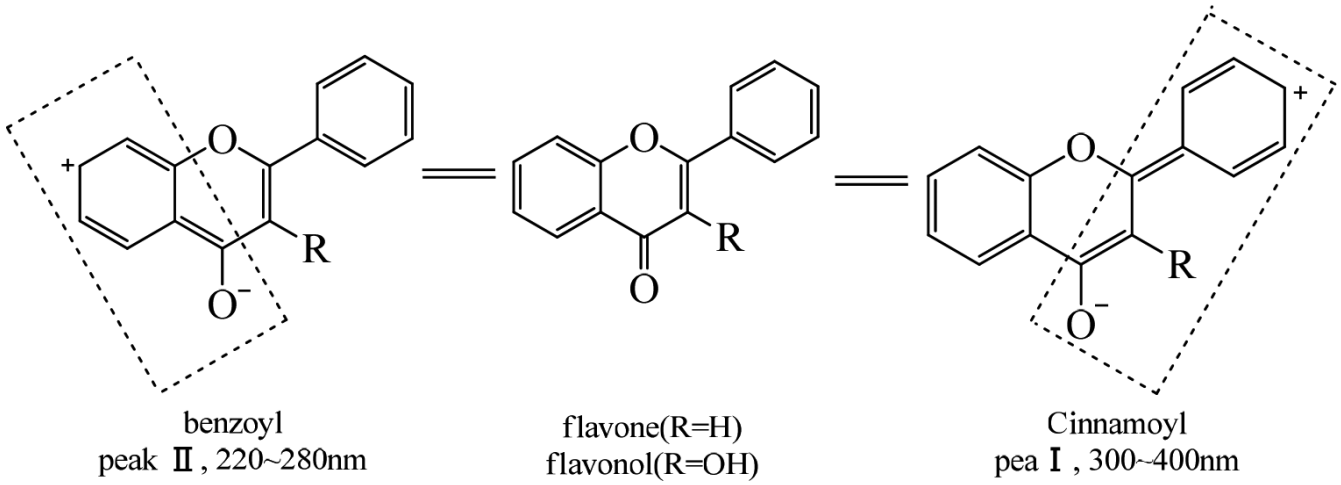


Figure II-3:UV spectrum of flavonoids

Table II-1:The spectral characteristics of UV-VIS spectrum of flavonoids [8].

Structure type	Band II (nm)	Band I (nm)
Flavone	250–280	304–350
Flavonol (3-OH is substituted)	250–280	328–357
Flavonol (3-OH is free)	250–280	358–385
Isoflavone	245–270	310–330 (shoulder peak)
Flavanone and flavanonol	270–295	300–330 (shoulder peak)
Chalcone	220–270 (weak peak)	340–390
Aurone	230–270 (weak peak)	370–430
Anthocyanidin	270–280	465–560

II-7-2 Mass Spectrometry :

MS is a very sensitive analytical method used to identify flavonoid conjugates or to perform partial structural characterization using microgram amounts of sample (Cuyckens and Claeys, 2004). Indeed, significant structural data can be obtained from less than 1 mg of the analyzed compound when different MS techniques are used in combination with chemical derivatization of the characterized compounds. A strategy for the combined application of different MS techniques and chemical derivatization are presented in Figure 2.2. Flavonoid glycosides are thermally labile compounds and the evaporation without decomposition of the analyte is impossible, even in the ion source of a MS, where high vacuum exists (about 3 × 10-5 torr). In this situation, soft ionization methods need to be applied for the analysis of this group of compounds, and the analyte molecules are ionized without evaporation in high vacuum (FAB or LSIMS, MALDI) or under atmospheric pressure (ESI, APCI). From normal mass spectra, information can be obtained about the molecular weight of the whole conjugate, the size of the sugar moieties attached to the aglycone, and the molecular weight of the aglycone [9]

In flavonoid analysis, MS is the state-of-the-art detection technique in LC.

Classical mass spectrometric gas-phase ionisation techniques such as electron ionisation (EI) and chemical ionisation (CI) are generally less suitable for polar, non-volatile compounds such as the flavonoids (Schuster, 1980).Atmospheric pressure ionisation interfaces (APCI) and electrospray ionisation (ESI)are used almost exclusively today. Both positive and negative ionisation are applied. ESI is more frequently used in flavonoid analysis, but APCI is gaining popularity and in some cases better responses are obtained in that mode [10].

II-7-3 Nuclear Magnetic Resonance:

NMR is a well-established and the most commonly used method for natural product structure analysis. The studies of flavonoid structures using ^{1}H-NMR were initiated in 1960s (Markham and Mabry, 1975) and along with ^{13}C-NMR have became the method of choice for the structure elucidation of these compounds. The chemical shifts and multiplicity of signals corresponding to particular atoms and their coupling with other atoms within the molecule allow for easy identification of the aglycone structure, the pattern of glycosylation, and the identity of the sugar moieties present. The literature of this topic is abundant and rapidly growing [11].

- ***Solvent Selection***

The first task when running any liquid-phase NMR experiment is the selection of a suitable solvent. Obvious though this sounds, there are a number of factors worth careful consideration before committing precious sample to solvent.

Your primary concern when selecting a solvent should be the *complete* dissolution of your sample. In general, we advise adhering to the simple old rule that 'like dissolves like'. In other words, if your sample is nonpolar, then choose a nonpolar solvent and vice versa., the vast majority of compounds can be dealt with using one of four basic solvents Nuclear magnetic resonance spectrum (NMR) is the most powerful method to elucidate the structures of flavonoids. Kinds of solvents, such as CDCl3, DMSO-d6, C5D5N, (CD3)2CO andCD3OD, could be employed while performing NMR experiments. DMSO-d6 is the optional solvent among them to perform NMR to flavonoids. Almost all kinds of flavonoids could be well dissolved in DMSO-d6, and the resonance signals of flavonoids are rarely overlapped by solvent peaks (about δ2.5). Furthermore, NMR signals of phenolic hydroxyl groups could be displayed clearly with DMSO-d6 as the solvent. The drawback of this solvent is high boiling point, which leads to difficulty in sample recovery [12].

- ***DEPT***

The first of these tools is the distortion less enhancement by *p*olarization *t*ransfer (DEPT) pulse sequence. There are a number of versions of this experiment which can be very useful for distinguishing the different types of carbons within a molecule. Of these, we have found the DEPT 135 sequence to be the most useful. In this experiment, the quaternary carbons are edited out of the spectrum altogether [13].

Methyl and methine protons naturally phase at 180◦ relative to the methylene carbons and the spectra are usually plotted with methyls and methines positive. (Note that should you encounter a signal that you cannot confidently assign to either a methyl or methine carbon, the DEPT 90 sequence may well be of use as it differentiates these carbons – methines appear positive and methyls are edited out of the spectrum but this technique can be considered obsolete if you have access to any of the 2-D proton–carbon correlated experiments discussed in Section 9.2.) [14].

This is demonstrated once more with our familiar morpholine compound in Spectrum 9.2. The DEPT sequences are of course, still relatively insensitive, though they are probably a little more sensitive than the standard 1-D, fully decoupled 13C spectrum. We find it convenient, particularly with complex molecules, to combine the 1-D ^{13}C spectrum with the DEPT-135 spectrum, which is plotted above it at the same expansion, of course! This enables you to differentiate the different types of carbon in your spectrum at a glance [14].

II-7-3-A Proton – Proton (Single Bond) Correlated Spectroscopy(2-D):

Two-dimensional $^{1}H–^{1}H$ COSY (correlation spectroscopy) experiments allow determination of the protons that are spin–spin coupled, and the spectrum shows couplings between neighboring protons (2JHH, 3JHH, and 4JHH) revealed as cross peaks in the spectrum. The $^{1}H–^{1}H$ DQF-COSY experiment is a modification of the standard 1H–1H COSY experiment. The main advantage of the DQF technique is that noncoupled proton signals are eliminated. The DQF technique eliminates the strong solvent signal and the often very strong H2O signal associated therewith, which may overlap with flavonoid sugar signals. The DQF-COSY experiment is routinely used in flavonoid analysis to assign all the sugar protons. The use of a ‘‘sequential walk’’ approach may provide information on the relative positions of individual proton signals along a spin system. The 2D homonuclear 1H–1H TOCSY (Total Correlation Spectroscopy) experiment identifies protons belonging to the same spin system. As long as successive protons are coupled with coupling constants larger than 5 Hz, magnetization is transferred successively over up to five or six bonds. The presence of heteroatoms, such as oxygen, usually disrupts TOCSY transfer. Since each sugar ring contains a discrete spin system separated by oxygen, this experiment is especially useful for assignments of overlapped flavonoid sugar protons in the 1D ^{1}H NMR spectrum. It must be understood that the crosspeak intensity is not an indicator of the distance between the protons involved, and that all expected correlations may not appear in a TOCSY spectrum. To avoid this latter problem it may be helpful to record a second spectrum with another mixing time [15].

II-7-3- B Proton–Carbon (Single Bond) Correlated Spectroscopy(2-D):

The most powerful techniques of all are undoubtedly the 2-D proton–carbon experiments (*H*eteronuclear *M*ultiple *Q*uantum *C*oherence/*H*eteronuclear *S*ingle *Q*uantum *C*oherence, or HMQC/HSQC; and *H*eteronuclear *M*ultiple *B*ond *C*orrelation, or HMBC) as they provide an opportunity to dovetail proton and carbon NMR data directly. Taking the HMQC and HSQC first both these techniques establish one-bond correlations between the protons of a molecule and the carbons to which they are attached. Both techniques are considerably more sensitive than a 1-D 13C spectrum, which might seem strange when you consider that the whole 2-D matrix is composed of a considerable number of 13C spectra. The secret of the superior signal/noise performance of these methods lies in the fact that they are both 'indirect detection' techniques. This means that the carbon signals are detected (indirectly) by the transfer of their magnetisation to the much more sensitive protons! A typical data matrix for an HMQC or HSQC might be composed of 256 increments in the carbon domain, each of 2 k points in the proton domain [16].

II-7-3- C Proton–Carbon (Multiple Bond) Correlated Spectroscopy(2-D):

Potentially even more useful, is the HMBC experiment. In this experiment, correlations are obtained between carbon atoms and protons that are separated by two and three bonds. Of course, the actual number of bonds separating the protons from the carbons is something of a red herring. What the spectrometer records are carbon-proton correlations for carbons that have protons couplings of specific magnitude. The sensitivity of the spectrometer to various sizes of proton-carbon coupling is controlled by one of the delays in the HMBC pulse sequence. This delay is selected on the basis of $1/2J$, where J is the coupling you wish to optimise for. A proton–carbon coupling of 10 Hz is a fairly typical value for the experiment, and thus the relevant delay would be set at $1/2 \times 10$, or 0.05 s. This means that the spectrometer sensitivity would be optimised for carbons with proton couplings of around 10 Hz. It does not mean that it will not detect carbons with smaller or larger proton couplings, just that the response shown will not be as intense. In practice, 3-bond proton couplings tend to be nearer to this value than are the 2-bond couplings and for this reason, the HMBC sequence is usually more sensitive to 3-bond than to 2-bond correlations. This has of course to be viewed within the context of the overall signal/noise for the experiment. If the signal/noise for the whole experiment is less than excellent, it is quite possible for some 2-bondcorrelations to slip through the net altogether. If you are wondering why the value of $1/2J$ is not used to even-up the response to 2- and 3-bond correlations, there are two important factors to consider. If this value was optimised for, say, 5 Hz proton couplings, then the spectra we would obtain would be further greatly complicated by 4-bond couplings which would start

to come through, since the *J* values for some 4-bond couplings are comparable with 2-bond values. Furthermore, as the value for *J* gets smaller, so the optimal delay required gets longer so that more and more signal gets lost to relaxation prior to acquisition and overall sensitivity for the experiment is lost. This incidentally partially explains why the technique is not as sensitive as HSQC in the first place (1-bond proton–carbon couplings are typically around 500 Hz, so the delay is extremely short and very little signal is lost) [17].

II-8-1Introduction:

Quantitative structure-activity relationships (QSAR) are used when there is little or no receptor information, but there are measured activities of (many) compounds. It is correlate chemical/biological activities with structural features or atomic, group or molecular (physico-chemical) properties.

The Quantitative Structure-Activity Relationships (QSAR) study has two steps:

a. Drawing of molecules: For drawing of structures of molecules the Chem-Draw software version 18.

b. Optimization: All molecular structure optimization calculations have was performed using the Hyper-Chem 08 software.

II-8-2 Chem-Draw:

Is a complete tool for chemists and biologists, integrating a whole range intelligent tools to facilitate the work of researchers, it is developed in1985 by David A. Evans and Stewart Rubenstein. It is an essential and preferred tool for illustrate chemical and biological concepts and It is imposed as a reference for the drawing of molecular structures. Because it is simple to use, powerful and draw intuitively and efficiently in two and three dimensions. [18]

- **Chem-Draw User Interface:**

By default, the user interface displays the toolbar, main menu and bar of commonly used documents, which make it easier for us to draw our molecules. The (FigureII-4) shows the Chem-Draw user interface.

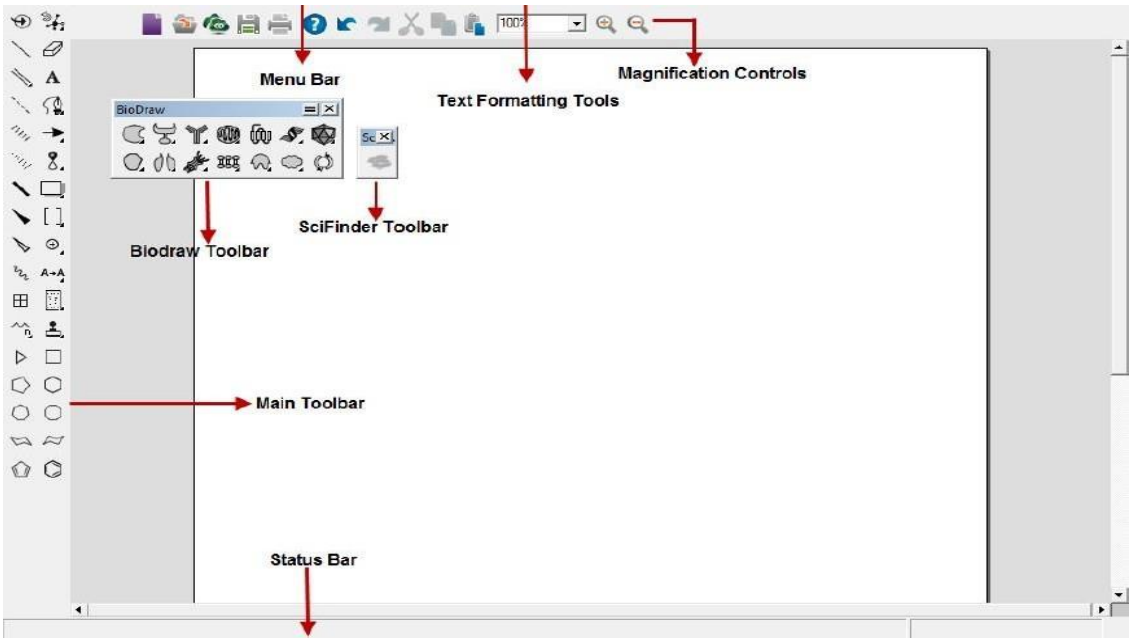


Figure II-4 Chem-Draw interface

- **Toolbars:**

In Chem-Draw, several toolbars are introduced, such as Search, Structure, Curves, etc. We use each in a specific task always for the purpose to draw our molecules in a more practical way. Chem-Draw, also allows us to display or hide a toolbar, depending on our needs by selecting it from the View menu.

- **The main toolbar:**

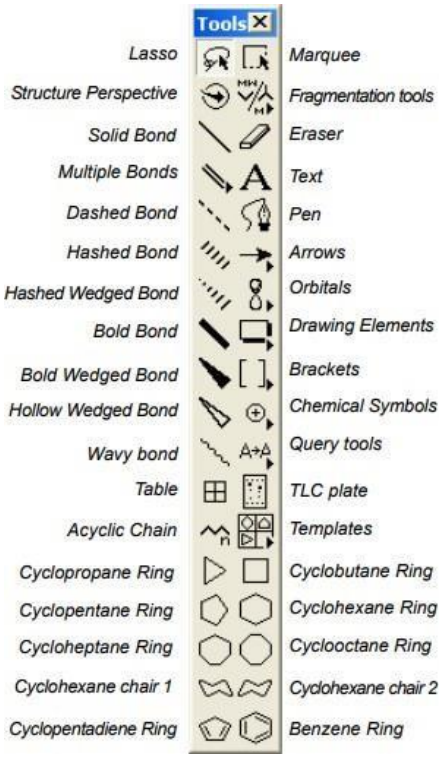


Figure II-5: main toolbar

The main toolbar shown in Figure 6 includes the most commonly used to draw structures. For example: The benzene cycle, instead of making 6 successive connections, among them 3 double connections, it just click on "benzene ring", the same for cyclohexane and cyclo octane…etc.

- **Recording:**

Once the drawing is completed, it will be saved in MOL format, then model the3D structure of the molecule using the Hyper-Chem software.

II-8-3 Hyper-Chem:

Hyper-Chem is a sophisticated molecular modeling environment, recognized for its quality, flexibility and ease of use. Unification of 3D visualization and animation with quantum chemistry, molecular mechanics and dynamics, Hyper-Chem puts more molecular modeling at your fingertips than any other program Windows. It includes all components of structure, thermodynamics, spectra and kinetics. [19]

- **Geometric optimization:**

1- Molecular mechanics MM+

Molecules already drawn using Chem-Draw software have been imported to Hyper-Chem.

First, prepare the molecule by adding the hydrogen atoms. (See Figure II-6)

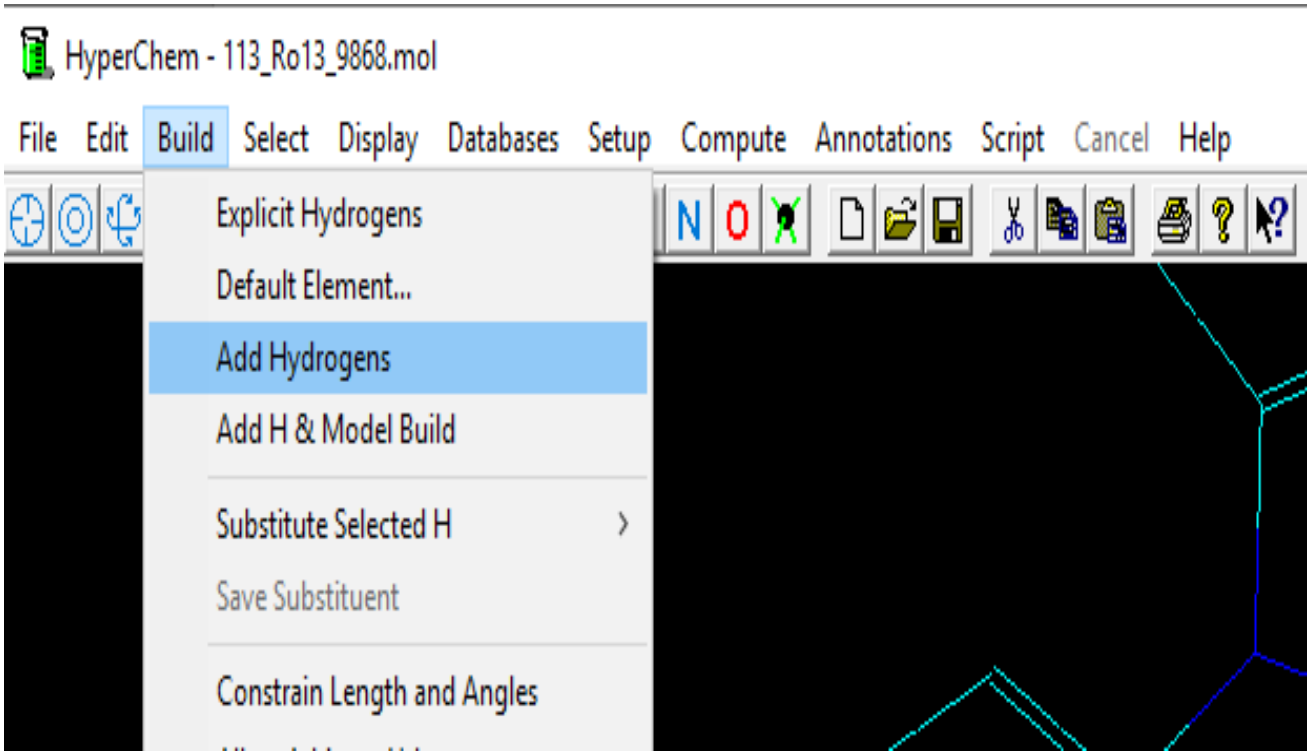


Figure II-6: addition of hydrogen

Now the molecule is ready to be optimized as follows: Selection of the "Setup" menu in the top line of the Hyper-Chem window then in the list, "Molecular Mechanics" is selected as shown in (Figure II-7).

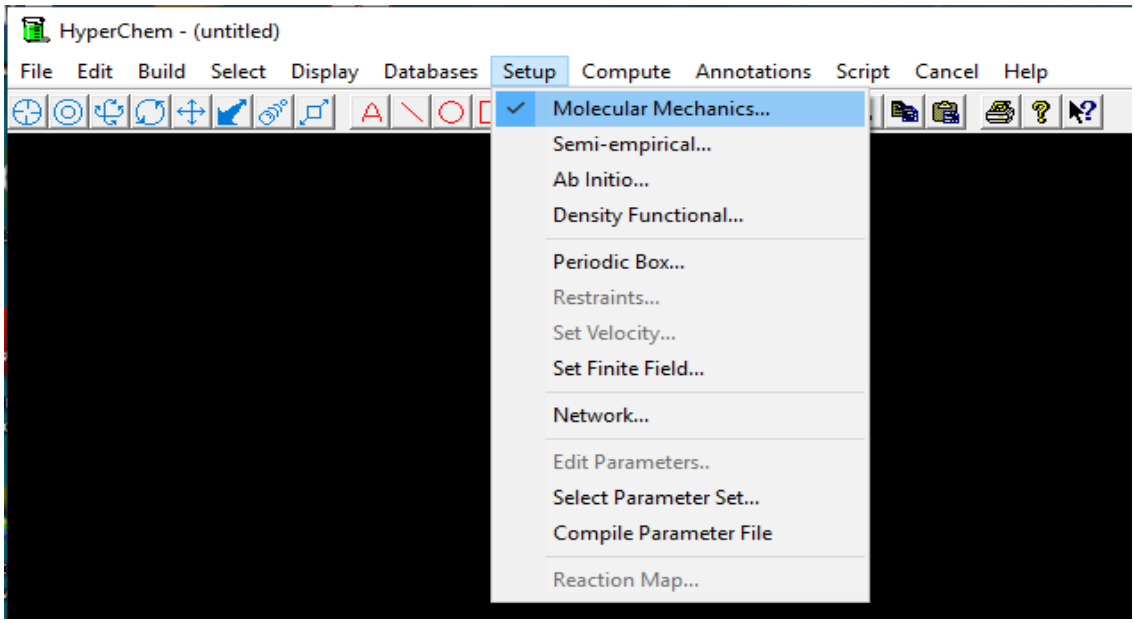


Figure II-7: optimization mode choices

In the "Molecular Mechanics Force Field" window that appears "MM +" is selected (See Figure II-8).

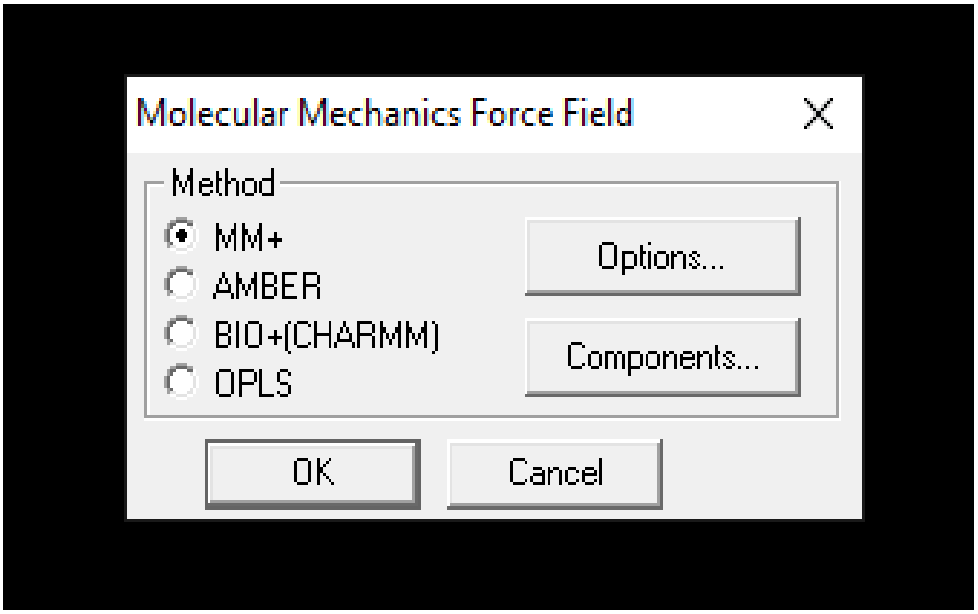


Figure II-8: choice of mm+ mode

The software is now ready to launch the geometry optimization (See Figure II-9).

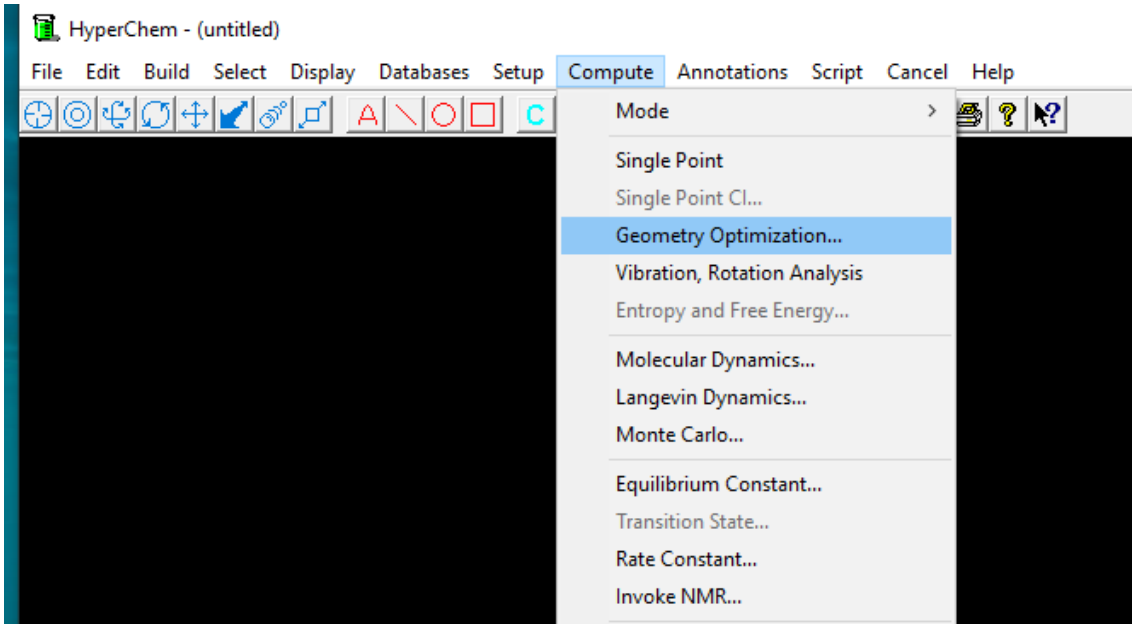


Figure II-9: launch of optimization

2-Semi Empirical PM3

We can now proceed to the second stage of optimization known as 'semiempirical"(See Figure II-10).

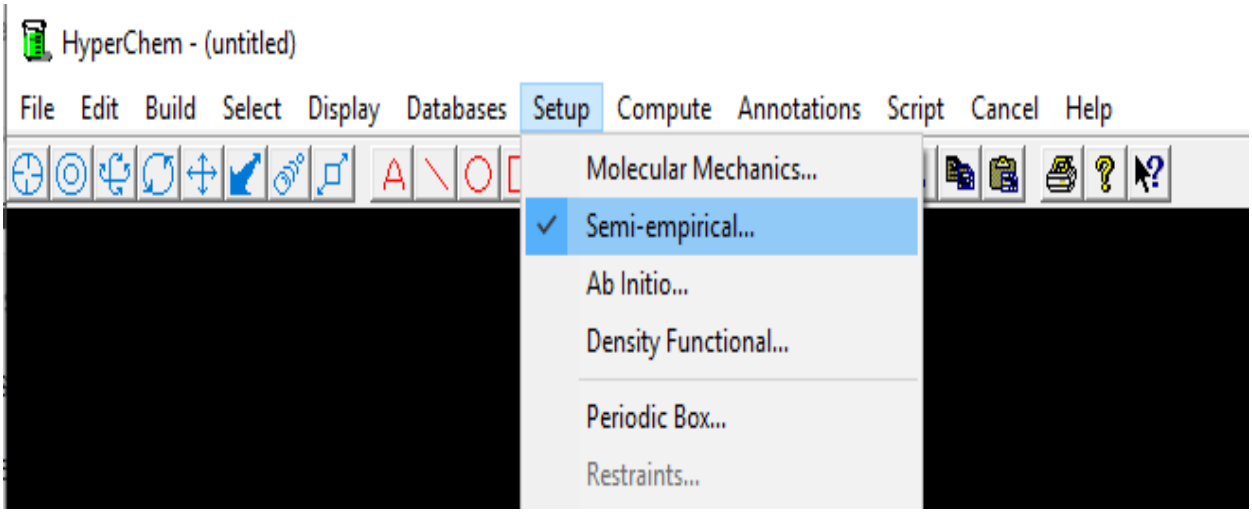


Figure II-10 choice of semi empirical method

A semi-empirical method window appears and you must choose the PM3 parameter as we have shown in (Figure II-11).

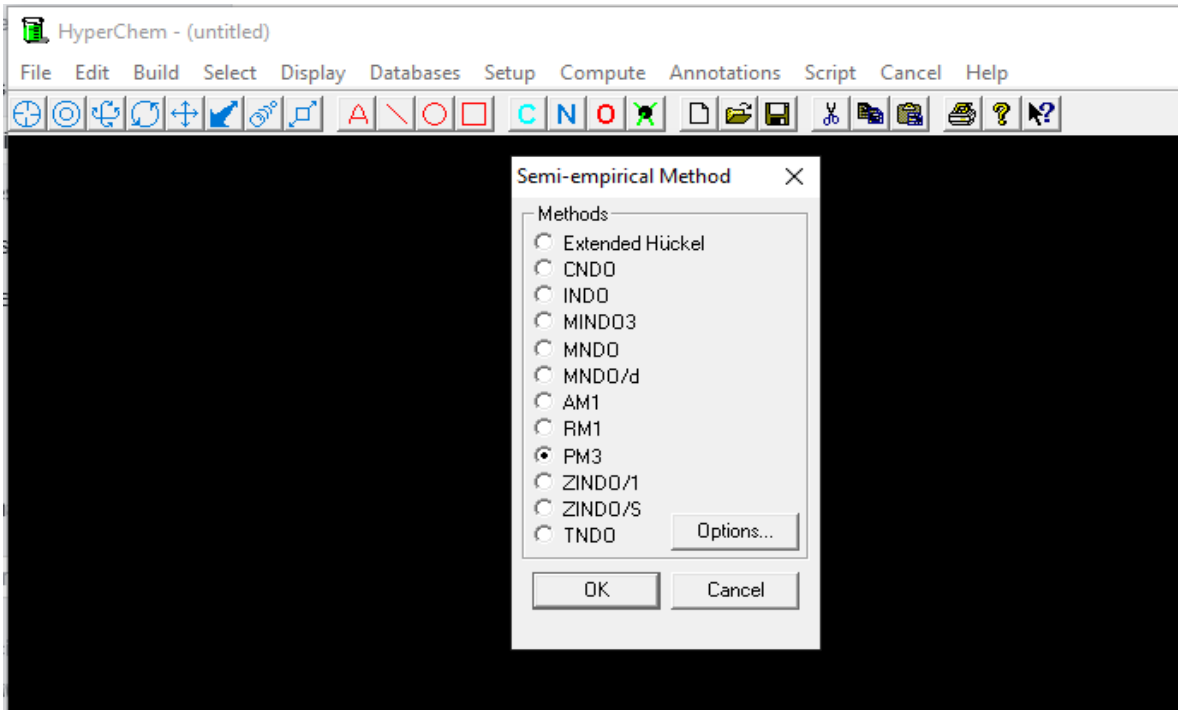


Figure II-11: PM3 mode selection

References

[1] Quezel P and Santa S. Nouvelle flore de l'Alg ´ erie et des ´regions d ´ esertiques m ´ eridionales. Ed. CNRS, Paris, 1963. ´

[2] Nedjimi B, Beladel B and Guit B. Biodiversity of halophytic vegetation in Chott *Zehrez* lake of Djelfa (Algeria). AmerJ Plant Sci, 2012, 3: 1527–1534. DOI: 10.4236/ajps.2012.311184 [3] K BOUHEDJAR, Toxicité des phénols : Une étude QSAR basée sur la méthode préconisée par l'OCDE, université Annaba décembre 2010

[4] ALLAOUI Messaouda. *Doctoral dissertation*. University kasdi Merbah Ouargla (*2015).*

[5] A.C. Crum-Brown and T.R. Fraser, —On the Connection Between Chemical Constitution and Physiological Action, Part I: On the Physiological Action of the Salts of the Ammonium Bases, Derived from Strychnia, Brucia, Thebia, Codeia, Morphia, Nicotia‖, *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 25, 1868, 151–203;

[6] A.C. Crum-Brown and T.R. Fraser, —On the Connection between Chemical Constitution and Physiological Action. Part II: On the Physiological Action of the Ammonium Bases derived from Atropia and Conia‖, *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 25, 1869, 693–739.

[7] J.A. Pople, —Nobel Lecture: Quantum chemical models‖, *Review of Modern Physics*, 71, 1999, 1267–1274.

[8] H. Meyer,La théorie de la narcose alcoolique. Premier message Quelle propriété des anesthésiques est la cause de leur effet narcotique, Archive pour Pathologie experimental et pharmacologie, 1899,109–118.

[9] E. Overton et G. Fischer, Études sur l'anesthésie en même temps, une contribution à la pharmacologie générale,1901.

[10] H. Fühner et E. Neubauer, "Immolation par des séries homologues de substances", Archive pour Pathologie experimental et pharmacologie,333-345, 1907.

[11] C. Hansch and T. Fujita, —p-σ-π Analysis. A Method for the Correlation of Biological Activity and Chemical Structure‖, *Journal of the American Chemical Society*, 86(8), 1964, 1616–1626.

[12] C. Hansch and E.J. Lien, —Structure-activity relationships in antifungial agents. A

survey‖, *Journal of Medicinal Chemistry*, 14(8), 1971, 653–670.

[13] S.Y. Tham and S. Agatonovic-Kustrin, ―Application of the artificial neural network in quantitative structure-gradient elution retention relationship of phenylthiocarbamyl amino acids derivatives‖, *Journal of Pharmaceutical and Biomedical Analysis*, 28(3), 2002, 581-590

[14] R.D. Cramer, D.E. Patterson, and J.D. Bunce, ―Comparative molecular field analysis (CoMFA): Effect of shape on binding of steroids to carrier proteins‖, *Journal of the American Chemical Society*, 110(18), 1988, 5959-5967.

[15] G. Klebe, U. Abraham, and T. Mietzner, ―Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological activity‖, *Journal of Medicinal Chemistry*, 37(24), 1994, 4130-4146.

[16] S Chtita, Modélisation de molécules organiques hétérocycliques biologiquement actives par des méthodes QSAR/QSPR. Recherche de nouveaux médicaments, 2017

[17] Guillaume F, Développement de modèles QSPR pour la prédiction despropriétés d'explosibilité des composés nitroaromatiques , 2010

[18] Kadrilege et Datacross. CHEMDRAW 18.0, LE DESSIN MOLÉCULAIRE PAR EXCELLENCE [enligne] https://ritme.com/fr/logiciels/chemdraw-chemoffice/ (consulté le 03/03/2022)

[19] MakoLab, Hypercube HyperChem Professional 8.0 [enligne] http://www.hyper.com/?tabid=360 (consulté le 03/03/2022)

Chapter III :
Results and discussion

Introduction

The identification and structural characterization of flavonoids and their conjugates isolated from plant material, as single compounds or as part of mixtures of structurally similar natural products, create some problems due to the presence of isomeric forms of flavonoid aglycones and their patterns of glycosylation. A number of analytical methods are used for the characterization of flavonoids. In many cases, nuclear magnetic resonance (NMR) analyses1D (^{1}H and ^{13}C) and 2D (COSY, HMQC, HMBC) are necessary for the unambiguous identification of unknown compounds; other instrumental methods(mass spectrometry, UV and IR spectrophotometry) applied for the identification of organic compounds fail to provide the information necessary to answer all the structural questions. Utilization of standards during analyses and comparison of retention times as well as spectral properties, especially when compounds are present in a mixture, is critical. An important area of research on flavonoids is the identification of their metabolites in plant.

Results and discussion :

III-1- Compound TR5I: The compound was extracted from butanol and was obtained as a yellow powder that was separated by CCM preparative [1].

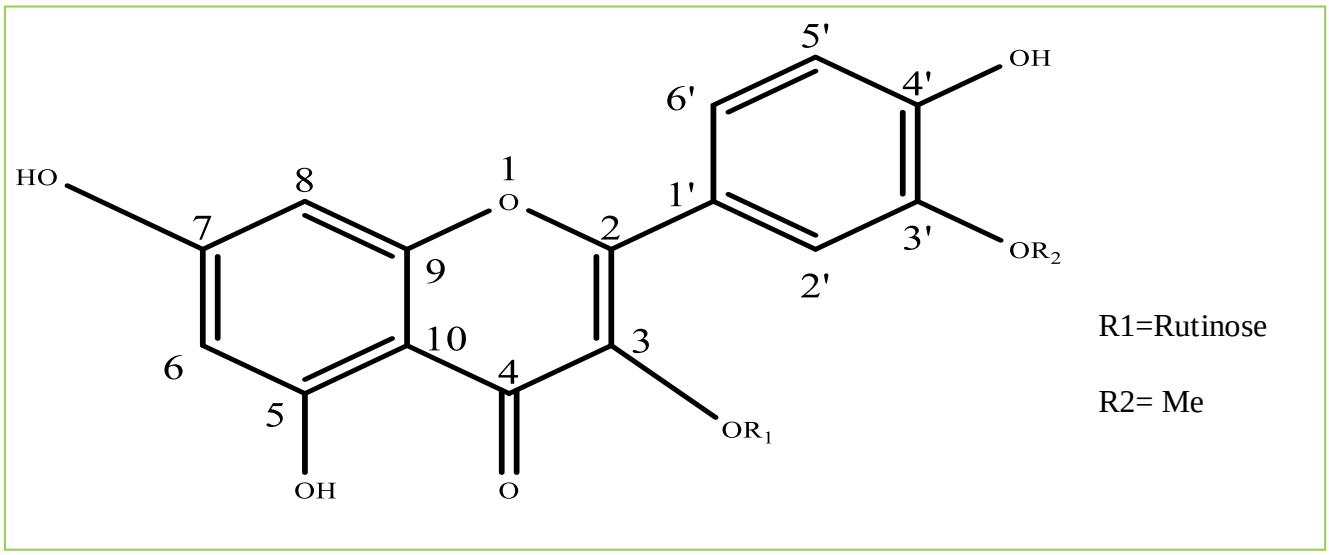


Figure III-1 : The partial structure of TR5I

Mass spectroscopy: The ESI MS spectrum of compound***TR5I***showed a **[M+Na]**$^{+}$at *m/z:*624+Na=647. (calcd 647. 55). and we confirmed with **[M -H]**$^{-}$***m/z*** : 624-H=623had the formula $C_{28}H_{32}O_{16}$.

NMR ^{1}H: The compound was obtained as a yellow powder. From the ^{1}H NMR spectrum signals in the weak domain characteristic of aromatic protons can be distinguished. more precisely of As flavonoid:

* Anomeric protons at 4.52 ppm (d. J = 1.3 Hz) and 5.4 ppm(d. J= 7.7 Hz) with coupling constants characteristic of the anomeric H configuration α and β respectively.

Peaks at 6.90 ppm (d. J= 8.3 Hz .H-5') 7.48 (dd. J= 1.9 and 8.45 Hz. H-6') and 7.99 (d. J= 1.8 Hz. H-2') are attributable to three protons characteristic of a 3,4-disubstituted B ring.

* Two broad singletons meta coupling related H-6 and H-8 appeared at 6.27 ppm(d. J = 1.55 Hz.H-8) and 6.02 ppm(d .J =1.55 Hz. H-6) indicating the bisubstitution of ring A..

* The absence of a single peak in the weak region attributable to H-3 of ring C.

* A three proton integration singlet at 3.84 ppm, characteristic of a methoxyl group(3H, s, 3′-OCH_3).

*A three-proton integrating singlet at 1.06 ppm (d. J = 6.15 Hz.3H-6''') characteristic of a methyl group specific to rhamnose.

Table III-1 : ^{1}H and chemical shifts (500MHz) in DMSO of the Compound TR5I

Position	*δ H (ppm)*	*Pick*	*J (Hz)*	*Integration*
6	**6.06**	**d**	**1.55**	**1**
8	**6.27**	**d**	**1.55**	**1**
2’	**7.99**	**d**	**1.8**	**1**
5’	**6.89**	**d**	**8.3**	**1**
6’	**7.48**	**dd**	**1.9-8.45**	**1**
3’- OMe	**3.84**	**s**		**3**
1”	**5.4**	**d**	**7.7**	**1**
2”	**3.119**	**s**		**1**
3”	**3.446**	**d**	**3**	**1**
4”	**3.404**	**d**	**3.1**	**1**
5”	**3.613**	**s**	/	**1**
6a” 6b”	**3.625** **3.319**	**d** **d**	**3.6** **3.45**	**2**
1”’	**4.43**	**sl**	/	**1**
2”’	**3.428**	**d**	**2.1**	**1**
3”’	**3.337**	**d**	**3.35**	**1**
4”’	**3.597**	**s**	/	**1**
5”’	**1.80**	**s**	/	**1**
6”’	**1.06**	**d**	**6.15**	**3**

NMR ^{13}C : In addition, the ^{13}C NMR spectrum showed 28 signals of the following carbons. As follows:

- Peaks δ 102.70 ppm (C-1") and δ 72.38 (C-2 "), 73.50 (C-3"), 68.72 (C-4 "), 73.90(C-5") and 65.54 (C-6 ") correspond to glucose.

- Peaks δ 100.45 ppm (C-1'"), δ 70.85 (C-2 '"), 71.09 (C- 3 '"), 71.66 (C-4'"), 68.38(C-5 '") and 18.35 (C-6'") correspond to rhamnose.

- The low-field shift of the signal δ 65.54 ppm assignable to C-6" relative to glucose, indicates that the C-6" carbon is the glycosylation site.

- A methoxyl group at 56.31 ppm.

Table III-2 : ^{1}H, ^{13}C NMR and DEPT 135°, DEPT 90° chemical shifts (500MHz) in DMSO of the Compound TR5I

	Position	*δ C (ppm)*	*δ H (ppm)*	*Pick*	*J (Hz)*	*Integration*	*DEPT 135°+90°*
isorhamnetin	**2**	***156.00***					***C***
	3	***133.29***					**C**
	4	***177.05***					**C**
	5	***161.43***					**C**
	6	***100.36***	**6.06**	**d**	**1.55**	**1**	**CH**
	7	***169.18***					**C**
	8	***94.79***	**6.27**	**d**	**1.55**	**1**	**CH**
	9	***157.22***					**C**
	10	***102.75***					**C**
	1'	***121.36***					**C**
	2'	***113.81***	**7.99**	**d**	**1.8**	**1**	**CH**
	3'	***147.48***					**C**
	4'	***150.19***					**C**
	5'	***115.63***	**6.89**	**d**	**8.3**	**1**	**CH**
	6'	***122.27***	**7.48**	**dd**	**1.9-8.45**	**1**	**CH**
	3'-OMe	***56.31***	**3.84**	**s**		**3**	**CH_3**
glucose	**1"**	***102.70***	**5.4**	**d**	**7.7**	**1**	**CH**
	2"	***72.38***	**3.119**	**s**		**1**	**CH**
	3"	***73.50***	**3.446**	**d**	**3**	**1**	**CH**
	4"	***68.72***	**3.404**	**d**	**3.1**	**1**	**CH**
	5"	***73.90***	**3.613**	**s**	**/**	**1**	**CH**
	6-a" **6-b"**	***65.54***	**3.625** **3.319**	**d** **d**	**3.6** **3.45**	**2**	**CH_2**
rhamnose	**1"'**	***100.45***	**4.43**	**sl**	**/**	**1**	**CH**
	2"'	***70.85***	**3.428**	**d**	**2.1**	**1**	**CH**
	3"'	***71.09***	**3.337**	**d**	**3.35**	**1**	**CH**
	4"'	***71.66***	**3.597**	**s**	**/**	**1**	**CH**
	5"'	***68.38***	**1.80**	**s**	**/**	**1**	**CH**
	6"'	***18.35***	**1.06**	**d**	**6.15**	**3**	**CH_3**

Ultraviolet-visible Absorption: The spectral series of the compound was carried out by

UV-visible spectrophotometry using characteristic reagents . shows :

***** Appearance of band I in methanol at λ_I = 360 nm leads us to a 3-substituted flavone or flavonol and as the ^{1}H NMR spectrum excluded a flavone structure, therefore the structure can only be a3substituted flavonol.

***** By adding a few drops of NaOH, we notice a bathochromic shift of the I band without the decrease of the optical density $\Delta\lambda_I$ (NaOH/MeOH)=60 nm, indicating an OH group in the 4' position. As well as the stability of the spectrum after five minutes indicates the absence of a 3,4 dihydroxy system.

*****Thus, the appearance of a new peak at 320 nm, indicating that position 7 has a free hydroxyl group.

To define the position of the two substituents: methoxyl and sugar, an acid hydrolysis (HCl, 6N) was performed.

hydrolysis (HCl, N) was performed. The UV spectrum of the aglycone recorded in methanol showed two bands: λ_{II} =260 and λ_I = 360 nm, which proves that the aglycone is a flavonol, where C-3 has become containing a hydroxyl group, which shows that the released sugar was located at the C-3 position and the methoxyl at the C-3' **[1]**.

COSY :According to the COSY spectrum, the following correlation spots can be distinguished, correlation spots can be distinguished:

-H- 2’ and H- 3’- OMe

-H-6' and H-5'

-H-8 and H-6

-H-1’' and H-2’'

-H-1’" and H-2”’

-H-2’" and H-6’"

HMQC: According to the HMQC experiment, each carbon is assigned its corresponding proton is assigned to each carbon:

between C- 6’ (δ 122.27 ppm) and H-6’ (δ 7.48 ppm)

•C- 5’ (δ 115.63 ppm) and H-5’ (δ 6.89 ppm)

•C- 2’ (δ 113.81 ppm) and H-2’ (δ 7.99 ppm)

•C- 1” (δ 102.70 ppm) and H-1” (δ 5.4 ppm)

• C- 6 (δ 100.36 ppm) and H-6 (δ 6.06 ppm)

•C- 1'" (δ 100.45 ppm) and H-1'" (δ 3.82 ppm)

•C- 8 (δ 94.79 ppm) and H-8 (δ 6.27 ppm)

• C- 6” (δ 65.54 ppm) and H-6”(a) (δ 3.6 ppm)

• C- 6” (δ 65.54 ppm) and H-6”(b) (δ 3.3 ppm)

• C-(3’-OMe) (δ 56.31 ppm) and H-(3’-OMe) (δ 3.84 ppm)

•C- 6”’ (δ 18.35 ppm) and H-6”’ (δ 1.06 ppm)

HMBC: The HMBC experiment shows the following correlations:

• C-2 (δ 156 ppm) shows 2 spots of correlations with

H- 2' (δ 7.99 ppm)

H-6’ (δ 7.48 ppm)

• C-3 (δ 133.29 ppm) shows 1 spot of correlation with

H-1” (δ 5.4 ppm)

• C-5 (δ 161.43 ppm) shows 1 spot of correlation with

H-6 (δ 6.06 ppm)

• C-6 (δ 100.36 ppm) shows 1 spot of correlation with

H-8 (δ 6.27 ppm)

• C-7 (δ 169.18 ppm) shows 2 spots of correlation with

H-6 (δ 6.06 ppm)

H-8 (δ 6.27 ppm)

• C-8 (δ 94.79 ppm) shows 1 spot of correlation with

H-6 (δ 6.06 ppm)

• C-9 (δ 157.22 ppm) shows 1 spot of correlation with

H-8 (δ 6.27 ppm)

• C-10 (δ 102.75 ppm) shows 2 spots of correlation with

H-6 (δ 6.06 ppm)

H-8 (δ 6.27 ppm)

• C-1’ (δ 121.36 ppm) shows 2 spots of correlation with

H-5’ (δ 6.89 ppm)

H-2’ (δ 7.99 ppm)

• between C-2’ (113.81ppm) and H-6’ (7.48 ppm)

• C-3’ (δ 147.48 ppm) shows 2 spots of correlation with

H-2’ (δ 7.99 ppm)

H-5’ (δ 6.89 ppm)

• C-4’ (δ 150.19 ppm) shows 3 spots of correlation with

H-2’ (δ 7.99 ppm)

H-5’ (δ 6.89 ppm)

H-6’ (δ 1.013 ppm)

• C-5’ (δ 115.63 ppm) shows spot of correlation with

H-6’ (δ 7.48 ppm)

• C-6’ (δ 122. 27 ppm) shows 2 spots of correlation with

H-2’ (δ 7.99 ppm)

H-5’ (δ 6.89 ppm)

• between C-6" (65.54) and H-1'" (4.43)

The correlations are shown in (Figure III- 2) :

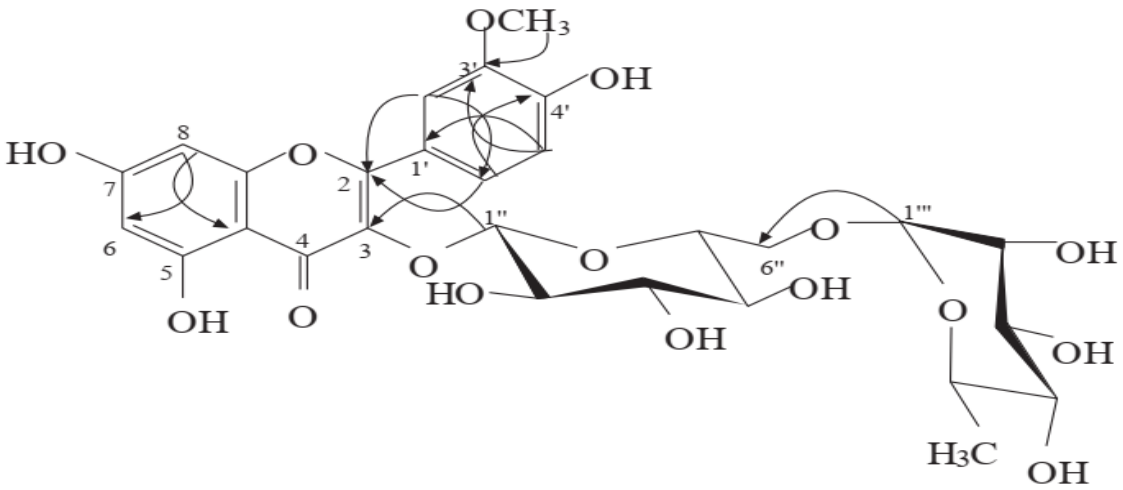
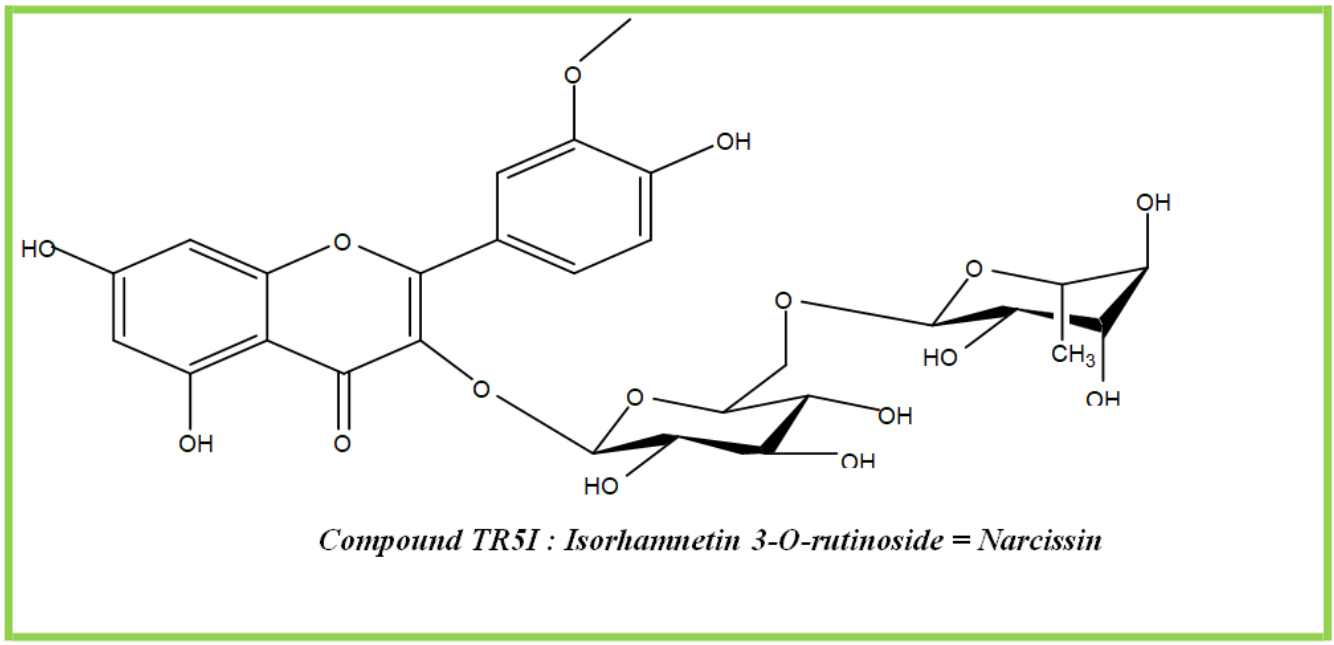


Figure III-2 : Selected HMBC correlations for compound TR5I

On the basis of these data, the compound is assigned as isorhamnetin 3-O-rutinosideor**isorhamnetin 3-*O*-α –L-rhamnopyranosyl - (1 →6)-β- D-glucopyranoside** also called ***Narcissin*.**

The chemical structure of TR5I is shown in (Figure III-3). was elucidated using spectroscopic data such as MS, UV-VIS, NMR 1D (1 H, 13C) and NMR 2D (COSY, HMQC, HMBC) are in agreement with the literature **[2]**.



Compound TR5I : Isorhamnetin 3-O-rutinoside = Narcissin

Figure III-3 : Structure of compound TR5I; isorhamnetin 3-O-rutinoside = narcissin

III- 2- Compound TR55 : The compound was extracted from butanol and was obtained as a yellow powder that was separated by CCM preparative [1].

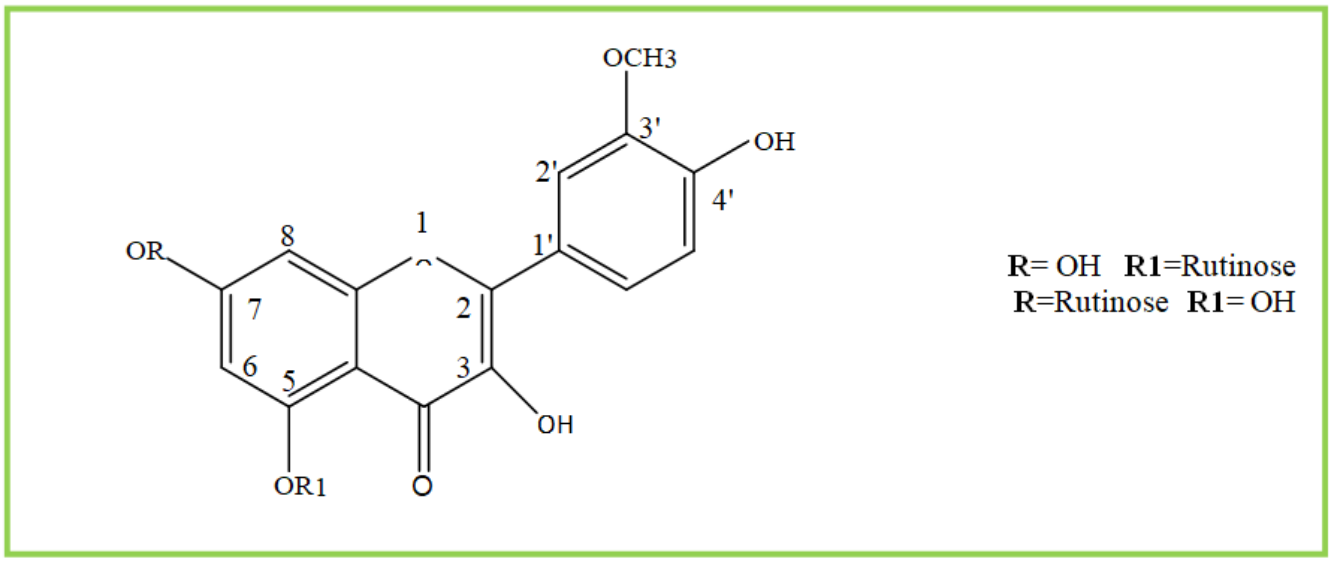


Figure III-4 :The partial structure of TR55

Mass spectroscopy: The ESI MS spectrum of compound ***TR55*** showed a**[M - H]**$^{-}$***m/z***:624-H =623. calcd 623.158had the formula $C_{28}H_{32}O_{16}$**[3]**

NMR ^{1}H: The compound was obtained as a yellow powder. From the ^{1}H NMR spectrum signals in the weak domain characteristic of aromatic protons can be distinguished. more precisely of As flavonoid:

- Anomeric protons at 4.42 ppm (sl. H-1”’) and 5.38 ppm (d. J= 7.55 Hz. H-1”) with coupling constants characteristic of the anomeric H configuration α and β respectively.

Peaks at 6.90 ppm (d. J= 7.6 Hz .H-5') 7.74 (d. J= 8.25 Hz. H-6') and 7.98 (sl. H-2') are attributable to three protons characteristic of a 3,4-disubstituted B ring.

*Two broad singletons at 6.26 ppm (s. H-8) and 6.05 ppm (s. H-6) indicating the bisubstitution of ring A.

*The absence of a single peak in the weak region attributable to H-3 of ring C.

*A three proton integration singlet at 3. 4 ppm, characteristic of a methoxyl group.(3H-(3’- OMe)).

*A three-proton integrating singlet at 1.06 ppm (s. 3H-6''') characteristic of a methyl group specific to rhamnose.

Position	*δ H (ppm)*	*Integration*	*Pick*	*J (Hz)*
2'	7.98	1	***sl***	/
5'	6.9	1	***d***	7.6
6'	7.74	1	***d***	8.25
8	6.26	1	***s***	/
6	6.05	1	***s***	/
1"	5.38	1	***d***	7.55
1"'	4.42	1	***s l***	/
3'- OMe	3.4	3	***s***	/
6"'	1.06	3	***s***	/

Table III-3 : ^{1}H and chemical shifts (500MHz) in DMSO of the Compound TR55

NMR ^{13}C: In addition, the ^{13}C NMR spectrum showed 28 signals of the following carbons

As follows:

- Peaks δ =102.77 ppm (C-1"), δ= 72.42 (C-2 "), 73.55 (C-3"), 70.84 (C-4 "), 73. 88(C-5") and 65.43 (C-6 ") correspond to glucose.

- Peaks δ =102.62 ppm (C-1'"), δ= 68.69 (C-2 '"), 71.09 (C- 3 '"), 71.69 (C-4'"), 68.27(C-5 '") and 18.35 (C-6'") correspond to rhamnose.

- The low-field shift of the signal δ 65.54 ppm assignable to C-6" relative to glucose, indicates that the C-6" carbon is the glycosylation site.

- A methoxyl group at 56.30 ppm.

The rhamnose C-1'". C-6" glucose attachment is deduced by the deblinding effect

This attachment of the two xucers, called rutinose, is noticed on the C-6" of glucose.

Table III-4 : ^{1}H, ^{13}C NMRand DEPT chemical shifts (500MHz) in DMSO of the Compound TR55

	Position	*δ C (ppm)*	*δ H (ppm)*	*Pick*	*J (Hz)*	*Integration*	*DEPT 135°+90°*
isorhamnetin	**2**	***161.37***					***C***
	3	***133.28***					**C**
	4	***180.23***					**C**
	5	***173.88***					**C**
	6	***100.39***	**6.05**	**s**	**/**	**1**	**CH**
	7	***176.97***					**C**
	8	***94.82***	**6.26**	**s**	**/**	**1**	**CH**
	9	***157.21***					**C**
	10	***102.77***					**C**
	1'	***121.24***					**C**
	2'	***113.79***	**7.98**	**s l**	**/**	**1**	**CH**
	3'	***147.50***					**C**
	4'	***155.94***					**C**
	5'	***115.66***	**6.9**	**d**	**7.6**	**1**	**CH**
	6'	***122.24***	**7.74**	**d**	**8.25**	**1**	**CH**
	3'-OMe	***56.30***	**3.4**	**s**	**/**	**3**	**CH_3**
glucose	**1"**	***102.77***	**5.38**	**d**	**7.55**	**1**	**CH**
	2"	***72.42***					**CH**
	3"	***73.55***					**CH**
	4"	***70.84***					**CH**
	5"	***73.88***					**CH**
	6a" 6b"	***65.43***				**2**	**CH_2**
rhamnose	**1"'**	***102.62***	**4.42**	**s l**	**/**	**1**	**CH**
	2"'	***68.69***					**CH**
	3"'	***71.09***					**CH**
	4"'	***71.69***					**CH**
	5"'	***68.27***					**CH**
	6"'	***18.35***	**1.06**	**d**	**5.9**	**3**	**CH_3**

Ultraviolet-visible Absorption: The spectral series of the compound was carried out by UV-visible spectrophotometry using characteristic reagents . shows :

***** Appearance of band I in methanol at λ_I = 360 nm leads us to a 3-substituted flavone or flavonol and as the ^{1}H NMR spectrum excluded a flavone structure, therefore the structure can only be a3substituted flavonol.

***** By adding a few drops of NaOH, we notice a bathochromic shift of the I band without the decrease of the optical density $\Delta\lambda_I$(NaOH/MeOH)=50 nm, indicating an OH group in the 4' position. As well as the stability of the spectrum after five minutes indicates the absence of a 4 hydroxyl system.

To define the position of the two substituents: methoxyl and sugar, an acid hydrolysis (HCl, N) was per formed .hydrolysis (HCl, N) was performed. The UV spectrum of the aglycone recorded in methanol

showed two bands: λ_{II} =245 and λ_I = 360 nm, which proves that the aglycone is a flavonol, where C-7 has become containing a hydroxyl group, which shows that the released sugar was located at the C-7 position and the methoxyl at the C-3' **[1]**.

COSY: According to the COSY spectrum, the following correlation spots can be distinguished

correlation spots can be distinguished:

-H-6' and H-5'

-H-8 and H-6

-H-2 ' and H-6 '

-H-1'' and H-2''

-H-1''' and H-2'''

HMQC: According to the HMQC experiment, each carbon is assigned its corresponding proton is assigned to each carbon:

between C- 6’ (δ 122.24 ppm) and H-6’ (δ 7.74 ppm)

• C- 5’ (δ 115.66 ppm) and H-5’ (δ 6.9 ppm)

• C- 2’ (δ 113.79 ppm) and H-2’ (δ 7.98 ppm)

• C- 1” (δ 102.77 ppm) and H-1” (δ 5.38 ppm)

• C- 6 (δ 100.39 ppm) and H-6 (δ 6.05 ppm)

• C- 1‴ (δ 102.62 ppm) and H-1‴ (δ 4.42 ppm)

• C- 8 (δ 94.82 ppm) and H-8 (δ 6.26 ppm)

• C- 6” (δ 65.43 ppm) and H-6”(a) (δ 3.6 ppm)

• C- 6” (δ 65.43 ppm) and H-6”(b) (δ 3.3 ppm)

• C-3’(O-Me) (δ 56.30 ppm) and 3H-(O-Me) (δ 3.4 ppm)

• C- 6”’ (δ 18.35 ppm) and H-6”’ (δ 1.06 ppm)

HMBC : The HMBC experiment shows the following correlations:

• C-2 (δ 156 ppm) shows 1 spot of correlations with

H- 2' (δ 7.99 ppm)

• C-1’ (δ 121.36 ppm) shows 1 spot of correlation with

H-2’ (δ 7.99 ppm)

• between C-2’ (113.81ppm) and H-6’ (7.48 ppm)

• C-3’ (δ 147.48 ppm) shows 3 spots of correlation with

H-2’ (δ 7.99 ppm)

H-5’ (δ 6.89 ppm)

H-6’ (δ 6.89 ppm)

• C-4’ (δ 150.19 ppm) shows 1 spot of correlation with

H-2’ (δ 7.99 ppm)

• C-5’ (δ 115.63 ppm) shows 1 spot of correlation with

H-6’ (δ 7.48 ppm)

• C-6’ (δ 122. 27 ppm) shows 2 spots of correlation with

H-2’ (δ 7.99 ppm)

H-5’ (δ 6.89 ppm)

• between C-6" (65.54) and H-1'" (4.43) .

• between C-1” (65.54) and H-2” (4.43) .

• C-4”’ (δ 122. 27 ppm) shows 1 spot of correlation with

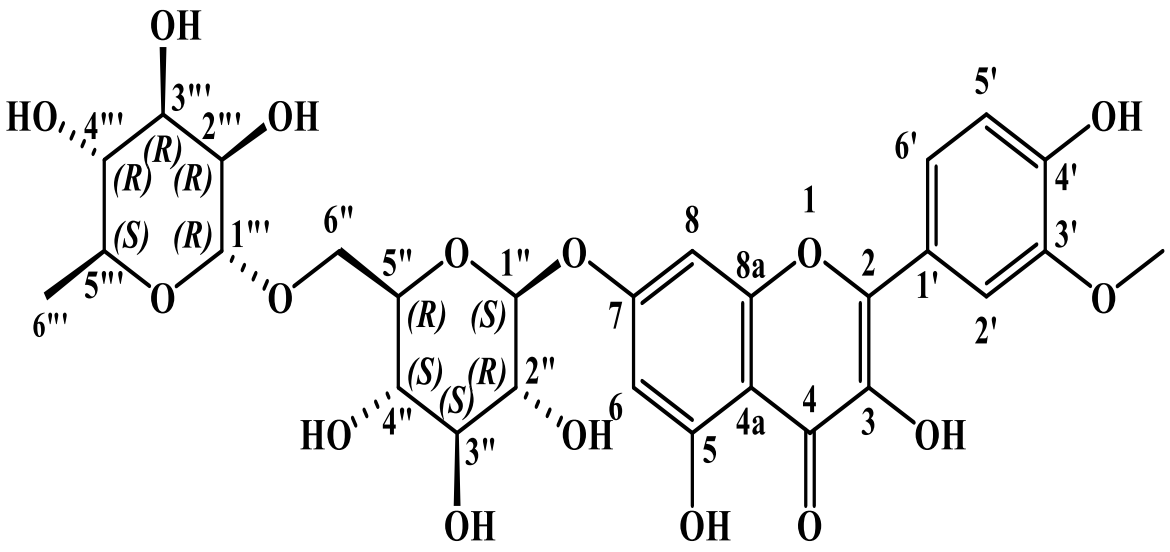
H-6”’ (δ 6.89 ppm)

• C-5”’ (δ 122. 27 ppm) shows 1 spot of correlation with

H-6”’ (δ 6.89 ppm)

On the basis of these data, the compound is assigned as isorhamnetin 7-Orutinoside or **isorhamnetin 7-*O*-α –L- rhamnopyranosyl - (1 → 6)-β- D- glucopyranoside**.

The chemical structure of TR55 is shown in (Figure III- 5). was elucidated using spectroscopic data such as MS, UV-VIS, NMR 1D (1 H, 13C) and NMR 2D (COSY, HMQC, HMBC) are in agreement with the literature **[4]**.

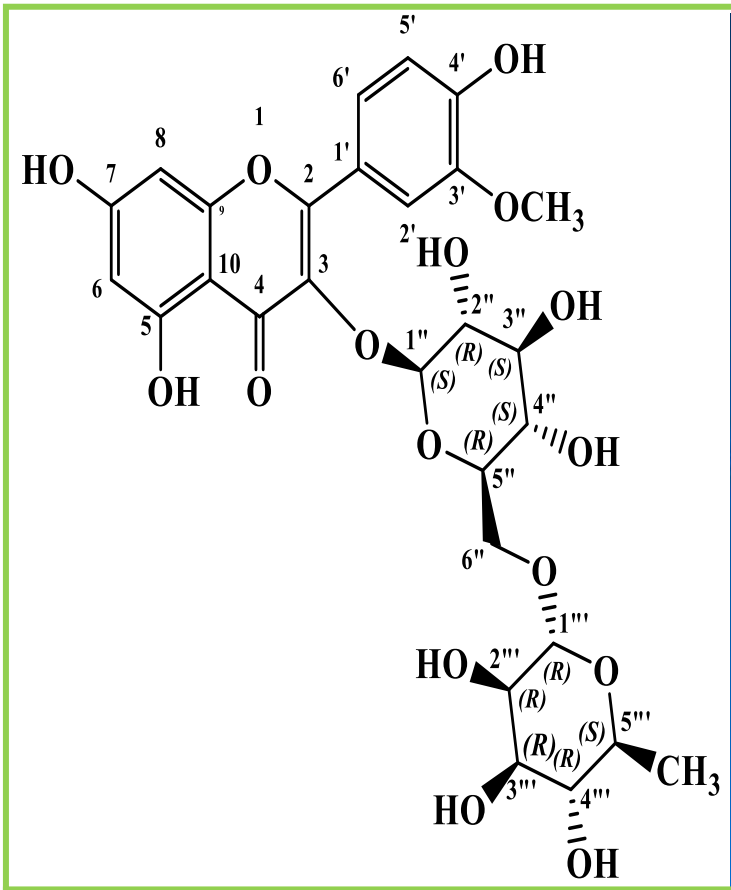


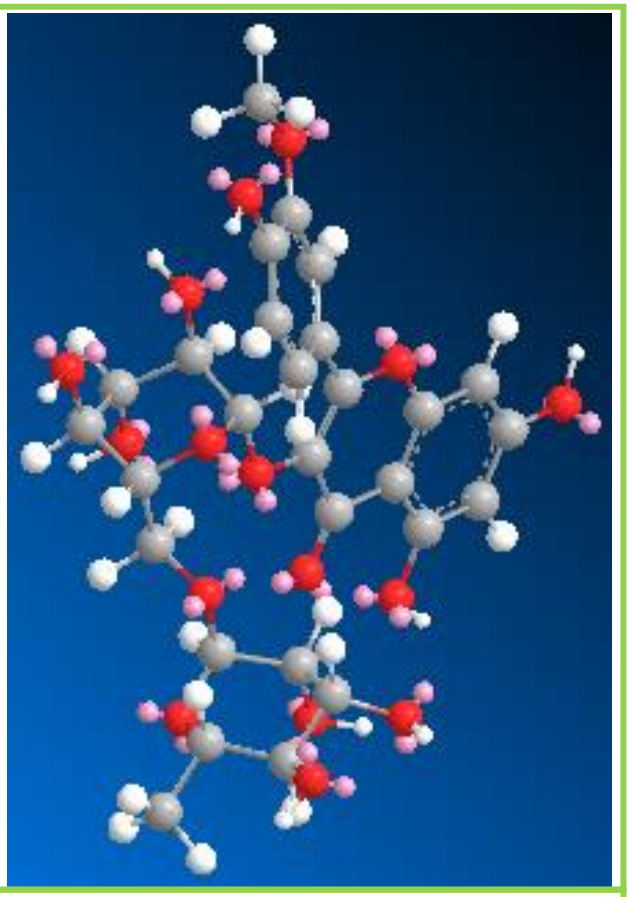
Compound TR55 : Isorhamnetin 7-O-rutinoside

Figure III- 5 : Structure of compound TR55; isorhamnetin 7-O-rutinoside

III-3 Structure of compounds from Cham Draw 1D and 3D :

III-3-1 Structure of compound TR5I:

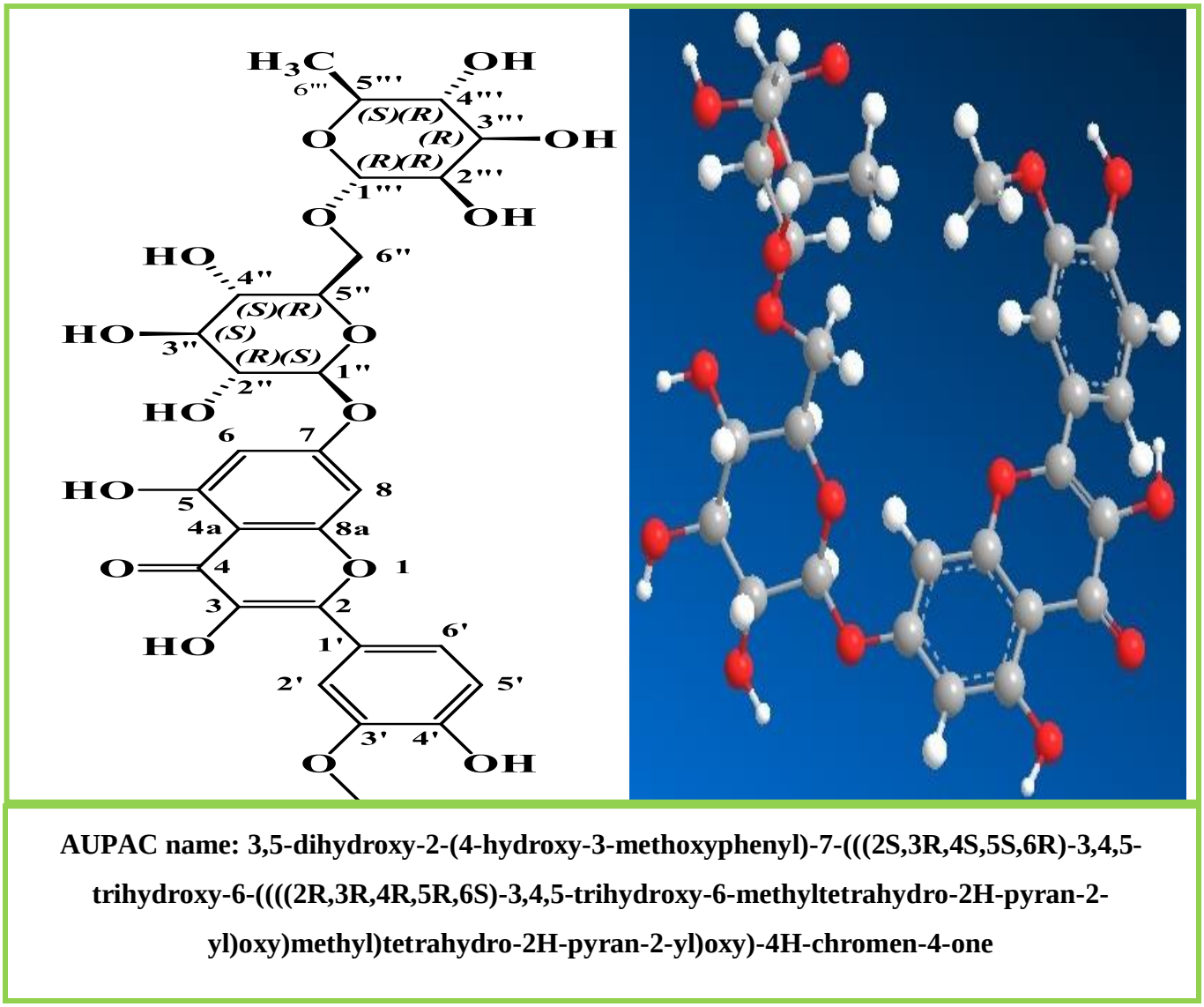




AUPAC name: 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-((((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one

Figure III-6 : Structure of compound TR5I from Cham Draw and AUPAC name

III-3-2 Structure of compound TR55:



AUPAC name: 3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-((((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one

Figure III-7 : Structure of compound TR55 from Cham Draw and AUPAC name

III-4Descriptors (Molecular properties of QSAR) :

III-4-1 Properties QSAR of Compound TR5I:

Table III-5 *:properties QSAR of Compound TR5I*

Properties	
Hydration energy (Kcal/mol)	**-44.77**
Log P	**-5.88**
Polarizability (Å³)	**56.88**
Refractivity) (Å³)	**151.24**
Molar Mass (amu)	**624.55**
Surface Area[Grid] (Å²)	**838.06**
Surface Area [Approx] (Å²)	**681.27**
Volume(Å³)	**1515.34**

III-4-2 Properties QSAR of Compound TR55

:*Table III-6 *:properties QSAR of Compound TR55

Properties	
Hydration energy (Kcal/mol)	**-44.43**
Log P	**-5.88**
Polarizability (Å³)	**56.58**
Refractivity (Å³)	**151.24**
Molar Mass (amu)	**624.55**
Surface Area[Grid] (Å²)	**840.60**
Surface Area [Approx] (Å²)	**707.02**
Volume(Å³)	**1466.97**

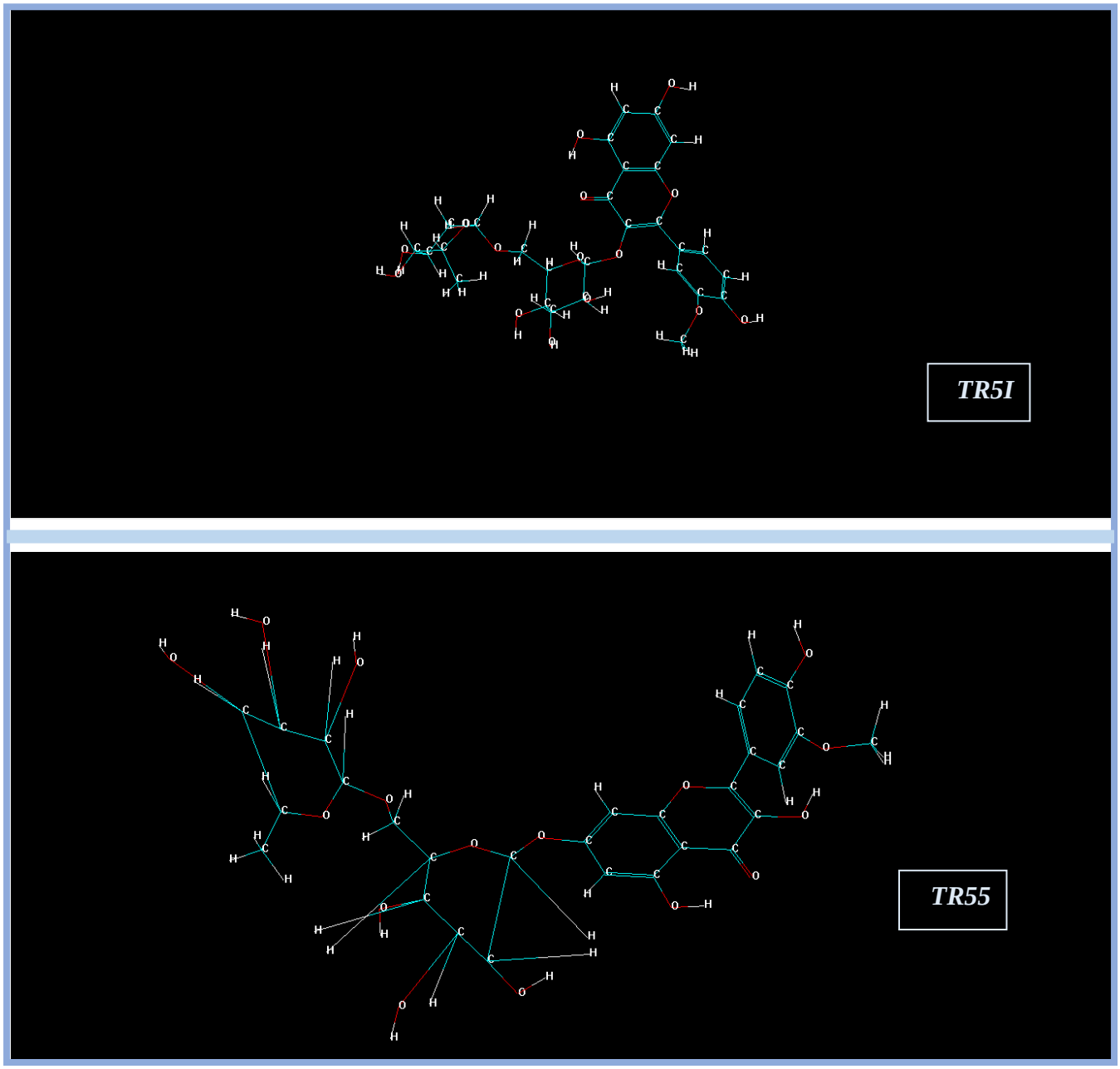


Figure III - 8 ; 3D of the compounds in Hyper Chem program

Reference

[1] ALLAOUI Messaouda. *Doctoral dissertation*. University kasdi Merbah Ouargla (*2015).*

[2] Boubaker, J., et al.*, Ethyl acetate extract and its major constituent, isorhamnetin 3-O-rutinoside, from Nitraria retusa leaves, promote apoptosis of human myelogenous erythroleukaemia cells.* Cell Proliferation, 2011. 44(5): p. 453-461.

[3] Sriseadka, T., S. Wongpornchai, and M. Rayanakorn, *Quantification of flavonoids in black rice by liquid chromatography-negative electrospray ionization tandem mass spectrometry.* Journal of agricultural and food chemistry, 2012. 60(47): p. 11723-11732.

[4] Abdullabekova, V.N., N.A. Yunuskhodjaeva, and M.A. Khodjaeva, *Implementation Chemical Research of the Woolly Erva Plants Growing in the Republic of Uzbekistan.* Annals of the Romanian Society for Cell Biology, 2021: p. 682-694.

General Conclusion:

In this study, We known two flavonoids (Isorhamnetin 3-Orutinoside and Isorhamnetin 7-Orutinoside) were identified by analysis using spectroscopic techniques, Ultra-Violet (UV), Nuclear Magnetic Resonance 1D (^{1}H-NMR,^{13}C-NMR), 2D (COSY, HMQC, HMBC) and Mass and define their QSAR descriptors. This compounds isolated from the (n-butanol) extract. of the aerial parts of plant ***T. nudatum***.

Finally, we hope to find the formulas of the remaining compounds and complete their modelling.

Abstract:

Traganum nudatum. (Chenopodiaceae family) is a herbaceous wild plant is used in medicinal plants widely used in Algerian traditional medicine. Traganum nudatum known locally as 'Damran' is used in traditional medicine to cure some diseases such as Diarrhea, wounds, rheumatism, dermatitis, and others. Phytochemical investigation of different extracts prepared from the aerial part of T. nudatum were collected from Touggourt (gamaa region) . Resulted to the isolation of two main flavonol glycosides from n-butanol extract. Using comprehensive spectroscopic methods. including 1D and 2D nuclear magnetic resonance (NMR). UV-Visible spectroscopy and mass spectroscopy, chemical structure of isolated compounds were determined as isorhamnetin-3-o-rutinoside (Narcissin) and isorhamnetin-7-o-rutinoside. and find QSAR descriptors for each compound.

Keywords :T. nudatum, Damran, n-butanol extract, spectroscopic methods, chemical structure, Narcissin, QSAR descriptors.

الملخص:

***Traganum nudatum*من عائلة *(chenopodiaceae)* هو نبات بري عشبيوهو من النباتات الطبية المستخدمة على نطاق واسع في الطب التقليدي الجزائري. يُستخدم *T. nudatum* المعروف محليًا باسم «الضمران» لعلاج بعض الأمراض مثل الإسهال والجروح والروماتيزم والتهاب الجلد وغيرها. أدت الدراسة الفيتوكيميائية لمستخلصات مختلفة تم إعدادها من الجزء الهوائي لنبات الضمران المأخوذ من منطقة تقرت (بالتحديد جامعة), إلى عزل عدة مركبات أهمها اثنين من الفلافونويدات الجليكوزيدية المستخلصة من البوتانول.**

باستخدام طرق التحليل الطيفي الشاملة، بما في ذلك الرنين المغناطيسي النووي *1D* و *2 D* *(NMR)* والتحليل الطيفي المرئي للأشعة فوق البنفسجية والتحليل الطيفي للكتلة، تم تحديد البنى الكيميائية للمركبات المعزولة على أنها *Isorhamnetin-3-O-rutinoside (Narcissin)* و *Isorhamnetin-7-O-rutinoside* إيجاد واصفات ال *QSAR* الخاصة بكل مركب.

الكلمات المفتاحية:

***T. nudatum*,الضمران, البوتانول ,طرق التحليل الطيفي, البنى الكيميائية,Narcissin ,واصفات ال QSAR**