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THE UNIVERSITY OF YAOUNDE I
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POSTGRADUATE SCHOOL OF SCIENCE, TECHNOLOGY AND GEOSCIENCES
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DOCTORAL RESEACH UNIT IN CHEMESTRY AND APPLICATIONS
****

ORGANIC CHEMISTRY DEPARTEMENT

DEPARTEMENT DE CHIMIE ORGANIQUE

LABORATORY OF BIOACTIVE NATURAL PRODUCTS

LABORATOIRE DES SUBSTANCES NATURELLES ET BIOACTIVES

Chemical study of the ethyl-acetate fraction from Methanol extract of the roots bark of *Newtonia griffoniana* (Baill.) Baker (Fabaceae)

A Dissertation Submitted in fulfilment of the requirements for the award of a Master of Science degree (MSC) in Organic Chemistry.

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(Professor, *UYI*)

Academic year : 2021-2022

DEDICATION

I dedicate this thesis to ;

My parents

- TALLA JEAN

  And

  - TOMNANG CATHERINE
- To all my sisters and brothers.

ACKNOWLEDGEMENT

I am grateful to you lord for you have done so much for us. I can't mention it all but in few words, I thank you for life, strength, joy, protection and sustenance you have given us throughout till the accomplishment of this work.

This work was carried out in the Bioactive Natural Products Laboratory (BiNaPLa) at the Higher Teacher Training College of The University of Yaoundé I under the direction of Professor KAPCHE WABO FOTSO Gilbert Deccaux my supervisor. I sincerely thank my supervisor for welcoming me in his research team, hence giving me an opportunity to work in a well-equiped laboratory throughout our journey.

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To all those who have contributed directly or indirectly to the realization of this work, may they receive here the expression of my gratitude.

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LIST OF ABREVIATIONS, SYMBOLS AND ACRONYMES

CC: Column Chromatography

TLC: Thin Layer Chromatography

$CDCl_3$:Deuterated Chloroform

COSY: Correlation Spectroscopy

***d**:* Doublet

dd: *Doublet of Doublet*

DEPT: Distortionless Enhancement by Polarisation Transfer

DMSO: Dimethyl Sulfoxide

EI: Electronic Impact

ESI: Electro Spray Ionisation

HMBC: Heteronuclear Multiple Bond Coherence

HSQC: Heteronuclear Single Quantum Coherence

Hz : Hertz

IC_{50} : Inhibitory mediane Concentration

IR: InfraRed

J: Coupling constant

CD_3OD: Deuterated methanol

MHz: MegaHertz

***N*:** *Newtonia*

ppm: Parts per million

^{13}C NMR: Carbon-13 Nuclear Magnetic Resonance

1D NMR: One-dimensional Nuclear Magnetic Resonance

^{1}H NMR: Proton Nuclear Magnetic Resonance

2D NMR: Two-dimensional Nuclear Magnetic Resonance

s: Singlet

t: Triplet

UV: UltraViolet

δ_C: Chemical shift of carbon

δ_H: Chemical shift of proton

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RESUME

Le présent travail porte sur l'extraction, l'isolement et la caractérisation des métabolites secondaires de la fraction à l'acétate d'éthyle des écorces des racines de *Newtonia griffoniana*, plante de la famille des Fabaceae, utilisée dans la pharmacopée traditionnelle Camerounaise pour le traitement de diverses maladies telles que l'anxiété et les troubles de sommeil.

Les écorces des racines de *Newtonia griffoniana* ont été récoltées le 14 Décembre 2022 à Batoufam dans la région de l'Ouest. Après découpage, séchage et broyage, la poudre obtenue (2,04 kg) a subi une extraction dans le méthanol à température ambiante pendant 72h et à produit 168.5g d'extrait brut. 158.5 g de cet extrait soumis à des méthodes chromatographiques usuelles (CC, CCM) a conduit à l'isolement de trois composés.

La Résonnance Magnétique Nucléaire à une dimension RMN 1D (^{1}H et ^{13}C) et à deux dimensions RMN 2D (HMBC, HSQC, COSY), ont permis de caractériser et d'identifier ces composés dont une chromone (5,7-dihydroxyl chromone), un triterpène pentacyclique (acide 3*β*-hydroxy-lup-20(29)-en-(28)-oique) et une flavonoïde (2*R*,3*R*)-3,4',5,7-tetrahydroxyflavan-4-one). Les structures de ces composés ont été confirmées par comparaison de leurs données spectroscopiques avec celles décrites dans la littérature.

Mots clés: *Newtonia griffoniana;* Fabaceae; Flavonoïdes, Triterpène, Chromone.

ABSTRACT

This work focuses on the extraction, isolation and characterization of secondary metabolites from the ethyl acetate fraction of the root barks of *Newtonia griffoniana*, a plant from the Fabaceae family used in folk medicine for the treatment of anxiety and sleep disorder. The root barks of *Newtonia griffoniana* was harvested on the 14 December 2022 at Batoufam in the west region. After cutting, drying and grinding, we obtained a powder of mass (2.04kg) which later underwent an extraction in the solvent methanol at room temperature and pressure for 72hr and resulted to 168.5g of crude extract. 158.5 g of the crude extract was subjected to usual chromatography methods (CC, TLC) and with the help of One dimensional (^{1}HNMR and ^{13}CNMR) and two dimensional (HMBC, HSQC, COSY) nuclear magnetic resonance analysis we were able to characterize and identify these compounds as a chromone (5,7-dihydroxyl chromone), a pentacyclic triterpene (3β-hydroxy-lup-20(29)-en-(28)-oic acid) and a flavonoid (2R,3R)-3,4',5,7- tetrahydroxyflavan-4-one). The structures of this compounds were confirmed by comparing their spectroscopic data with those described in the literature.

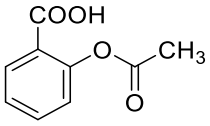
Key words: *Newtonia griffoniana*, Fabaceae, Flavonoids, Triterpene, Chromone

GENERAL INTRODUCTION

INTRODUCTION

Plants have been exploited for their medicinal purposes for thousands of years and have played a significant role in maintaining human health and improving the quality of human life (**Samuelsson, 2004; Dzoyem *et al*., 2013**).

Nowadays, plants continue to bring new materials in medicine for example in the field of oncology with *Catharantus roseus* commonly referred as Madagascar Periwinkle which is a plant that contains molecules for the therapeutic treatment of cancers (**Moudi *et al.*, 2013**). In addition, plants have been vital in drug discovery and drug development for instance in the discovery of drugs such as Aspirin (**i**) and Morphine (**ii**) which humanity can no longer do without because every day they save lives and relief pain.



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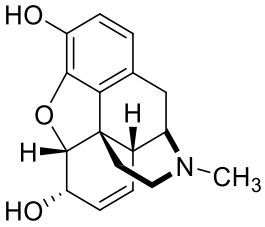
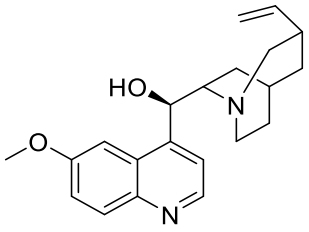


(ii)

Although medicine has experienced considerable growth in recent years, it is important to emphasize that the world is increasingly faced with an insufficiency or even absence of treatment faced to certain pathogens. This may be due to resistances developed by certain pathogenic agents such as parasites, viruses, bacteria with regard to existing drugs and also to the undesirable side effects of certain drugs in the organism like Quinine (**iii**) used in other times for the treatment of malaria. To this, we add the emergence of new diseases, including one of the most recent the corona virus (Covid-19) which has claimed many victims in the world since the year 2019. That is why the search for new, effective and less toxic active ingredients from natural sources remains relevant.



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In the same line, the University of Yaounde I through some specialized laboratories in Organic Chemistry and Natural Substances including BiNaPLa have set the aim of isolating

and characterizing active ingredients responsible for the therapeutic virtues of medicinal plants and which can be used in the manufacture of new drugs.

With the view of bringing in our contribution to this vast field of research, the laboratory of bioactive natural products of the Higher Teacher Training College of The University of Yaounde I has set itself objectives of isolating and studying secondary metabolites of Cameroonian medicinal plants. Thus, our master's work in this laboratory was focused on the <<Chemical study of the ethyl acetate fraction of the root barks of *Newtonia griffoniana* (Fabaceae) >>. The choice of this plant is motivated by the fact that primary, to the best of our knowledge the stem bark of this plant is used in folk medicine for the treatment of anxiety and sleep disorder, secondly few chemical and pharmacological studies have been done on *Newtonia griffoniana* and finally the wide uses of the plants of the genus *Newtonia* in folk medicine in Cameroon for the treatment of anxiety ,sleep disorder, diarrhoea. **(Djiogue *et al* ., 2015**).

However can we isolate from this plant compounds with activities that justifies their uses in folk medicine? to answer this question,we have set ourselves the general objective to isolate and characterize secondary metabolites from the ethyl-acetate fraction from Methanol extract of the roots bark of *Newtonia griffoniana* . Hence to achieve this objective, some specific objectives have been formulated and consist of harvesting of the roots, preparation of crude extract by maceration, fractionation and isolation of compounds, purification and characterization of secondary metabolites. This work is subdivided into three main chapters as follow:

The first chapter entitled the literature review which relates to the generalities on ethnobotanical aspects, ethnobotanical uses, previous chemical and biological studies carried on *Newtonia griffoniana*;

The second chapter entitled results and discussion, concerns the outcome of our day-to-day work carried out in the laboratory and

The third chapter which presents the material and the experimental methods used to obtain these results.

CHAPTER 1 : LITTERATURE REVIEW

I-Botanical review

I-1.Generalities on the Fabaceae family

The Fabaceae commonly known as the legume, pea, or bean family are a large and agriculturally important family of flowering plants made up of dicotyledenous plants of the Fabales order. They include trees, shrubs, perennial or annual herbaceous plants which are easily recognized by their fruit and their compound stipulate leaves (**Judd *et al* ., 2002**). This family owes her name because their flowers have the look of a butterfly **(Takhtajan, 1996).** This family is divided into three sub families which are ; Caesalpinoideae, Mimosoideae, Faboideae the largest sub family (**Lavin *et al* ., 2005**).

The Fabaceae is the third largest family of plants in the world as counted by its total number of species and has about 20,000 species distributed in 765 genera including *Newtonia* our genus of interest (**Christenhusz *et al*., 2016**).

I-2.Generalities on the *Newtonia* genus

Bailon in 1888 described the genus *Newtonia* with the specie type *Newtonia linsignis* Baill which originates from Gabon and has undergone many modifications. It is in 1992 that Boutique on the occasion of their review of Mimosaceae from the Zaire, Burundi and Rwanda restored the genus *Newtonia* (**Villers, 1990**). The plants of the *Newtonia* genus include trees and shrubs. Their identification is based on the following characteristics: their leaves are bipinnate provided with glands at the insertion of pinnae and leaflets. The inflorescence is in spikes or spike like racemes and caducous bracts; the flowers are small. The pods and seeds are narrowly oblong, flattened and leathery (**Gilbert, 1952)** .

The genus *Newtonia* belongs to the Fabaceae family and the sub family of Mimosoideae. This genus originates from Africa and includes about 16 species in the world (**Brenan, 1959**) and about 14 in Africa which are: *Newtonia griffoniana, Newtonia aubrevillei*, *Newtonia. camerunensis, Newtonia devredii, Newtonia Hildebranditii, Newtonia leucocarpa*, *Newtonia paucijuga*, *Newtonia scandens*, *Newtonia buchanani, Newtonia ellioti, Newtonia. duparquetiana, Newtonia grandifolia, Newtonia erlangeri, Newtonia grandulifera* (**Villers, 1990***).* **Table 1** illustrates the distribution of some species of *Newtonia* in Africa.

Table 1: Geographical distribution of the *Newtonia* genus in Africa

Species	Biological type	Country	References
Newtonia hildebrandtti	Shrub	Kenya and Tanzania	**Villers, 1990**
Newtonia paucijuga	Tree	East of South Africa	
Newtonia erlangeri	Tree	Costal regions of Somalia, Kenya, Tanzania	
Newtonia griffoniana	Tree	Gabon; Cameroon	
Newtonia buchananii	Tree	Angola, Cameroon, Kenya, Malawi, Mozambique, Rwanda	
Newtonia scandens	Tree	Cameroon	
Newtonia camerunesis	Tree	Cameroon (Bamenda and mount Bamboutous)	**Boutique, 1952**
Newtonia glandulifera	Tree	Gabon	

I-3. The *Newtonia griffoniana* specie

I.3.1. Description of the *Newtonia griffoniana* specie

Newtonia griffoniana (figure 1) is a large tree of tropical forest that can reach a height of 25 to 35 m. The trunk is usually straight and cylindrical void of branches and can reach a height of 12 m, it can measure 70 to 80 cm of diameter with foot hills at the base. The surface of its bark is smooth, with a pale grey to greyish brown color. The internal back has a yellowish pink with a translucent yellowish drain. It also has young short haired twigs with a dense and reddish-brown colour (**Villers, 1990**).

Their leaves are bipinnately compound with two pairs of opposite pinnae, stipulate absent or indistinct or indefinite. Its petiole and spine together reach a height of 4 cm long ,with short glands between each pair of pinnae, the leaf axes are provided with gland between each pair of penne, the numerous leaflets are small (**Villers, 1990**).

Its flowers are submersible arranged in narrow spikes or racemes, flowers are bisexual and regular, the calice is bell shaped with valve segments. The tube of the calice measures 1 mm long, it is hairy outside and whitish in its upper part, the anthers are often provided at the top with a gland carried on a short and quickly caducous foot, the carpels forms a hairy, unilocular and has many ovulated ovary. The pods are narrowly oblong flattened reddish brown in colour containing two to four seeds. The seed is oblong, flat, 7-9 cm long including the brown papery wing that surrounds them attached to the pod at one end (**Villers, 1990**).

A B C

Figure 1: Leaves, pod and tree of *Newtonia griffoniana* (A; Leaves, B; Pod, C; Tree) (Villers, 1990)

I.3.2. Systematic position of *Newtonia griffoniana*

The first botanical classification based on morphology of the sexual organs of plants and using binomial nomenclature system for a given specie was established in 1753 by Carl Linné. Subsequently and in particular in the 19th century, other phylogenic classifications taking into account the plant's total morphological characteristics emerged following Darwin's work relating to the theory of the evolution of living species. Recent scientific advances in the knowledge of molecular charateristics of species have led to the current classification which is used by the vast majority of botanists **(Judd *et al*., 1999).** According to taxonomy, the genus *Newtonia* belongs to the order of Fabales, the subfamily of Mimosoideae and the family of Fabaceae, Leguminosae or Mimosaceae. **Table 2** gives the position of *Newtonia griffoniana* in the evolutionary classification systems **(APG III, 2009**) and **figure 2 ,** the different locality where *Newtonia griffoniana* was harvested in Cameroon.

Table 2: APG III systematic position(APGIII, 2009)

Kingdom	Plantae
Phylum	Fabidea
Order	Fabales
Family	Leguminosea
Sub family	Mimosaceae
Genus	*Newtonia*
Species	*Newtonia griffoniana*

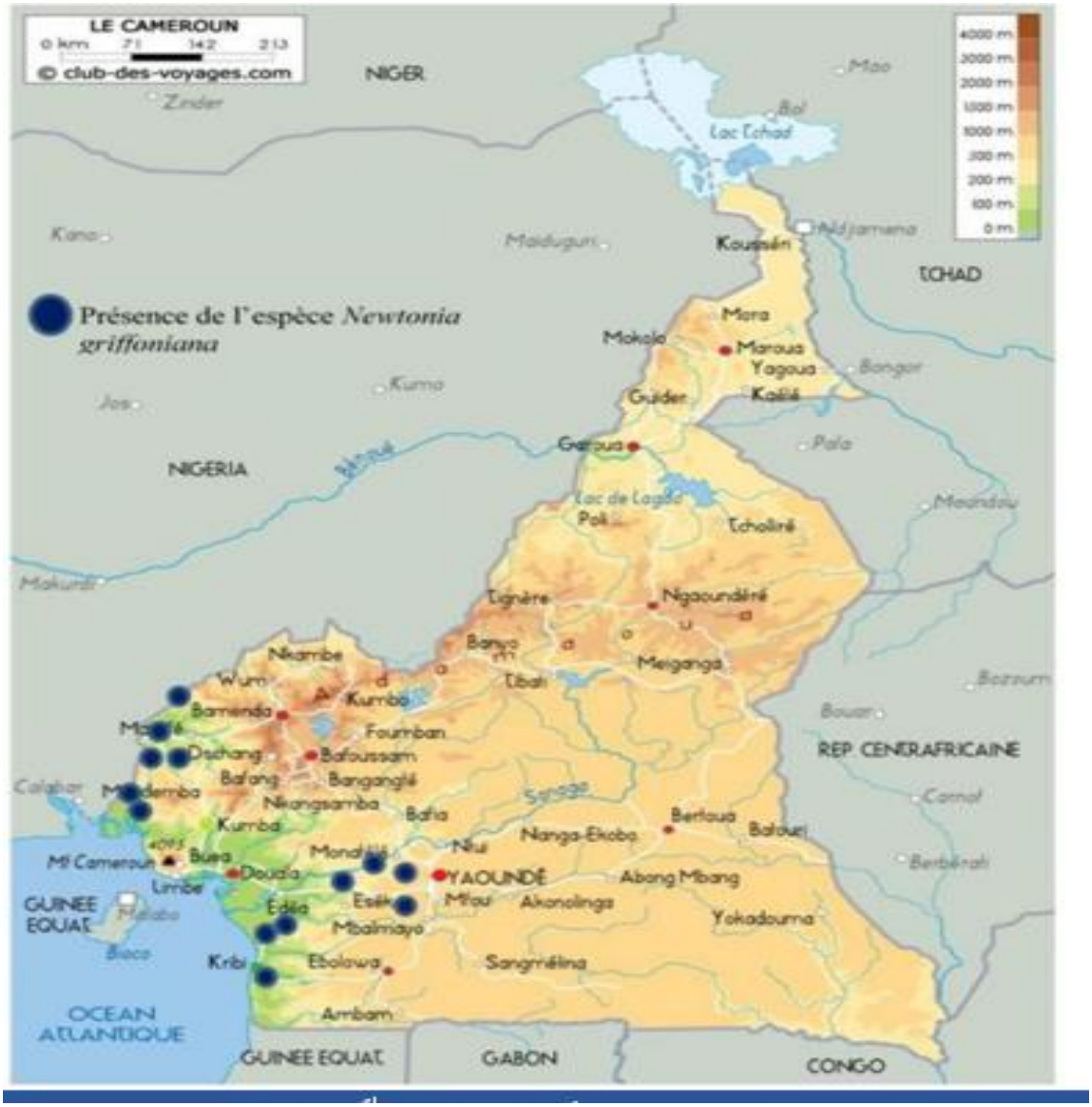


Figure 2: Different localities where *Newtonia griffoniana* was harvested in Cameroon in Cameroon

I-4 Some uses of the *Newtonia* genus

The genus *Newtonia* has not yet been studied widely, but we have some few uses enumerated below.

- **Therapeutic uses**

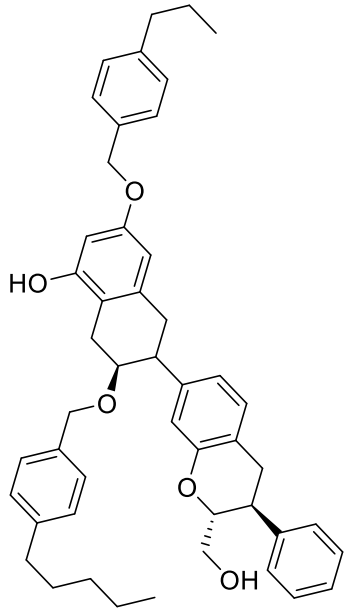
Newtonia giffoniana is a tree from the central African rainforest, whose bark extracts are used in Cameroonian folk medicine for the treatment of anxiety and sleep disorders (**Kinyok *et* al., 2017**). *Newtonia hildebrandtii* and *Newtonia buchananii* are used for the treatment of wounds and skin disorders and for a stomach upset (**Fratkin, 1996; Kariba *et al.*, 2001**). Maceration and infusion of *Newtonia buchanani and Newtonia hilderbrandtii* are used in human and veterinary medicine for the treatment of gastro-intestinal disorders, including diarrhoea (**Katlego *et al.*, 2021).**

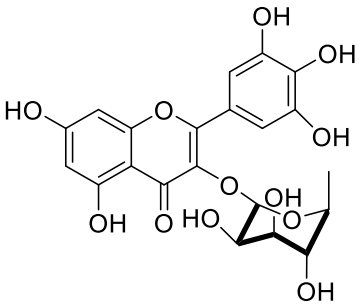
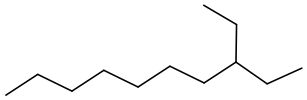
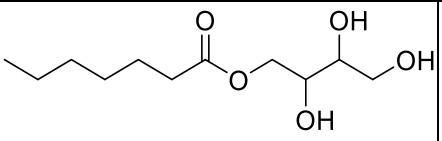
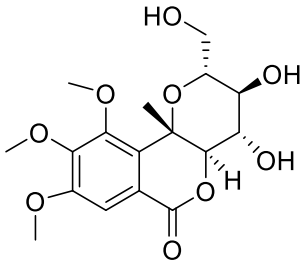
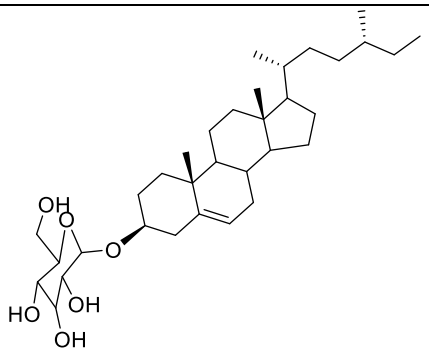
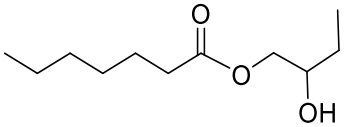
II-Previous chemical and biological studies on the genus Newtonia

II.1. Chemical studies

Some few studies have been done on the genus *Newtonia* leading to the isolation and characterisation of various classes of secondary metabolites (table 3)

Table 3: Structure of compounds isolated from plants of the *Newtonia* genus

Class of metabolites	Structures of compounds	Compound names	Sources	References
Flavonoids	 **1**	(7S)-6-((2S,3S)-3-(hydroxymehyl)-2,3-dihydrobenzy oxy (1,4) dioxin-6-yl)-7-((4-pentylbenzyl)oxy) 5,6,7,8- tetrahydron alphthalen-1-ol	Leaves and stems of *Newtonia griffoniana*	**Kinyok *et al.*, 2017**

	 **2**	Myricetin-3-*O*-rhamnoside	Bark of trunk of *Newtonia hildebrandtti* and *Newtonia Buchananii*	Katlego et al ., 2020
Alcane	 **3**	3-ethylnonane	Leaves and stems of *Newtonia griffoniana*	**kinyok *et al*., 2017**
Erythritol ester	 **4**	Newtonoate		
Isocoumarine	 **5**	Tri-*O*-methylnorbergenin	Leaves and stems of *Newtonia griffoniana*	**kinyok *et al*., 2017**
Saponins	 **6**	3-*O*-*β*-*D* glucopyranoside sitosterol		
Monoglyceride	 **7**	Heptacosan up to 2,3 dihydroxypropyle		

II-2.Previous biological studies on the plants of the genus *Newtonia*

The methanol extract of *Newtonia griffoniana* trunk bark has anxiolytic activity slightly higher than diazepam (100 and 150 mg/Kg BW; and newtonate the major compound also

showed anxiolytic activity (3 and 15 mg /Kg BW) (**Djiogue *et al.*, 2015**). Petroleum ether, dichloromethane and methanol extracts of the trunk bark of *Newtonia hildelbranndtii* presented anti-fungal and anti-bacterial activities (**Katelgo *et al.*, 2020).** *Newtonia buchananii* extracts has promising activity against Gram-positive and Gram-negative bacteria implicated in causing diarrhoea (MIC = 20 - 80 μg/ml). The acetone leaf extract was subjected to bioassayguided fractionation to isolate and characterize bioactive compounds using chromatographic techniques, NMR spectroscopy and mass spectrometry. Two isolated flavonoids(1,2) have good antibacterial activity (MIC = 62.5 to 250 μg/mL). Compound **2** (myricetin-3-*O*-rhamnoside) was evaluated for antiviral activity and mechanism of action against H1N1 *Influenza* virus as a model organism. It was effective against *Influenza* virus in co-penetration combined treatment, thereby confirming the efficacy of this antiviral compound against viral attachment **(Motlhatlego *et al.*, 2018**). *Newtonia buchananii* and *Newtonia hildebrandtii* were selected based on good preliminary antimicrobial activity and low cytotoxicity to evaluate their efficacy against diarrhoea by evaluating antimicrobial activities and safety of the leaf, stem and seed extracts *Newtonia buchananii* showed strong antimicrobial effect against *Pseudomonas aeruginosa* with MIC=20 μg/mL and moderate activity of 40 μg/mL against *Bacillus cereus* while *Newtonia hildebrandtii* had the highest antibacterial activity against *Bacillus cereus* with MIC=80 μg/mL. The extracts were relatively non-toxic with IC_{50} values of 30-750 μg/mL. Selectivity index values as high as 25 were reached. The acetone extracts of both *Newtonia buchananii* and *Newtonia hildebrandtii* had good antimicrobial activity against a range of bacteria and fungi strains implicated in diarrhoea (**Motlhatlego *et al.*, 2017).**

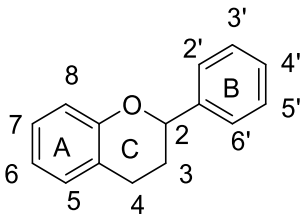
To the best of our knowledge, very few chemical studies have been performed on the genus *Newtonia*. However, several studies done on the plants belonging to the Fabaceae family led to the isolation and characterization of some secondary metabolites belonging to several classes. The most common of which are flavonoids (**Salwa *et al.*, 2001**), terpenoids (monoterpenes, diterpenes, triterpenes, sesquiterpenes), saponins, steroids (**Muhammad *et al.*, 2001**), alkaloids (**Huxtable, 1990, Keeler, 1989**), and coumarins (**Estévez-Braun and Gonnzalles, 1997**).

II-3. Chemical studies on the Fabaceae family

II .3.1.Flavonoids

II.3.1.1.Generalities

The term flavonoids refers to a very wide range of natural compounds belonging to the polyphenol compounds (**Lee *et al*., 2004**). They are considered as almost universal plant pigments. These various substances are found at the same time in free form or in the form of glycosides. They are found in a very general way in all vascular plants where they can be localized in various organs: roots, stems, woods, leaves, flowers and fruits (**Marfak, 2003**). Flavonoids including: flavones, flavanols, flavanones, flavanonols and anthocyanins (**Lee *et al*., 2004**).The basic structure of flavonoid is showed by structure 8 **(Balasundram *et al*., 2006).**



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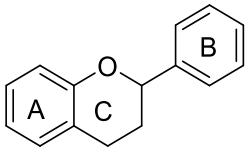
II-3.1.2. Flavonoids classification and biosynthesis

a. Classification of flavonoids

The term flavonoid encompasses a very wide range of natural polyphenolic compounds. It is the structure of the central heterocycle and its degree of oxidation which makes it possible to distinguish the different classes of flavonoids (**Marais *et al*., 2007**). Structurally, flavonoids fall into several classes of molecules including flavanones, flavones, flavanols, dihydroflavonols, isoflavones, chalcones, aurones, anthocyanins and tannins (**Heller *et* Forkman, 1993**). Flavonoids are classified according to several criteria which include the presence or not of a double bond in position 2 and the presence or absence of hydroxyl group in position 3 and also the location of the hydroxyl groups most often in the 2', 3', 4' and 5'positions as well as in position 5 and 7. They contain several classes amongst which : isoflavones which comes from a transposition of an aromatic nucleus (from C-2 carbon to C-3 carbon); the flavones and flavanols of an oxidation (formation of the double bond on ring C) respectively of the flavanones and dihydroflavonols **(Marfak, 2003**).



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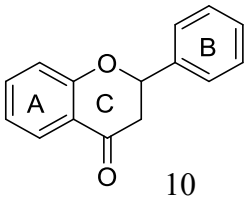
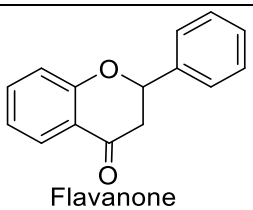
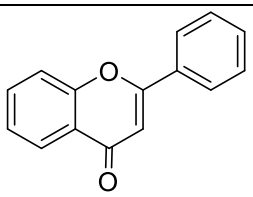
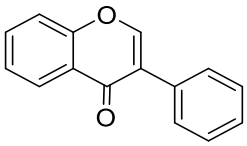
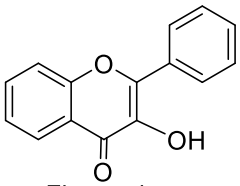
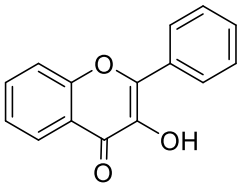
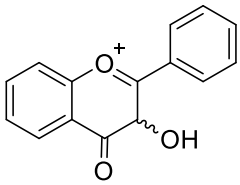
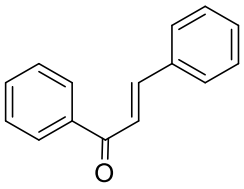
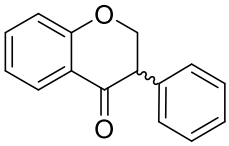
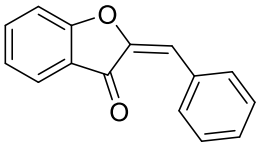


10

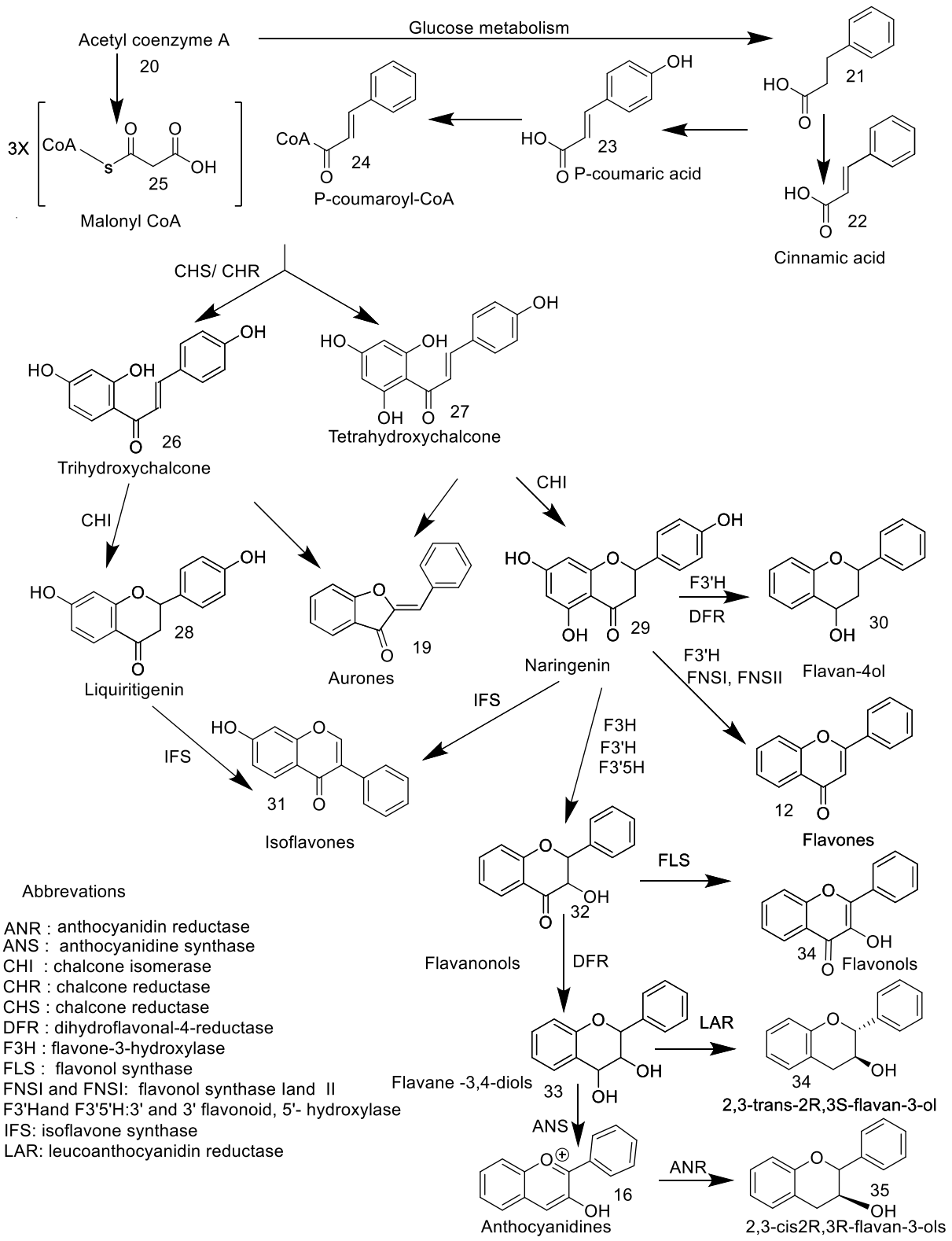
Table 4 presents the different sub-classes of flavonoids.

Table 4 : Different sub-classes of flavonoids

 Flavanone **11**	 Flavone **12**	 Isoflavone **13**
 Flavonol **14**	 Dihydroflavonol **15**	 Anthocyanidine **16**
 Chalcone **17**	 Isoflavanone **18**	 Aurones **19**

b. Biosynthesis of flavonoids

Flavonoids represent a large group of natural compounds that are important because of their structural diversities, their physiological properties and their mixed biosynthetic origin (**Yong, 1992**). They come from the condensation of cinnamate Coenzyme A and malonate Coenzyme according to two pathways: the polymalonic acetic and the shikimic pathway. The product of the reaction is chalcone, precursor of all classes of flavonoids (**Marais *et al.*, 2007**).This chalcone is metabolized into different classes of flavonoids (**Yong, 1992; Watchueng, 2004**). Compounds of each subclass are distinguished by the position and nature of substituents. **Scheme 1** shows the biosynthesis of the different classes of flavonoids.

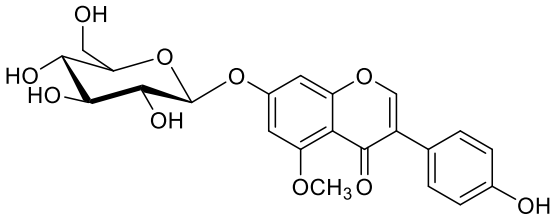
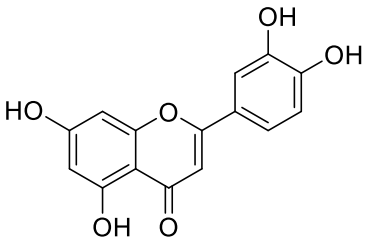
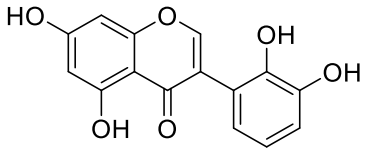
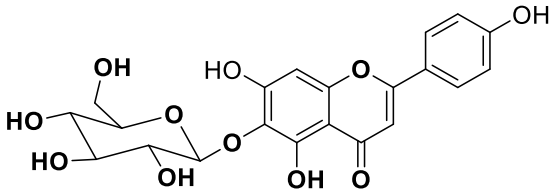
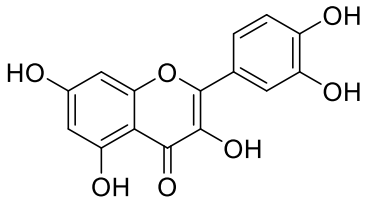


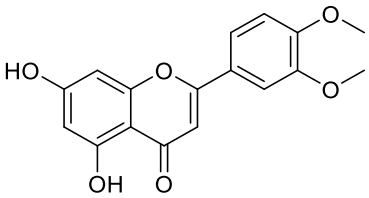
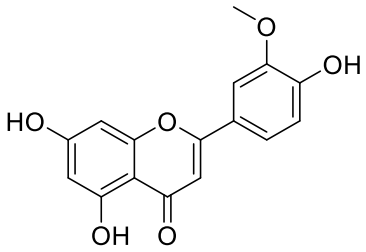
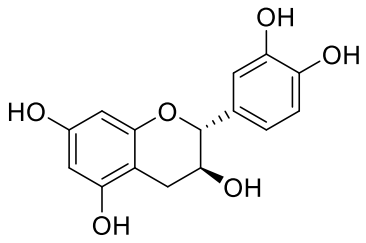
Scheme 1: Biosynthesis of flavonoids (Marais et al., 2007).

II.3.1.3. Structure of some flavonoids isolated from Fabaceae family

Flavonoids isolated from the some plants of the Fabaceae family (**table 5).**

Table 5: Structures of some flavonoids isolated from Fabaceae family

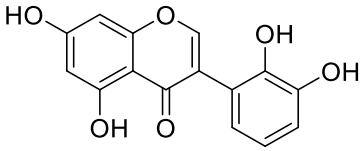
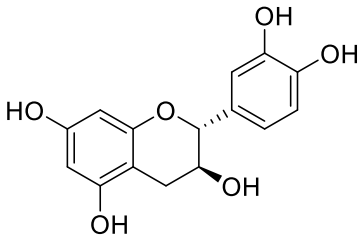
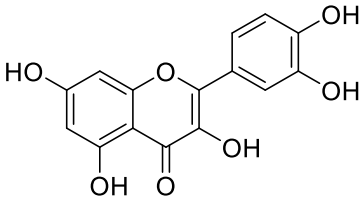
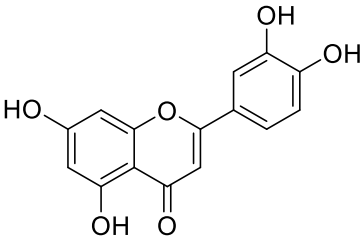
Structures of compounds	Compound names	Sources	References
 **36**	7-*O*-*β*-D glucopyranoside genistein	*Genista morisii*	**Galchi *et al*., 2002**
 **37**	Luteolin		
 **38**	Eriodictyol		
 **39**	Isovitexin	*Vepris grandifolia*	
 **40**	Quercetin	*Tabem aemontana*	

 **41**	5,7-dihydroxy 3',4'-dimethoxy flavone		
 **42**	Chysoeriol	*Ajuga iva*	**Ghéddira *et al.*, 1991**
 **43**	Catechin	Leaves of *Annona muricata*	**Nawwar *et al.*, 2012**

II.3.1.4. Pharmacological properties of flavonoids

Flavonoids constitute a group of very important substances which are of great interest in many fields. Commonly used in the pharmaceutical industry, they are endowed with estrogenic, antiallergic, anti-radical, analgesic, antiplasmodial, anti thrombic, hepatoprotective properties (**Williams *et al.*, 1995**). Flavonoid drugs are used in the treatment of cramps, capillary fragility disorders, cancer (**Ango, 2006).** Below are biological activities of some flavonoids in **Table 6.**

Table 6: Biological activities of some flavonoids.

Compounds	Biological activities	References
 **38**	Isoflavones widely spread in vegetables potray analgesic, anti-inflammatory, antipyretic and anti-tumor properties.	**Pandey *et al.*, 2009**
 **43**	This compound is found in tea leaves and is responsible for their antioxidant and anticancerogenic properties	**Ahmad *et al.*, 1997**
 **40**	It prevents the oxidation of lipids, cardiovascular diseases. Increases mucus secretion from gastric cells so as to protect cells against low stomach pH	**James *et al.*, 1994**
 **37**	This compound has the ability to induce apoptosis, to prevent carcinogenesis in animal models, to reduce tumor growth *in vivo* and to sensitize tumor cells to the cytotoxic effects of some anticancer drugs suggest that this flavonoids has cancer chemopreventive and chemotherapeutic potential. It also presents antioxidant, antimicrobial and anti - inflammatory activities.	**Lopez *et al.*, 2009**.

II.3.1.5. Structural elucitdation of flavonoids

The structural elucidation of flavonoids is essentially based on the different spectroscopic techniques such as one (^{1}H, ^{13}C, DEPT), and two dimensional (COSY, HMBC, HSQC) Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS) with different types of ionization including, Electron Impact (EI), Chemical Ionization (CI) and Accelerated Atom Bombardment (FAB) as well as Infrared Spectroscopy (IR) and Ultraviolet Visible Spectroscopy (UV/VIS) which remain methods of choice for this type of compound (**Mabry *et***

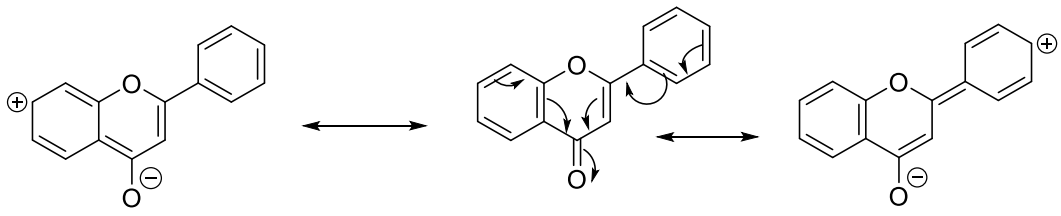
***al.*, 1970; Harborne, 1966**). These methods give important indications on the nature of flavonoids and its mode of fragmentation. However, fluorescence under UV light and their Rf values in different solvent systems give useful information.

Infared Spectroscopy (IR)

Infrared spectroscopy compared to other techniques presents little informations but is useful for the elucidation of the structure of flavonoids. In fact, most flavones, isoflavones and hydroxylated chalcones or dihydrochalcones exhibit a maximum broad band absorption between 3200 and 3500 cm^{-1} due to hydroxyl groups. In addition, an intense band absorption characteristic for the carbonyl group of these compounds is observed around 1680 cm^{-1} and is moved to 1620-3500 cm^{-1} when the carbonyl group is chelated with a hydroxyl group. On the IR spectrum of flavonoids an absorption band is also observed around 1600 cm^{-1} because of the aromatic double bonds **(Markham, 1982).**

- **Absorption of UV radiation**

The action of flavonoids in plants results in part from their flittering effect and from their strong absorption in the UV spectrum **(Harbone and Williams, 2000; Kong *et al*., 2003).** UV spectra of flavonoids exhibit two main absorptions bands in the 240-400nm region. The band I (300-395 nm) is considered as being associated with the absorption of the cinnamoyl part (nucleus B) of the flavonoid and the band II (240-280 nm) to that of the benzoyl part. Several factors can affect the absorption spectrum and the extinction coefficient in flavonoid. The increase in the number of hydroxyls on the aglycone part causes a bathochromic shift of the absorption bands towards higher large values **(Harbone and Williams, 2000).**



44 **45** **46**

240-295nm (bande II) benzoyle Chromophore

300-340 nm (bande I) cinnamoyle chromophore

Scheme 2: UV values of the maximum absorption bands taken in methanol or ethanol of flavonoids.

Table 7 illustrates the relationship between the absorptions and the type of flavonoids.

Table 7: Relationship between maximum UV absorption and type of flavonoids (Markham and Geiger., 1966).

Class of flavonoids	Absorption UV-Visible Spectrum	
	BAND II	BANDI
Flavonol	250-280	330-385
Flavone	250-280	310 -350
Flavanone and dihydroflavanol	275-295	300-330
Flavanol	270-280	
Chalcone	230-270	340-390
Aurone	230-270	380-430
Anthocyane	270-280	465-560
Isoflavone	245-275	310-330

In the absence of hydroxyl in position 3, in the case of the flavones, the wavelength of the band I is 20 to 30 nm shorter. Both methylation and glycosylation in particular on the hydroxyls in position 3,5,7,4', causes a hypsochrome displacement towards shorter wavelengths. However, the nature of the sugar generally has no effect (**Markham and Geiger, 1993**). For acylation, few data exist on the effect of this reaction on the spectral properties of flavonoids.

- **Mass spectrometry**

Mass spectroscopy is a technique that allows the determination of the molecular mass of the aglycone as well as the number and nature of the hydroxyl or methoxyls substituents (**Nielsen and Moller, 1970**). Breaks of chemical bonds within the molecular on gives characteristics fragment ions. These provide useful information in particular in the substitution of the nuclei A and B (**Hvattum and Ekeberg, 2003**). Different ionization techniques exist; the most recent of which are gentle ionization techniques which are :electron spray ionization (ESI), atmospheric pressure chemical ionization (APCI), electronic impact ionization (IE), Higher Resolution Ionization Electronics (HRIE), Fast Atom Bombardement (FAB). In this last techniques, the molecular ion is not always observable. We usually observe the ion corresponding to the pseudo molecular ion $[M+H]^+$. Other ions can be formed when there are salt impurities or by addition of sodium chloride NaCl (we obtain the sodium adduct ion $[M+Na]^+$) or potassium adduct (we obtain the ion $[M+K]^+$). This information makes possible to deduce the molecular weight of the compound studied. Flavonoids have been widely studied

in Mass Spectrometry in particular the glycosylated derivatives (**Hvattum and Ekeberg, 2003).**

- **Nuclear magnetic resonance spectroscopy (NMR)**

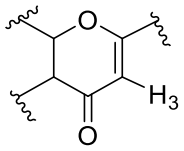
Nuclear magnetic resonance spectroscopy finds great use in determination of flavonoid structures (**Markham and Geiger., 1993).** It's a precise and effective method but require a large quantity of the product which limits its use in relation to other analytical methods.

- **Proton Nuclear Magnetic Resonance spectroscopy (^{1}H NMR)**

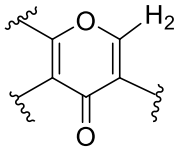
It facilitates the structural analysis of organic compounds as well as those of macromolecules. For each class of flavonoids the characteristic value of spectral parameters are noted , this makes it possible to define the ranges of chemical shifts and coupling constants. These values depend on the type of cycle A, B, C.

Cycle A exhibit mostly meta coupled proton system appearing between 6.0 and 6.9 ppm as well as chelated hydroxyl between 12.5 and 13.8 *ppm* (**Mabry *et al.*, 1970**). Cycle B on the other hand presents ortho and meta coupled proton system, H_3' and $H_{5'}$. Proton signals are generally shielded and appear between 6.1 and 6.7 *ppm* while those of $H_{2'}$ and $H_{6'}$ protons are unshielded and appear between 6.7 and 7.9 *ppm* .In addition, the substitution are mostly made on the $C_{3'}$ carbons, $C_{4'}$ and $C_{5'}$(**Mabry *et al.*, 1970**).

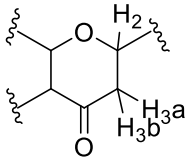
C-ring proton signals of flavonoids (**Table 8**) appear in various chemical shift ranges depending on the type of substitutions



Flavone



Isoflavone

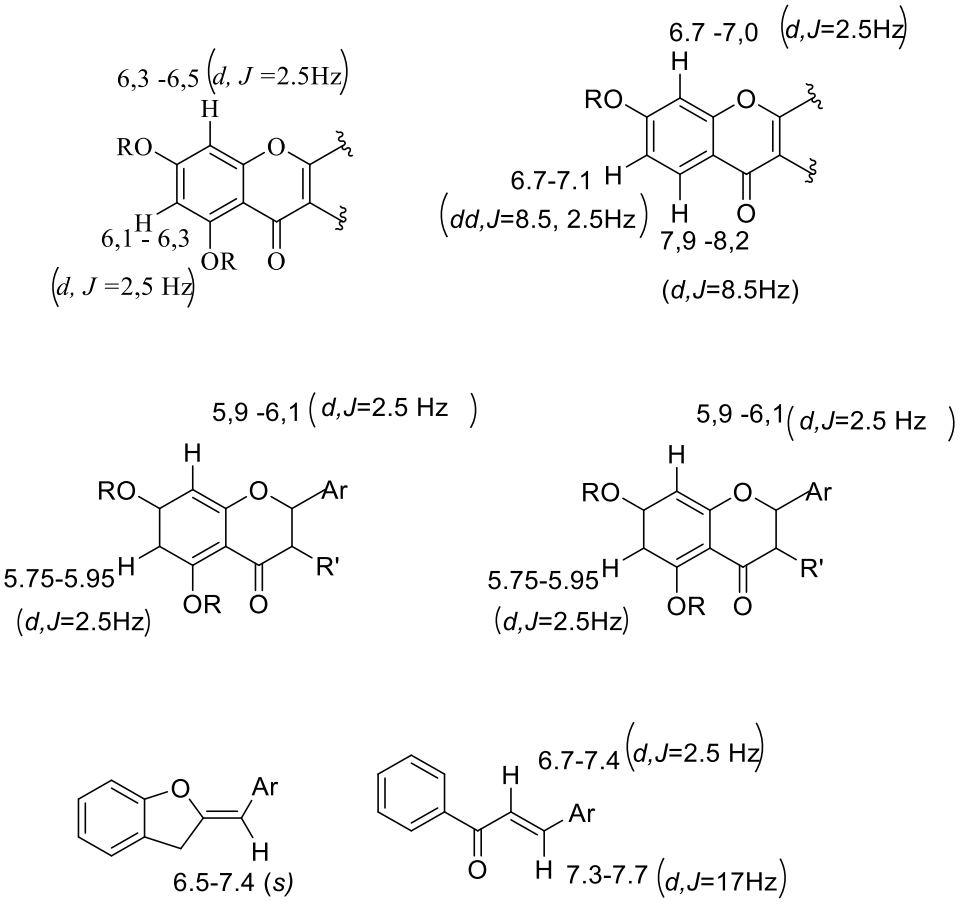


Flavanone

Table 8: ^{1}H NMR of the C ring of flavonoids

Type of flavonoid	H2	H3
Flavanones	5.1 -5.5 (*dd, J* =3. 12 Hz)	2.5-2.8(*dd, J* =17.3 Hz)
Dihydroflavonols	4.9 -5.1 (*d, J* =11 Hz)	4.4-4.6(*d, J* =11 Hz)
Flavones		6.3-6.7(*s*)
Isoflavanones	4.5 (*dd, J* =5-6,11-12 Hz) 4.4 (*dd, J* =5-6,11-12 Hz)	4.1(*dd, J* =5-6,11-12 Hz)
Isoflavones	7.6-7.9 (*s)*	

The chemical shifts of methoxyl protons depends on their position. They appear between 3.7- 4.0 *ppm*. The A-ring protons usually appear in the strong fields compared to cycle B. Proton H_3 in flavones gives singlet around 6.3 *ppm* which overlaps with the chemical shifts of the H_6 and H_8. In Flavones 5,7-dioxides, H_6 and H_8 appear as a doublet with a meta coupling constant of 2.5 H_Z and can be easily distinguished in DMSO- d_6.The benzylic proton of the aurones appears between 6.37 and 6.94 *ppm*, the exact position depends on its oxidation degree.



Scheme 3: Some ^{1}HNMR data of flavonoids

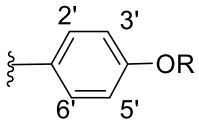
The 4'-substituted (oxygenated) flavonoids yield a pair of doublets with a coupling constant of *J* =8.5Hz,for protons of ring B.

Table 9 gives the chemical shift of ring B of 4'-oxygenated flavonoid characteristics protons.

Table 9 : ^{1}HNMR data of ring B protons 4'-oxygenated flavonoids

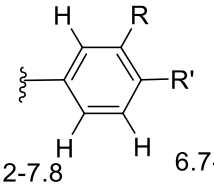
Class	H2'/H6'	H3'/H5'
Flavanone	7.1 -7.3	6.5 -7.1
Dihydrofavonols	7.2-7.4	6.5- 7.1
Isoflavones	7.2-7.5	6.5-7.1
Chalcones	7.4-7.6	6.5-7.1

Aurones	7.6-7.8	6.5-7.1
Flavones	7.7-7.9	6.5-7.1
Flavonols	7.9-8.1	6.5-7.1



3',4'-disubstituted flavonoids give an ABC system for B-ring protons .

7.3-7.9 (*d, J* = 2.5Hz)



7.2-7.8 (*dd,J* =8.5 and 2.5Hz) 6.7-7.1 (*d, J* =2.5Hz)

In the case of flavones, flavanols and aurones, the $H_{6'}$proton overlaps with the $H_{2'}$proton but is easily distinguished from the coupling constant. In the case of isoflavones, flavones and dihydroflavonols, $H_{2'}$, H_5 and $H_{6'}$ appears as a complex multiplet in the region 6.7-7.1 *ppm*. The 3',4',5'-trioxygenated flavonoids on the other hand reveal the proton $H_{2'}$ and $H_{6'}$ in the form of a singlet of two protons between 6.5-7.5 *ppm* (**Mabry *et al.*,1970**).

- **Carbon nuclear magnetic resonance (^{13}C NMR)**

The ^{13}C NMR of flavonoids is of paramount importance in their structural determination. The chemical shifts of the different types of carbons of a flavonoid (**Dongo, 2001;Ternai *et al.*, 1976**) are listed in **Table 10**.

Table 10 : Chemical shifts (in *ppm*) of the different types of carbon in a flavonoid (Mabry et al., 1970).

Types of carbon	Chemical shift in ppm
Carbonyl (4-Keto,acyl)	210-170
Aromatic and oxygenated ethylenics	165-155(absence of oxygenated substituents in ortho /para) 150-130 (presence of oxygenated substituents ortho/para)

Oxygenated aliphatics : flavanones (C2), flavanonols (C2,C3), isoflavanones(C2), flavanols(C3), isoflavanes(C2)	83-69
Aromatic and non-oxygenated ethylenics	135-125 (absence of oxygenated substituents in ortho/para) 125-90 (presence of oxygenated substituents ortho/para)
Dioxymethylene	104-100
$O\underline{C}H_3$	63-55
$C\text{-}\underline{C}H_3$, $CO\text{-}\underline{C}H_3$	20-17
Prenyl	21(CH_2),122(CH=),131(C=), 18(CH_3)

Moreover, the chemical shifts of the central carbons C_2, C_3, C_4, (**Table 11)** are of a considerable utility for the determination of the class of the flavonoid (**Mabry *et al.*, 1970; Markham, 1982**).

Table 11: Chemical shifts of the central carbons of some flavonoids (Mabry et al., 1970)

Types of flavonoids	C2	C3	C4
Flavones	160.5- 165.5	103.9-112.5	176.3- 183.5
Flavonol free 3-OH 3-OH chelated or glycosylated	145.4- 147.1 151.1 -156.4	136.0-138.1 133.5-141.	172.4- 176.7 173.6 -180.0
Isoflavones	149.8 -155.4	119.3-125.9	174.5-180.6
Dihydroflavonols	78.3 - 83.6	70.9-72.5	189.7-198.4
Flavanones	75.0 -83.0	42.0-44.8	189.5 -196.7
Isoflavanones	71.5-71.7	50.9-51.5	190.4-190.6
Catechins	78.1-81.2	65.1-66.6	28.0-28.1
Isoflavanes	69.6-70.3	31.4-32.5	30.1-31.5
Chalcones	136.5-145.8	116.6-128.1	188.6-195.0
Aurones	146.1-147.7	116.6-119.9	182.5-182.7

The presence of a substituent on the A or B ring of a flavonoid induces a sensitive variation in chemical shift which not only affects the carbon on which it is fixed but also the

neighbouring carbons (ortho, meta, para). We can thus distinguish from the ^{13}C NMR spectrum a meta-dihydroxylated flavonoid from its ortho dihydroxylated isomer.

In meta dihdroxylated flavones, flavanones, isoflavones, isoflavanones on ring B the two aromatic carbon bearing hydroxyl groups resonate between 151-159*ppm* while with the ortho isomer, the two carbons resonate between 141-149 *ppm*. Most natural flavonoids are hydroxylated at C_5, C_7, $C_{3'}$, $C_{4'}$or $C_{5'}$ due to biogenetic reasons (**Mabry *et al*.,1970**)

II.3.2. Triterpenes

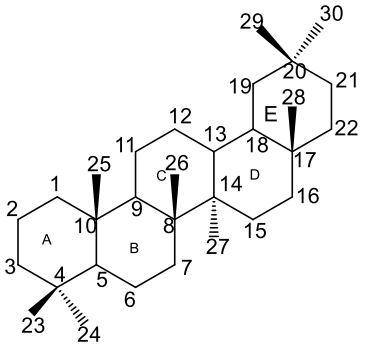
II.3.2.1. Generalities

Triterpenes are a class of secondary metabolites composed of 30 carbons atoms. At the biosynthetic level, the 200 different skeletons known to date and isolated from natural sources come from the cyclization of 3S-2,3-epoxysqualene. When the cyclization is incomplete, we obtain monocyclic terpenes, bicyclic or tetracyclic triterpenes. When they are complete, we obtain pentacyclic triterpenes which are divided into several groups (**Boiteau *et al*.,1964).**

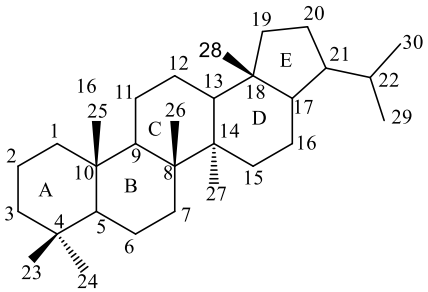
Starting from the general formula we distinguish several forms of the terpenes according to the number of carbon atoms. The unit of numeration is based on the first terpenoid isolated in 1850 which was a C10. Depending on the number of isoprene units that make up the terpenes, we distinguish: hemiterpenes (C-5), monoterpenes (C-10), sesquiterpenes (C-15), diterpenes (C-20), sesterpenes (C-25), triterpenes (C-30), tetraterpene (C-40). Pentacyclic aliphatic (Squalene), tetracycliques triterpenes with pentacyclic triterpenes are composed of six isoprene units arranged either with five rings with six carbon atoms or with five rings with the last ring containing five carbon atom on the first carbon of this alky radical (**Bruneton, 1999**). In our subsequent work, we would base our focus on pentacyclic triterpenes.

II.3.2.2. Nomenclature:numerotation of the carbon chain

The numbering of pentacyclic triterpene begins with the cycle A and goes back towards the cycle E. In the case of compounds containing a ring E with five carbon atoms supporting an alky chain, the numbering continues on the first carbon of this alkyl radical .The numbering of the methyl groups is then done from ring A to ring E and the least number is attributed to the groups in the β. We have as an example the case of oleane and hopane (**Bioteau et *al.*, 1964**).



47



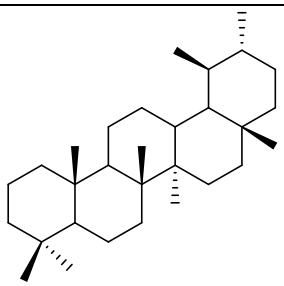
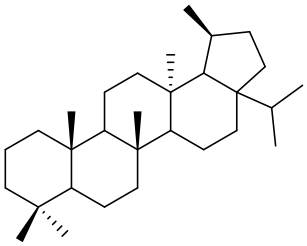
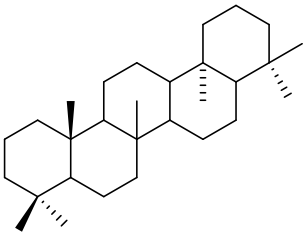
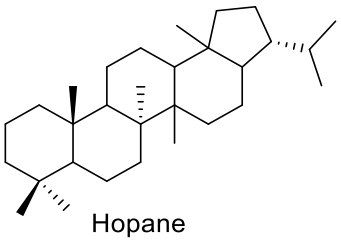
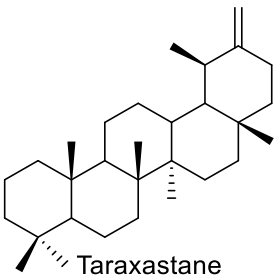
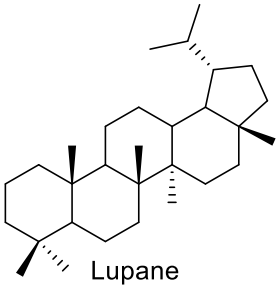
48

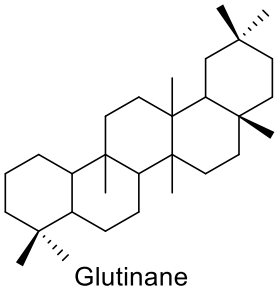
II.3.2.3. Classification and biosynthesis of pentacyclic triterpenes

a. Classification of Pentacyclic triterpenes

Pentacyclic triterpene are classified according to the position occupied by the methyl group as illustrated in table 12

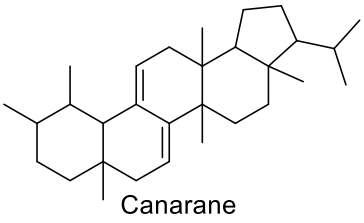
Table 12 : Structure of some types of pentacyclic triterpenes (Wansi, 2000; Wafo *et al.*, 2010)

 Ursane **49**	 Friedomadeirane **50**	 Serratane **51**
 Hopane **52**	 Taraxastane **53**	 Lupane **54**



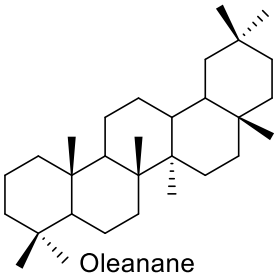
Glutinane

55



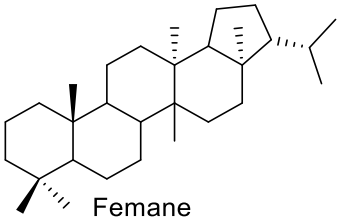
Canarane

56



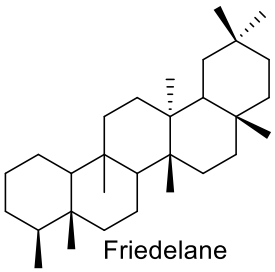
Oleanane

57



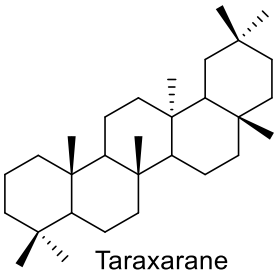
Femane

58



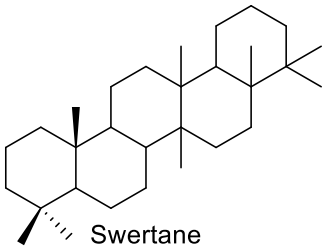
Friedelane

59



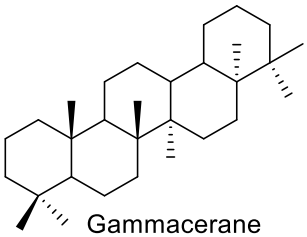
Taraxarane

60



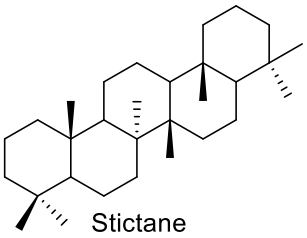
Swertane

61



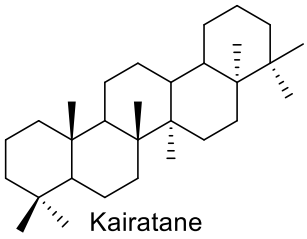
Gammacerane

62



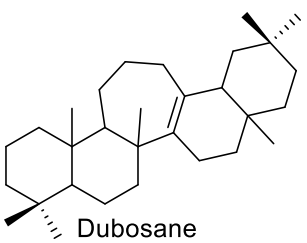
Stictane

63



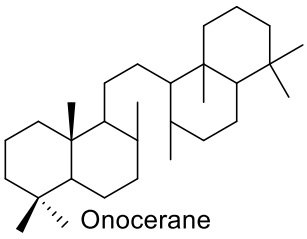
Kairatane

64



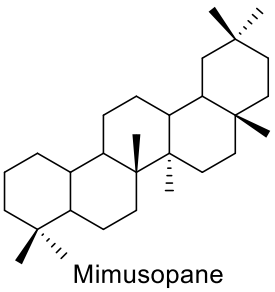
Dubosane

67



Onocerane

65



Mimusopane

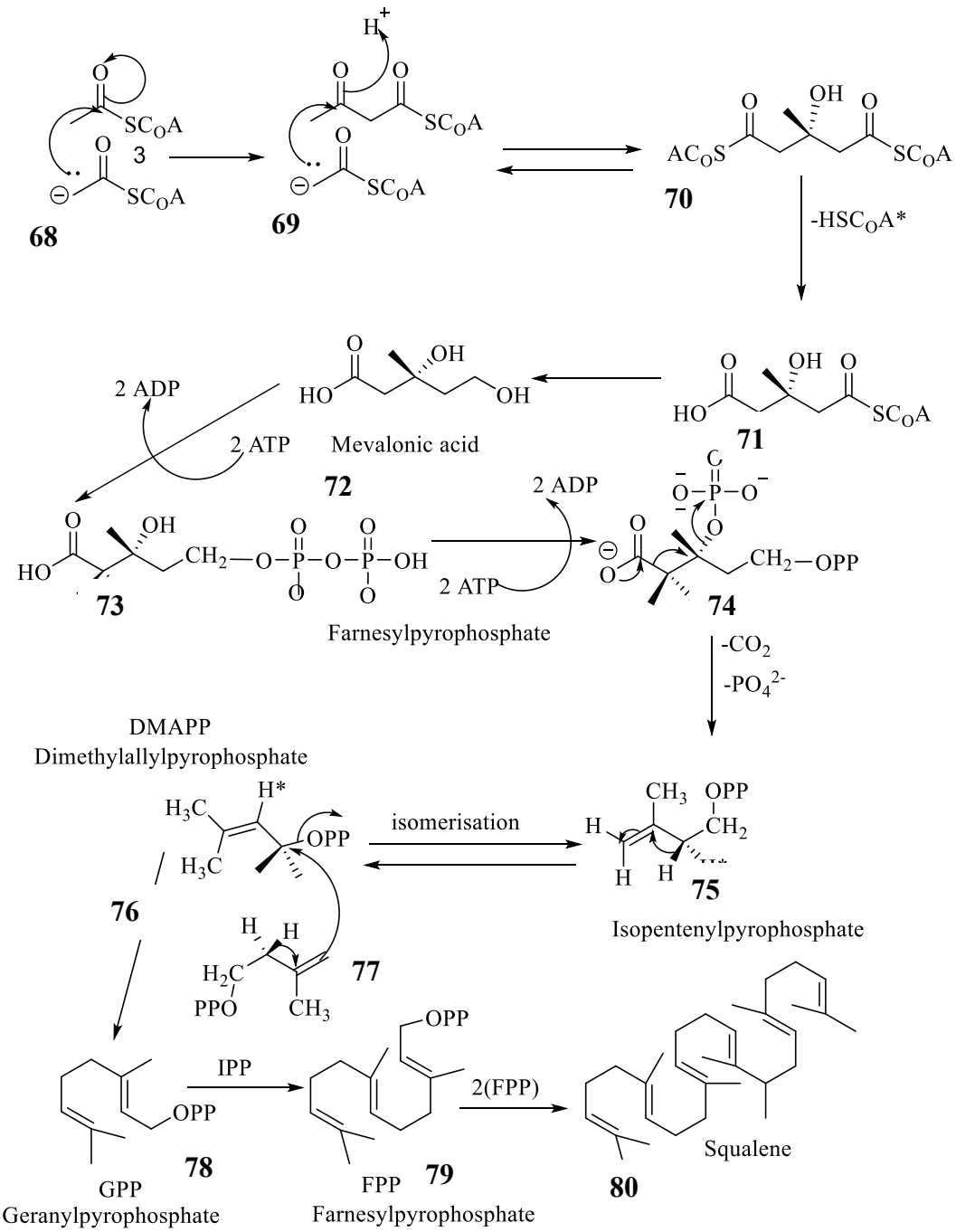
66

b. Biosynthesis

The biosynthesis of pentacyclic triterpenes is done in two stages ;

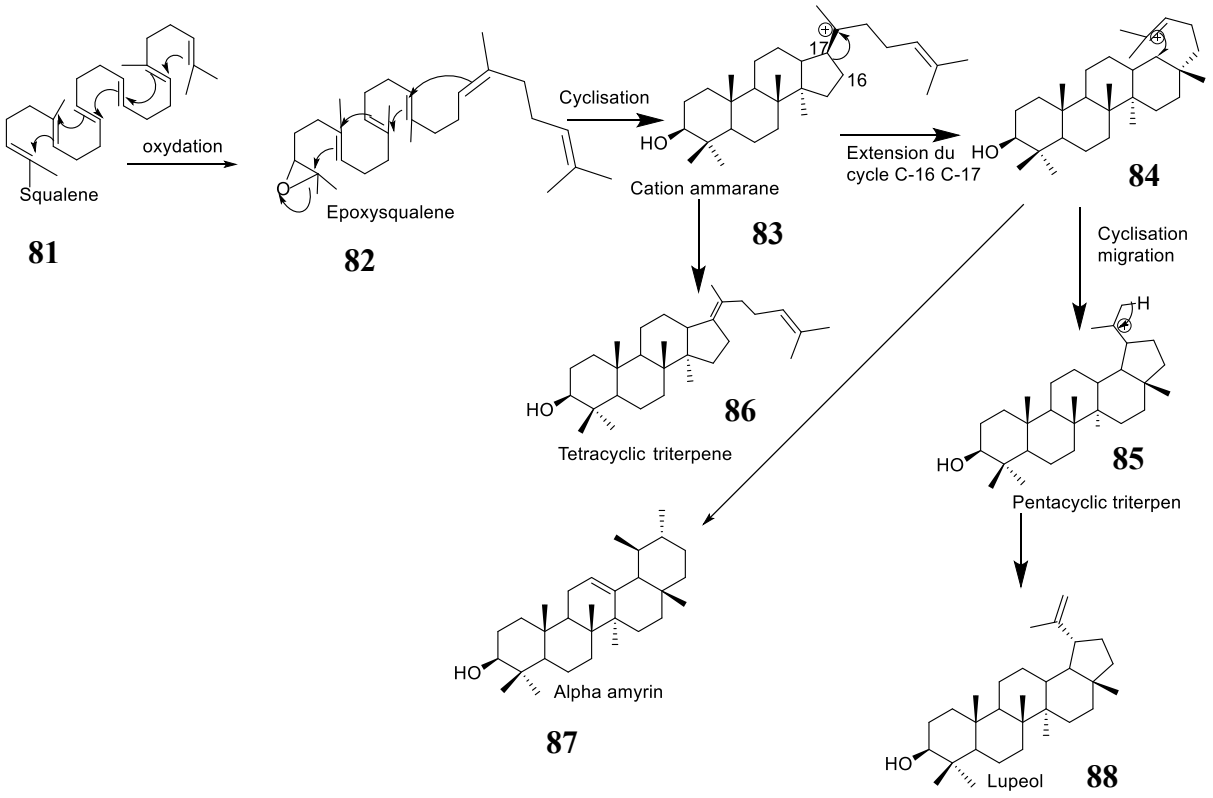
Biosynthesis of Squalene

The biosynthesis of squalene is made by the mevalonic pathway from acetic acid and more precisely from its actif equivalent acetyl coenzyme A. This leads to mevalonic acid through successive aldolisation reactions. This acid undergoes a double phosphorylation in the presence of Adenosin Triphosphate (ATP) to lead to mevalonic acid -5-pyrophosphate which in turn decarboxylase to give a precursor C_5 isopentenyl pyrophosphate (IPP), the pyrophosphate of geranyl and farnesyl which undergoes a dimerization to squalene. Scheme 4 presents the biosynthesis pathway of squalene **(Dewick, 2002).**



Scheme 4 : Biosynthesis of squalene (Dewick, 2002).

Squalene undergoes an oxidation reaction to form epoxysqualene which later undergoes cyclization and other series of reactions which leads to the formation of triterpenes (scheme 5).



Scheme 5 : Biosynthesis of some pentacyclic and tetracyclic triterpenes (Bruneton, 1999).

II.3.2.4. Structures of some pentacyclic triterpenes isolated from plants of the Fabaceae family

Pentacyclic triterpenes are very numerous in the Fabaceae family. **Table 13** presents the structures of some pentacyclic triterpenes isolated from plants of the Fabaceae family.

Table 13 : Structures of some triterpenes isolated from species of the Fabaceae family

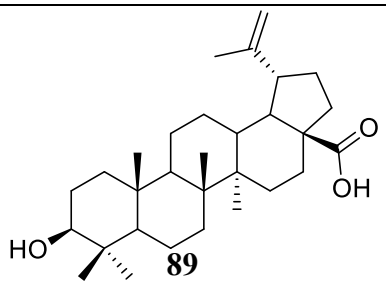
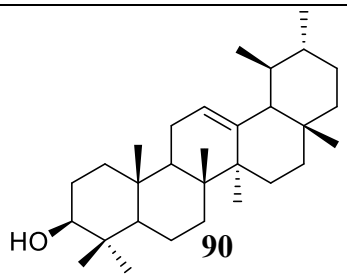
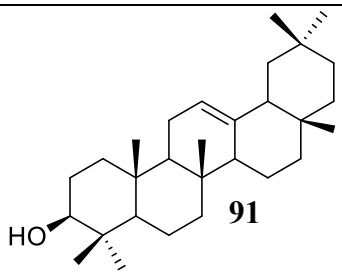
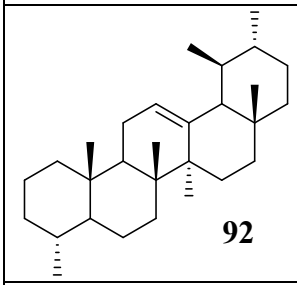
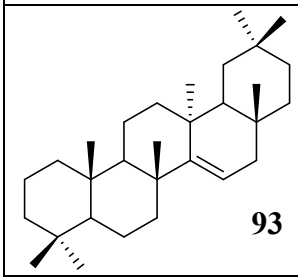
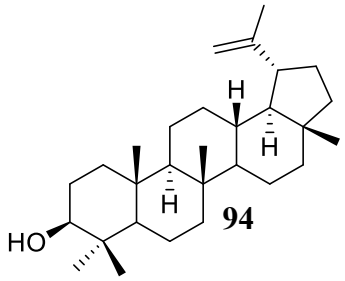
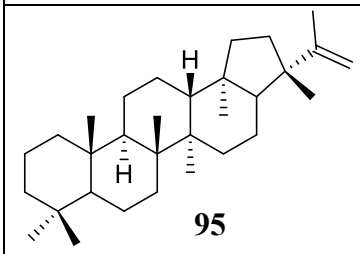
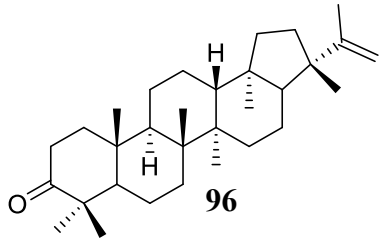
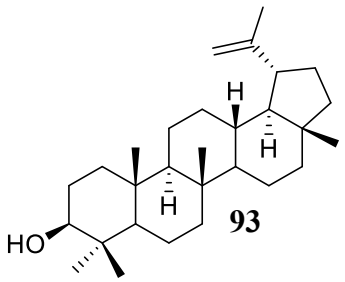
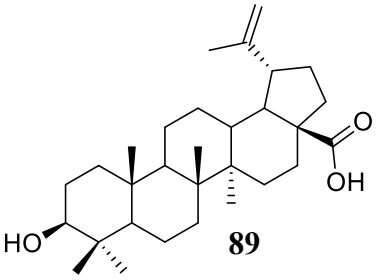
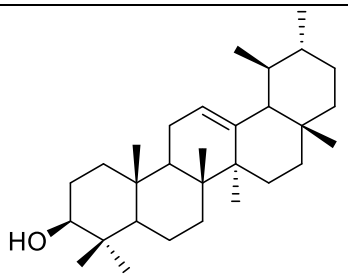
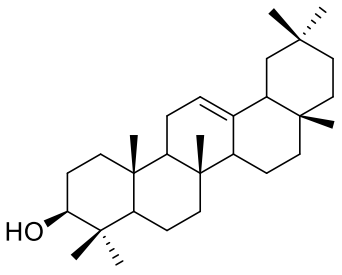
Structures of metabolites	Compounds names	Sources	References
 **89**	Betulinic acid	Roots of *Sesbania grandifolora*	**Noviany and Osman 2021**
 **90**	*α*-amyrin		
 **91**	*β*- amyrin	Leaves of *Astragalus propinqus*	**Venkata *et al* 2013**
 **92**	24-nor-urs-12-ene	*H. Scoparium*	**Rullkotter *et al.*, 1982**
 **93**	Taraxer-14-ene		

Table 13 (continued)			
Structures of metabolites	Compounds names	Sources	References
 **94**	Lupeol	*Machaerium brasiliene*	**Silva *et al.*, 2020**
 **95**	Hopene		**Souza *et al*., 2012**
 **96**	Hopanone		

II.3.2.5. Biological properties of triterpenes

Triterpenoids and triterpene glycosides are the basis for the synthesis of several contraceptives and anti-inflammatories. They are also essential in the pharmaceutical industry since they are used as raw materials. They are also endowed with anti-tumor properties (**Mercer *et al* ., 1976)**. The activities of some triterepenes are listed in **Table 14**.

Table 14 : Biological activities of some pentacyclic triterpenes

Compounds	Biological activities	References
 **93**	Has a great potential in the prevention and treatment of cancers including liver cancer, lung cancer, colorectal cancer, bladder cancer, and osteorsarcoma, protection of the heart, liver, and skin.	**Vijay, 2014**
 **89**	antibacterial, antiviral , antimalarial effects	**Yogeeswari *et al.*, 2005**
 **90**	Presents antimicrobial, antifungal and anti-inflammatory activities	**Carretero *et al.*, 2008**
 **91**		

II.3.2.6. Structural analysis of pentacyclic triterpenes

Given the considerable biological Importance of triterpenes, their separation, identification and quantification are done by the combined use of several spectroscopic techniques.

- **Infrared (IR) spectroscopy**

IR spectroscopy is mainly used to determine the type of functional groups, the modes of substitution of the aromatic nuclei. All functional groups such as carbonyls, phenolic hydroxyls, phenyls possess corresponding IR absorptions. With regard to triterpenes, the absorption band of hydroxyl groups appear in the region 3200-3300 cm^{-1}. Carbonyl groups are in the region 1700-1750 cm^{-1} (**Manguro *et* Wagai, 2016**).

- **Proton Nuclear Magnetic Resonance (^{1}H NMR)**

^{1}H NMR compared to ^{13}C NMR is more sensitive. The proton is present at 99.9% in nature while the natural abundance of ^{13}C is only at 1.1%. All the organic metabolites that contain protons are likely to give signals.

The ^{1}H NMR spectra of pentacyclic triterpenes are very complex and difficult to interpret. This is essentially due to the presence of several groups of methynes, methylenes and methyls in strong fields, region between 0.5 and 2 *ppm*. However, the methyl signals are easily discernible because they appear in the form of intense doublets or singlets depending on the case. Chemical shifts of methyl greater than 1.5 *ppm* are often indicative of methyl bound to an olefinic double bond and those bond to an heteroatom appear above 2.80 *ppm* (**Wang *et al.*, 2015**).

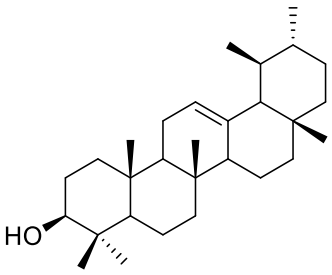
- **Carbon Nuclear Magnetic Resonance (^{13}C NMR)**

It provides useful information and necessary information to better identify the molecule. Generally, this technique makes it possible to highlight all the carbons of the molecule. The analysis is based on observed chemical shifts as a function of environment of each carbon atom (**Benguerba, 2008**). In fact, it allows the possibility to distinguish the different types of triterpenes skeletons. So for the series of taraxeranes, the double bond is positioned on the carbons C_{14} and C_{15} which resonate around 158.1 and 117.0 *ppm* respectively for taxerol. Otherwise for the olean-12-ene series the double bond is on the carbon C_{12} and C_{13} whose chemical shift are around 122 and 145 ppm respectively (**Mahato and kundu, 1994**) while for those in urs-12-ene, these carbons resonate at 124.5 ppm and 138 ppm respectively. For lup-20(29)-enes, the carbon C_{20} and C_{29} resonate at 150.0 and 109.0 *ppm* respectively (**Culioli *et al.*, 2003**). The carbonyl, carboxylic acids and alcohol groups exhibit signals on the ^{13}C NMR

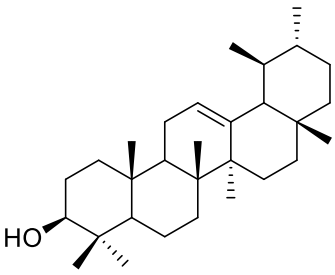
around (210 *ppm* for a carbonyl, 170 *ppm* for a carboxylic acid and 70 *ppm* for an alcohol) (**Hamimed, 2009**). The chemical shifts of some triterpenes derivatives are presented in table 15

Table 15 : Chemical shifts (in ppm) of some specific carbons of some triterpene derivatives (Kapche, 2000).

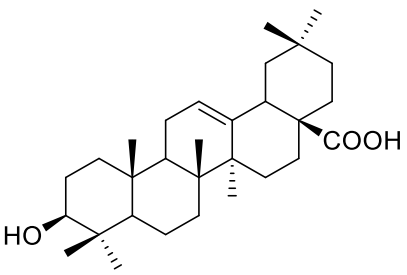
Triterpene derivatives	*β*-amyrin (91)	Oleanolic Acid (89)	Urs-12-ene (92)	α- amyrin (90)	Lup-20(29)-en (98)	Betulinic Acid (89)
C2	27.4	27.3	52.5	27.2	27.4	27.6
C3	78.7	79.0	46.2	78.8	47.7	78.9
C4	38.7	38.7	43.6	38.7	44.8	39.0
C12	122.7	121.7	124.3	124.3	25.1	25.6
C13	143.4	145.0	139.3	139.3	38.6	38.2
C16	23.4	27.0	26.6	26.6	35.6	32.6
C17	46.6	32.5	33.7	33.7	42.9	56.3
C18	41.3	47.2	58.9	58.9	48.2	47.1
C20	30.6	31.1	39.6	39.6	47.9	49.2
C27	26.0	26.0	23.3	23.3	14.5	14.7
C28	181.0	28.4	28.1	28.1	18.0	178.9
C29	33.2	33.2	23.3	17.4	109.2	109.4
C30	23.6	23.6	21.3	21.3	19.3	19.4



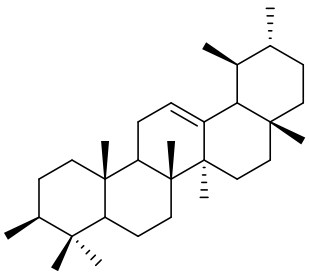
B-amyrin (**91**)



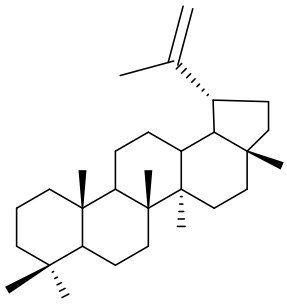
α-amyrine (**89**)



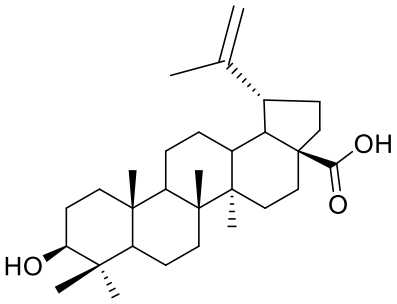
Oleanolic acid (**97**)



Urs-12-ene (**92**)

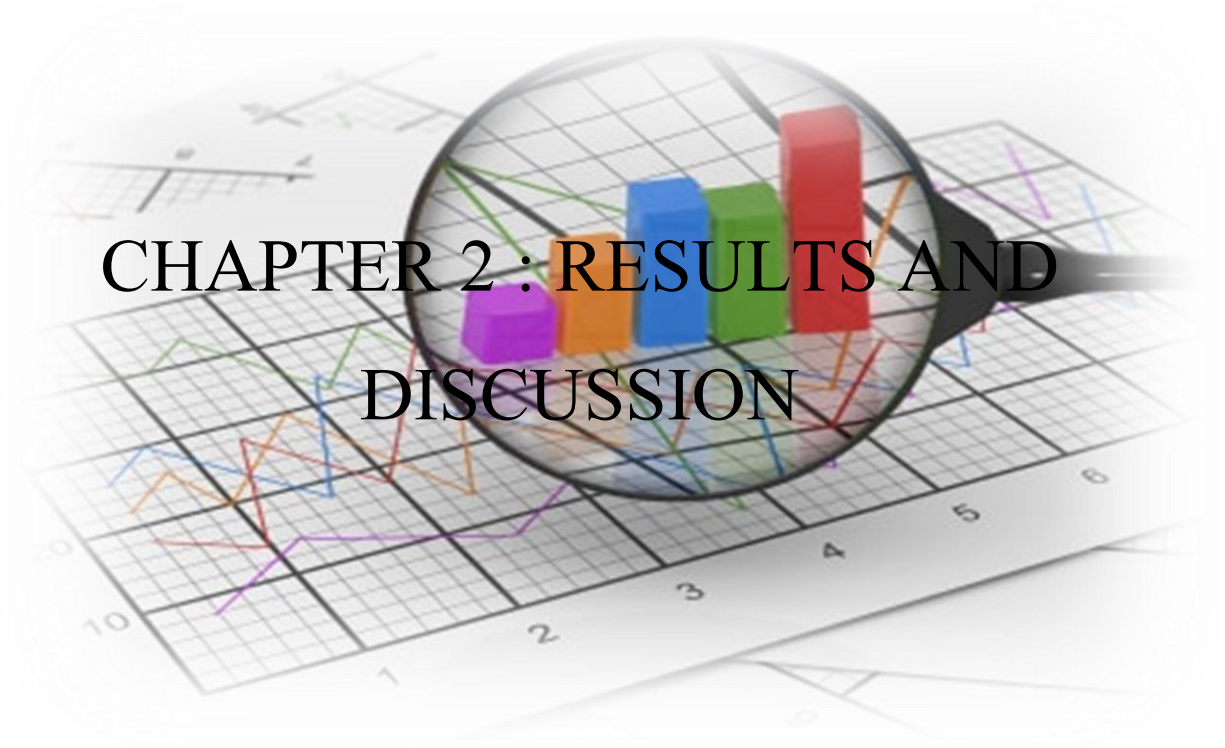


Lup-20(**29**)-ene (**98**)



Betulinic acid (**89**)

Given the wide uses of the plant of the same family as well as *N. griffonania*, and also their diverse pharmacological properties, we undertook the chemical study of *N. griffoniana* . This led to some results which are going to be presented and discussed in chapter 2

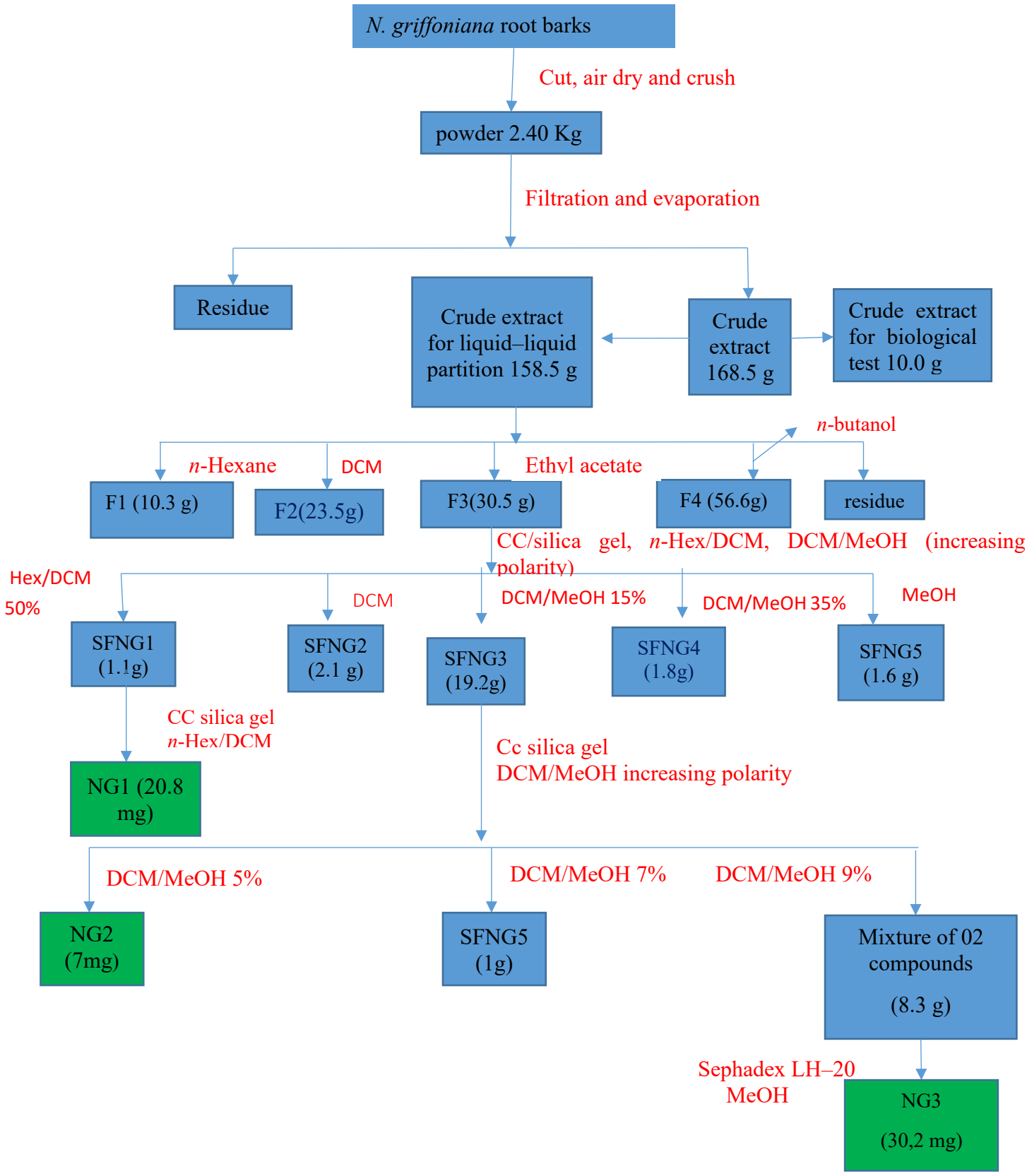


CHAPTER 2 : RESULTS AND DISCUSSION

INTRODUCTION

The root barks of *Newtonia griffoniana* were harvested on December 14th 2021 in the west region of Cameroon precisely in the village Batoufam. The identification of the collected plant material was done by Mr NANA Victor a retired botanist at the National Herbarium of Cameroon.

After cutting, drying and crushing, the root barks powder gave 2.04 Kg which was then extracted by maceration in methanol for 72 hours at room temperature. The solution obtained was filtered and concentrated under reduced pressure using a rota–vapor device which allowed us to obtain 168.5 g of brown crude extract. 158.5 g were subjected to a liquid–liquid partition in increasing polarity using *n*-hexane, DCM, ethyl acetate and *n*-butanol to lead to four fractions labelled F1-F4. 10.3 g (F1), 23.5 g (F2), 30.5 g (F3) and 56.6g (F4). On the basis of their TLC we have chosen to focus our study on the ethyl acetate fraction. Series of Silica gel column chromatography on this fraction using the system of solvents *n*-Hex/DCM and DCM/MeOH for elution in order of increasing polarity led to the isolation of 03 compounds namely NG1, NG2 and NG3. Their structures were deduced from the elucidation of their 1D (^{1}H,^{13}C) and 2D (COSY, HSQC, HMBC) spectra and by comparison of spectral data with those described in the litterature.The experimental protocol is resume in scheme 6.



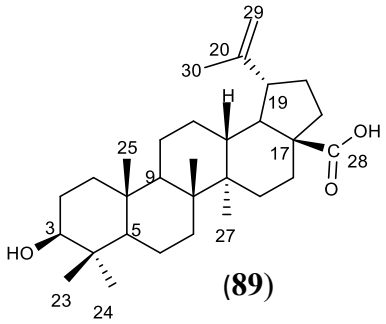
Scheme 6 : **Protocol of extraction, isolation and purification of compounds from the roots of *Newtonia griffoniana***

II-STRUCTURAL STUDY OF ISOLATED COMPOUND

I.1. Identification of compound NG1

Compound NG1 was obtained in the form of white powder in the mixture *n*-Hex/CH_2Cl_2 1:1 (V/V) and is soluble in pyridine.It gives a positive response to the Libermann Buchard test characteristic of triterpenes.

Analysis of the 1D (^1H, ^{13}C) and 2D (HSQC, COSY, HMBC) NMR spectra allowed us to assign to NG2 the structure (89) corresponding to general molecular formular $C_{30}H_{48}0_3$ containing 7 double bond equivalent.



(**89**)

Its ^1H NMR (Pyridine-d_5, 600 MHz, figure 3) spectrum shows in the down field the signals of two doublets of protons appearing at δ_H 4.97 (*d, J* = 2.5 Hz, 1H) and δ_H 4.79 (*d, J* = 2.5 Hz, 1H) attributable to the two exo-methylene protons H-29a and H-29b, This spectrum also exhibits the signal of an hydroxymethine proton at δ_H 3.48 (*dd, J* = 9.0, 7.1 Hz, 1H) attributable to proton H-3 and in the up-field the signal of the allylic methyl group proton at δ_H 1.81 (*s,* 3H) attributable to proton H-30 and finally the signals of 5 angular methyl appearing respectively δ_H 1.25 (*s,* 3H), δ_H 1.09 (*s,* 3H), δ_H 1.08 (*s*, 3H), δ_H 1.03 (*s,* 3H), δ_H 0.84 (*s*, 3H).

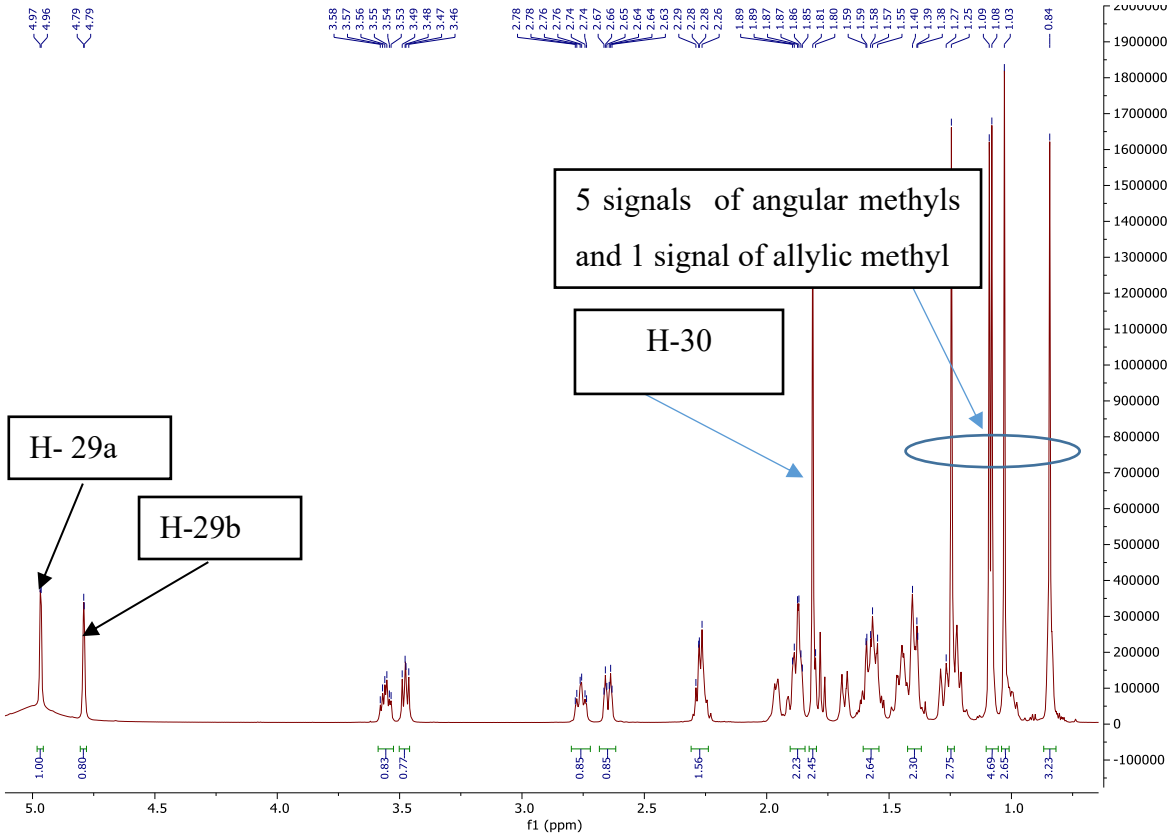


Figure 3: ^{1}H NMR spectrum of NG1 (Pyridine-*d*$_5$, 600 MHz)

Furthermore, the ^{13}CNMR (Pyridine-*d5*, 600 MHz, figure 4) spectrum of this compound revealed signals of 29 carbon atoms which were distinguished as six methyls,eleven methylenes (one vinylic and 10 aliphatics respectively), six methines and seven quaternary carbons (one carbonyl, one vinylic, five aliphatics respectively. The types of carbon patterns were determined based on the DEPT experiment (135 sequence pulses) (figure 5). These signals are listed as follow :

- 7 Quaternary carbon atoms at δ_C 178.7 (C-28), δ_C 151.1 (C-20), δ_C 56.5 (C-17), δ_C 42.7 (C-14), δ_C 40.9(C-8), δ_C 39.4 (C-4), δ_C 37.3 (C-10);
- 6 methine Carbon atoms at δ_C 77.9 (C-3), δ_C 55.7 (C-5), δ_C 50.8 (C-18), δ_C 49.6 (C-9), δ_C 47.6 (C-19), δ_C 38.4 (C-13);
- 11 methylene carbon atoms at δ_C 109.7 (C-29), δ_C 39.0 (C-1), δ_C 37.4 (C-22), δ_C 34.6 (C-7), δ_C 32.7 (C-16), δ_C 31.0 (C-21), δ_C 30.0 (C-15), δ_C 28.1 (C-2), δ_C 25.9 (C-12), δ_C 21.0 (C-11), δ_C 18.6 (C-6);
- 6 methyl carbon atoms at δ_C 28.5 (C-23), δ_C 19.2 (C-30), δ_C 16 .1(C- 24), δ_C 16.2 (C-25) ,δ_C 14.7 (C-27).

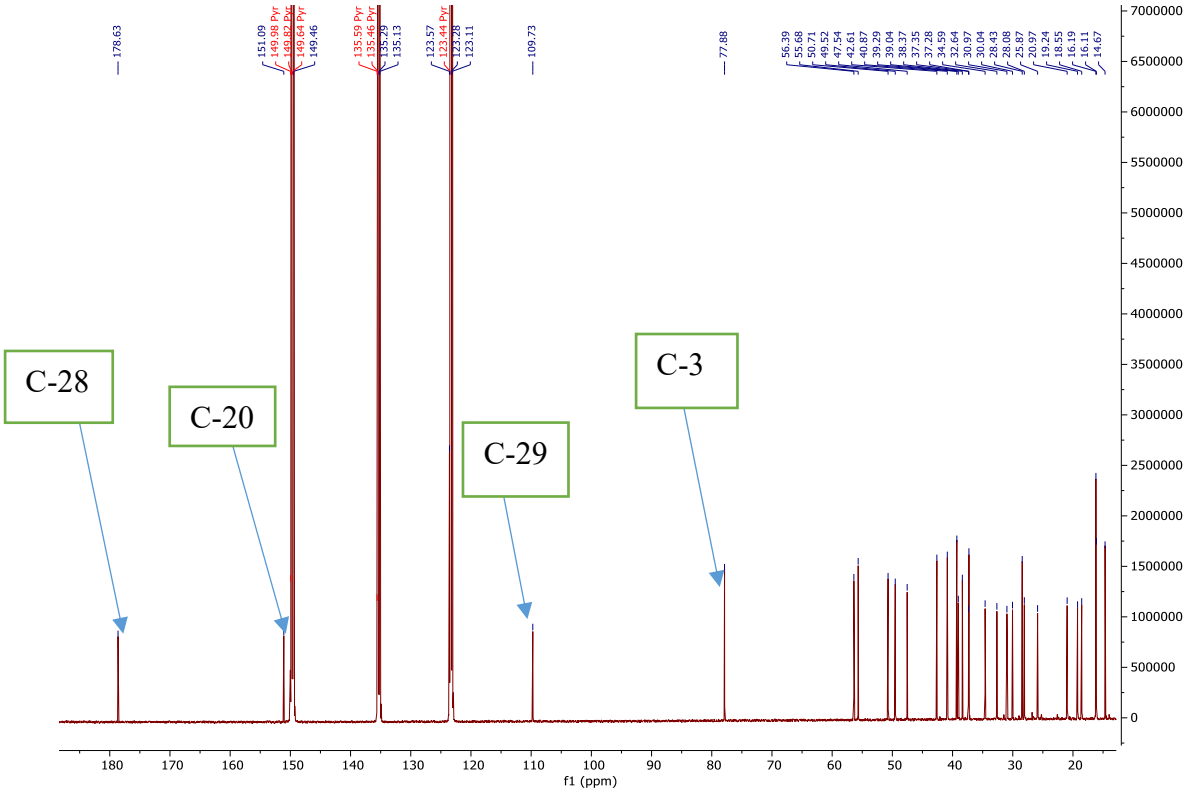


Figure 4 : ^{13}C NMR spectrum of NG1 (Pyridine-d_5, 150 MHz)

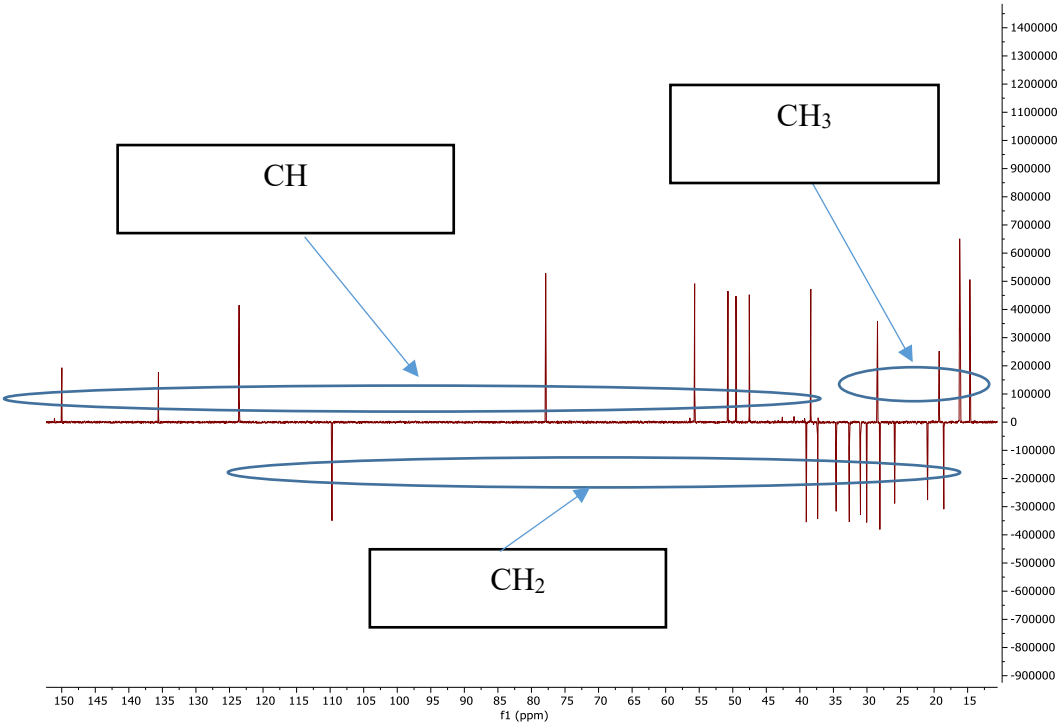


Figure 5 : DEPT 135 spectrum of compound NG1 (Pyridine-d_5, 150 MHz)

The position of different groups were confirmed by the HMBC (figure 6) spectrum through the correlations observed between :

- The proton at δ_H 3,48 (H-3) and the carbon at δ_C 39,01 (C-1); the proton at 1,87 (H-2) and the carbon at δ_C 77,9 (C-3) confirm the position of the oxymethine at C-3.
- The proton at δ_H 3.48 equally confirms the position of the two methyl group at C-4 through the correlation between δ_C 28.5 (C-23), δ_C 16.1 (C-24).
- The protons at δ_H 4.94 and 4.75 (H- 29) and the carbons at δ_C 19.2 (C-30), δ_C 151.1 (C-21), δ_C 47.6 (C-19) confirms the position of the double bond .
- The proton at δ_H 2.27 (H-20) and the carbons at δ_C 56.7 (C-17), δ_C 178.7 (C-28); the proton at 2.65 and the carbons at δ_C 56.5 (C-17), δ_C 178.7 (C-28) enables us to confirm the position of the acide group at C-17; again, the proton 2.27 and the carbon at δ_C 151.1 (C-20), 47.6 (C-19) enable us to confirm the position of the double bond at (C-20–C-29).

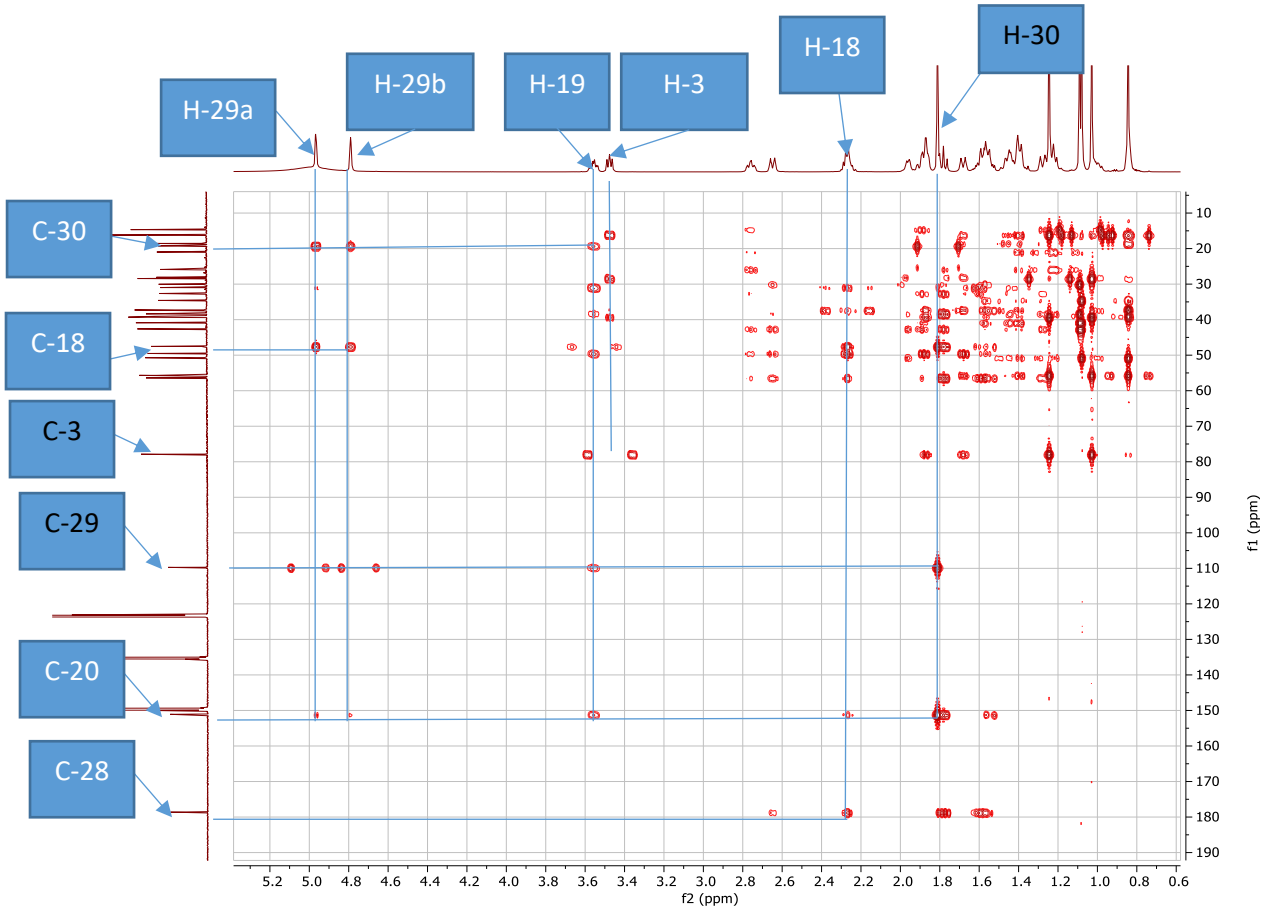
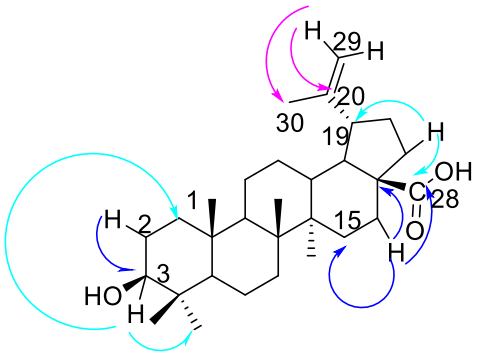


Figure 6 : HMBC spectrum NG1 (Pyridine-*d*5, 150 MHz)

Some HMBC correlations are presented below



Its COSY spectrum (figure 8) coupled with its HSQC (figure) enable us to confirm some correlations like that between the proton at δ_H 4.94 and 4.75 which are respectively H-29a and 29-b hence confirming the position of the double bond at C-20. The proton at δ_H 1.87(H-2) and 3.48 (H-3) confirming the position of the oxymethyne at C-3.

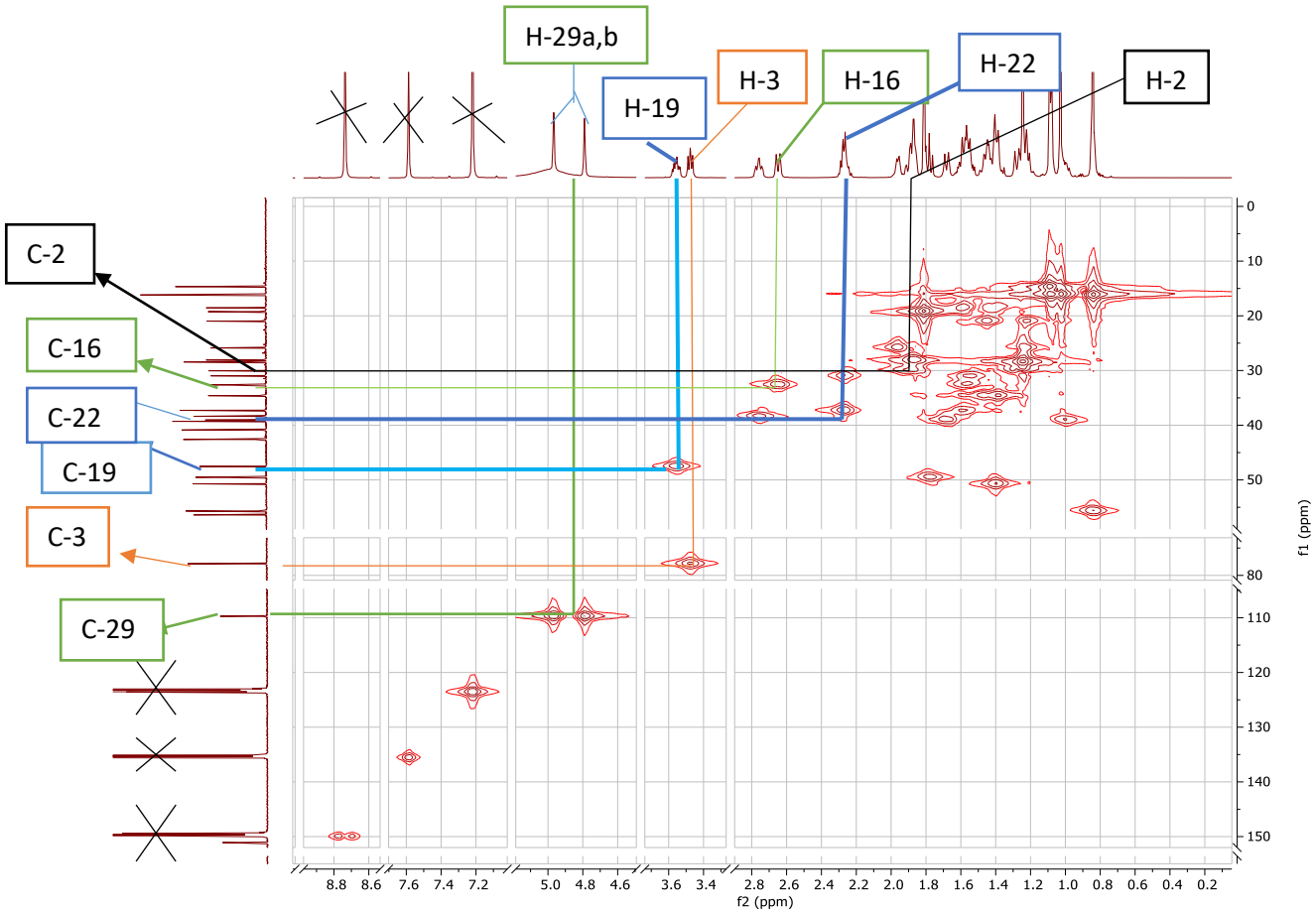


Figure 7 : HSQC spectrum NG1

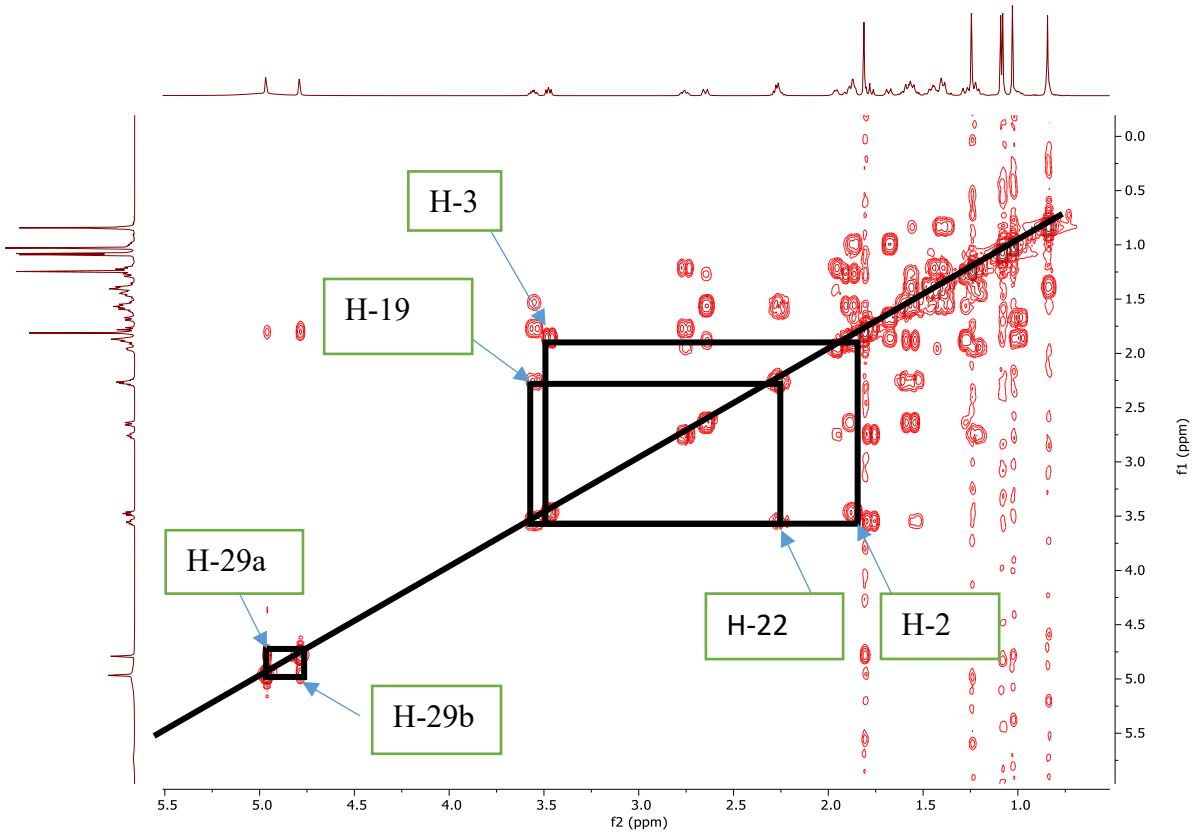
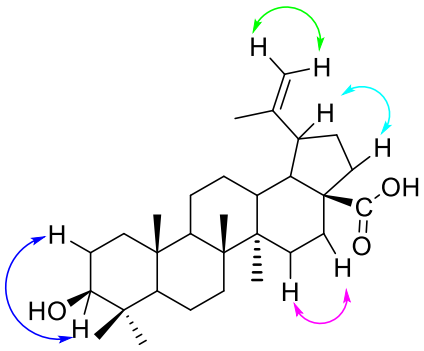
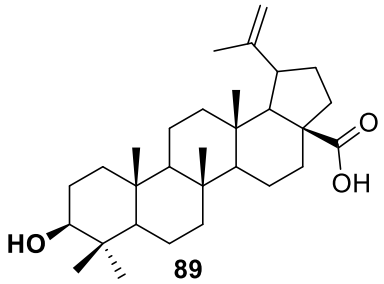


Figure 8 : **COSY spectrum of NG1**

Some cosy correlations of the compound NG1 are presented below:



The results indicated that compound NG1 is a lupane type triterpene based on its spectroscopy data (1D and 2D NMR) with comparison of literature data **(Noviany and osman 2012**) (**Table 16**). Compound NG1 is readily identified as **3*β*-hydroxy–lup-20(29)-en-(28)-oic acid** (88), a pentacyclic triterpene previously isolated by Ibrahim and collaborators in 2019 from the stem bark of *Milletia richardiana* of the Fabaceae family.



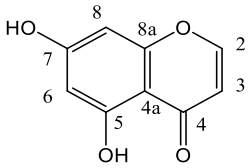
89

Table 16: ^{1}H NMR and ^{13}C NMR data of the compound NG1 compared with that of 3β-hydroxy-lup-20(29)-en-(28)-oic acid (Scholichin *et al.*, 1980)

Carbon Number	NG1 ^{13}C NMR 150MHz,pyridine-d_5 ^{1}H NMR 600MHz, DMSO pyridine-d_5		3β-hydroxy-lup-20(29)-en-(28)-oic acid ^{13}C NMR 100 MHz ,DMSO-d_6 ^{1}H NMR 400 MHz, DMSO-d_6	
	δ_C	δ_H (*m;J* in Hz)	δ_C	δ_H (*m ;J* in Hz)
1	39.0	/	39.1	/
2	28.1	/	28.0	/
3	77.9	3.48 (1H *dd*, 9.0 ,7.1,)	77.7	3.17 (*m)*
4	39.4	/	39.4	/
5	55.7	/	55.8	/
6	18.6	/	18.8	/
7	34.6	/	34.8	/
8	40.9	/	40.9	/
9	49.6	/	49.5	/
10	37.3	/	37.6	/
11	21.0	/	21..3	/
12	25.9	/	25.9	/
13	38.4	/	38.5	/
14	42.7	/	42.9	//
15	30.0	/	30.1	/
16	32.7	/	32.6	/
17	56.5	/	56.3	/
18	50.8	/	50.8	/
19	47.6	/	47.5	/
20	151.1	/	151.2	/
21	31.0	/	31.0	/
22	37.4	/	37.2	/
23	28.5	1.25 (3H ,*s*)	28.9	0.87 (s)
24	16.1	1.09 (3H, *s*)	16.8	0.65 (s)
25/26	16.2		16.6	0,76 (s) ; 0.93 (s)
27	14.7	0.84 (3H,*s*)	15.9	0.87 (*s*)
28	178.7	/	178.1	/
29	109.7	4.97 (1H,*d,* 2.5) 4.79 (1H, dd, 2.5, 1.5)	110.4	4.56 (brs) ;4.69 (brs)
30	21.0	1.81 (3H, *s*)	19.8	1.64 (s)

I.2. Identification of compound NG2

Compound NG2 was isolated in the form of colourless flaky crystal in the system n-Hex/CH_2Cl_2 3/7 (V/V) and is soluble in dimethyl sulfoxide (DMSO). It has a positive respond to the ferric chloride test characteristics of phenolic compounds. The 1D (^1H,^{13}C) and 2D (HSQC, COSY, HMBC) NMR data allowed us to assign to NG2 structure 99 corresponding to the molecular formular $C_9H_6O_4$ containing 7 double bonds equivalent.



(99)

On its ^1H NMR (DMSO-d_6, 600 MHz, figure 9**)** spectrum, we observed the signals of a chelated proton at δ_H 12.71 (*s,* 1H). Four protons in the aromatic region : two meta-coupled doublets at δ_H 6.36 (*d, J* =2.1 Hz, 1H) and δ_H 6.20 (d, *J* =2.1 Hz, 1H) which are characteristic of the protons H-6 and H-8 of chromenes, respectively. We also observe two signals at δ_H 8.19 (d, *J* =5.9 Hz, 1H) and δ_H 6.28 (d, *J* =5.9 Hz, 1H) with vicinal coupling which can be assigned to the protons H-2 and H-3.

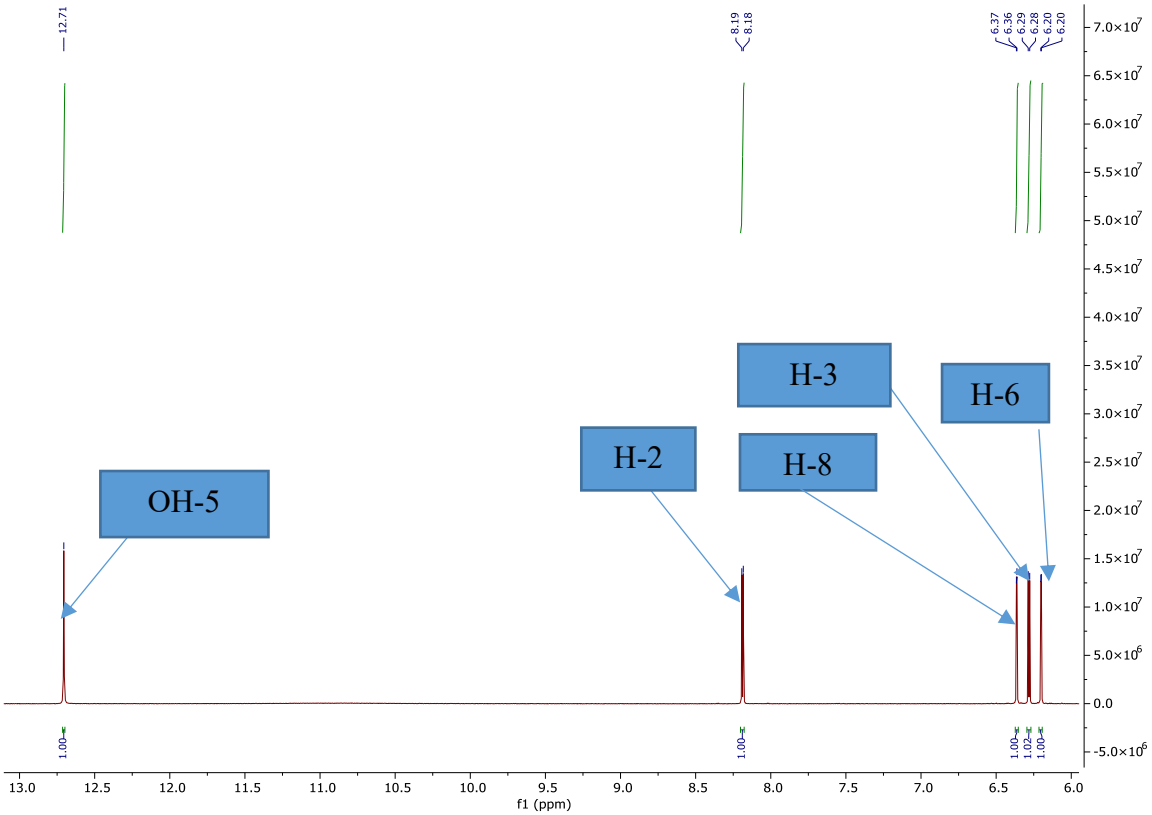


Figure 9: ^1H NMR spectrum of NG2 (DMSO-d_6, 600 MHz)

Its ^{13} C NMR (DMSO-d6, 150 MHz, figure 10) spectrum presents the signals of 9 carbon atoms with aromatic carbons distributed at δ_C 165.0 (C-7), 162.1 (C-5), 158.2 (C-8a), 105.3 (C-4a), 99.5 (C-6), 94.5 (C-8), olefinic carbons at δ_C 157.9 (C-2). 110.9 (C-3) and a carbonyl at δ_C 181,7 (C-4).

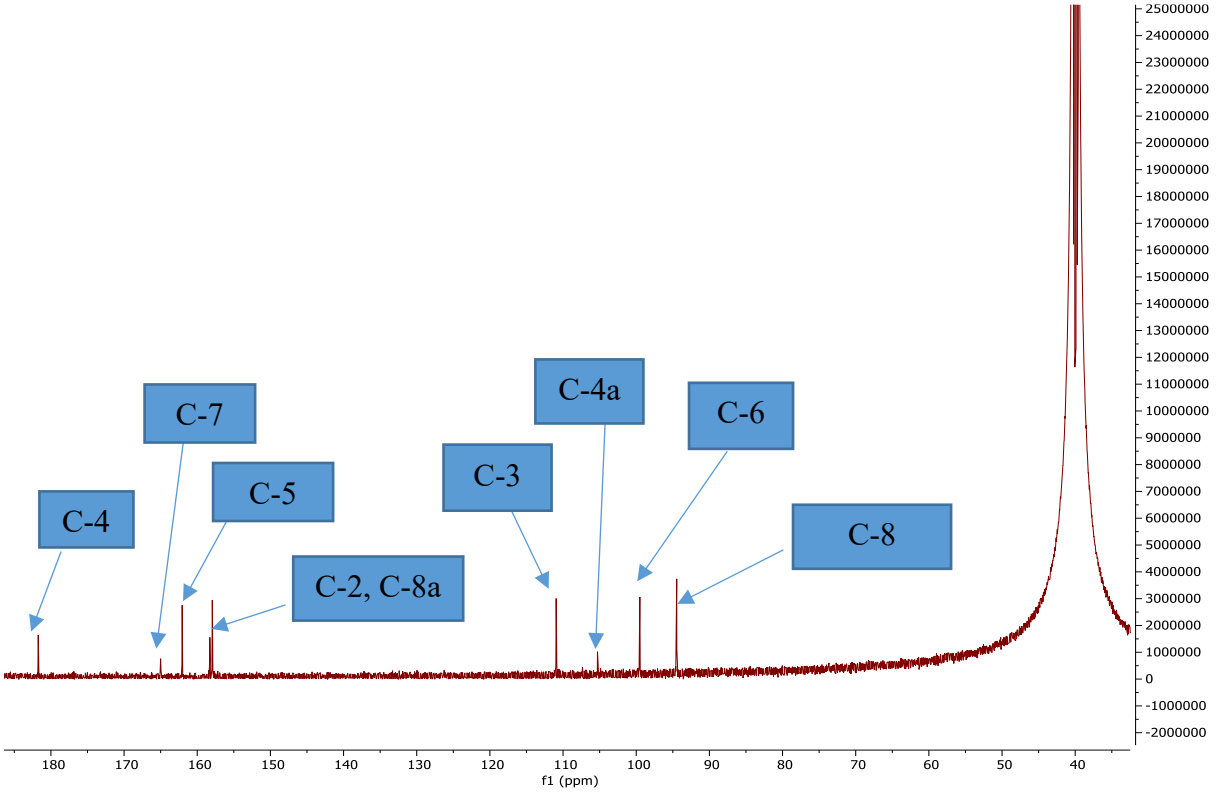


Figure 10 : ^{13}C NMR spectrum of NG2 (DMSO-d_6, 150 MHz).

Its COSY spectrum (figure 11) shows homonuclear correlation between olefinic protons H-2 and H-3. Moreover we also observe correlation between proton H-6 and H-8 confirming the meta-coupling nature of these two protons.

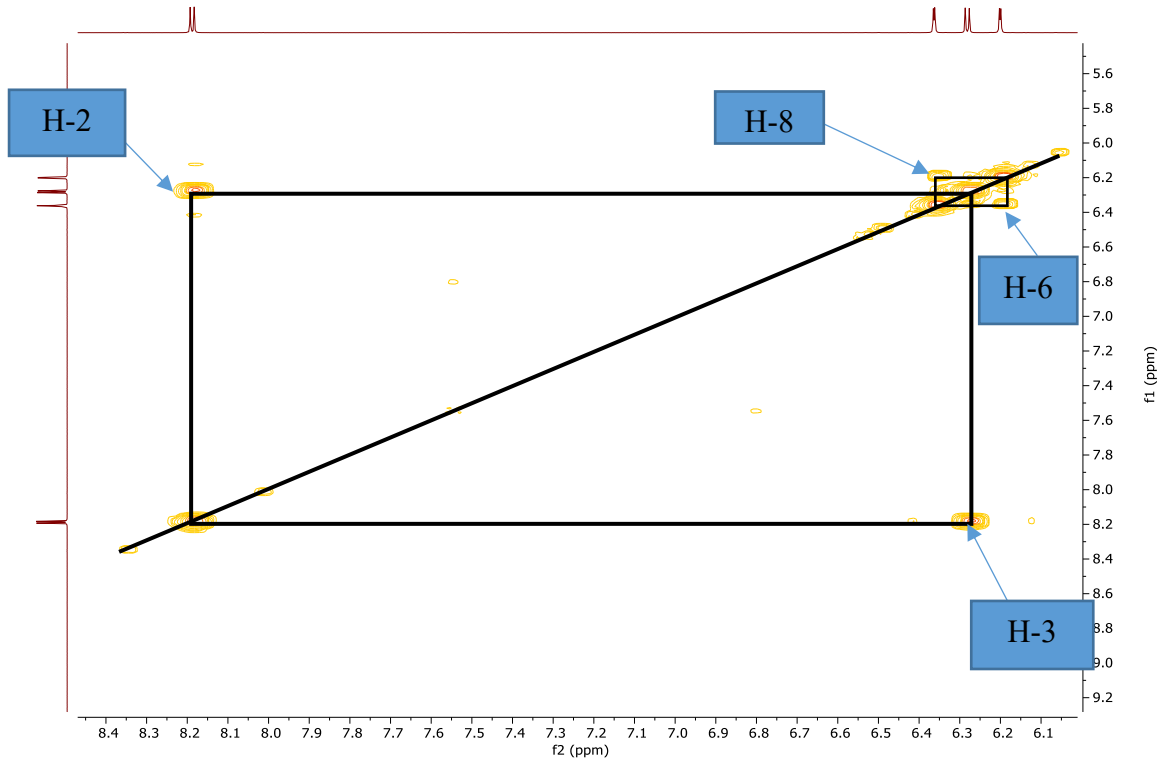


Figure 11: COSY Spectrum of NG2

The analysis of its HSQC spectrum (figure 12) allowed us to attach each proton to its carbon.

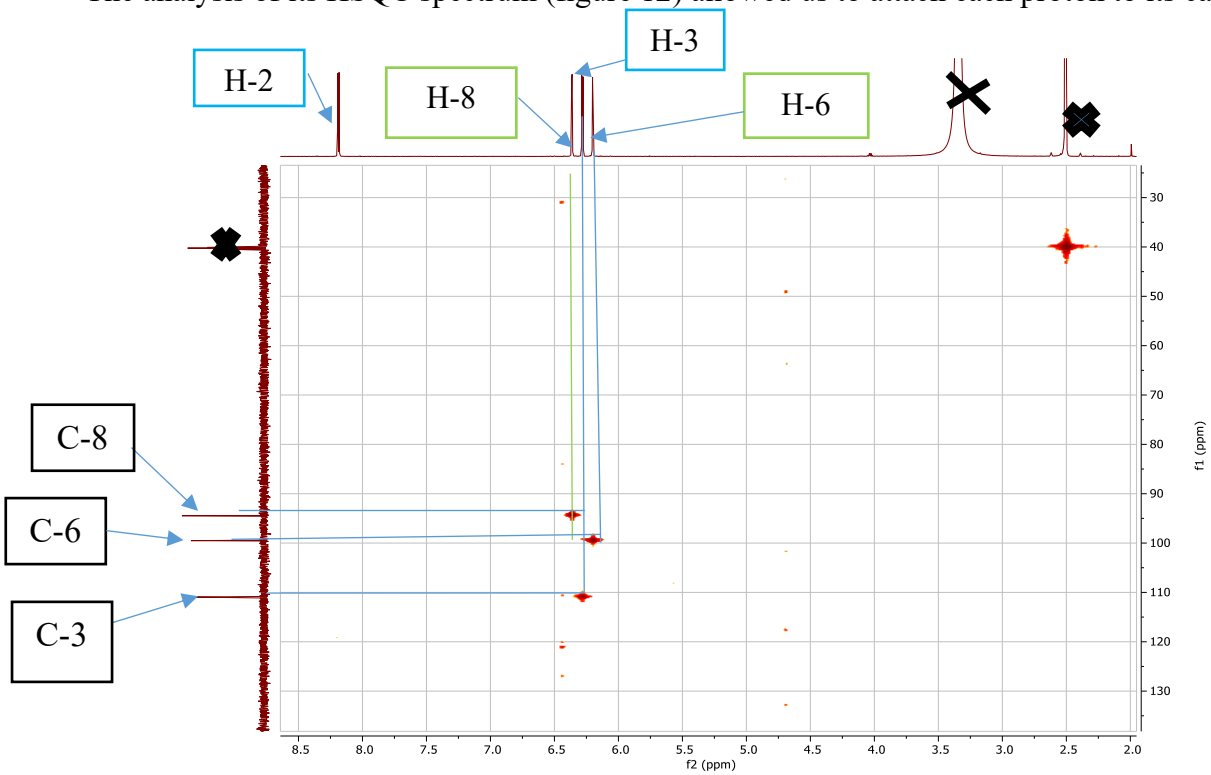


Figure 12 : HSQC Spectrum of NG2

The analysis of its HMBC (figure 13) spectrum enables us to confirm the structure and attribute the chemical shift of some quaternary carbon. In fact, we observe;

- A ^{2}J correlation between the proton at δ_H 8.19 (H-2), δ_H 6.28 (H-3) and the carbon at δ_C 181.7 corresponding to carbon C-4. Hence confirming the position of the carbonyl group.
- A ^{2}J correlation between the proton at δ_H 6.36 (H-5), δ_H 6.20 (H-6) and the carbon at δ_C165.0 on one hand and on the other hand a ^{2}J correlation between the proton at δ_H 6.20 and the carbon at δ_C 162.1 confirming the bearing of two oxyaryls on the carbon C-7 and C-5 respectively.
- A ^{3}J correlation between the proton at δ_H 6.28 , δ_H 6.20 and carbon at δ_C 105.3 on one hand and on another hand a ^{2}J correlation between the proton at δ_H 6.36 and carbon at δ_C 158.2 corresponding to the carbons C-4a and C-8a respectively.

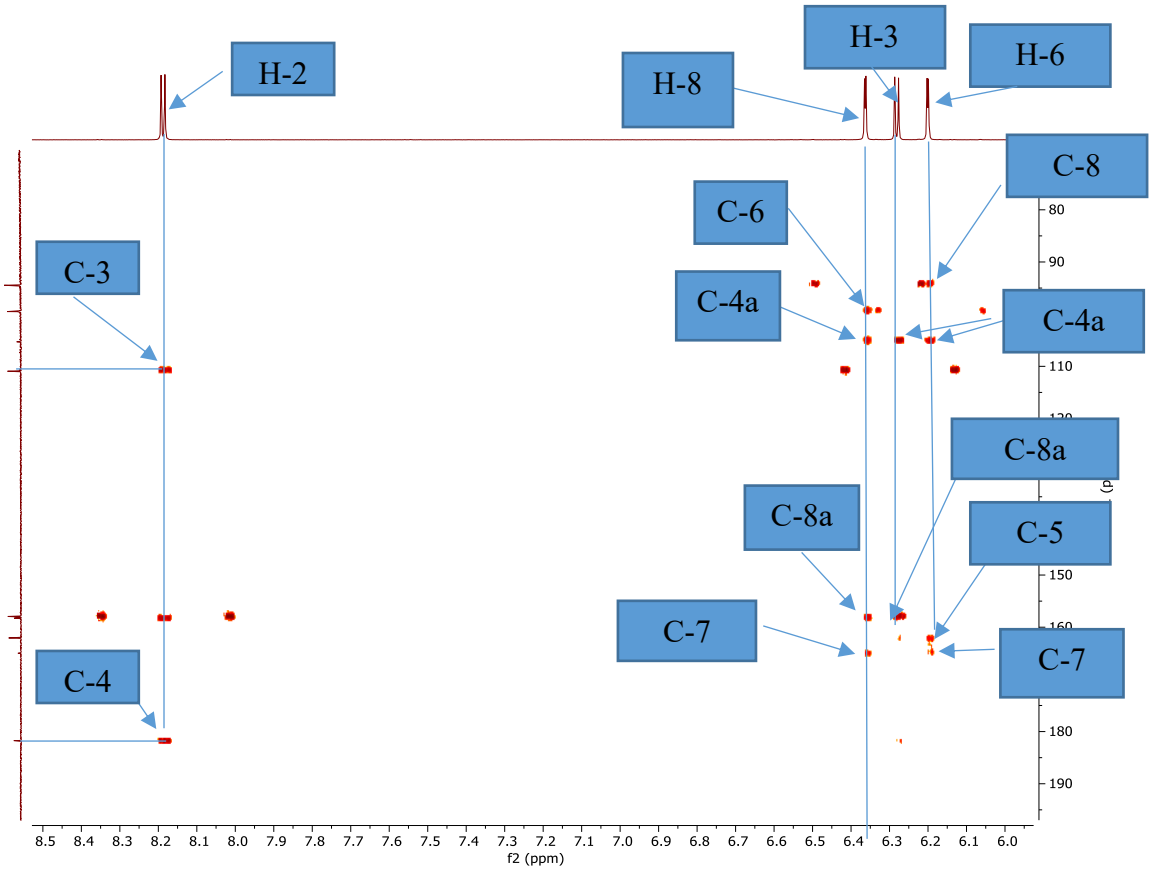
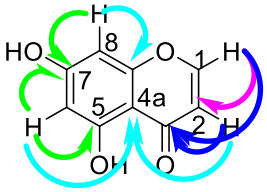


Figure 13 : HMBC Spectrum of NG2

Some correlations of HMBC spectrum are represented below :



The analyses of 1D and 2D NMR spectral data and the comparison with data described in the literature (**Table 17**) allowed us to identify NG2 as 5,7-dihydroxylchromone which was synthesized in 1902 by Kostanecki and collaborators.

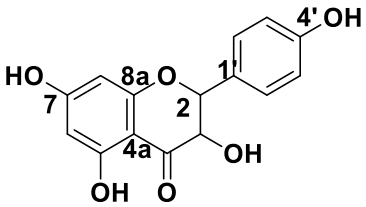
Table 17 : ^{1}H NMR (600 MHz, DMSO-*d*$_6$) and ^{13}C NMR (150 MHz, DMSO-*d*$_6$) data of the compound NG2 compared with those of 5,7-dihydroxylchromone (Zhucan *et al*., 2014)

N°	NG2		5,7-dihydroxylchromone	
	^{13}C NMR (150 MHz, DMSO-*d6*) δ_C	^{1}H NMR (600 MHz, DMSO-*d6*) δ_H (*m* ;*J* in Hz)	^{13}C NMR (100MHz,DMSO-*d6*) δ_C	^{1}H NMR (400 MHz, DMSO-*d6*) δ_H (*m* ;*J* in Hz)
2	157.9	8.19 (1H *d,* 5.9).	157.94 (C-2)	8.19 (1H, *d,* 3.6)
3	110.9	6.28 (1H *d,*5.9)	110.95 (C-3)	6.28 (1H *d,* 3.6)
4	181.7		181.75 (C-4)	
4a	105.3		105.37 (C-4a)	
5	162.1	12.71 (1H, *s,* OH-5)	162.07 (C-5)	12.70 (1H, *s*, 5-OH)
6	99.5	6.20 (1H *d,* 2.1)	99.46 (C-6)	6.20 (1H, *s,*)
7	165.0		164.80 (C-7)	10.88 (1H, *s*, 7-OH)
8	94.5	6.36 (1H *d,* 2.1)	94.43 (C-8)	
8a	158.2		158.26 (C-8a)	

I.3.Identification of NG3

Compound NG3 was obtained in the form of a pale yellow powder in the mixture CH_2Cl_2/MeOH and is soluble in MeOD. It has a positive respond to the Shinoda test characteristic test of flavonoids.

The analysis of its spectral data, namely the 1D NMR (^{1}H, ^{13}C) and 2D NMR (HMQC, HMBC, COSY) spectra enabled us to assign the structure below with the corresponding molecular formular $C_{15}H_{12}O_6$ containing 10 double bonds equivalent.

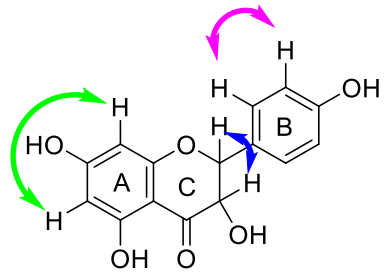


(100)

The analysis of the proton spectra (figure 14) shows us:

- The signals of four aromatic protons forming the system AA'BB' at δ_H 7.38(*dd*, 2H) , δ_H 6.85 (*dd*, 2H) which corresponds to proton H-2'/6', H-3'/5' characteristic of para disubstituted aromatic nucleus of ring B of flavonoids.
- The signals of two trans coupling aliphatic protons at δ_H 4.56 (*d, J* =11.0 Hz, 1H) and δ_H 5.00 (*d, J* =11.6 Hz ,1H) corresponding to proton H-3 and H-2 respectively.
- The signals of two meta coupled aromatic Protons forming the system AB at δ_H 5.90 (*d, J* = 2.1Hz, 1H) and δ_H 5.95 (*d, J* =2.1 Hz, 1H) which are proton H-8 and H-6 respectively.

  All these protons are showing strong correlation on the COSY spectrum (figure 15) as represented below



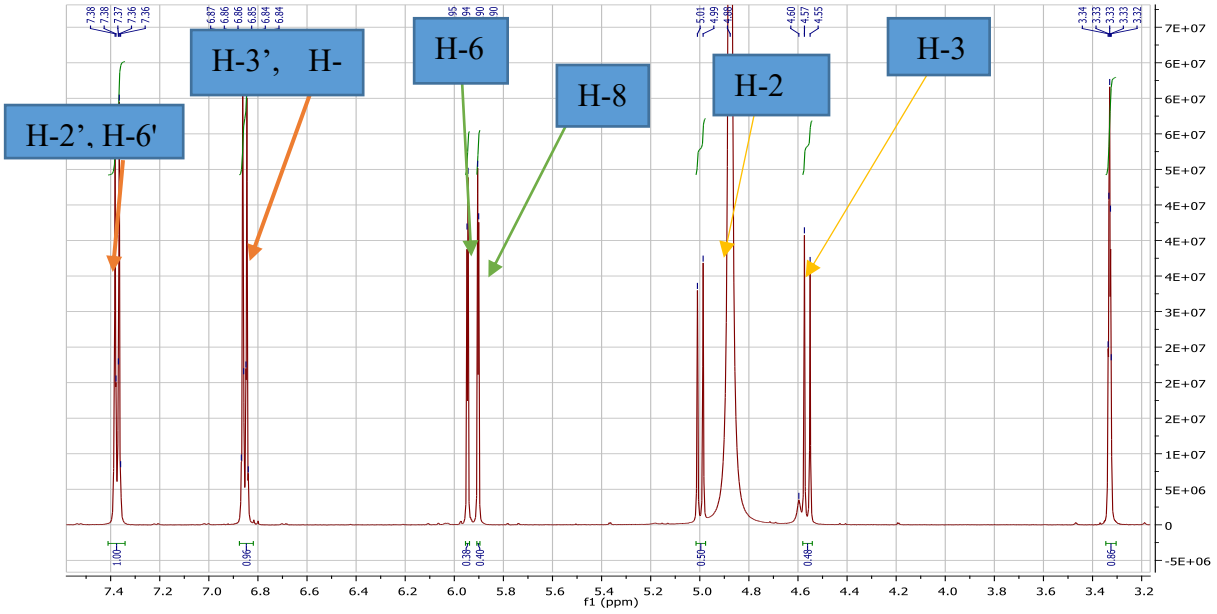


Figure 14 : ^{1}H NMR spectrum of NG3 (500 MHz, CD_3OD)

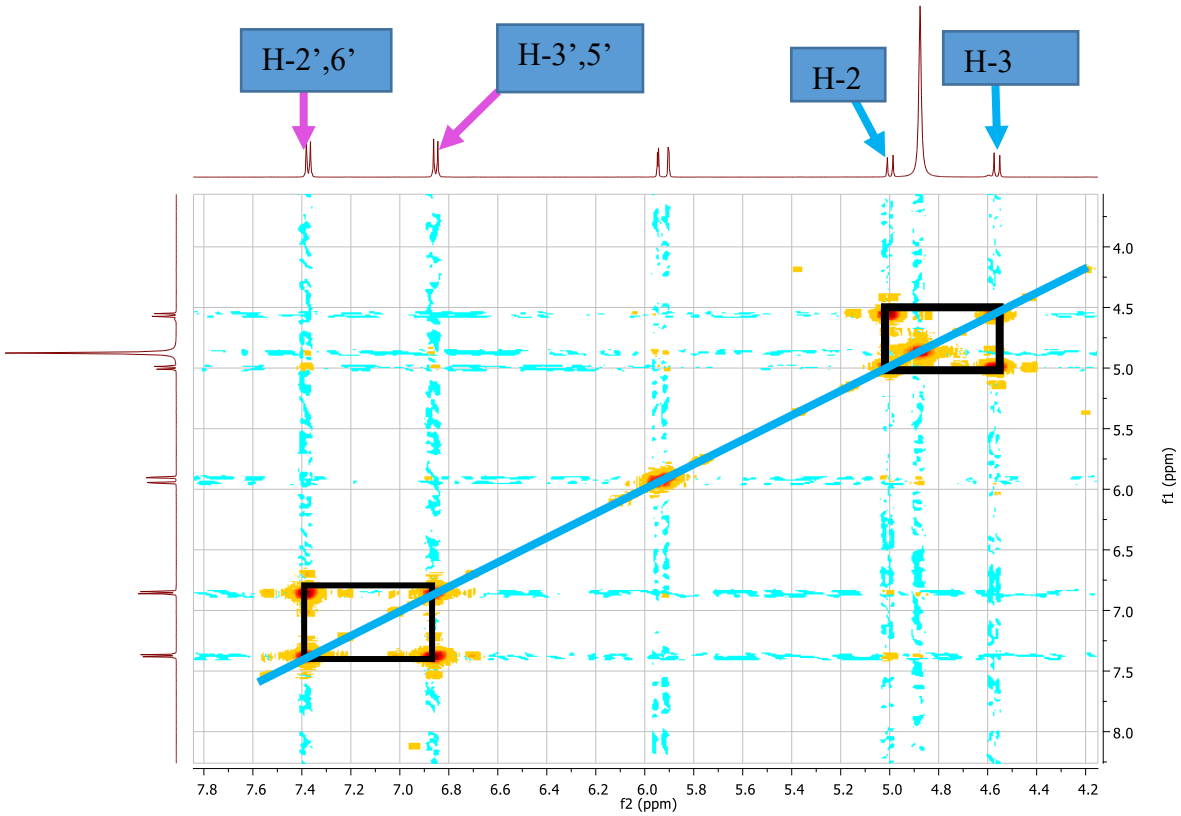


Figure 15 : **COSY spectrumNG3**

The analysis of the ^{13}C NMR spectrum (figure 16) coupled with HSQC (figure 17) shows:

- The signals at δ_C/δ_H 83.5/4.56, 72.3/5.00, 197.1 which corresponds to the chemical shifts of the central carbon (C-2, C-3, C-4) which are characteristic of a dihydroflavonol skeleton (**Grayer, 2009**).
- The signals δ_C/δ_H 197.1(C-4),three carbons carrying the hydroxyl group at δ_C 164.0(C-7), δ_C 167.4 (C-5), δ_C 157.8.
- The signals of 04 methines at δ_C/δ_H 95.0/5.95, 96.0/5.90, 129.0/7.38, 114/6.85.
- The signals of 04 oxyaryl at δ_C 163.2, δ_C 167.4, δ_C 163.9, δ_C 163.2.
- The signals of 02 non oxygenated sp^2 quaternary carbon at δ_C 127.9, δ_C 100.5

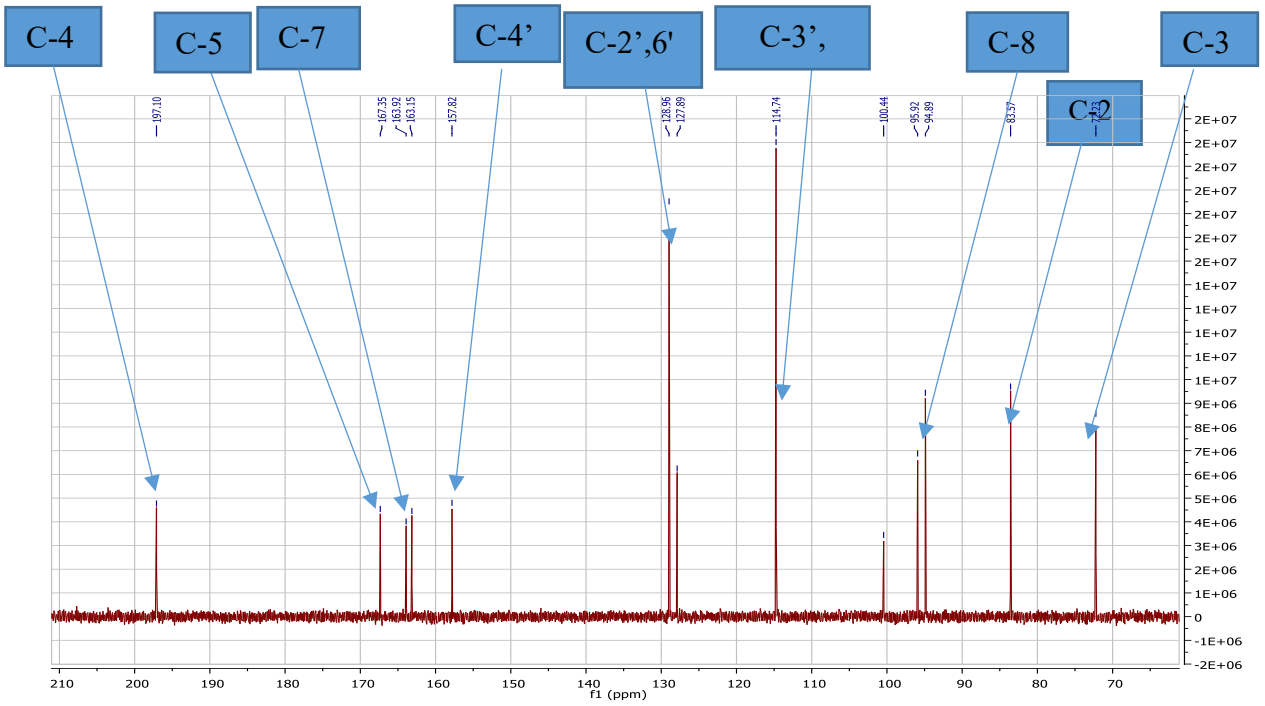


Figure 16 : ^{13}C NMR spectrum of NG3 (125 MHz, CD_3OD)

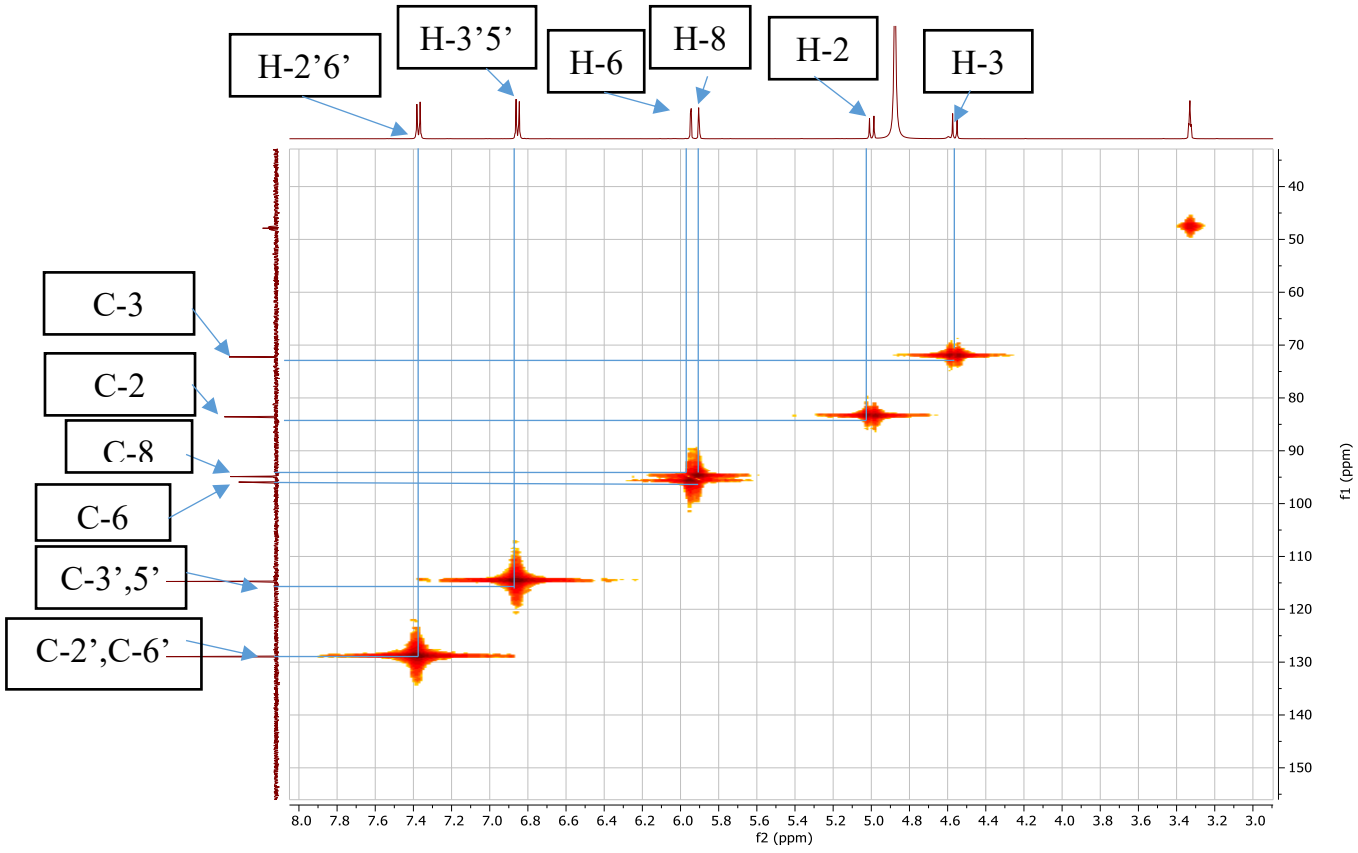


Figure 17 : HSQC spectrum of NG3 (CDOD)

The analysis of the HMBC spectrum (figure 18) enable us to attribute the chemical shift of some quaternary carbon. In fact, we observe :

- The correlation between the protons at δ_H 5.90, δ_H 5.95 and the oxyaryl at δ_C 167.4 corresponding to the carbon C-7.
- A ^2J correlation between the protons at δ_H 5.90 and the carbon at δ_C 163.2 in one hand, δ_H 5.95 and δ_C 163.9 corresponding respectively to the chemical shift value of carbon C-5 and C-9.
- A ^2J and ^3J correlation between the proton at δ_H 6.87, δ_H 7.38 and the carbon at δ_C 157.8 corresponding to the carbon C-4'.
- A ^2J and ^3J correlation between the proton at δ_H 6.87, δ_H 7.38 and the carbon at 127.9 corresponding to carbon C-1'.

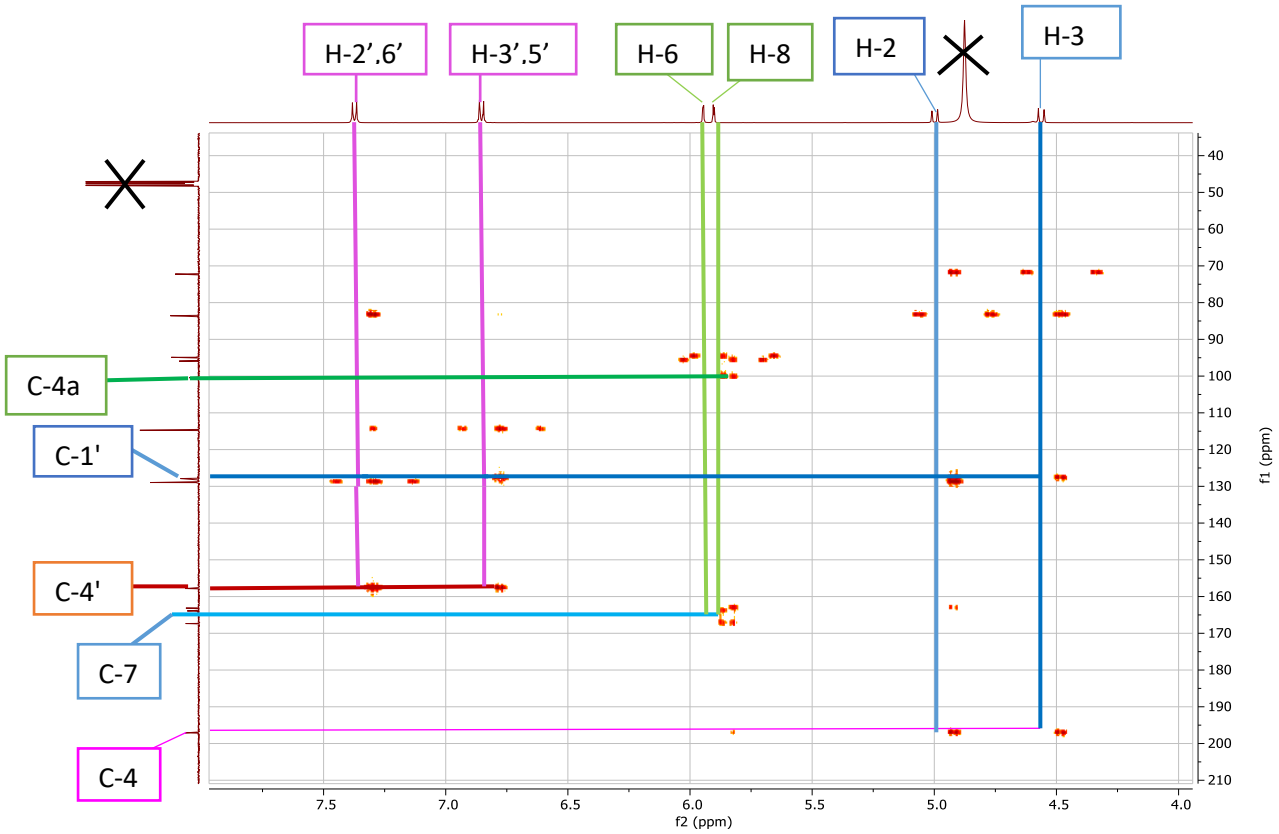
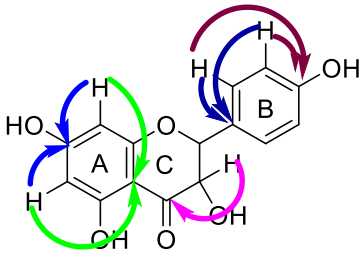
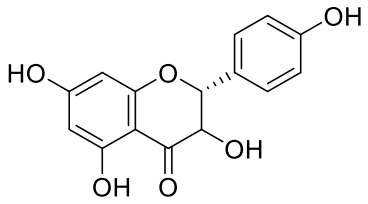


Figure 18 :HMBC spectrum of compound NG3

Some correlations of the HMBC spectrum are presented below:



On the basis of all these spectroscopic data and those reported in the literature NG3 was identified (2*R*,3*R*)-3,4',5,7-tetrahydroxyflavan-4-one (100) previously isolated from the plant ***Pistacia integerrima*** **(Abdur *et al*., 2015)**.



(100)

Table 18 : ^{1}H NMR and ^{13}C NMR data of the compound NG3 compared with that of (2*R*,3*R*)-3,4',5,7-tetrahydroxyflavan- 4-one (Abdur *et al*., 2015)

Position	NG3 ^{1}H NMR (500MHz,CD_3OD) ^{13}C NMR (125 MHz, CD_3OD)		(2*R*,3*R*)-3,4',5,7-Tetrahydroxyflavan- 4-one ^{1}H NMR(600MHZ, CD_3OD) ^{13}C NMR(CD_3OD, 150MHz	
	δ_C	δ_H (*m;J* Hz)	δ_C	δ_H (*m;J*Hz)
1	-		-	
2	83.5	5.00(1H,*d*, 11.6)	83.4	5.32 (1H, *d*, 2.0)
3	72.3	4.56 (1H ,*d*, 11.0)	71.2	5.78 (1H, *d*, 2.0)
4	197.1	/	193.0	/
4a	100.5	/	104.0	/
5	163.2	/	161.9	/
6	96.0	5.95 (1H,*d*, 2.1)	104.1	6.23 (1H, *d*, H-6, 8.)
7	167.4	/	165.7	
8	95.0	5.90 (1H,*d*,2.1)	95..5	6.85 (1H, *d*, H-8, 8.8)
8a	163.9	/	158.5	/
1'	127.9	/	130.9	/
2'/ 6'	129.0	7.38(2H,*dd*)	129.9	7.11 (1H, *d*, H-2', 8.8)
3'/ 5'	114.7	6.85 (2H,*dd*)	117.0	6.82 (1H,*d* ,H-10.2)'
4'	157.8	/	158.5	/

II- Discussion

During this chemical study 03 known compounds were isolated, characterised and identified as; a pentacyclic triterpene of a lupane type namely 3*β*-hydroxy-lup-20(29)-en-(28)-oic acid (betulinic acid), a chromone named as 5,7-dihydroxylchromone and a flavonoid belonging to the class dihydroflavonol named (2*R*,3*R*)-3,4',5,7-tetrahydroxyflavan-4-one.

The previous chemical studies on the Fabaceae family enabled the isolation of these classes of compounds from different parts of plants in different genus of this family.

From the literature review, a variety of biological activities have been ascribed to betulinic acid including anti-malarial effect, a high anti-HIV activity and specific cytotoxicity against a variety of tumour cell lines (**Robert *et al.*, 2004**). It has been demonstrated that (2*R*,3*R*)-3,4',5,7-tetrahydroxyflavan-4-one posseses anti lieishmanicidal activities. The activity was majored on the leishmanal species (*Leishmania major)* and the results obtained were, for a concentration of 5 μg of compound we had a percentage effect of 68.09 ± 0.76 and an IC_{50} value of 30.43 ± 0.32 in (μM). Betulinic acid also posseses anti-inflammatory, analgesic, muscle relaxant, sedative and anti- pyretic activity (**Zahoor *et al.*, 2018**). These scientific results confirm the traditional usage of the specie *Newtonia griffoniana.*

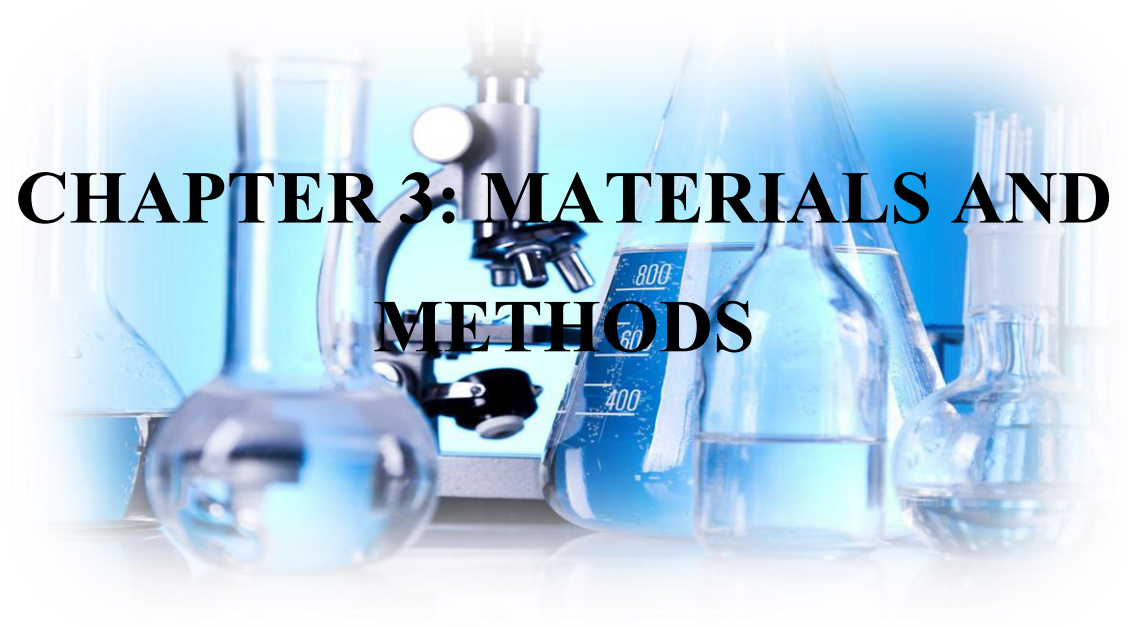
GENERAL CONCLUSION AND PERSPECTIVES

In summary, the main aim of this work was to isolate and characterize compounds from the ethylacetate fraction of the roots bark of *Newtonia griffoniana*.

The present work with the help of usual chromatographic method led to the isolation of three compounds indexed NG1, NG2 and NG3.These compounds were identified and characterised on the basis of spectroscopic data (^{1}HNMR, ^{13}CNMR, HMBC, HSQC, COSY) compared with those found in literature as being 5,7-dihydroxyl chromone, 3*β*-hydroxy-lup-20(29)-en-(28)-oic acid a pentacyclic triterpene, and (2*R*,3*R*)-3,4',5,7-tetrahydroxyflavan-4-one, a flavonoid, respectively.

We suggest in the continuation of our work to

- Continue the study of the remaining fractions and extend our study to the other parts of the species.



CHAPTER 3: MATERIALS AND METHODS

I. EQUIPMENT AND PLANT MATERIAL

I.1 Equipment

The development of our work required, in addition to the standard glassware of the chemistry laboratory, the following equipment

- COBOS electronic balances, model D-6000-SX with a precision of 1/10 (for weighing plant material and crude extract) and ACCULAB SARTORIUS GROUP ALC with a precision of 1/1000 (for weighing the compounds obtained).
- A separating funnel with a capacity of 2000 ml for liquid-liquid separation;
- Glass tubes of approximately 80 cm in height and 6.5 cm in diameter (for the large fractions) for column chromatography; the purification of the compounds was carried out on an open column of 35 cm in height and 3 cm in diameter on one hand, and on the other hand a column of 50 cm in height and 1 cm in diameter.
- Chromatography was carried out on a MERCK silica gel-coated column with a particle diameter of 0.004-0.063 mm ;
- Analytical chromatography was carried out on TLC plates consisting of aluminium supports using aluminium sheets 0.2 mm thick and 20 x 20 cm in size, coated with silica gel type 60 F254. These plates were developed in glass vessels containing solvent systems of varying polarity;
- The stationary phases used were MERCK silica gel and Sephadex type LH-20.
- TLC plates were developed using a UV lamp type VL-6.LC (24 watt power and providing light at wavelengths of 254 and 356 mm), then sprayed with a 35% sulphuric acid solution under host, followed by heating in an oven at a temperature of approximately 110°C ;
- The elution systems were chosen according to the nature and polarity of the compounds to be separated.
- Evaporation and recovery of the eluting solvent was performed using a HEIDOLPH VV2000 rotary evaporator;
- ^{1}H and ^{13}C NMR spectra were recorded using a BRUKER spectrometer operating at 600 MHz (^{1}H) and 150 MHz (^{13}C) with tetramethylsilane (TMS) as internal standard.

I.2 Plant material

The roots barks of the *Newtonia griffoniana* was collected on the 14th December 2022 at Batoufam in the west Region; the identification of this species was carried out by Mr. Nana Victor, a retired botanist at the National Herbarium of Cameroon.

II. EXTRACTION, ISOLATION AND CHARACTERISATION

II.1 Extraction

The roots barks of *Newtonia griffoniana* were cut, dried and then ground to obtain 2.04 Kg of powder. This powder was macerated for 72 h at room temperature in 10 litres of methanol. After filtration and evaporation of the solvent,168.5 g of crude extract was obtained. One part of this extract, i.e 158.5 g, was degraded by differential solubilisation, successively with pure hexane, DCM, ethyl acetate and *n*-butanol to give four fractions of respective masses 10.3 g, 23.5 g, 30.5 g, 56.6 g and the other part was reserved for biological tests.

II.2 Isolation and purification of compounds

II.2.1. Investigation of the ethyl acetate fraction (F2)

The ethyl acetate fraction (30.5 g) was fixed on silica and separated by column chromatography. Elutions were performed with the n-Hexane/DCM and DCM/MeOH systems in order of increasing polarity. 200 fractions of volume 200 mL each were collected, concentrated and grouped on the basis of analytical TLC as shown in the **Table 19** below**.**

Table 19: Chromatogram of the F2 fraction

Elution system	Fraction	Observation
n-Hex/DCM 70:30 (V/V)	1-13	Mixture of at least four compounds
n-Hex/DCM 65:35 (V/V)	14-40	Mixture of at least five compounds
n-Hex/DCM 50:50 (V/V)	41-61	Mixture of at least three compounds including NG1
Pure DCM	62-77	Mixture of at least five compounds
DCM/MeoH 97:3 (V/V)	78-93	Mixture of at least four compounds
DCM/MeoH 95:5 (V/V)	94-104	Mixture of at least four compounds
DCM/MeoH 93:7 (V/V)	105-125	Mixture of at least six compounds
DCM/MeoH 91:10 (V/V)	125-145	Mixture of at least five compounds
DCM/MeoH 87:15 (V/V)	146-165	Mixture of at least five compounds including NG3 and NG2
DCM/MeoH 75:25 (V/V)	166-180	Mixture of many compounds
DCM/MeoH 65:35 (V/V)	181-190	Mixture of many compounds
MeOH	190-200	Trail

II.2.2. Treatment of the sub fractions

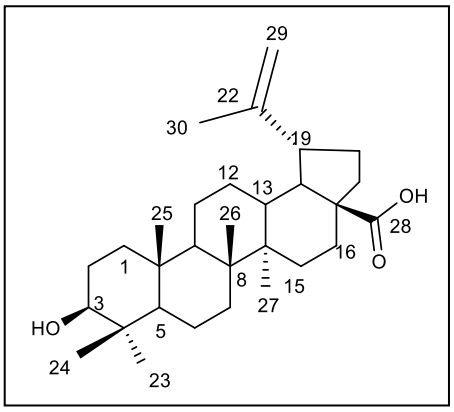
We obtain five fractions SFNG1 -SFNG5. Subsequently, we treated two fractions which are SFNG1 and SFNG3.

The sub-fraction SFNG1 obtained in pure *n*-Hex/DCM (50%) was purified by column chromatography with silica as stationary phase and *n*-Hex/DCM system in order of increasing polarity as mobile phase. After this purification, we isolated 20.8mg of the indexed compound NG1 in the *n*-Hex/DCM 30% system. This compound is in the form of white powder soluble in pyridine.

After purification on silica gel of the sub-fraction SNFG3 obtained in the system DCM/MeOH 10%, we obtained 7mg of the indexed compound NG2 in the mixture DCM/MeOH 5% soluble in the solvent DMSO, a subfraction denoted SFNG5 of mass 1 g and a mixture of two compound of mass 8.3 g obtained at polarities 7% and 9% respectively. The mixture underwent a purification on Sephadex LH 20 column and after the purification, we obtained the indexed compound NG3 with a mass of 30.2 mg.soluble in MeOH.

III. PHYSICO-CHEMICAL AND SPECTROSCOPIC CHARACTERISTICS OF THE ISOLATED COMPOUNDS

III.1 Compound NG1



3β-hydroxy-lup-20(29)-en(28)-oic acid

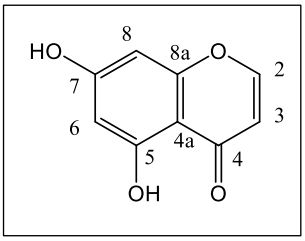
- Molecular formula : $C_{30}H_{48}0_3$
- Physical appearance: white powder

Molar mass : 456 g/mol

- Solubility solvent: pyridine
- ^{13}C NMR spectrum (150 MHz, pyridine-d_5)
- ^{1}H NMR spectrum (600 MHz, pyridine-d_5)

Libermann-Buchard test: Positive

III.2 Compound NG2



5,7-dihydroxyl chromone

- Molecular formula: $C_9H_6O_4$

Physical appearance: Colourless flaky crystal

- Molar mass: 178 g/mol

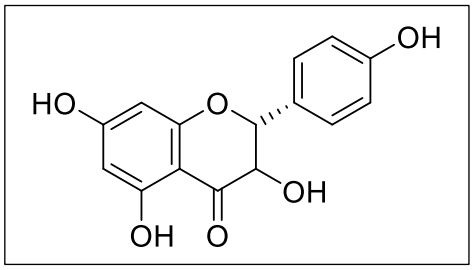
- Solubility solvent : DMSO
- ^{13}C NMR spectrum (150 MHz ,DMSO-d_6): Figure 8
- ^{1}H NMR spectrum (600 MHz, DMSO-d_6): Figure 10:

Ferric chloride test: Positive

III.3 Compound NG3

(2*R* ,3*R*)-3.4',5,7-tetrahydroxyflavan-4-one

Molecular formula: $C_{15}H_{12}O_6$



- Physical appearance: Pale yellow powder
- Molar mass:288 g/mol
- Solubility solvent: CD_3OD
- ^{13}C NMR spectrum (125 MHz, CD_3OD)
- ^{1}H NMR spectrum (500 MHz, CD_3OD)

-Schinoda test: positive

IV. CHEMICAL CHARACTERISTIC TESTS CARRIED OUT

❖ Schinoda test

Purpose: to identify flavonoids

Reagents: alcohol, hydrochloric acid (HCl), magnesium chips

Procedure: dissolve 1mg of the product in 1ml of ethanol placed in a test tube: add five drops of concentrated hydrochloric acid then some magnesium chips

Observation: the flavonoids give in the presence of Schinoda's reagent an effervescence followed by a brick red or purplish red coloration.

❖ Libermann-Bürchard test

Purpose: identification of triterpenes and sterols.

Reagents: Acetic anhydride, H_2SO_4.

Procedure: a small amount of compound was dissolved in 1ml of methylene chloride; to the solution obtained, a few drops of acetic anhydride were added, then sulphuric acid.

Observation: the solution changed from a colourless to a purplish red colour.

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