REPUBLIC OF CAMEROON
Peace-Word-Fatherland
****
THE UNIVERSITY OF YAOUNDE I
****
POST GRADUATE SCHOOL FOR SCIENCES, TECHNOLOGY AND GEOSCIENCES
****

REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie
****
UNIVERSITE DE YAOUNDE I
****
CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES, TECHNOLOGIE ET GEOSCIENCES
****

DEPARTMENT OF ORGANIC CHEMISTRY

DEPARTEMENT DE CHIMIE ORGANIQUE

DOCTORAL RESEARCH UNIT FOR CHEMISTRY AND APPLICATIONS

UNITE DE RECHERCHE ET DE FORMATION DOCTORALE EN CHIMIE ET APPLICATIONS

Isolation and structural elucidation of biflavonoids from the roots of *Garcinia gnetoides* (Clusiaceae) as potent anti-HIV agent: insight from molecular docking simulation

A Dissertation submitted in partial fulfilment of the requirements for the award of Master degree in Organic Chemistry

By

MEWOLO MIASSE SOPHIE AUDREY

Registration number: **19V2384**

BS_C in chemistry

Under the supervision of

Dr MESSI Angélique Nicolas

Senior lecturer

University of Yaounde I

ACADEMIC YEAR: 2023-2024

REPUBLIC OF CAMEROON
Peace-Word-Fatherland
****
THE UNIVERSITY OF YAOUNDE I
****
POST GRADUATE SCHOOL FOR SCIENCES, TECHNOLOGY AND GEOSCIENCES
****

REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie
****
UNIVERSITE DE YAOUNDE I
****
CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES, TECHNOLOGIE ET GEOSCIENCES
****

DEPARTMENT OF ORGANIC CHEMISTRY

DEPARTEMENT DE CHIMIE ORGANIQUE

DOCTORAL RESEARCH UNIT FOR CHEMISTRY AND APPLICATIONS

UNITE DE RECHERCHE ET DE FORMATION DOCTORALE EN CHIMIE ET APPLICATIONS

Isolation and structural elucidation of biflavonoids from the roots of *Garcinia gnetoides* (Clusiaceae) as potent anti-HIV agent: insight from molecular docking simulation

A Dissertation submitted in partial fulfilment of the requirements for the award of Master degree in Organic Chemistry

By

MEWOLO MIASSE SOPHIE AUDREY

Registration number: **19V2384**

BS_C in chemistry

Under the supervision of

Dr MESSI Angélique Nicolas

Senior lecturer

University of Yaounde I

ACADEMIC YEAR: 2023-2024

DEDICATION

TO

MY PARENTS

Mr. MIASSE HERMAN OLIVIER and Mrs. NGONO NDZULI EVELYNE

ACKNOWLEDGEMENTS

The present study was carried out in the Laboratory of Pharmacochemistry of Natural Substances and Phytoprotection (LPNSP), directed by Professor Dieudonné Emmanuel PEGNYEMB, Rector of University of Bertoua and current Head of the Department of Organic Chemistry of the University of Yaounde I. It was also carried out in the Laboratory of Chemistry of Natural Substances (LCNS) directed by Professor Raphael GHOGOMOU TIH. I am indebted to them for welcoming me in their laboratory team.

Genuinely, I appreciate my supervisor Dr Angelique Nicolas MESSI for his endless assistance, encouragements, advices and task of scrutinizing this work.

Also, my appreciations go to Professor Karl Von ESCHWEGE for the opportunity to record my compounds' spectra at the Department of Chemistry in the University of the Free State South Africa.

My gratefulness is equally expressed to my laboratory elders: Dr Carine KWENE, Dr Gregoire TENE, Mr Roger BALEBA, Mrs Raissa DZUKONG, Mr Abdias SEBDOU, Mr Tite KOUAM, Mrs Sostelle FOMENE, Mr Gendarme MBAT, Mr Stany TSOMENE and Mr Oscar BILONG for their guidance, support and encouragements throughout my laboratory tasks.

Much love and respect to my laboratory colleagues: Mrs Tafrie MVEPEWO, Mrs Cathy HYONKEU MBAKOP and classmates for their availability and support in the course of this academic year.

Besides, I am very grateful to my family and my loved ones especially: Mr Simon NDIMA, Mr Pascal ABENA, Mrs Marguerite MESSINA and Mr Marc ESSOMBA for their advices, moral and financial supports throughout my academic years.

TABLE OF CONTENT

LIST OF ABBREVIATIONS AND SYMBOLS

δc	Chemical shift of Carbon (ppm)
δ_H	Chemical shift of Proton (ppm)
AIDS	Acquired Immunodeficiency Syndrome
APT	Attached Proton Test
CC	Column Chromatography
CCD	Catalytic Core Domain
$CDCl_3$	Deuterated chloroform
$(CD_3)_2CO$	Deuterated acetone
C_5D_5N	Deuterated pyridine
cDNA	Complementary DNA
COSY	Correlation Spectroscopy
d	Doublet
Dd	Doublet of doublets
Ddd	Doublet of doublets of doublets
DEPT	Distortionless Enhancement by Polariztion Transfert
DMSO	Deuterated dimethyl sulfoxide
DMSO-d_6	Dimethyl sulfoxide hexadeuterated
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl 1-picrylhydrazyle
ESI	Electrospray Ionization
EtOAc	Ethyl acetate
FAB	Fast Atom Bombardement
G.	*Garcinia*
GG	*Garcinia gnetoides*
g	Gram
HIV	Human Immunodeficiency Virus
HIV-I	Human Immunodeficiency Virus Type I
HMBC	Heteronuclear Multiple Bond Correlation
HRESIMS	High Resolution Electrospray Ionization Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence

Hz	Hertz
IC_{50}	Half-maximal Inhibitory concentration
IN	Integrase enzyme
IR	Infrared spectroscopy
J	Coupling constant
LAV	Lymphadenopathy-associated virus
LGA	Lamarckian Genetic Algorithm
m	Multiplet
MeOH	Methanol
MeOH-d_4	Methanol deuterated
$[M+H]^+$	Protonated molecule
Ms	Mass spectrometry
MHz	Megahertz
m/z	Mass per electronic charge
NMR	Nuclear Magnetic Resonance
Ppm	Parts per million
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
s	Singlet
STD	Sexually Transmitted Diseases
t	Triplet
TLC	Thin Layer Chromatography
TMS	Tetramethyl silane
WHO	World Health Organization

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

LIST OF APPENDICES

ABSTRACT

The main objective of this research is to isolate biflavonoids from the roots of *Garcinia gnetoides*, having a potential inhibitory effect against HIV-I integrase enzyme. To achieve our objective, we resorted to the phytochemical study of the acetone extract of these roots, from which six pure compounds were isolated. Only two of these compounds (GGB and GGF) were fully characterized and elucidated using spectrometric (HR-ESI mass spectrometry) and spectroscopic methods (^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMBC, HSQC and ROESY). From that, GGB and GGF were identified as: **6"-*O*-acetyl-3′-*O*-methylorientin-[I-(3)-II-(6)]-6"-O-acetyl-3'-O-methylorientin** and **6"- (I) – *O* - acetyl vitexin-[I-(3)-II-(3')]-orientin** respectively. Their inhibitory activity against HIV-I integrase and their respective cytotoxicity concentration were evaluated. According to the results, GGF (IC_{50}= 3.1 µM) presented a noteworthy inhibitory effect against HIV-I integrase *in vitro* but was shown cytotoxic (CC_{50}= 5.2 µM) meanwhile, GGB was shown inactive. A pure competitive inhibitor of HIV-I integrase named Chicoric acid was used as control during these evaluations. In order to validate the therapeutic effect of these two biflavonoids, *in silico* investigations were carried out. The upshot revealed that, GGF and GGB have lower binding scores (**-8.92 Kcal/mol** and **-6.4 Kcal/mol** respectively) compared to Chicoric acid (**-5.56 Kcal/mol**).

Key words: *Garcinia gnetoides*, HIV-I integrase, biflavonoids.

RESUME

Cette recherche a pour objectif d'isoler les biflavonoïdes des racines de *Garcinia gnetoides* ayant un effet inhibiteur contre l'enzyme intégrase du VIH-I. Pour atteindre notre objectif, nous avons eu recours à l'étude phytochimique de l'extrait à l'acétone de ces racines qui a conduit à l'isolement de six composés purs. Deux de ces composés (GGB et GGF) ont été entièrement caractérisés et élucidés à l'aide des méthodes spectrométriques (HR-ESI spectrométrie de masse) et spectroscopiques (RMN ^{1}H, RMN-^{13}C, COSY ^{1}H-^{1}H, HMBC, HSQC et ROESY). Celles-ci ont conduit à l'identification de GGB et GGF comme étant : **6"-*O*-acetyl-3′-*O*-methylorientine-[I-(3)-II-(6)]-6"-O-acetyl-3'-O-methylorientine** et **6"-(I)-*O*-acétyl vitexin-[I-(3)-II-(3')]-orientine** respectivement. L'activité inhibitrice de GGB et GGF contre l'enzyme intégrase du VIH-I, ainsi que leur concentration cytotoxique ont été évalués *in vitro*. Ces résultats ont montré que GGF (IC_{50}= 3.1 µM) a meilleur effet inhibiteur mais est cytotoxique (CC_{50}= 5.2 µM) tandis que GGB est inactive. Un inhibiteur compétitif de l'intégrase du VIH-I nommé acid Chicorique a été utilisé comme témoin durant ces évaluations. Dans le but de valider l'effet thérapeutique de ces deux biflavonoïdes, les investigations *in silico* ont été réalisées. Ces résultats ont révélé que GGF et GGB ont le score de liaison le moins élevé (**-8,92 Kcal/mol** et **-6.4 Kcal/mol** respectivement) comparer à l'acide Chicorique (**-5.56 Kcal/mol**).

Mot clés : *Garcinia gnetoides*, l'enzyme intégrase du VIH-I, biflavonoïdes.

INTRODUCTION

Human immunodeficiency virus (HIV) remains a leading global health concern. In 2023, there were 39.9 million people across the globe living with HIV of which, 38.6 million were adults and 1.4 million were children. Approximately 76,000 children and 560,000 adults died from AIDS related causes, mostly due to inadequate access to HIV prevention, care and treatment services. Out of these 39.9 million, a staggering 25.6 million people are in Sub-Saharan Africa which has been the hardest hit by the HIV epidemic. In Cameroon, the number of people living with HIV was estimated at 480232 **(WHO, 2024)**.

HIV-1 integrase plays a pivotal role in the integration of viral DNA into the cellular genome. The research development of highly active antiretroviral therapies (HAARTs) has helped to control HIV pandemic. These medications are meant to target different steps of the HIV life cycle to prevent viral replication. **(Marina and Robert, 2016).** Unfortunately, their prolonged use is compromised as a result of the development of virus resistance, unavailability and the lack of a curative effect **(Tessa *et al.*, 2024).**

The above information underscores the need to explore novel therapeutic strategies such as the incorporation of medicinal plants for the management and treatment of viral diseases and related opportunistic infections. Many studies reported that flavonoids are effective in suppressing HIV replication. The presence of free hydroxyl groups in some of these flavonoids could be responsible for their anti-HIV effect. Biflavonoids are a subclass of flavonoids and are naturally formed by two identical or nonidentical flavonoid units. Moreover, the bioactivities of biflavonoids are stronger than those of flavonoid hence have advantages over monomeric flavonoids since biflavonoids are able to survive first-pass metabolism, which inactivates most flavonoids **(Messi *et al.*, 2022).**

Garcinia species contains a broad range of biologically active secondary metabolites especially biflavonoids and prenylated xanthones. These metabolites were associated to biological activities such as cytotoxicity, anti-HIV and anti-oxidant **(Bruna *et al.*, 2020)**. Some biflavonoids isolated from *Garcinia* exhibited anti-HIV activity such as; Amentoflavone, Morelloflavone, Morelloflavone-7''-*O*-glycoside and Volkensiflavone **(Yuh-meei *et al.*, 1997).** *Garcinia gnetoides* was selected as the subject of investigation. This choice was motivated by the fact that, up till date, no phytochemical studies as well as the assessment of the inhibitory effects on HIV-1 integrase enzyme have been previously investigated. Besides, *Garcinia gnetoides* may contain biflavonoids with anti-HIV properties.

General objective

The backbone of this study is mainly aimed at isolating and characterizing biflavonoids from the roots of *Garcinia gnetoides* capable of inhibiting HIV-I integrase enzyme thus preventing viral replication.

Specific objectives:

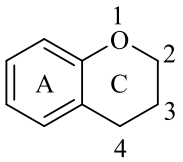
- Isolate biflavonoids from the roots of *Garcinia gnetoides* using organic solvents in order of increasing polarity.
- Characterize these biflavonoids using spectroscopic and spectrometric analysis methods.
- Evaluate the inhibitory effect of these biflavonoids against the HIV-I integrase enzyme *in vitro*.
- Validate the anti-HIV activity of these biflavonoids by molecular docking studies against HIV-I integrase.

CHAPTER I: LITERATURE SURVEY

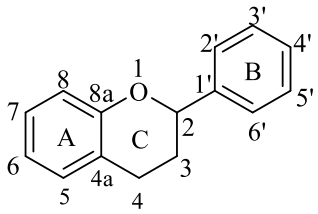
I.1. GENERALITIES ON FLAVONOIDS

Flavonoids are low molecular weight bioactive polyphenols which plays an essential role in photosynthesizing cells **(Cushine and Lamb, 2005).** The original "flavonoid" research started in 1936, when a Hungarian scientist Albert Szent-Gyorgi was uncovering a synergy between pure vitamin C and unidentified cofactors from the peels of lemons, which he first called "citrin," (later it was referred to as "vitamin P") **(Murray, 1998).** Flavonoids display an extensive range of structures and are responsible for the significant organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties. They also contribute to the nutritional qualities of fruits and vegetables **(Karak, 2019).**

Flavanoids constitute one of the largest classes of naturally occurring plant products mostly phenols and exist either in the free state or as their respective glycosides **(Sarita *et al.*, 2009).** Flavanes 2 (Basic chemical structure of Flavonoids) possess a C_6–C_3–C_6 carbon framework or more specifically a phenyl benzopyran skeleton (the benzopyran ring system is also called chroman 1). The three rings of the phenyl benzopyran nucleus are labeled as A, B and C (pyran) rings as shown on **Figure 1 (Alok *et al.*, 2010).**



1



2

Figure 1: Benzopyran 1 and Flavane chemical skeleton 2 **(Alok *et al.*, 2010)**

I.1.1. Structure and classification of flavonoids

Flavonoids can be subdivided into different subgroups depending on the carbon of the C ring on which the B ring is attached. Flavonoids in which the B ring is linked at position **3** of the C ring are called isoflavanes 4 meanwhile those in which the B ring is linked at position **4** are called neoflavanes 5. The location of the B ring is linked at position **2** can be subdivided into several subgroups on the basis of the structural features of the C ring. These subgroups are: Flavone 6, Flavonol 7, Flavanone 8, Flavanonol 9, Flavanol or Catechin 10, Anthocyanins 11 and Chalcones 12 **(Panche *et al.*, 2016).** Their respective structure is shown on **Figure 2.**

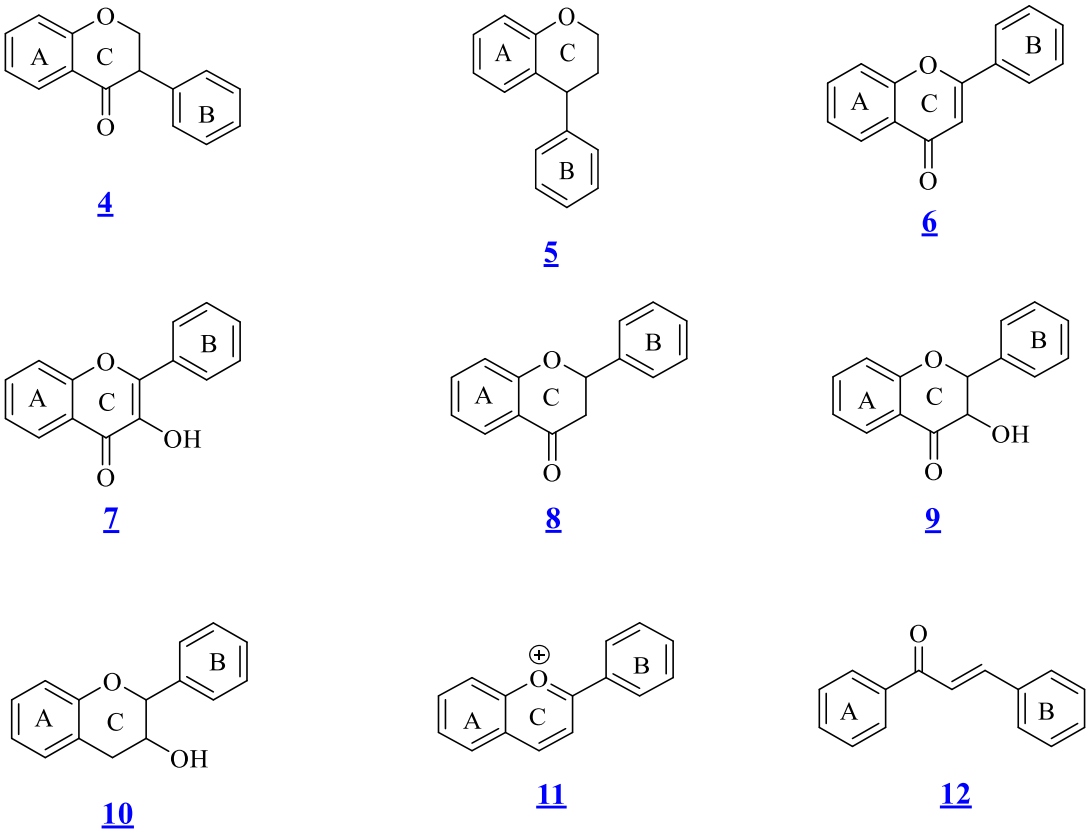


Figure 2: Classes of flavonoid monomers **(Panche *et al.*, 2016).**

Some food sources containing different classes of flavonoids are given on **Table 1**. Being phytochemicals, flavonoids cannot be synthesized by humans and animals.

Table 1: Subclasses of flavonoids and their occurrence in foods

Flavonoid subclass	Examples of compounds	Food source	References
Flavonol	Kaempferol, Quercetin and Myricetin	Onion, red wine, apples and cherries	**Stewart *et al.*, 2000**
Flavone	Chrysin, Apigenin and Luteolin	Red wine and tomato skin	**Middleton, 1998**
Flavonone	Naringin, Naringenin and Hesperidin	Citrus fruits and lemons	**Miyake *et al.*, 2000**
Flavanol	Catechin, Epicatechin and Epigallocatechin	Apple and tea	**Stewart *et al.*, 2000**
Isoflavone	Genistein and Daidzein	Soya beans and Legumes	**Kaufman *et al.*, 1997**
Anthocyanidins	Apigenidin, Cyaniding and Delphinidin	Cherries, Raspberry and strawberry	**Kreft *et al.*, 1999**

Flavonoids are often hydroxylated at position 3, 5, 7, 3', 4' and/or 5'. One or more of these hydroxyl groups can be methylated, acetylated, prenylated, sulphated or glycosylated **(Eva *et al.*, 2006)**.

I.1.2. Flavonoid's glycosides

In principle, any hydroxyl group on flavonoid skeleton can be glycosylated, but some positions are favored in nature. According to the glycosidic linkage pattern, natural flavonoid glycosides are divided into O-glycosides and C-glycosides **(Liangqin *et al*. 2022).**

I.1.2.1. C-glycoside

Flavonoid C-glycosides are linked by the anomeric carbon of sugar and flavonoid skeleton through a C-C bond. Natural C-glycosides have been detected on flavones, flavonols, isoflavones and chalcones. The most important sub-class is flavone C-glycoside. The C-glycosides of flavonoid are predominantly linked at C-6 or C-8 of ring A. A few are found to be linked to other positions, like C-3 **(Hussain *et al.*, 2008).** The most widely distributed aglycones of flavonoid C-glycoside are apigenin and luteolin. Flavonoid C-glucosides are the most abundant type. Mono-C-glycosyl and di-C-glycosyl flavonoid are the top two structures in this type of chemical **(Choo *et al.*, 2012).**

I.1.2.2. O-glycoside

The O-glycosides have sugar substituents bound to a hydroxyl group of the aglycone, usually located at 3 or 7. The O-bonding occurs more frequently than the C-bonding **(Eva *et al.*, 2006).**

O and C-glycoside can be differentiated by using soft ionization techniques with low fragmentation energy (usually FAB-MS). Glycosides from O-glycosides undergo heterolysis of their hemi-acetal O-C bonds, giving rise to Y_i^+ ions. Meanwhile, C-glycosides only produce $[M+H]^+$ ions **(Cuyckens *et al.*, 2000).**

Glycosides can improve the stability of compounds, increase water solubility, improve the specific targeting property of drugs, possess better bioavailability than their aglycones *in vivo*, reduce toxic and side effects. C-glycosides show more diversified biological activities than O-glycosides, because C-C covalent bonds are steadier and resistant to acid, alkali and enzymatic hydrolysis **(Liangqin *et al*. 2022).**

I.1.3. Biosynthesis of Flavonoids

Flavonoids are products of both the shikimic acid and acetate pathway being formed by the condensation of a phenyl- *y* -propanoid precursor with 3 malonyl coenzyme A unit. Chalcones works as an important intermediate for majority of flavonoid compounds in the

plant kingdom **(Sarita *et al.*, 2009)**. The biosynthetic pathway of the different classes of flavonoids can be described according to **Figure 3**.

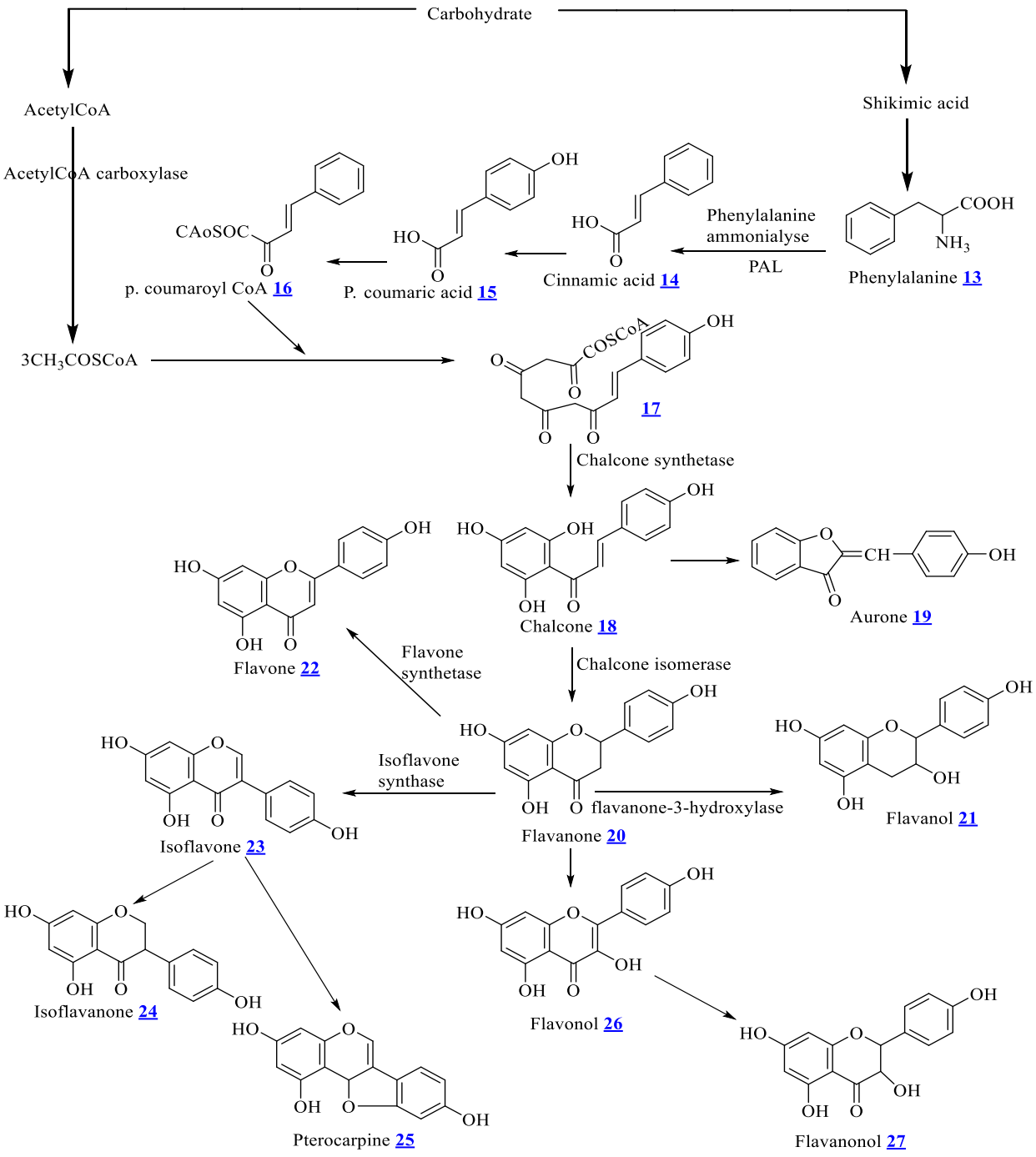


Figure 3: General biosynthetic pathway of the basic skeletons of the different classes of Flavonoids **(Harbone and Williams, 2000)**

I.2. GENERALITIES ON BIFLAVONOIDS

Biflavonoids are flavonoid dimers generally made up of flavone-flavone, flavone-flavanone, flavanone-flavanone dimers, but rarely of chalcone and isoflavone dimers **(Weniger *et al.*, 2006).**

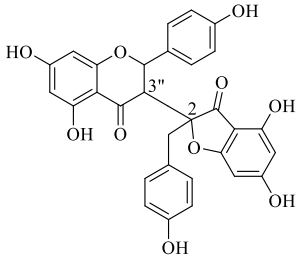
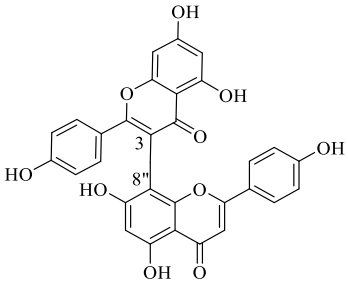
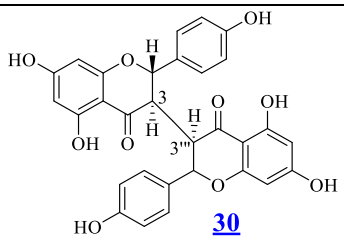
I.2.1. Types of Biflavonoids

The bonds between flavonoid dimers can either be a C-C (C-biflavonoid) or C-O-C (Biflavonoid ether) bond type. Depending on the linkage uniting flavonoids units, three main types of biflavonoids can be enumerated as shown below;

I.2.1.1. C-C type

These are biflavonoids with a C-C linkage. They are in large number and so can be divided into: 2-3", 2'-8", 2'-6", 3-3", 3-3'", 3-6", 3'-6", 3-7", 3-8", 4-6", 4'-8", 5-5", 6-6", 6-8", 7-7" and 8-8". Some examples of C-C type biflavonoids are presented on **Table 2.**

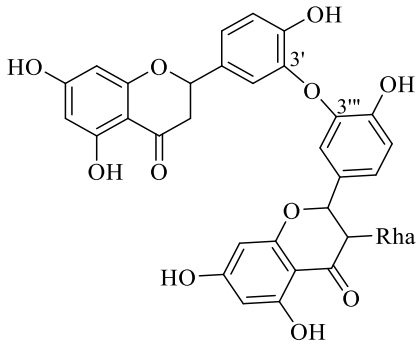
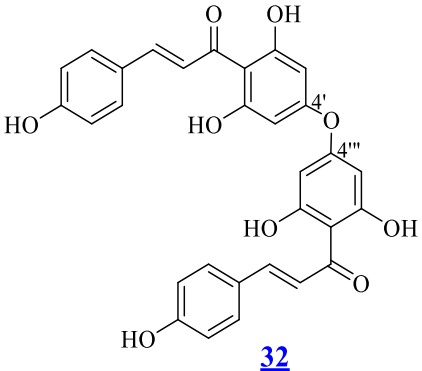
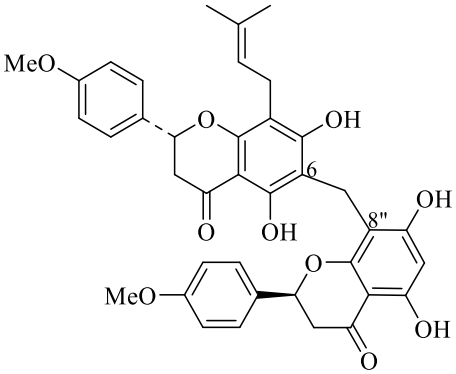
Table 2: Some examples of C-C type biflavonoids

C-C type	Name	Structure	Reference
2-3"	Linobiflavonoid	 **28**	**(Gontijo *et al.*, 2017)**
3-8"	3-8" Biapigenin	 **29**	**(Nonaka *et al.*, 1983)**
3-3'"	Chamaejasmine	 **30**	**(Yun-yun *et al.*, 2015)**

I.2.1.2. C-linear fragment-C type

These are biflavonoids consisting of a C-*O*-C, C-C-C or other linear fragment connection (that is: 3'-*O*-3''', 3-*O*-4''', 4'-*O*-4''', 6-*O*-7'', 6-C-8'' and 8-C-8''). Some examples of C- linear fragment-C type biflavonoids are presented on **Table 3**.

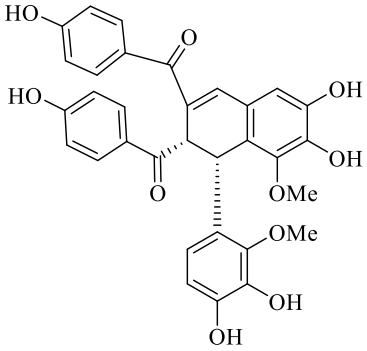
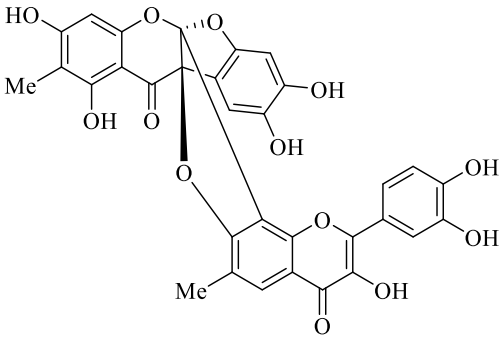
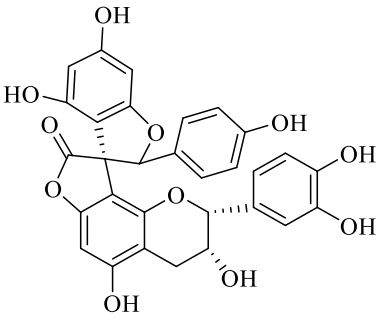
Table 3: Some examples of C-linear fragment-C type biflavonoids

C-linear fragment-C type	Name	Structure	References
3'-*O*-3'''	Sparinaritin	 31	**(Adjapmoh *et al.*, 2016)**
4'-*O*-4'''	Achyrobichalcone	 32	**(Carini *et al.*, 2013)**
6-C-8''	Bosistoabiflavone	 33	**(Parsons *et al.*, 1993)**

I.2.1.3. Complex bioflavonoids

They include the simple ring type (C-C and C-C, C-C and C-C-C, C-O-C and C-O-C), the bicyclic type and spiroflavonoids. Some examples of C-C type biflavonoids are presented on **Table 4**.

Table 4: Some examples of complex biflavonoids

Complex biflavonoids	Name	Structures	References
Simple ring type (C-C and C-C)	Licobichalcone	 **34**	**(Bai *et al.*, 2003)**
Bicyclic type	Baeckein F	 **35**	**(Ren *et al.*, 2019)**
Spiroflavonoids	Absienol A	 **36**	**(Gontijo *et al.*, 2017)**

I.2.2. Biosynthesis of Biflavonoids

Several flavonoid polymers have been characterized as constituents of different plants. After experiments on phenolic radical couplings in the laboratory, a rechercher named Jackson and his collaborators attributed the biosynthesis of biflavonoids to the oxidative coupling of two naringin radicals. The existence of biflavonoids could be explained by condensation reactions based on the theory of dimerization of two radicals from flavonoid units **(Jackson *et al.*, 1971)**. Thus, through this mechanism **(Figure 4)**, we find radicals that are precursors of all natural biflavonoids. These radicals, which are formed by the loss of a hydrogen atom of a phenol function on the flavonoid, are designated by Rα, Rβ, Rɣ, Rδ.

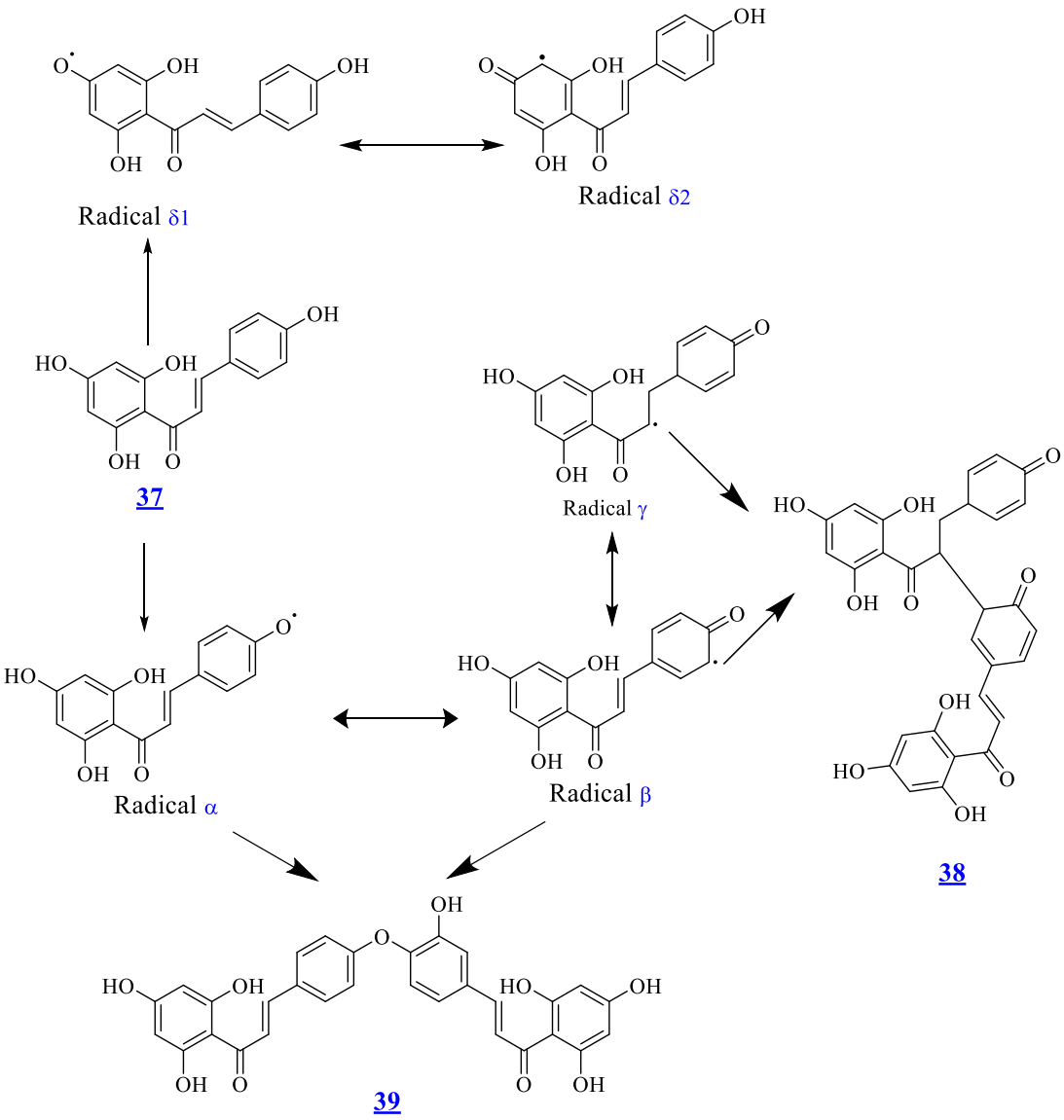


Figure 4: Mechanism of formation of biflavonoids precursors **(Jackson *et al.*, 1971)**

I.3. METHODS USEFUL IN THE DETERMINATION OF FLAVONOID STRUCTURES

I.3.1. Mass spectrometry

A mass spectrometer is a universal detector that can achieve very high sensitivity and provide information on the molecular mass and structural features. More detailed structural information can subsequently be obtained by resorting to tandem mass spectrometry (MS/MS) in combination with collision-induced dissociation (CID) **(Filip and Magda, 2003)**.

Gas chromatography (GC)/MS is not widely used in flavonoid analysis owing to the limited volatility of flavonoid glycosides. Since the development of Electrospray ionization (ESI) and liquid chromatography (LC)/MS coupling became more efficient and easier to use, making it by far the most popular technique for flavonoid nowadays **(McGhie and Markham, 1994)**. The highest sensitivity is obtained using ESI in the negative ion mode with an eluent system consisting of an acidic ammonium acetate buffer. This results in limited fragmentations, making it most suited to infer the molecular mass of the separated flavonoids especially in cases of low concentrations **(Cuyckens and Claeys, 2002)**.

The peak at the highest *m/z* ratio is not always the molecular ion species in the positive mode ($[M+H]^+$) and the negative mode ($[M-H]^-$) because adducts with solvent and/or acid molecules and also complexes $[2M+H]^+$ or $[2M-H]^-$ can be generated **(Schieber *et al.*, 2002).**

I.3.2. Nuclear magnetic resonance spectrum (NMR)

Nuclear magnetic resonance spectrum (NMR) is the most powerful method to elucidate the structures of flavonoids. Different solvents, such as $CDCl_3$, DMSO-d_6, C_5D_5N, $(CD_3)_2CO$ and CD_3OD could be employed while performing NMR experiments. DMSO-d_6 is the best solvent among them to perform NMR on flavonoids because almost all kinds of flavonoid could be well dissolved in DMSO-d_6 and the resonance signals of flavonoids are rarely overlapped by its solvent peaks. Furthermore, NMR signals of phenolic hydroxyl could be displayed clearly with DMSO-d_6 as the solvent. The drawback of this solvent is high boiling point, which leads to difficulty in sample recovery **(Weisheng *et al.*, 2017)**. NMR experiments can be performed in one dimension (1D) or two dimensions (2D) depending on the complexity of the system being studied.

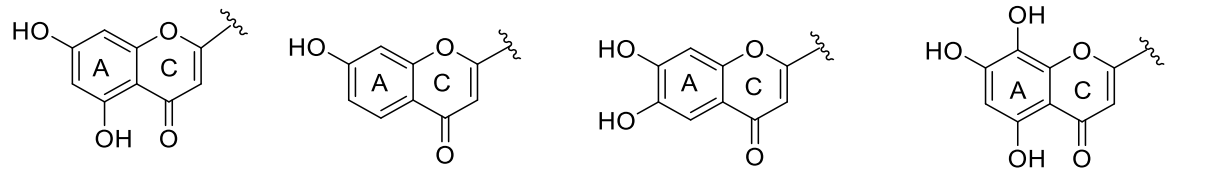
I.3.2.1. 1D NMR

One-dimensional (1D) NMR spectroscopy includes: Proton (^{1}H) and Carbon (^{13}C) NMR spectroscopy.

- **^{1}H-NMR Spectrum**

It provides information of chemical shifts, coupling constants and proton number of the analyte. The types of flavonoids, substitution modes, number and configurations of glycosyls could be determined via ^{1}H NMR spectrum.

The possible substitution modes on ring A of flavonoids are: 5,7-dioxygenation, 7-oxygenation, 6,7-trioxygenation and 5,7,8-trioxygenation as shown on the **Figure 5** (using flavone as example).



5,7-dihydroxyflavone 7-hydroxyflavone 6,7-trihydroxyflavone 5,7,8-trihydroxyflavone

Figure 5: Substitution modes of ring A

5,7-Dihydroxyl flavonoids are mostly common. For this type of substitution, the signal of H-8 ranges from δ_H 5.7-6.9 as a doublet meanwhile the signal of H-6 is always at the higher field than H-8. The signals of both H-6 and H-8 shift move to lower fields after the glycosylation of 7-OH.

The signal of H-5 in 7-hydroxyl substituted ring A is shown to be a doublet since vicinal coupling exists between H-5 and H-6. Additionally, the chemical shift is at low field because of the shielding effect of carbonyl at C-4 **(Mabry *et al.*, 1970).** The chemical shifts of protons on ring A are summarized on **Table 5**.

Table 5: Chemical shifts of protons on ring A **(Fang, 2006; David, 2021)**

Substitution mode	Flavonoid type	H-5	H-6	H-8
5,7-Dihydroxyl	Flavone, flavonol, isoflavone	-	δ_H 6.0-6.2 (d) J= 2-3 Hz	δ_H 6.3-6.5 (d) J= 2-3 Hz
	Flavanone, flavanonol	-	δ_H 5.75-5.95 (d) J= 2-3 Hz	δ_H 5.9-6.1 (d) J= 2-3 Hz
7-Hydroxyl	Flavone, flavonol, isoflavone	δ_H 7.9-8.2 (d) J= 2-3 Hz	δ_H 6.7-7.1 (dd) J= 2-3 Hz and 6-10 Hz	δ_H 6.7-7.0 (d) J= 6-10 Hz
	Flavanone, flavanonol	δ_H 7.7-7.9 (d) J= 2-3 Hz	δ_H 5.7-6.0 (dd) J= 2-3 Hz and 6-10 Hz	δ_H 5.9-6.1 (d) J= 6-10 Hz

Generally, signals of protons on ring B are showed at slightly lower field and the chemical shifts are usually ranging from δ_H 6.7-8.1. The substitution modes and structural information of protons could also be determined via the chemical shifts and coupling constants on ring B. They are shown on **Figure 6 and Table 6** below:

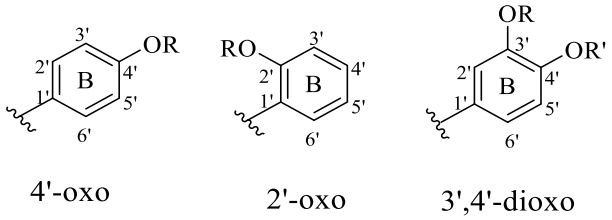


Figure 6: Substitution modes of ring B **(Weisheng *et al.*, 2017)**

Table 6: Chemical shifts of protons on ring B of various substituent modes **(Fang, 2006)**

Substituent mode	H-2′	H-3′	H-4′	H-5′	H-6′
2′-Oxygenated	-	δ_H 6.8-6.9 (m or dd)	δ_H 7.2 (m or dd)	δ_H 6.8-6.9 (m or dd)	δ_H 7.4-7.5 (m or dd)
4′-Oxygenated	δ_H 7.1-8.1(1H, d, *J*=8.0Hz)	δ_H 6.5-7.1 (1H, d, *J*=8.0Hz)	-	δ6.5-7.1 (1H, d, *J*=8.0Hz)	δ_H 7.1-8.1 (1H, d, *J*=8.0Hz)
3′,4′-Oxygenated	δ_H 7.2-7.8(1H, d, *J*=2.0Hz)	-	-	δ_H 6.7-7.1 (1H, d, *J*=8.0Hz)	δ_H 6.7-7.7(1H, dd, *J*=8.0,2.0Hz)

Chemical shifts of protons on common substituents and ^{1}H-NMR characteristic protons on ring C are described on **Table 7 and 8** respectively.

Table 7: Chemical shifts of protons on common substituents **(Weisheng *et al.*, 2017)**

Proton type	Chemical shift
Phenolic hydroxyl	δ_H 10.0-14.0
Methyl	δ_H 2.3-2.5 (3H, s)
Methoxy	δ_H 3.5-4.1 (3H, s)
Terminal protons of glycosyl	δ_H 4.5-5.5

Table 8: Chemical shifts and coupling constants of protons on ring C of common flavonoids **(Weisheng *et al.*, 2017)**.

Flavonoid type	H-2	H-3
Flavone	-	δ_H 6.3-6.8 (s)
Isoflavone	δ_H 7.6-7.8 (s)	-
Chalcone	δ_H 6.7-7.4(d, *J*=17Hz)	δ_H 7.3-7.7(d, *J*=17Hz)
Flavanone	δ_H 5.0-5.5(dd, *J*=11.5Hz)	δ_H 2.3-2.8(2H) (dd, *J*=17.11Hz)
Flavanonol	δ_H 4.8-5.0(d, *J*=11Hz)	δ_H 4.1-4.3(d, *J*=11Hz)
Aurone	Exocyclic proton: δ_H 6.5-6.7 s	-

- **^{13}C NMR Spectrum**

The types of flavonoids, number, connections and glycosyl positions of carbon atoms could be spotted from ^{13}C-NMR spectra. The characteristic signals of carbons in ring C permit the identification of different types of flavonoids **(Table 9)**. Also, common substituents may exhibit a certain characteristic carbon signals range of chemical shift as shown on **Table 10**.

Table 9: Chemical shifts of carbons in ring C of flavonoids **(Heller and Forkman, 1988)**

Flavonoid type	C-2(δ ppm)	C-3(δ ppm)	C-4(δ ppm)
Flavone	160.0-165.5	104.0-112.0	175.0-184.0
Isoflavone	149.8-156.5	120.3-126.0	174.5-182.2
Flavanone	75.0-80.5	42.0-44.6	189.5-197.2
Flavanonol	82.7-83.5	71.2-73.0	188.0-197.0
Chalcone	136.9-145.4	116.8-128.1	188.0-194.6
Aurone	146.1-147.7	111.0-113.3	180.5-182.7
Flavan-3-ol	77.5-82.7	65.1-68.2	27.5-28.6

Table 10: Chemical shifts of carbons of common substituents on flavonoids **(Munekazu *et al.*, 1980)**

Substituent	Chemical shift
$-CH_3$	δc 20-30
$-OCH_3$	δc 55-57
Terminal carbon of glycosyls O-glycosides C-glycosides	δc 95-105 δc 71-78

- **Glycosides of flavonoids**

In plants, flavonoids are often present as O- or C-glycosides. The O-glycosides have sugar substituents bound to a hydroxyl group of the aglycone and are usually located at position 5 or 7 whereas, the C-glycosides have sugar groups bounded to a carbon of aglycone, usually 6-C or 8-C. The most common carbohydrates are rhamnose, glucose, galactose and arabinose **(Cuyckens *et al.*, 2000).**

Generally, the chemical shift of anomeric protons of glycosyls are at δ_H 4.5-5.5 in ^{1}H-NMR. The anomeric carbons of O-glycosides are at δc 95-105 meanwhile, for C-glycosides, they are at δc 71-78. Furthermore, the number of glycosyls could be determined by combined analysis of ^{1}H and ^{13}C-NMR spectra **(Jane *et al.*, 1981).**

Glycoside monomers predominantly exist as two isomeric pyranoses (α and β anomers). The α-anomer has a *J* coupling constant ranging from 2-4 Hz whereas, for β-anomer ranges from 7-10 Hz. As the α and β anomers are diastereomeric, they generally have a distinguishable NMR spectrum. In fact, the influences of both solvent and temperature on glycosides are readily determined by NMR **(William, 2003)**. **Figure 7 and Table 11** show the carbon and proton chemical shift of α-/β- D-Glucose and α-/β-D-Galactose respectively.

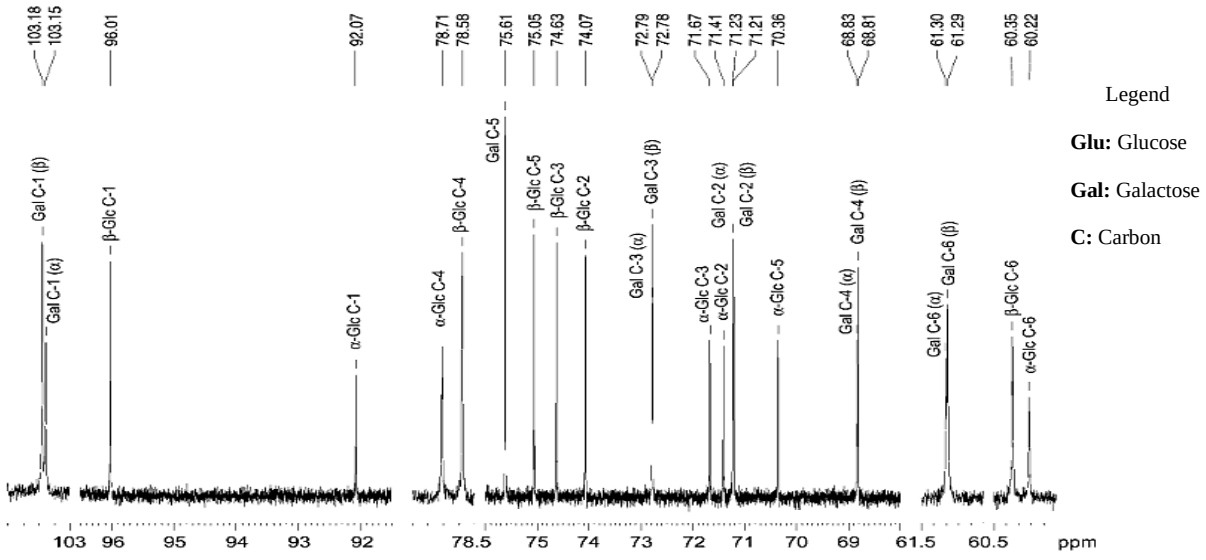


Figure 7: Carbon chemical shifts of α-/β- D-Glucose and α-/β-D-Galactose **(William, 2003)**

Table 11: Proton chemical shifts of α-/β- D-Glucose and α-/β-D-Galactose **(Mattias *et al.*, 2003)**

Type of glycosyl	H-1"	H-2"	H-3"	H-4"	H-5"	H-6a", 6b"
β-D-glucose	δ 5.21	δ 3.51	δ 3.69	δ 3.39	δ 3.81	δ 3.82, 3.74
α-D-glucose	δ 4.62	δ 3.22	δ 3.46	δ 3.38	δ 3.44	δ 3.87, 3.70
β-D-galactose	δ 4.58	δ 3.50	δ 3.94	δ 3.94	δ 3.71	δ 3.80, 3.72
α-D-galactose	δ 5.26	δ 3.82	δ 3.87	δ 4.00	δ 4.10	δ 3.80, 3.72

Glycosides can at best be distinguished through their characteristic *J* coupling constant values as shown on **Figure 8**.

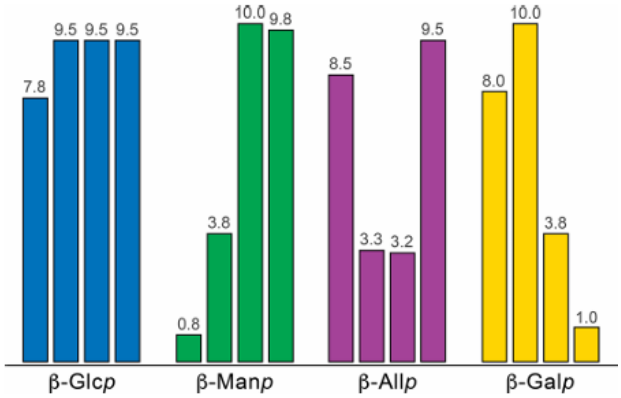


Figure 8: Representation of the $^{3}J_{H1,H2}$, $^{3}J_{H2,H3}$, $^{3}J_{H3,H4}$ and $^{3}J_{H4,H5}$ coupling constant values (left to right, respectively) of *β*-anomeric of some monosaccharides using bar charts. *J* coupling constants values (Hz) are indicated at the top of each bar **(Carolina and Goran, 2023).**

I.3.2.2. 2D NMR

The two-dimensional (2D) NMR is an advanced spectroscopic technique that builds upon the capabilities of one-dimensional (1D) NMR by incorporating an additional frequency dimension. Some 2D NMR spectrum could be described as follows;

- **COSY (Correlation spectroscopy):** Provides information about scalar coupling between protons in a molecule through bonds.
- **ROESY (Rotating-Frame Overhauser Enhancement Spectroscopy):** Provides information about the spatial proximity of protons in a molecule (Protons close to each other in space but not bonded).
- **HSQC (Heteronuclear Single Quantum Coherence):** Correlates proton and heteronuclear (example; Carbon or Nitrogen) atom single bond coupling. It is useful for determining the location of functional groups for assigning carbon resonances.
- **HMBC (Heteronuclear Multiple Bond Correlation):** Provides information about the long-range proton-heteronuclear couplings through two to four bonds. It helps to determine the connectivity and relative positions of functional groups in complex molecules.
- **TOCSY (Total Correlation Spectroscopy):** Shows all proton-proton correlations in a molecule, regardless of scalar couplings. It is particularly useful for determining the connectivity in small molecules.

I.4. GENERALITIES ON GENUS *Garcinia*

Genus *Garcinia* is the largest genus within the family Clusiaceae (formely Guttiferae) and nearly comprised of 250 species worlds over. They are generally small or medium sized evergreen trees distributed in pantropical regions, with high richness in South-East Asia **(Shameer *et al.*, 2016).** The plants of this genus have multiple applications in culinary, pharmaceutical and industrial fields. Also, it is ornamental with a dense canopy of green leaves and red-tinged tender emerging leaves **(Hemshekhar *et al.*, 2011).** The genus is native to Asia and Africa. *Garcinia* species plants contain a broad range of biologically active metabolites **(see I.5.)** which had received considerable attention due to the chemical compositions of their extracts. Compounds isolated from this genus have been shown to exhibit several biological activities such as: anti-inflammatory, antioxidant, antihistaminic, antiulcerogenic, antimicrobial and anti-HIV **(Bruna *et al.*, 2020).**

I.4.1. Geographical distribution of *Garcinia's* species

About 35 species are common in India and are endemic to the evergreen forests of Western Ghats and North-Eastern region of India **(Hemshekhar *et al.*, 2011).** In Africa, *Garcinia* is mainly found in countries going from Senegal, Angola, Democratic Republic of Congo to Tanzania and Cameroon.

Figure 9: Distribution map of *Garcinia* species in the world **(Shameer *et al.*, 2016)**

The herbarium samples conserved in the National Herbarium of Cameroon indicate the presence of about 21 species of *Garcinia* **(Guedje and Fankap, 2001).**

Table 12: Different species of *Garcinia* present in Cameroon **(Guedje, 2002)**

SPECIES OF *Garcinia* (G.)	**REGIONS**									
	ES	AD	FN	NO	CE	WE	NW	SW	LT	SO
G. afzelii			×		×					
G. barteri		×								
G. brevipedicellata					×			×	×	
G. chromocarpa								×		
G. ovalifilia	×	×		×					×	
G. mannii		×			×				×	
G. epunctata		×			×			×	×	×
G. polyanta	×				×		×	×		
G. gnetoides				×	×				×	
G. elliotii								×		
G. nobilis								×		×
G. mangostana						×				
G. tinctoria						×				
G. conranana					×	×				
G. preussii	×				×			×		
G. lucida					×			×	×	×
G. kola	×							×		
G. staudtii									×	
G. smeathmannii					×	×	×			
G. letestii									×	
G. punctata	×				×			×	×	×

Legend **ES:** East, **AD:** Adamawoua, **FN:** Far North, **NO:** North, **CE**: Center, **WE:** West, **NW:** North West, **SW:** South West, **LT:** Littoral and **SO:** South

According to **Table 12**, *Garcinia*'s species are mostly present in the Center region and the South-west region.

I.4.2. Botanical description of Genus *Garcinia*

Genus *Garcinia*'s plant parts can be described as follows;

I.4.2.1. Fruits

Garcinia are berries with walls that are thick or thin and woody. No *Garcina* species has edible fruit wall. The color of the fruits is normally greenish when young, turning

yellowish, reddish or dark purplish when mature. The shape of the fruits varies from globose, ovoid (flask-shaped) to ellipsoid. Fruit swig ranges from very small (less than 1 cm) to large (more than 7 cm) **(Nazre *et al.*, 2018).**

Figure 10: Fruit morphology of *Garcinia* species in the Western Ghats: **A.** *G. rubro-echinata*, **B.** *G. imberti*, **C.** *G. wightii*, **D.** *G. travancorica*, **E.** *G. morella*, **F.** *G. talbotii*, **G.** *G. pushpangadaniana*, **H.** *G. indica* and **I.** *G. gummi-gutta* **(Zixi *et al.*, 2018).**

I.4.2.2. Flowers

The flowers are fleshy, unisexual and tetramerous. Also,they are usually small, fasciculate and axillary or terminal. Their color varies from greenish-yellow to dark red **(Guedje *et al.*, 2000).**

Figure 11: Images of flowers from representative species of *Garcinia* and its segregate genera: **A.** *Garcinia. asterandra*, **B.** *G. parvifolia*, **C**. *G. atroviridis*, **D.** *G. hombroniana*, **E.** *G. afzelii*, **F.** *Pentaphalangium latissimum*, **G.** *G. prainiana*, **H.** *Ochrocarpos parvifolius*, **I.** *G. prainiana*, **K.** *G. xanthochymus*, **L.** *G. verrucosa*, **M.** *G. conrauana*, **N.** *Rheedia. aphanophlebia*, **O.** *G. smeathmannii*, **P.** *Rheedia macrophylla*, **Q.** *G. livingstonei* **(Sweeny, 2008)**

I.4.2.3. Leaves

They are leaves deep-green in color; 5-8 cm and 2-3 cm broad, simple, exstipulate with acute apex and the venation is intercostal. The microanatomy of leaves showed the presence of rubiaceous stomata only on the abaxial side. The anatomical characteristics of petiole shows 6-12 mm long **(Priya and Hari, 2018)**.

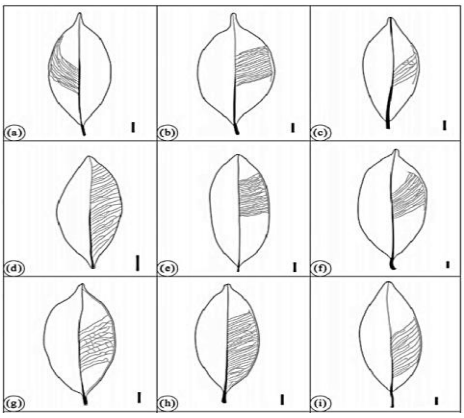


Figure 12: Leaf architecture of *Garcinia* species : **A.** *G. afzelii*, **B.** *G. brevirostris*, **C.** *G. cadelliana* **D.** *G. calcicola*, **E.** *G. caloneura*, **F.** *G. celebica*, **G.** *G. multifora*, **H.** *G. pallida* and **I.** *G. tonkinensis* **(Zixi *et al.*, 2018).**

I.4.2.4. Tree bark

Garcinia are generally small to medium-sized trees reaching 10-35 cm diameter at breast height (dbh) and are 5-25 m tall when mature, rarely reaching 50 cm dbh and 30 m tall **(Nazre *et al.*, 2018).** The color of the tree bark can be yellowish or brownish depending on the species **(Guedje *et al.*, 2007).**

Figure 13: Tree bark exudates in *Garcinia* species in the Western Ghats: **A.** *G. rubro echinata*, **B.** *G. imberti*, **C.** *G. wightii*, **D.** *G. travancorica*, **E.** *G. morella*, **F.** *G. talbotii*, **G.** *G. pushpangadaniana*, **H.** *G. indica* and **I.** *G. gummi-gutta* **(Shameer *et al.*, 2016).**

I.4.3. Uses of Genus *Garcinia*

Genus *Garcinia* can be used both traditionally and socio-economically as described below;

I.4.3.1. Traditional uses of *Garcinia*

Various parts of plants of Genus *Garcinia* (stem bark, fruits, roots and leaves) are diversly used as traditional medicines.

In Thailand, *Garcinia mangostana*, one of the best-known tropical fruits is used as a native anti-inflammatory, anti-diarrheal and for treatment of dysentery **(Yapwattanaphun *et al.*, 2002).** The pericarps, seeds and leaves of *Garcinia indica* have been used to treat inflammatory disorders and rheumatism in China **(Fengke *et al.*, 2021).** Also, the fruits and leaves of *Garcinia cowa* are used for the improvement of blood circulation and as an expectorant for the treatment of cough and indigestion while the roots are used for fever relief **(Panthong *et al.*, 2006).**

In Liberia, the roots of *Garcinia epunctata* mixed with palm wine form an excellent aphrodisiac. The bark is chewed to fight cough and the leaves are eaten in the form of soup against stomach disorders in Congo Brazzaville. Also, a decoction of its leaves is used in Ivory Coast to soothe toothaches **(Burkill, 1997).**

In Cameroon, the seeds and the barks of *Garcinia* are the most commonly used for multiple treatments as shown on **Table 13**.

Table 13: Traditional uses of *Garcinia*'s species in Cameroon **(Guedje *et al.*, 2000)**

Medical issue	Parts used	*Garcinia* Species
Gastroenteritis	Seeds and Stem bark	*G. kola, G. mannii, G. lucida*
Oral pathologies	Branches	*G. kola, G. afzelii*
Eye conditions	Stem bark	*G. mannii*

I.4.3.2. Socio-economic uses of Genus *Garcinia*

Almost every plant part was found to be useful in these different categories: building materials, cosmetic, food products, medicine, spices, veterinary medicine and wood **(Guedje *et al.*, 2020).**

Mangosteen, Brindle berry and Kokum (*G. mangosteen*, *G. gummi-gutta* and *G. indica* respectively) are tropical fruits and a rich source of nutrients, minerals, vitamins, and dietary fibers. They are important tropical fruits naturally occurring in Asia, Africa, South America, Australia and Polynesia. The fruits of *Garcinia* with medicinal and nutraceutical properties have been used since ancient times in traditional medicinal practices **(Hosakatte *et al.*, 2018)**.

In Nigeria, *Garcinia*'s trunk, because of its firmness and strength, is used for the manufacture of furnitures, bridges and art objects, thus boosting the country's economy **(Waterman and Crichton, 1980)**.

In Cameroon, *Garcinia* has a great economic importance because it can be used in various ways as shown on **Table 14**.

Table 14: Socio-economic uses of Genus *Garcinia*'s species in Cameroon **(Guedje *et al.*, 2000)**

Equipment	Parts used	Species
Pestle	Taproot	*G. kola*
Hunting baits	Seeds	*G. lucida*
Construction equipment	Trunk	*G. kola*

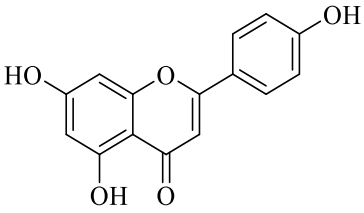
I.5. PREVIOUS PHYTOCHEMICAL STUDIES AND MAIN SECONDARY METABOLITES ISOLATED FROM GENUS *Garcinia*

Plants of the genus *Garcinia* produce structurally various secondary metabolites such as flavonoids, xanthones, benzophenones, biflavonoids, biphenyls, phloroglucinols, depsidones and terpenoids. The rich diversity in chemical structures made the genus *Garcinia* attractive for the phytochemists. **(Anu *et al.*, 2016)**.

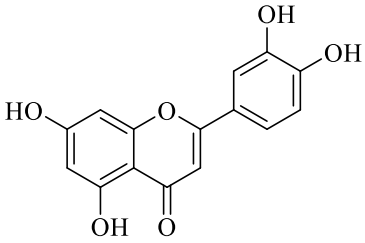
I.5.1. Flavonoids

Several flavonoids have been isolated from different parts of plants of *Garcinia*'s species ; Apigenin **40** and Luteolin **41** both belonging to the subclass flavone have been isolated from the leaves of *G. morella* **(Hosakatte *et al.*, 2020),** Quercetin **42** and Kaempferol **43** which are both flavonol were isolated from the stem bark of *G. atrviridis* **(Wen-Nee *et al.*, 2016)**, Irigenin **44** is an isoflavone isolated from the leaves of *G. nervosa* **(Mohd *et al.*, 1994)**, a new flavone-8-C-glycoside : 2", 6"-Di-O-acetylvitexin **45** isolated from the leaves of *G. mckeaniana* **(Thi *et al.*, 2022)** , a flavanone **46** isolated from the fruits of *G. preussii* **(Bernadette *et al.*, 2014)**, Vitexin **47** a flavone isolated from the *G. morella* **(Bhargav *et al.*,**

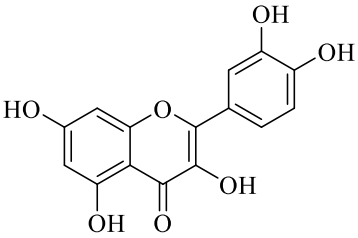
2023) and Epicatechin 48 , a flavanol isolated from the trunk bark of *G. celebica* **(Elfita *et al.*, 2009).**



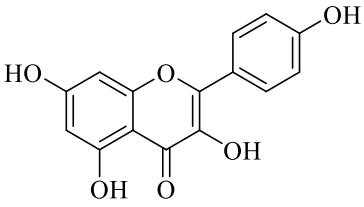
40



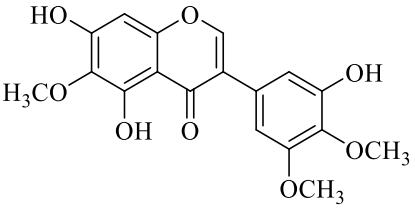
41



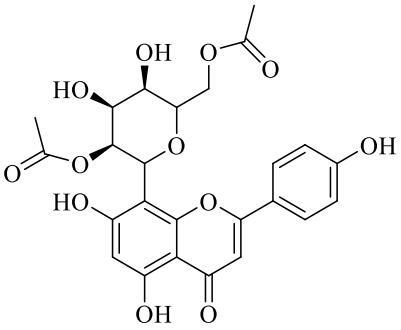
42



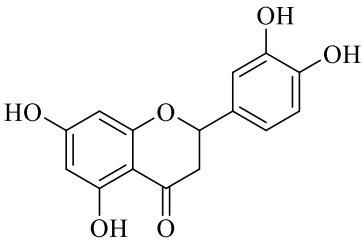
43



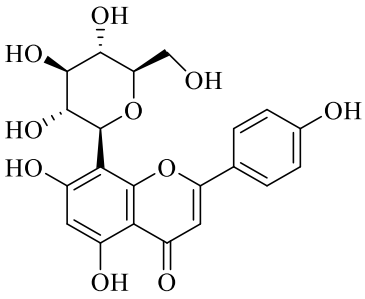
44



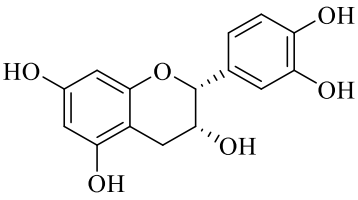
45



46



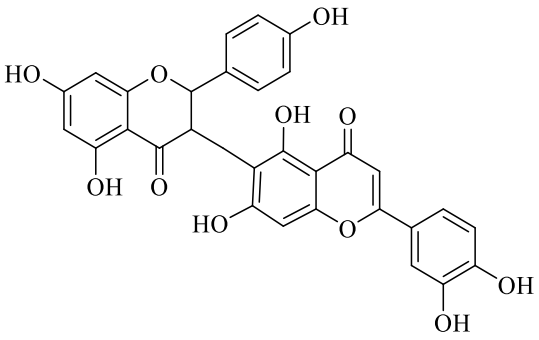
47



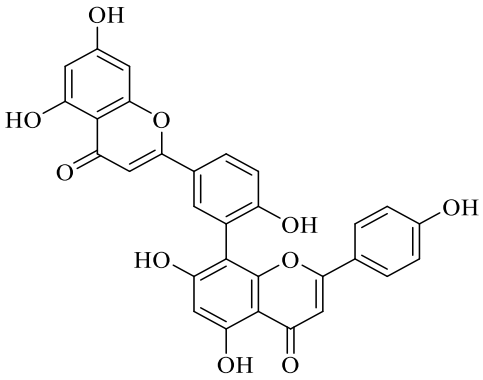
48

I.5.2. Biflavonoids

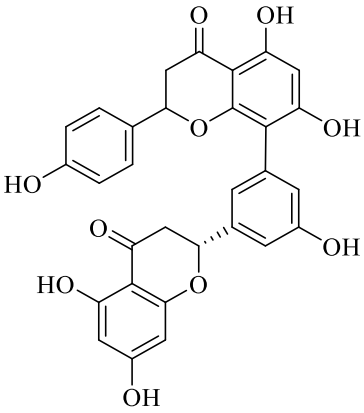
Biflavonoids can be isolated from different plant part of genus *Garcina*'s species: Macrophylloflavone 49 an isolated from the stem bark of *G. macrophylla* **(Hawa *et al.*, 2020)**, Amentoflavone 50 and Volkensiflavone 51 isolated from the leaves of *G. bakeriana* **(Ahmed *et al.*, 2013) ,** Biapigenin 52 isolated from the fruits of *G. livingstonei* **(Hui *et al.*, 2010)**, Brevipedicelone E 53 isolated from the leaves of *G. brevipedicellata* **(Akongwi *et al.*, 2019),** Manniflavanone 54, Garcinia biflavonoid 2 55 were isolated from the stem bark of *G. buchananii* **(Savannah *et al.*, 2023)** and dulcisbiflavonod A 56, isolated from the leaves of *G.dulcis* **(Saelee *et al.*, 2015).**



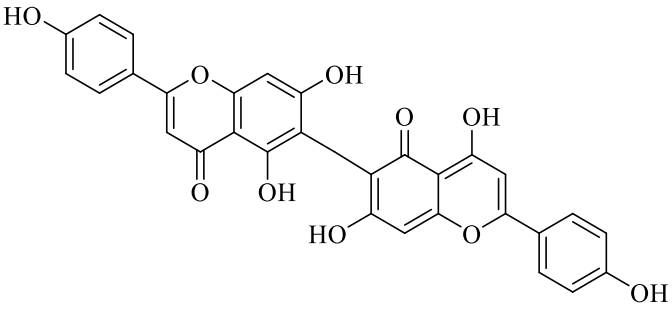
49



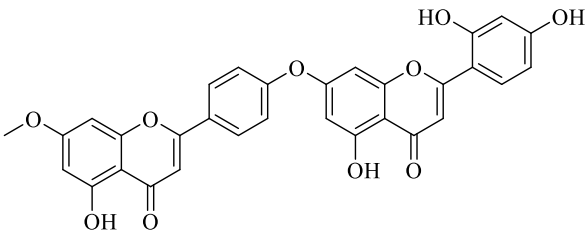
50



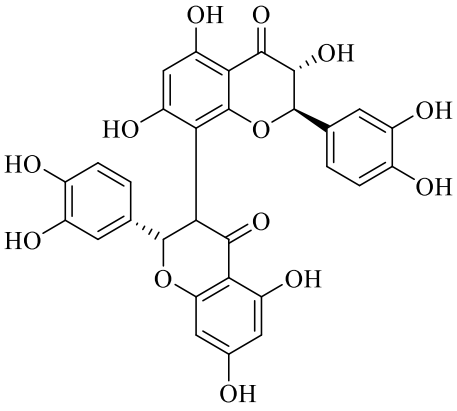
51



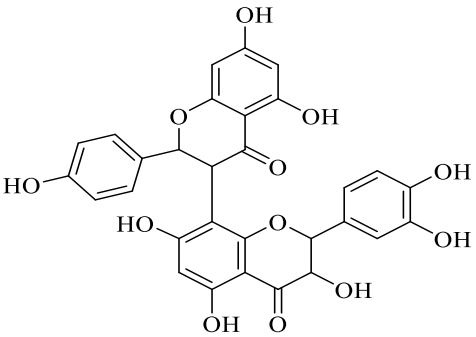
52



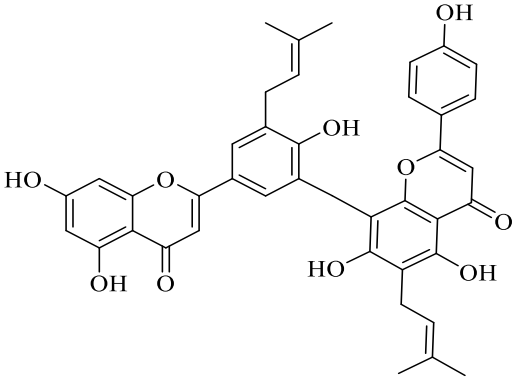
53



54



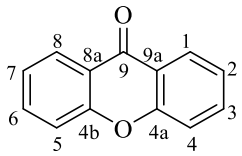
55



56

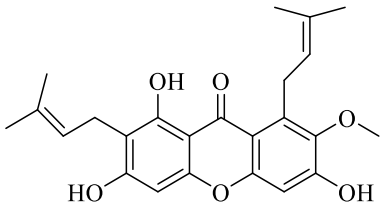
I.5.3. Xanthones

Xanthone is a class of oxygenated heterocyclic compounds which has as basic skeleton xanthone-9-one or dibenzo-*y*-pyrone. In general, it has a yellow coloration **(Wang *et al.*, 2003).**

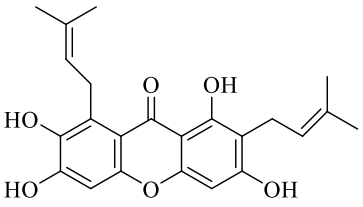


57

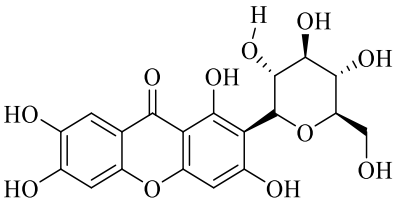
The number of the carbon atom on the basic skeleton follows the biogenetic convention as carbon 1 to 4 are assigned to the acetate derivative of ring A while carbon 5 and 8 to the shikimic derivative of ring B **(Panda *et al.*, 2013).** The previous phytochemical studies have made it possible to characterize the different subclasses of xanthones (oxygenated xanthones, glycolyzed xanthone, prenylated xanthone and furannoxanthone) in plants of genus *Garcinia*; α-mangostin **58** and γ-mangostin **59** isolated from the fruit hull of *G. mangostana* **(Lih-geeng *et al.*, 2007),** Magiferin **60** and Allanxanthone **61** isolated from the leaves of *G. mackeaniana* **(Nguyen *et al.*, 2019).**



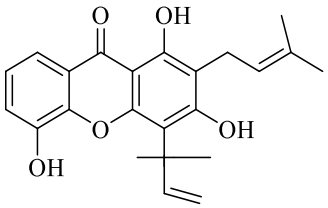
58



59



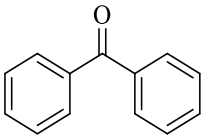
60



61

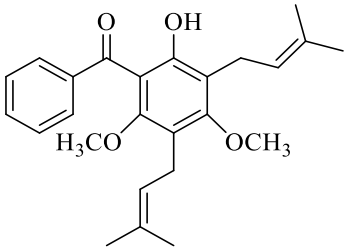
I.5.4. Benzophenones

Benzophenones isolated from *Garcinia* are either simple benzophenones or polyprenylated benzophenones. **(Zhang *et al.*, 2010)**.

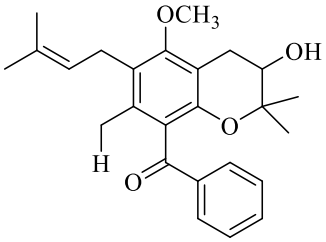


62

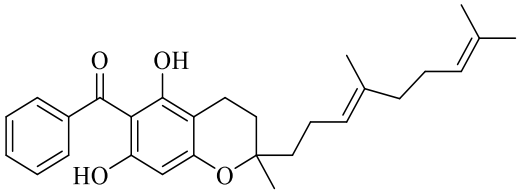
The following benzophenones were isolated from the plants of Genus *Garcinia*; Mytriaphenone A 63 and Pseudoguttiaphenone A 64 was isolated from the stem bark of *G. pseudoguttifera* **(Sadaquat *et al.*, 2020),** Bethamianone 65 isolated from the stem bark of *G. benthamiana* **(Irene *et al.*, 2015)** and Garcinol 66 isolated from the hexane extract of *G. indica* **(Chaoquin *et al.*, 2017)**



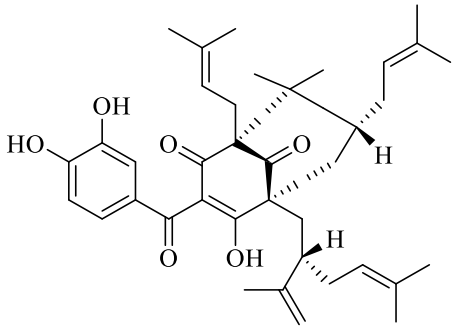
63



64



65

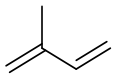


66

I.5.5. Terpenoids

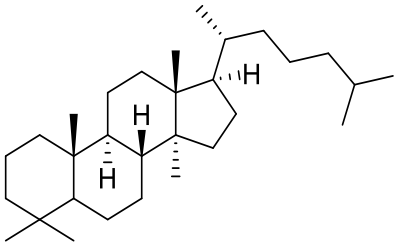
Terpenoids constitute one of the largest and structurally diverse groups of naturally occurring compounds. They play key roles in plant growth-development, response to the environment and physiological processes. They belong to a class of natural products derived from mevalonic acid (MVA) which are composed of a plurality of isoprene (C5) structural units 67. Based upon the number of isoprene units, these have mainly classified into

monoterpene (C10), sesquiterpene (C15), diterpene (C20), triterpene (C30) tetraterpene (C40), and polyterpene (C > 40) **(Wenqiang *et al.*, 2020).**

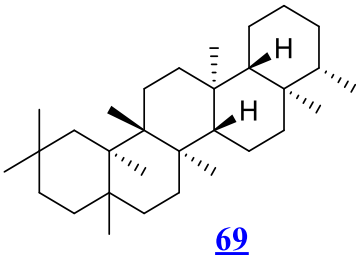


67

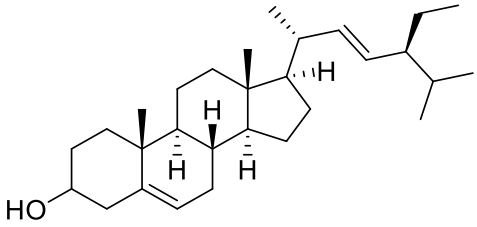
Structurally, triterpenes may be divided into linear ones (mainly derivatives of squalene), tetracyclic and pentacyclic (containing respectively four and five cycles) as well as the two and tricyclic ones **(Shamsul *et al.*, 2020).** According to previous phytochemical studies, some triterpenes were isolated from the plants of genus *Garcinia*: Lanostanes **68** isolated from the leaves of *G. hombroniana* **(Vatcharin *et al.*, 2005),** Friedlin **69** and Stigmasterol **70** were isolated from the leaves of *G. penangiana* **(Mohd, 2005).**



68



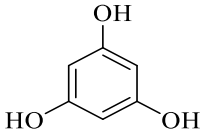
69



70

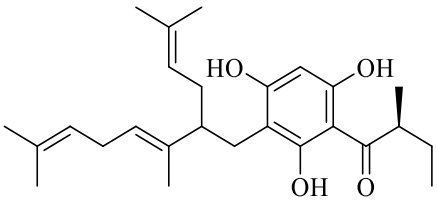
I.5.6. Phloroglucinols

Phloroglucinol compounds include synthetic or semi synthetic moieties and > 700 naturally occurring compounds. This is an important class of natural products containing 1,3,5-trihydroxy benzene as basic skeleton **(Inder *et al.*, 2009).**

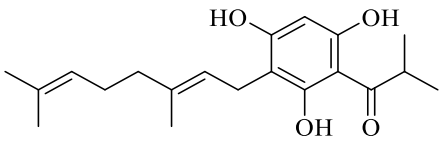


71

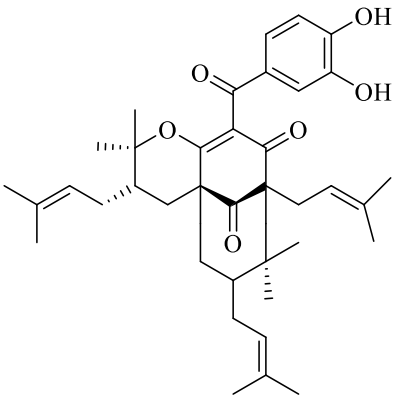
Some Phloroglucinols isolated from plants of genus *Garcinia* include; Dauphinol A **72** and 3'-methylhyperjovoinol B **73** isolated from the roots of *G. dauphinensis* **(Rolly *et al.*, 2018)** and Paucinochymol D **74** isolated from the fruits of *G. paucinervis* **(Xue *et al.*, 2020).**



72



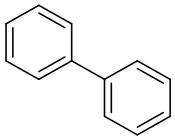
73



74

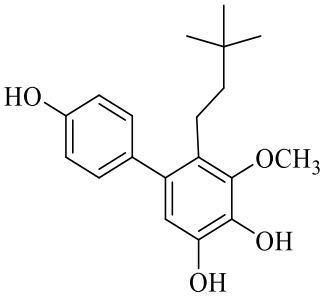
I.5.7. Biphenyls

Biphenyls, as the name suggests, are two conjoined benzene rings attached through their 1,1′-positions. They are white crystals with a characteristic pleasant smell. Their structural analogs are used as synthetic intermediate for the production of other organic compounds such as emulsifiers, optical brighteners, crop protection products, plastics and pharmaceuticals. Their structural skeleton is as shown below **(Zenish *et al.*, 2017).**

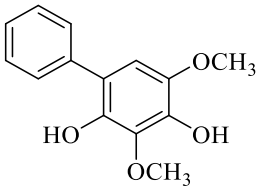


75

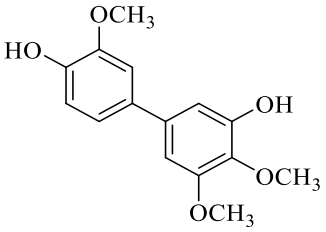
The following biphenyls were isolated from plants of genus *Garcinia*'s species; Schomburgbiphenyl 76 from the trunk bark of *G. schomburgkiana* **(Chutichot *et al.*, 2013),** Garcinuntabiphenyl A 77 from the roots of *G. nuntasaenni* **(Suppisak *et al.*, 2018),** Garciosines A 78 from the leaves of *G. speciosa* **(Phanruethai *et al.*, 2018)** and Oblongifoliagarcinines A 79 from the leaves and stem bark of *G. oblongifolia* **(Xu *et al.*, 2008).**



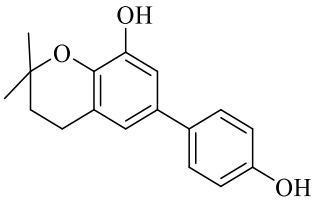
76



77



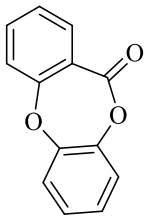
78



79

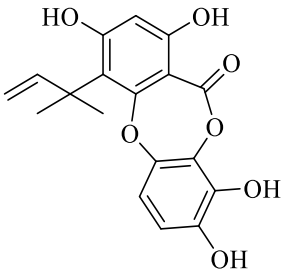
I.5.8. Depsidones

Depsidones are polyphenolic polyketides featuring a tricyclic framework that have a central seven-membered lactone ring; 11H-dibenzo[b,e][1,4]dioxepin-11-one. **(Maan *et al.*, 2023).**

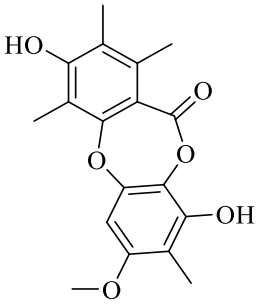


80

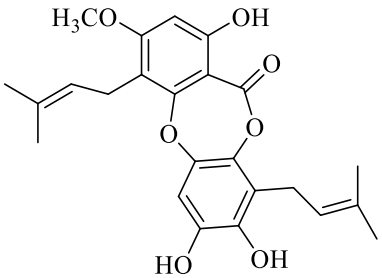
Previous phytochemical studies shown that Depsidone were isolated from the plants of *Garcinia*'s species; Garcinisidone H 81 from the bark of *G. celebica* **(Thai *et al.*, 2016),** Polyanthadepsidone A 82 isolated from the leaves of *G. polyantha* **(Alain *et al.*, 2017)** and Schomburgdepsidone B 83 from the roots of *G. schomburgdepsidone* **(Edwin *et al.*, 2016).**



81



82



83

I.6. PREVIOUS BIOLOGICAL ACTIVITIES OF GENUS *Garcinia*

It is proven by extensive research that some bio-compounds from *Garcinia* species exhibit a wide range of biological and pharmacological activities as indicated below;

I.6.1. Anti-oxidant activities

The methanolic extract of *Garcinia penducalata* has a high amount of phenolic content and its IC_{50} values of DPPH, oxygen and nitric oxide scavenging activities were 50.23µg/ml, 66.06 µg/ml and 63.02 µg/ml respectively. This provides the extract with a good anti-oxidant activity **(Munmee *et al.*, 2012).** Also, the anti-oxidative effect displayed by Garcinol 66 was demonstrated using cell-free assays. It was shown to be able to quench $OH^{\cdot -}$ at an IC_{50} of 0.32 µM thus preventing induced DNA damage **(Chaoquin *et al.*, 2017).**

I.6.2. Anti-inflammatory

Two xantones α- and γ-mangostins were isolated from ethyl acetate extract of the fruit hull of *Garcinia mangostana* and both significantly inhibited nitric oxide (NO). The IC_{50} values for the inhibition of NO production α- mangostins 58 and γ-mangostins 59 were 12.4 and 10.1 µM respectively. The anti-inflammatory effects of α- and γ-mangostins were evaluated by carrageenan-induced paw edema in mice that was used as an acute model of inflammation **(Lih-geeng *et al.*, 2007)**.

Few investigations have been conducted so far on the chemical composition of the volatile oils from *Garcinia* species. The fruit-peel volatile of *Garcinia brasiliensis* constituted mainly of sesquiterpenes was evaluated in terms of anti-inflammatory activity based on the gradual edema of rat paw upon application of the inflammatory agent carrageenan. The biological assay revealed that the inflammatory process was inhibited by 25 ± 5%. This inhibition is not directly related to the inhibition reactive oxygen species (ROS) **(Felipe *et al.*, 2008)**.

I.6.3. Anti-HIV

Garcinia species could be the possible source of lead compounds and anti-HIV drug candidates. The ethanol extracts of the fruits of *Garcinia livingstoneii* and *Garcinia semseii* were evaluated using an anti-HIV-1 infectivity assay and showed significant anti-HIV-1 activity with EC_{50} values of 2.25 ± 0.51 and 0.93 ± 0.67 µg/ml respectively **(Magadula and Suleimani, 2010).**

Morelloflavone **86**, isolated from *Garcina multiflora*, demonstrated a significant antiviral activity against HIV-1 (strain LAV-1) in phytohemagglutinin-stimulated primary human peripheral blood mononuclear cells at an EC_{50} value of 6.9 µM and a selectivity index value of approximately 10 **(Yuh-meei *et al.*, 1997).**

γ-mangostin **59** from the ethanol extract of *Garcinia mangostana* L. (Guttiferae) showed potent inhibitory activity against HIV-1 protease with an IC_{50} value of 4.81 ± 0.32 µM **(Shao-xing *et al.*, 1996).**

I.7. DESCRIPTION OF *Garcinia gnetoides*

This part is concerned with the geographical distribution, botanical description, vernacular names and ethnobotanical uses of *Garcinia gnetoides*. The previous phytochemical studies carried out on *Garcinia gnetoides*, its biological and pharmacological properties were equally associated to this part.

1.7.1. Geographical distribution of *Garcinia gnetoides*

Garcinia gnetoides is native to western and central tropical African countries mostly: Gabon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria, Sierra Leone and Cameroon **(Marc and Gilles, 2012).**

Garcinia gnetoides can be found in five regions of Cameroon: South region at Kribi, Lolodrof and Bipindi, South-west at Ekundukundu and Ekowong, Littoral at Edea and Mbanga, North-west at Metchum and Center region at Eseka and Mefou **(riha.african-herbaria.org, 2024).**

Figure 14: Geographical distribution of *Garcinia gnetoides* **(POWO, 2024)**

I.7.2. Botanical description of *Garcinia gnetoides*

Garcinia gnetoides is primarily known for its striking foliage and woody growth. It possesses the following identifiable traits:

I.7.2.1. Fruits: *Garcinia gnetoides'* fruits are typically round or oval, juicy and often orange to yellow in color. They are not edible but are a vital food source for various wildlife in their natural habitat **(Marc and Gilles, 2012).**

Figure 15: Fruit of *Garcinia gnetoides* **(westafricanplants, 2024)**

I.7.2.2. Flowers: They are small and yellowish-white in color, growing in axillary or terminal clusters **(Botanical realm, 2024).**

Figure 16: Flower of *Garcinia gnetoides* **(WCVP, 2024)**

I.7.2.3. Leaves: The leaves are thick, dark green and glossy with an entire margin **(Botanical realm, 2024).**

Figure 17: Leaf of *Garcinia gnetoides* **(westafricanplants, 2024)**

I.7.2.4. Tree bark: *Garcinia gnetoides* is an evergreen tree that can grows up to 17 meters tall. The straight, cylindrical bole can be up to 25 cm in diameter **(Useful Tropical Plants, 2014).** It can be found primarily in wet tropical areas **(POWO, 2024).**

Figure 18: Tree bark of *Garcinia gnetoides* **(WCVP, 2024)**

According to the general classification of the Clusiaceae, *Garcinia gnetoides* can be classified as shown on **Table 15**.

Table 15: Classification of *Garcinia gnetoides* **(POWO, 2024)**

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnolidae
Order	Malpighiales
Family	Clusiaceae
Genus	*Garcinia*
Species	*Garcinia gnetoides*

I.7.3. Vernacular names of *Garcinia gnetoides*

Garcinia gnetoides and *Garcinia granulata* are both synonyms of *Garcinia quadrifaria* **(Marc and Gilles, 2012)**. **Table 16** shows the different vernacular names of *Garcinia gnetoides* depending on the locality.

Table 16: Vernacular names of *Garcinia gnetoides* **(JSTOR, 2024)**

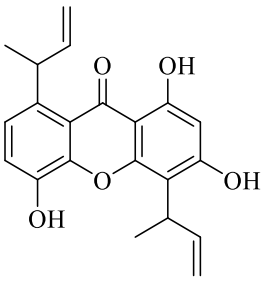
Country	Vernacular name
Ivory coast	"Akye"
Ghana	"Fante"
Nigeria	"Bokyi"

I.7.4. Ethnobotanical uses of *Garcinia gnetoides*

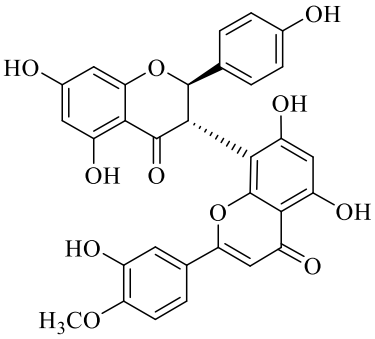
A decoction of the leaves of *Garcinia gnetoides* is used as a purgative. In Africa, it is used for cancer and malaria treatment**.** Some local cultures use *Garcinia gnetoides'* tree for traditional rituals and practices **(POWO, 2024)**.

I.7.5. Previous phytochemical studies on *Garcinia gnetoides*

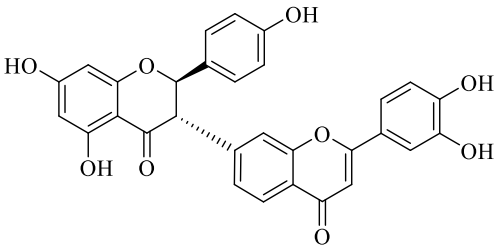
Up till date, phytochemical studies were only carried out on the stem bark of *Garcinia gnetoides* from which novel xanthone (1,3,5-trihydroxy-4,8-di(3,3-dimethylallyl) xanthone **84**) and two biflavonoids (3-O-methylfukugetin **85** and Morelloflavone **86**) were isolated **(Peter and Raouf, 1982).**



84



85



86

I.7.6. Biological and pharmacological properties of *Garcinia gnetoides*

To the best of our knowledge, no biological investigations were carried out on *Garcinia gnetoides* up till date.

CHAPTER II: RESULTS AND DISCUSSION

II.1. EXTRACTION AND ISOLATION OF BIFLAVONIDS FROM THE ROOTS OF *Garcinia gnetoides*

Garcinia gnetoides was selected to as the subject of investigation for several criterias:

- *Garcinia* species are known for their therapeutic properties as they are rich sources of secondary metabolites including xanthones, benzophenones, lactones, phenolic compounds (especially flavonoids and biflavonoids) and organic acids **(Bruna *et al.*, 2020)**.
- Up till date, phytochemical studies were only carried out on the stem bark of *Garcinia gnetoides* from which a novel xanthone **(84)** and two biflavonoids **(85 and 86)** were isolated **(Peter *I* and Raouf, 1982)**. This motivated the recherche to be carried on the roots of *Garcinia gnetoides* since it was reported that, flavonoids and biflavonoids are mostly concentrated in soil solutions **(Diptesh *et al.*, 2023)**.
- A thin layer chromatography was carried out on the Acetone extract and Methanol extract of roots of *Garcinia gnetoides*. The TLC plate of the Acetone extract presented more polar compounds than the Methanol crude extract. Hence, the Acetone extract was chromatographed for the isolation of these polar compounds. **See Appendix 3 and 4**.
- However, no biological investigations were carried out on *Garcinia gnetoides*' plant parts up till date.

II.1.1. Phytochemical analysis

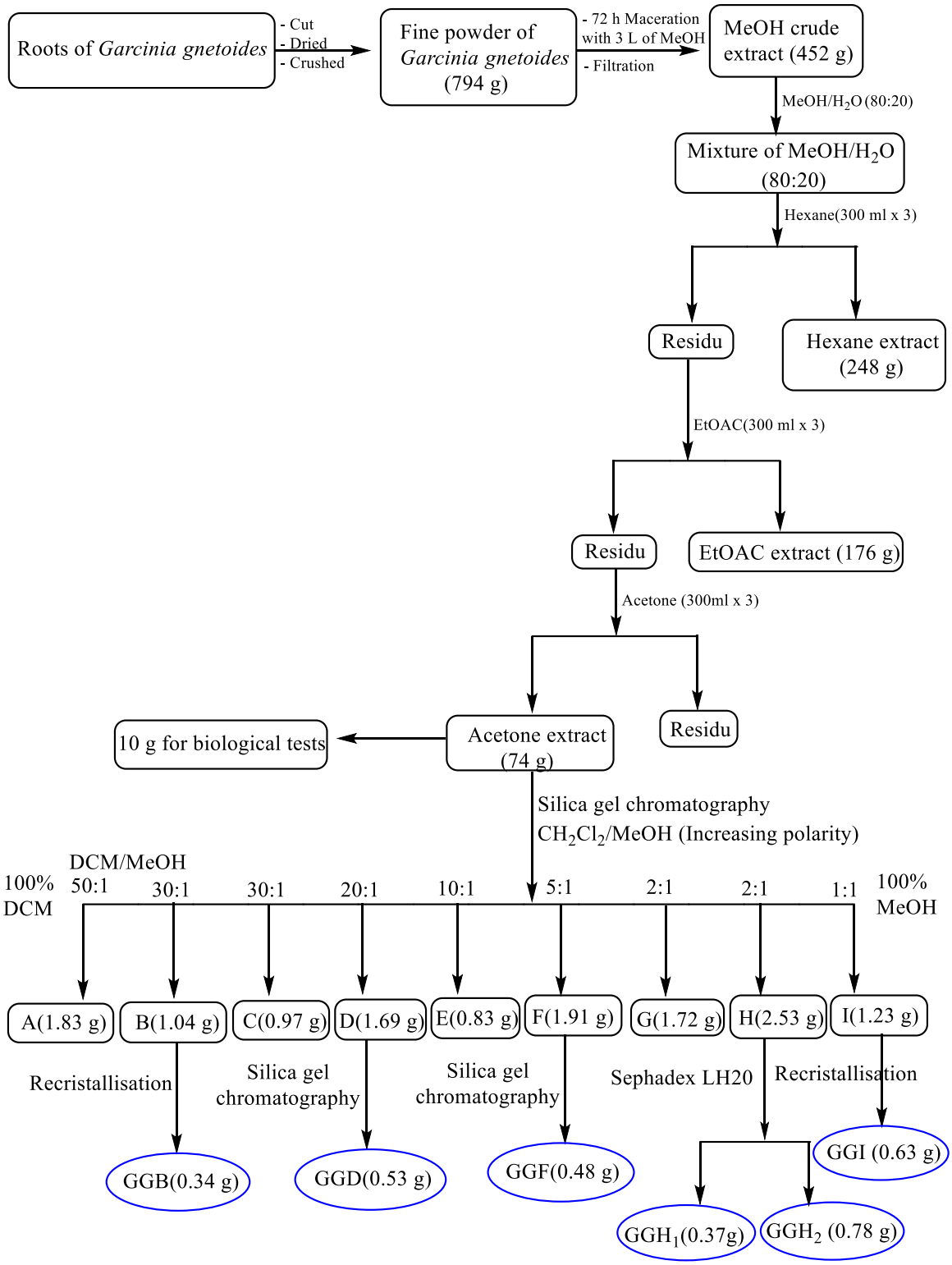
II.1.1.1. Extraction from roots of *Garcinia gnetoides*

The roots of *Garcinia gnetoides* were cut, air dried and crushed into a yellowish fine powder of mass 794 g then subjected to maceration for 72 hours at room temperature using 3 L of MeOH. It was then concentrated using a rotary vapor (BUCHI 461 water bath) and dried in an oven, resulting into a crude extract of mass 452 g. This crude extract was mixed with MeOH/H_2O in the ratio 80 :20 then successively partitioned with solvents in increasing polarity (that is: Hexane, EtOAc and Acetone) which were concentrated to obtain their respective extract (that is: 248 g, 176 g and 74 g respectively).

II.1.1.2. Isolation of constituents from the roots of *Garcinia gnetoides*

10 g were removed from the Acetone extract for biological tests. The remaining 64 g of the Acetone extract of the roots of *Garcinia gnetoides* was chromatographed in silica gel

with CH_2Cl_2 / MeOH as eluent. The elution process was carried out in increasing polarity from 50:1 to the ratio 1 :1. **Scheme 1** materializes the above experimental protocol.



Scheme 1: Experimental protocol for the extraction and isolation of compounds from the roots of *Garcinia gnetoides*

II.2. DATA ANALYSIS

This part presents the structural elucidation of compound GGB and GGF using their respective mass spectrum, 1D and 2D NMR spectra.

II.2.1. Structural elucidation of GGB (Compound 87)

GGB was obtained as an amorphous yellow powder and gave a positive reaction to Shinoda's and Molish's tests, characteristic of phenol and sugars respectively. The HR-ESIMS$^+$ spectrum of GGB **(Figure 19)** showed a pseudo-molecular ion peak at *m/z* 1007.2447 $[M + H]^+$ which corresponds to the molecular mass of $C_{48}H_{47}O_{24}$ (calculated 1007.2463) and has 26 unsaturation sites. The TLC plate presents compound GGB as a pure compound (**Figure 20**).

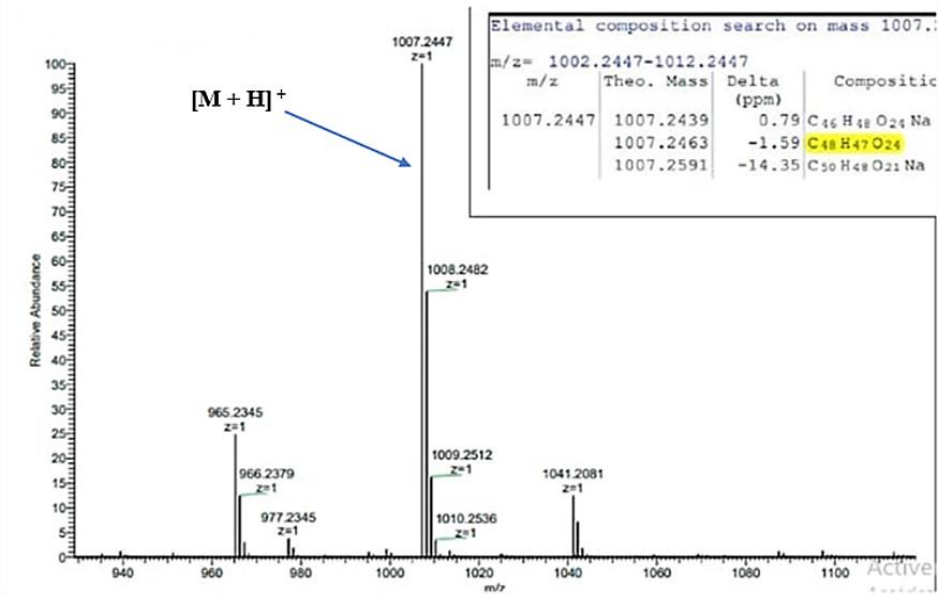


Figure 19: HR-ESI$^+$ Mass spectrum of GGB

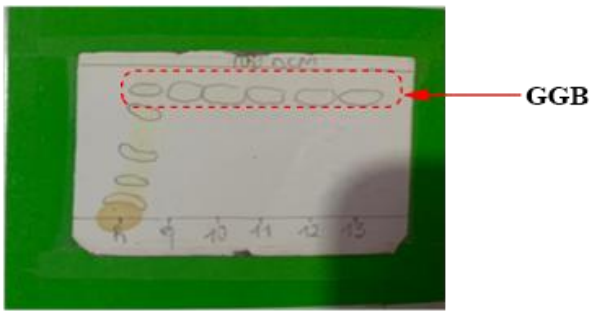


Figure 20: TLC plate of GGB

The ^{1}H NMR (**Figure 21**) permitted the analyzes of the spin systems found in GGB.

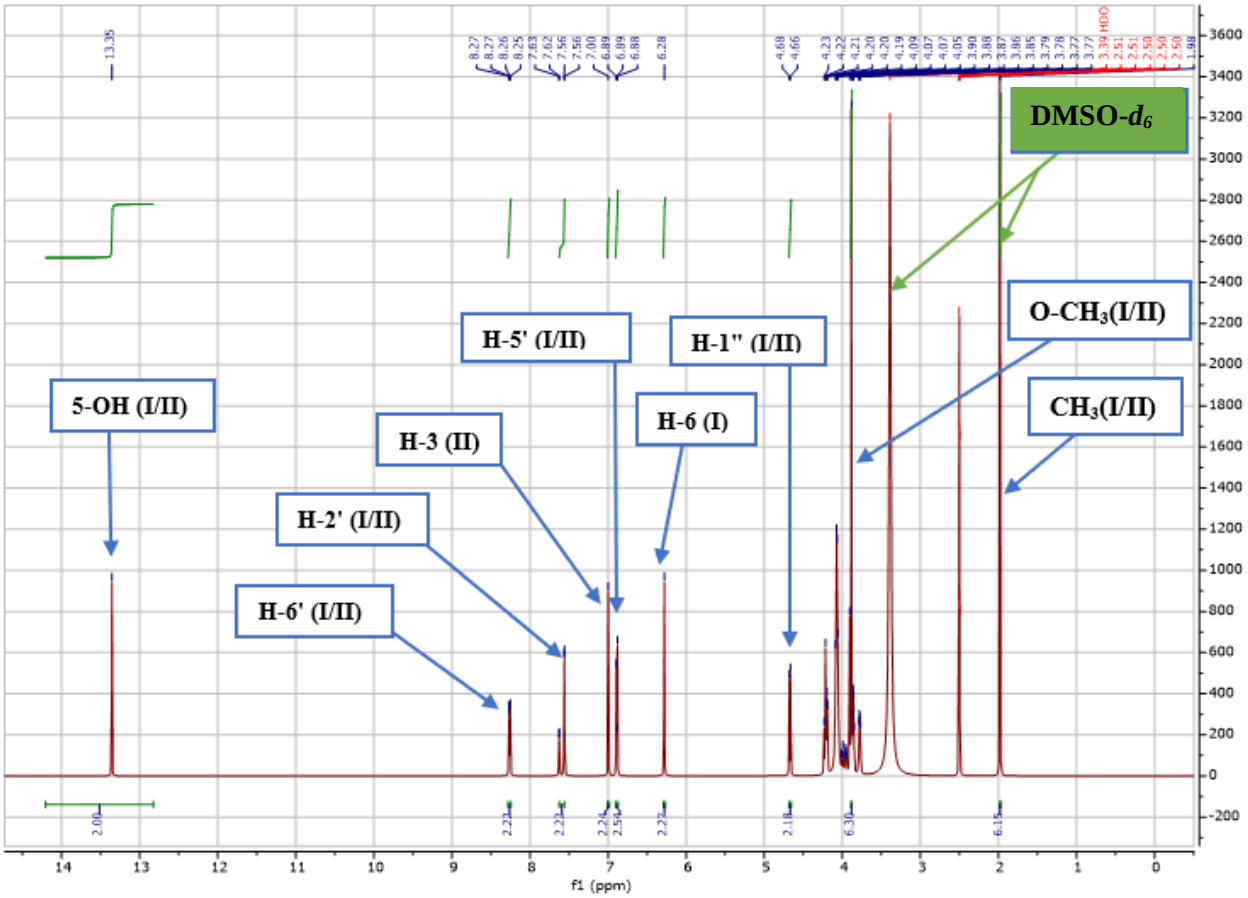


Figure 21: ^{1}H NMR Spectrum (600 MHz, DMSO-d_6) of GGB

The ^{1}H-NMR spectrum of GGB **(Figure 21 and Table 17)** revealed 46 proton atoms, showing a singlet signal integrating for two protons at δ_H 13.35 (2H, s, 5-OH (I/II)), ascribed to the proton of the chelated hydroxyl in peri of the carbonyl group. Also, this spectrum revealed one olefinic resonance at δ_H 7.00 (1H, s, H-3(II)), characteristic to flavone of unit II **(Mariana et *al.*, 2013)** and a singlet signal at δ_H 6.28 (1H, s, H-6(I)), assigned to the proton on the penta-substituted ring A(I) of flavone unit I. Furthermore, still in the aromatic region, the ^{1}H-NMR spectrum exhibited two ABX-proton system types at δ_H 8.27 (2H, dd, J = 8.2, 2 Hz, H-6′ (I/II)), δ_H 7,56 (2H, d, J = 2 Hz, H-2′ (I/II)) and δ_H 6.89 (2H, d, J = 8.2 Hz, H-5′(I/II)), attributable to ring B(I) and B(II). These systems gave both flavone units a Luteolin based-structure. The up-field portion of this spectrum presented a methoxy proton resonance at δ_H 3.88 (6H, s (I/II)), indicating the presence of two methoxy groups. Moreover, an anomeric proton signal at δ_H 4.67 (2H, d, J = 9.8 Hz, H-1(I/II)), characteristic to a β-linked sugar moiety (**Weisheng et *al.*, 2017**) integrates for two anomeric protons showing up two sugar moieties. Regarding the $^{3}J_{H3,H4}$ coupling constant value (J =3.7Hz), the two sugar moieties observed here are galactopyranosides **(Carolina and Goran, 2023).** Besides, a

singlet signal at δ_H 1.98 (6H, s (I/II)) was observed, suggesting the presence two acetyl groups.

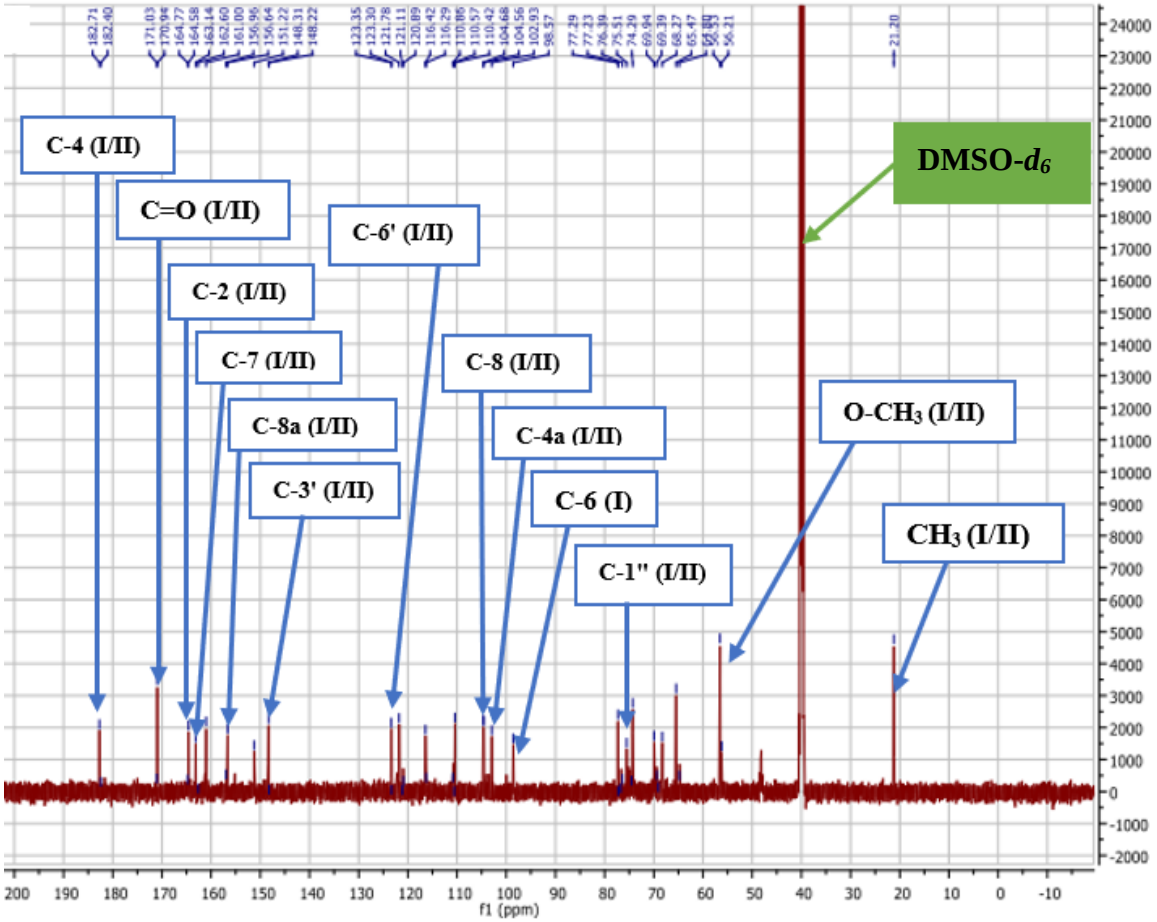


Figure 22: ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of GGB

The ^{13}C-NMR data of GGB **(Figure 22 and Table 17)** disclosed 48 carbon resonances, comprised of four carbonyl carbon atoms among which; two ketone carbonyl carbon atoms at δc 182.7ppm and 182.4 ppm (confirming the presence of two flavone units) and two ester carbonyl carbon atoms at δc 171.0 ppm and 170.9 ppm were spotted. Twenty other quaternary carbon atoms and eight sp^2 carbon atoms were identified. Forbye, the ^{13}C-NMR spectrum showed sixteen sp^3 carbon atoms among which twelve carbon atoms resonated between δc 65.4-77.3 ppm and 64.8-77.2 ppm, warranting the presence of two galactopyranoside units (**William *et al.*, 2003**). These chemical shift values are almost identical to those of a C-galactopyranoside moiety (**Jane *et al.*, 1981**). Furthermore, two methoxy groups (at 56.5 ppm and 56.2 ppm) and two acetyl groups (at 21.2 ppm) were spotted.

An HSQC 2D experiment made it possible to assign most of the carbon atoms. These findings supported a flavone-flavone based biflavonoid structure for compound GGB.

The above information led us to define the following substructures:

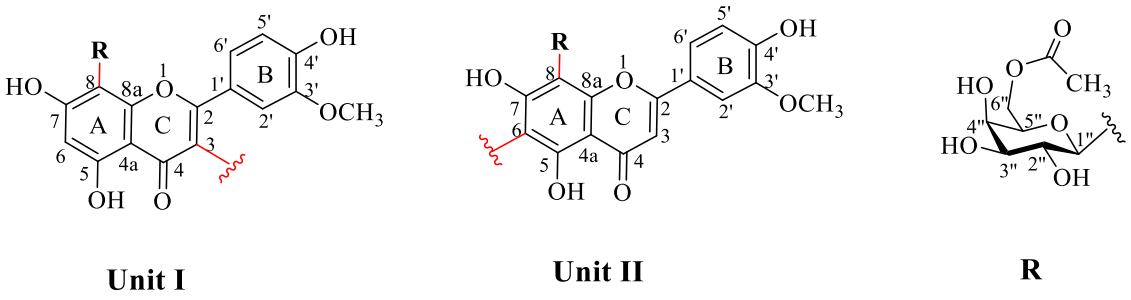


Figure 23: Substructures found in GGB

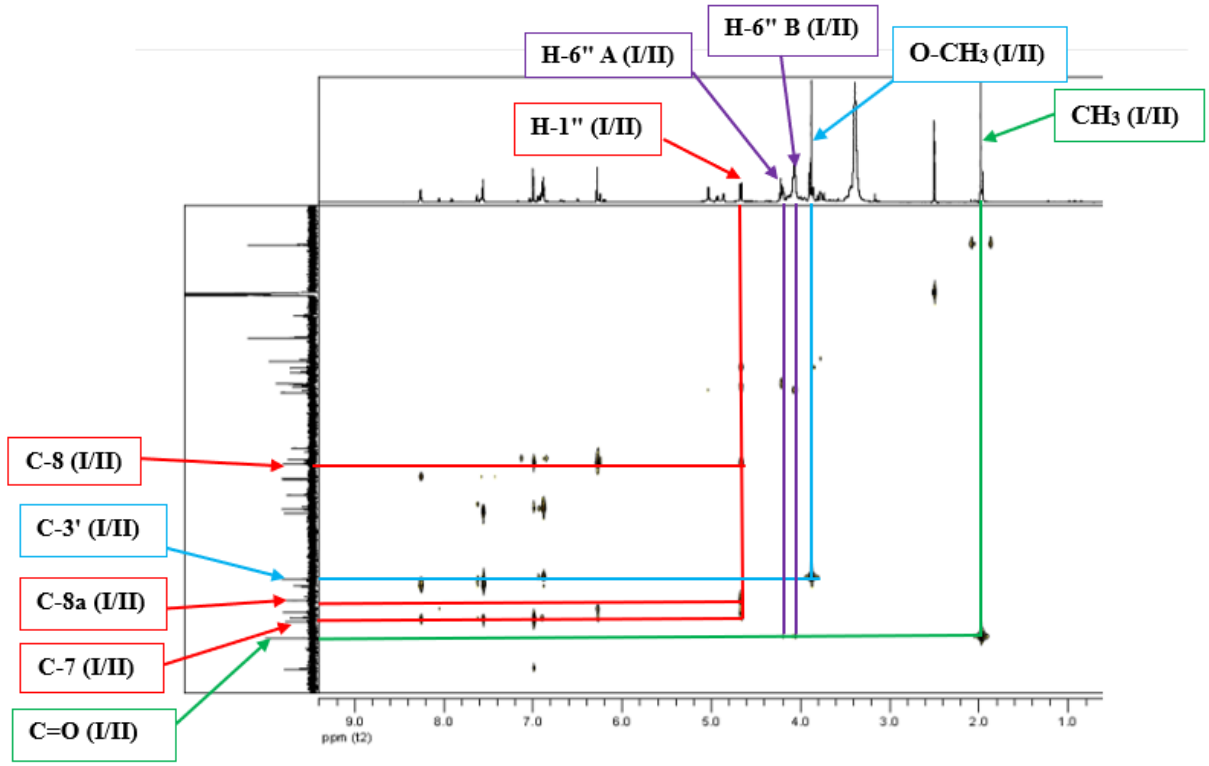


Figure 24: Some key correlations of HMBC spectrum of GGB

The 2D HMBC cross peaks **(Figure 24)** of the methoxy protons at δ_H 3.88 correlate each with C-3 (δc 148.3) in unit I and C-3 (δc 148.2) in unit II. The position of the methoxy groups was also supported by the ROESY spectrum (**Figure 26**) which showed B-ring cross peaks at MeO-3'/H-3' in unit I and II. Furthermore, HMBC correlations in unit I and II were observed between the anomeric proton at δ_H 4.68 ppm and carbon atom resonances at δc 163.1 ppm (C-7), δc 156.6 ppm (C-8a) and δc 104.6 ppm (C-8). These correlations indicated that the C-galactopyranoside moiety is attached at C-8 of flavone unit I and II each. This data suggested an Orientin (8-C-galactopyranoside luteolin) based skeleton for GGB. Moreover, the location of the acetoxy group at C-6" of unit I and II was established due to the correlation

between the diastereotopic protons H-6" (at δ_H 4.21 ppm and 4.09 ppm) and the ester carbonyl carbon atom at δc 171.0 ppm (C=0 (I)) in both units.

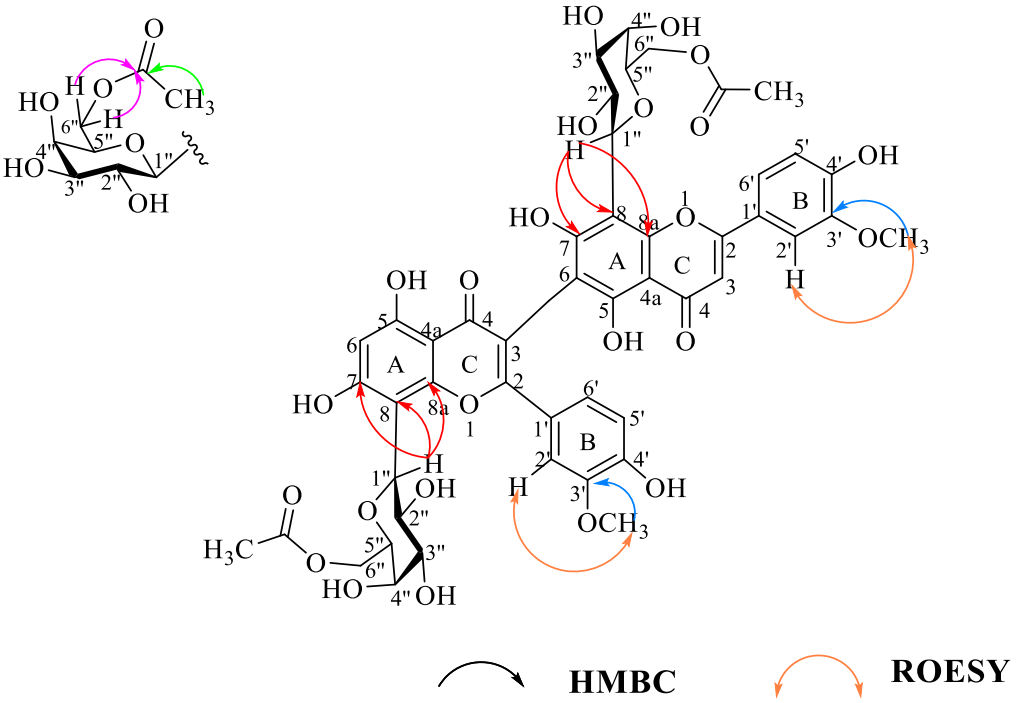


Figure 25: Some correlations observed in HMBC and ROESY spectrum

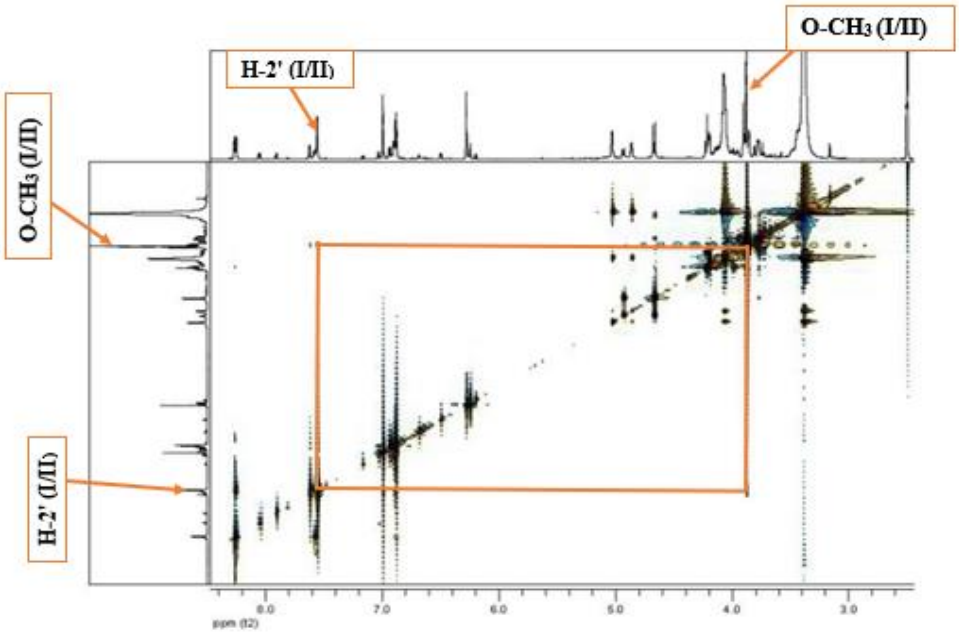
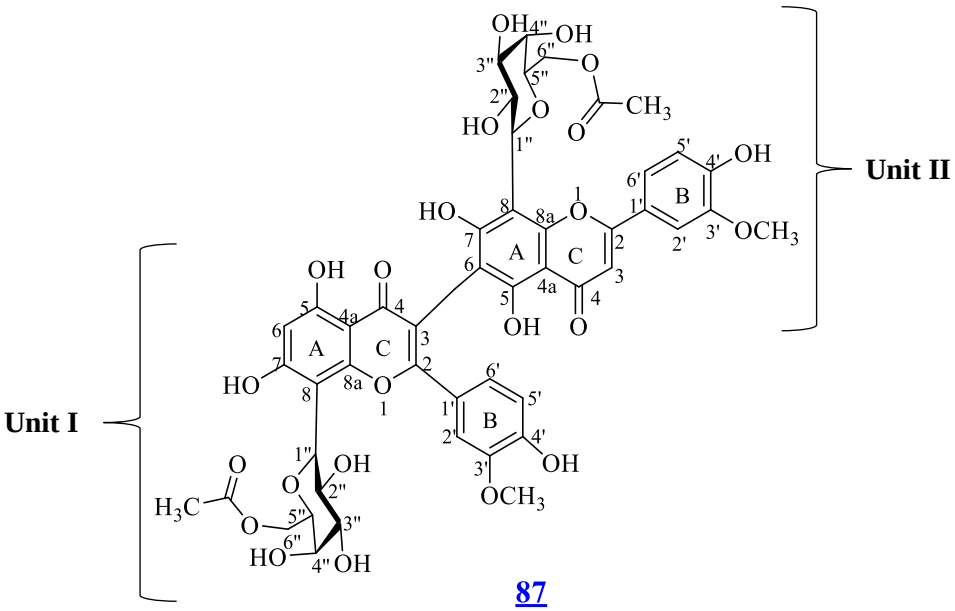


Figure 26: ROESY spectrum of GGB

The presence of one olefinic resonance observed at δ_H 7.00 ppm (1H, s, H-3(II)), characteristic of flavone unit II and a singlet signal at δ_H 6.28 ppm (1H, s, H-6 (I)), assigned to the proton in the pentasubtituted ring A of unit (I) suggested that, the junction between unit

I and II is C-3 (I)-C-6 (II). These observations were confirmed by the ^{13}C NMR data of GGB which showed only one carbon resonance at δc 98.5 ppm (C-6 (I)) compared to that of GGF, which indicated two carbon signals at δc 99.4 ppm (C-6 (I) and C-6 (II)). On the basis of the above information, GGB was identified as: **6"-*O*-acetyl-3′-*O*-methylorientin-[I-(3)-II-(6)]-6"-O-acetyl-3'-O-methylorientin** (Compound 87).



87

The ^{1}H (δ_H in ppm and *J* values in Hz) and ^{13}C (δc in ppm) assignments of compound **87** are summarized on **Table 17**.

Table 17: ^{1}H NMR (600MHz, DMSO-d_6) and ^{13}C NMR (150MHz, DMSO-d_6) data of GGB

Position	**δ_H (ppm) (*J* values in Hz)**		**δ_C (ppm)**	
Unit I/II	**Unit I**	**Unit II**	**Unit I**	**Unit II**
2	-	-	164.7, C	164.5, C
3	-	7.00 (1H) s	110.8, CH	104.5, CH
4	-	-	182.7, C	182.4, C
4a	-	-	102.9, C	102.9, C
5	13.35 (1H) s	13.35 (1H) s	161.0, C	161.0, C
6	6.28 (1H) s	-	98.5, CH	110.4, CH
7	-	-	163.1, C	162.6, C
8	-	-	104.6, C	104.6, C
8a	-	-	156.9, C	156.6, C
1′	-	-	121.7, C	121.1, C
2′	7.56 (1H) d (2)	7.56 (1H) d (2)	121.1, CH	120.9, CH
3′	-	-	148.3, CH	148.2, C
4′	-	-	151.2, C	151.1, C
5′	6.89 (1H) d (8.2)	6.89 (1H) d (8.2)	116.4, CH	116.2, CH
6′	8.27 (1H) dd -8.2, 2)	8.27 (1H) dd (8.2, 2)	123.3, CH	123.3, CH
1"	4.68 (1H) d (9.8)	4.66 (1H) d (9.8)	75.5, CH	75.5, CH
2"	4.23 (1H) m	4.22 (1H) m	74.2, CH	74.2, CH
3"	4.05 (1H) m	4.04 (1H) m	76.3, CH	76.3, CH
4"	4.94 (1H) m	4.94 (1H) m	69.9, CH	69.3, CH
5"	3.79 (1H) m	3.78 (1H) m	77.2, CH	77.2, CH
6" (A)	4.21 (1H) m	4.09 (1H) m	65.4, CH	64.8, CH
6" (B)	4.20 (1H) m	4.7 (1H) m	65.4, CH	64.8, CH
3-OMe	3.88 (3H) s	3.88 (3H) s	56.5	56.2
H_3CCOCO-6″	1.98 (3H) s	1.98 (3H) s	21.2, CH_3	21.2, CH3
H_3C**OC**O-6″			171.0, C	170.9, C

II.2.2. Structural elucidation of GGF (Compound 88)

GGF was obtained as yellow powder and gave a positive reaction to Shinoda's and Molish's tests, characteristic of phenol and sugars respectively. The HR-ESIMS$^+$ spectrum of GGF **(Figure 26)** showed a pseudo-molecular ion peak at m/z 907.2302 [M + H] $^+$ which corresponds to the molecular mass of $C_{44}H_{43}O_{21}$ (calculated 907.2302) and has 24 unsaturations sites. The TLC plate presents compound GGF as a pure compound (**Figure 27)**.

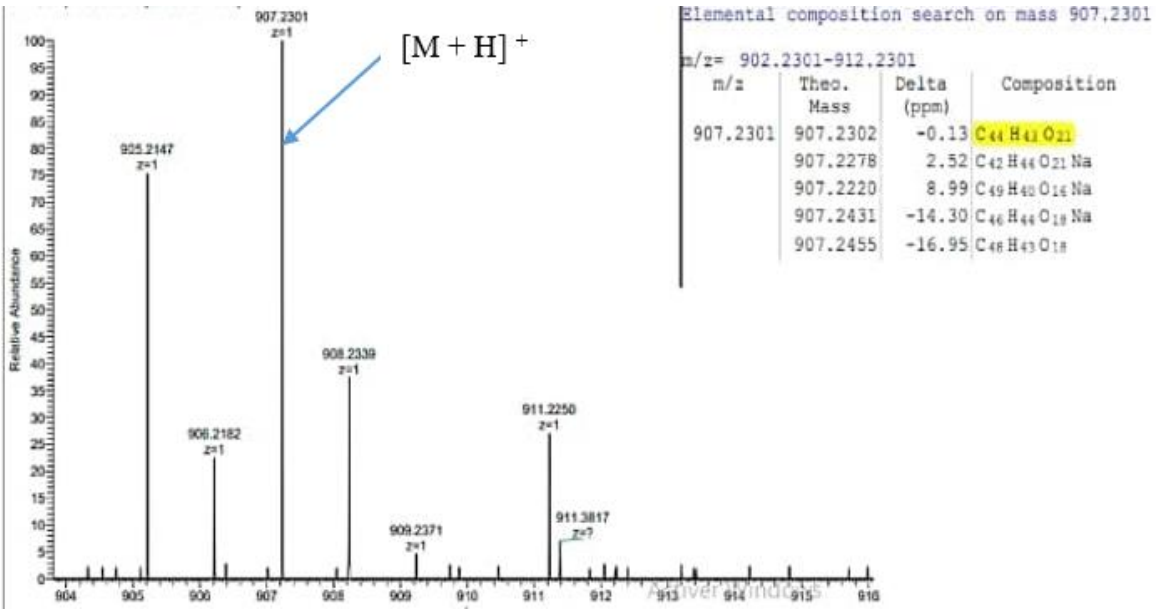


Figure 27: HR-ESI$^+$ Mass spectrum of GGF

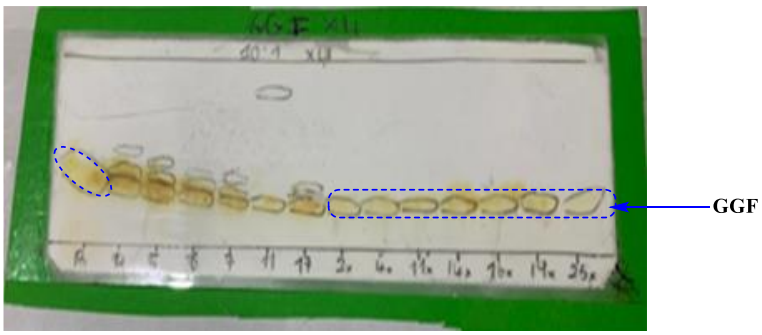


Figure 28: TLC plate of GGF

The ^{1}H NMR and ^{1}H-^{1}H COSY (**Figure 29 and 30**) permitted the analyses of different spin systems found in GGF.

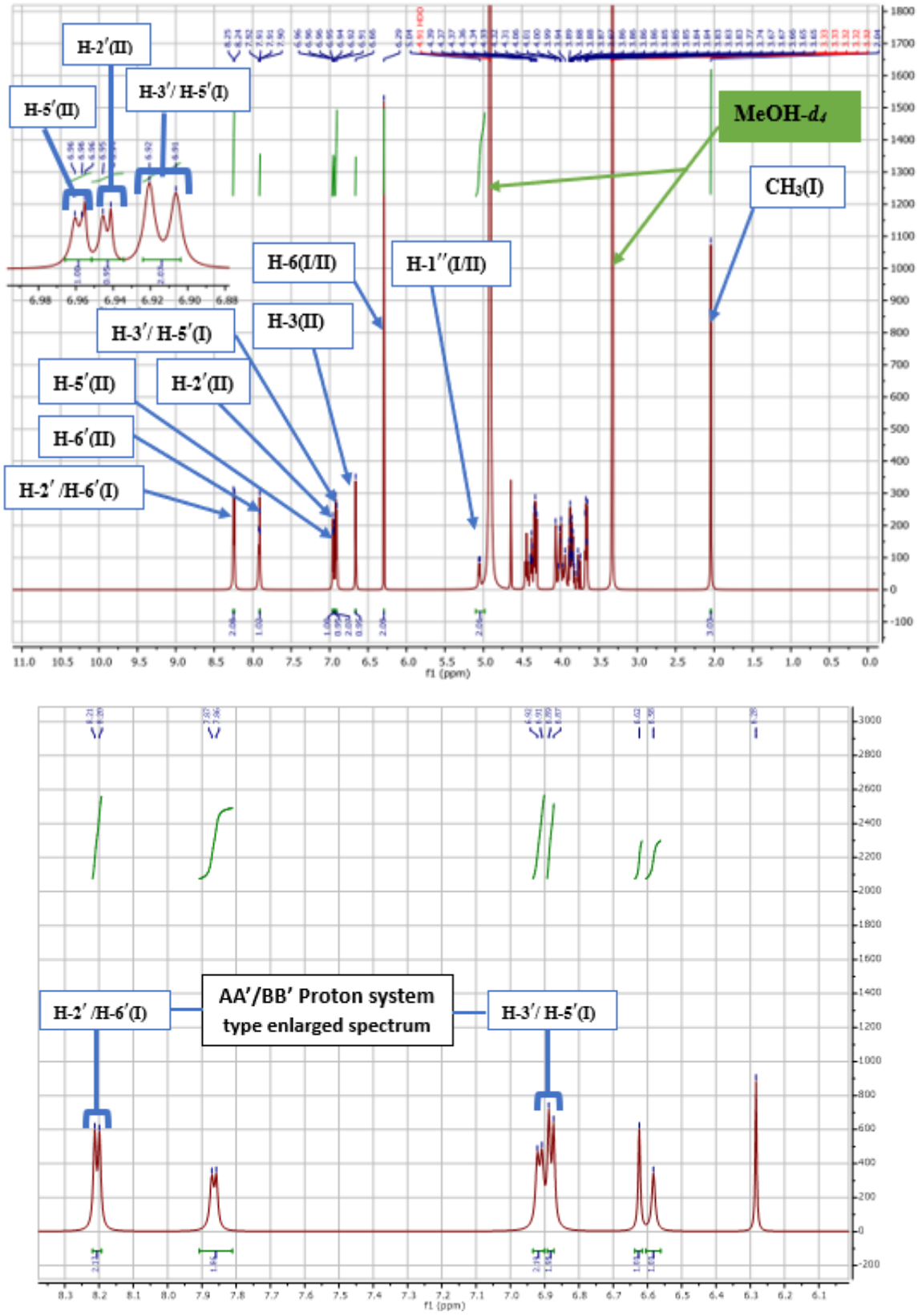


Figure 29: ^{1}H NMR spectrum (600 MHz, MeOH-d_4) of GGF

The ^{1}H-NMR spectrum of GGF **(Figure 29 and Table 18)** divulged 42 protons, showing one olefinic resonance at δ_H 6.66 (1H, s, H-3 (II)), characteristic to flavone of Unit (II) **(Mariana et *al.*, 2013)** and a singlet signal integrating for two protons at δ_H 6.29 (2H, s, H-6 (I/II)), assigned to the proton on the penta-substituted ring A(I) and ring A (II) respectively. Also, the ^{1}H-NMR spectrum exhibited characteristic resonances of an aromatic AA'/BB' proton system type at δ_H 8.24 (2H, d, J = 8.3 Hz, H-2'/H-6') and δ_H 6.91 (2H, d, J = 8.3 Hz, H-3'/H-5'), attributable to ring B of the flavone unit (I) (close to an Apigenin based structure). Another aromatic ABX-type proton system observed at δ_H 7.91 (1H, dd, J = 8.0, 1.7 Hz, H-6'), δ_H 6.96 (1H, d, J = 8.0 Hz, H-5') and δ_H 6.95 (1H, d, J = 1.7 Hz, H-2') was ascribed to ring B (II) of flavone unit (II) (similar to a Luteolin based structure). These two systems were confirmed by ^{1}H-^{1}H COSY spectrum **(Figure 30)**. The up-field portion of this spectrum exhibited a doublet signal, integrating for two anomeric protons at δ_H 5.04 (2H, d, J =8.0 Hz, H-1" (I/II)) and trait of a β-linked sugar, showing up two sugar moities **(Weisheng *et al.*, 2017)**. The $^{3}J_{H3,H4}$ coupling constant value (J = 3.6 Hz) suggested that, the two sugar moieties observed here are galactopyranosides **(Carolina and Goran, 2023).** Moreover, a singlet signal at δ_H 2.04 (3H, s) was spotted, suggesting the presence of an acetyl group.

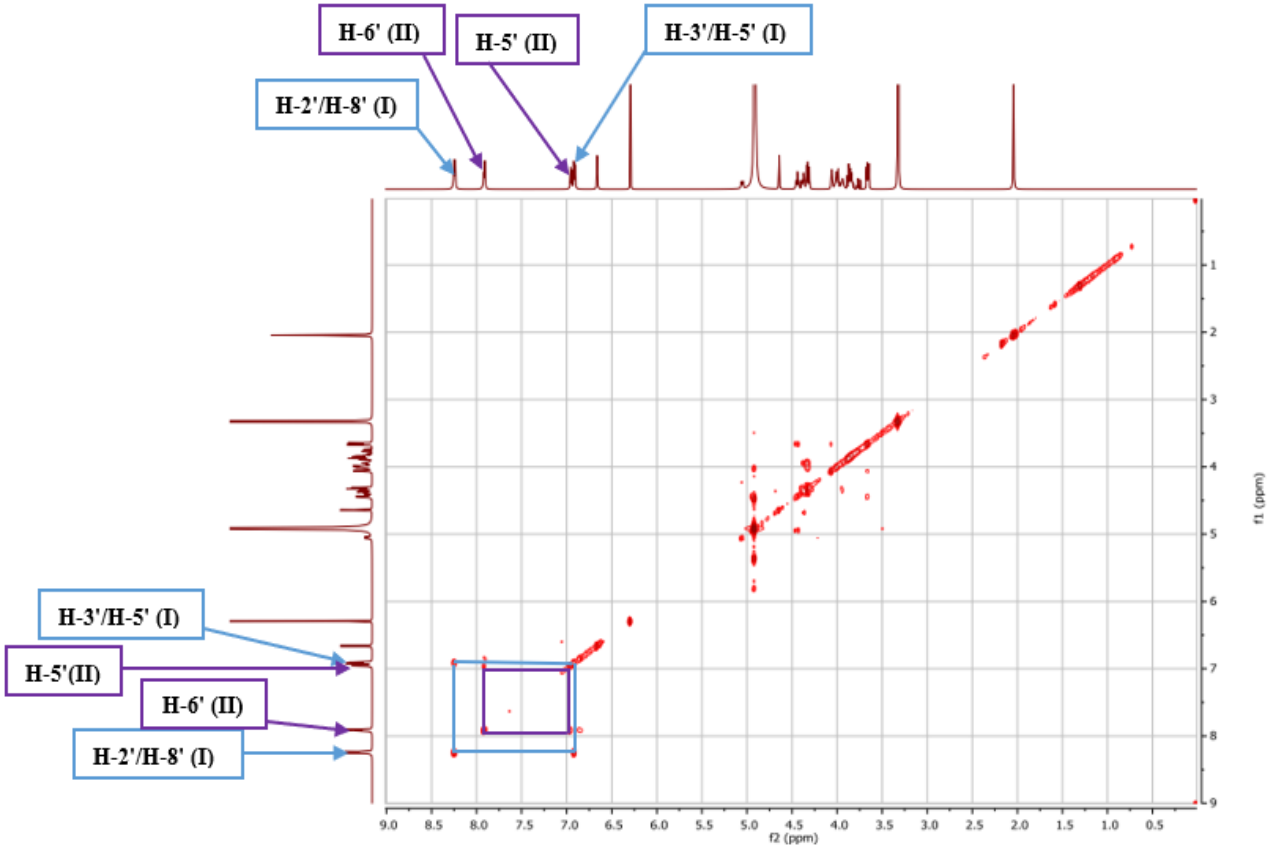


Figure 30: COSY spectrum (600MHz, MeOH-d_4) of GGF

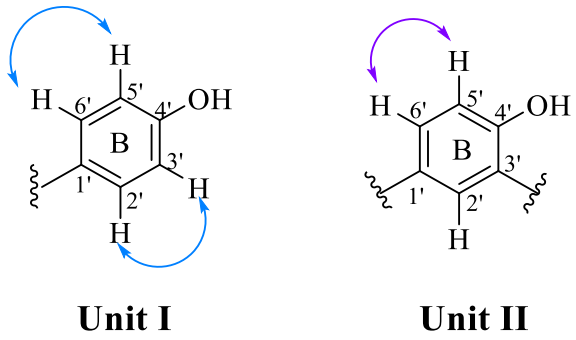


Figure 31: COSY correlations observed in GGF

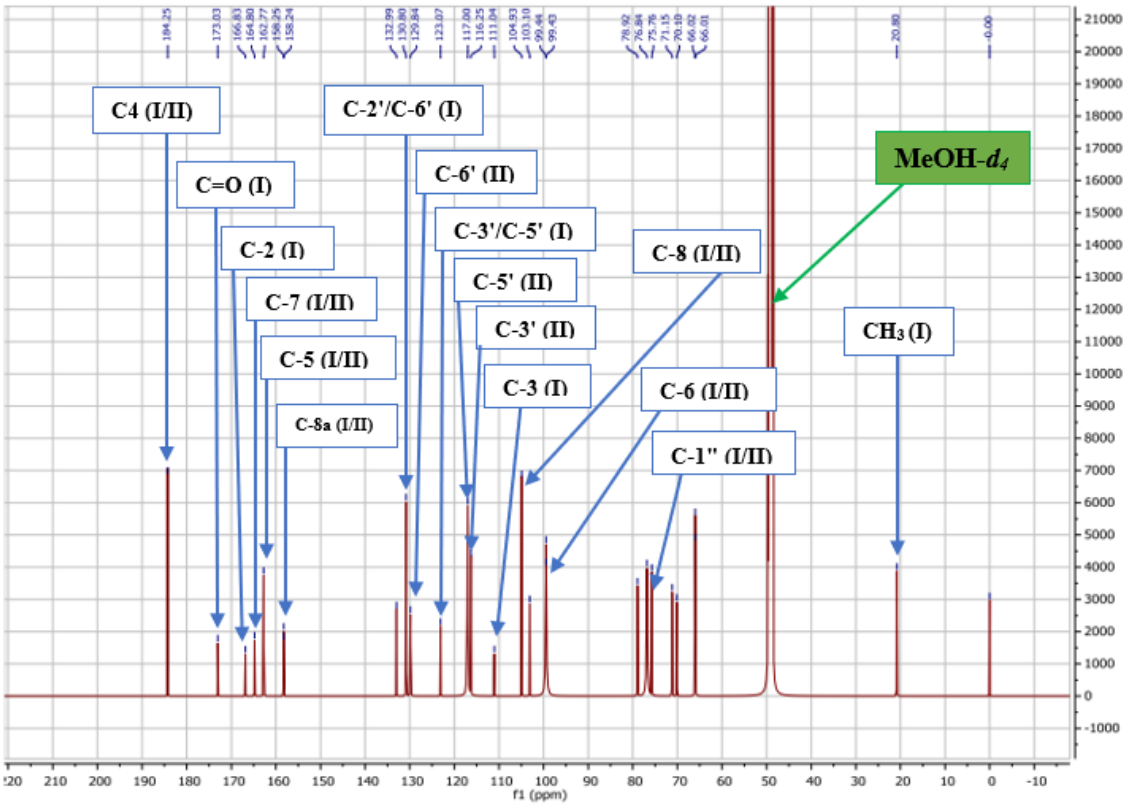


Figure 32: ^{13}C-NMR spectrum (150MHz, MeOH-d_4) of GGF

The ^{13}C-NMR data of GGF **(Figure 32 and Table 18)** disclosed 44 carbon signals, consisting of three carbonyl carbon atoms among which; two ketone carbonyl carbon atoms (resonating at δc 184.2 ppm and confirming the presence of two flavone units) and one ester carbonyl carbon atom (resonating at δc 173.0 ppm) were observed. Forbye, eighteen other quaternary carbon atoms and ten sp^2 were identified in this spectrum. Furthermore, thirteen sp^3 were descried, out of which twelve carbon signals ranging from *δ*c 66.0 to 78.9 were assigned to the two sugar residues. This confirmed that GGF is a biflavone *C*-digalactopyranoside **(Jane et *al.*, 1981; William, 2023).** The remaining sp^3 carbon signal was identified at *δ*c 20.8ppm, approving the presence of an acetyl group.

From the above information, we defined the following substructures:

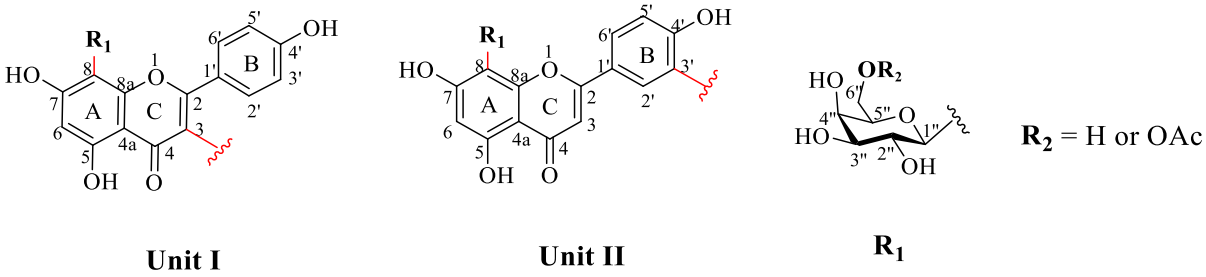


Figure 33: Substructures of GGF

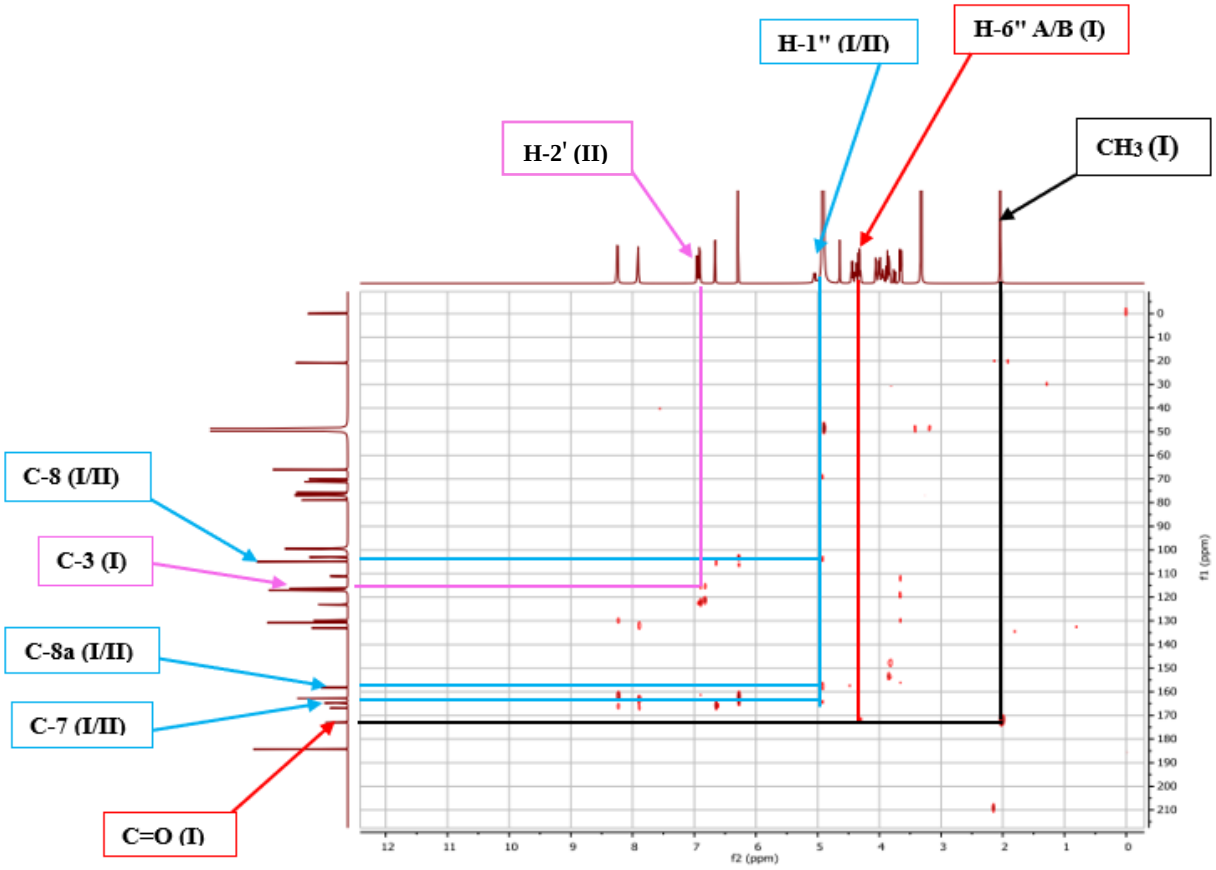


Figure 34: Some key HMBC correlations observed in GGF

The HMBC spectrum **(Figure 34)** showed correlations between the anomeric proton at δ_H 5.04 ppm and carbon atom resonances at δc 164.8 ppm (C-7), δc 158.2 ppm (C-8a) and δc 104.9 ppm (C-8) in unit I and II. These correlations indicated that the C-galactopyranoside moiety is attached at C-8 of flavone unit I and II respectively. Withal, the location of the acetoxy group at C-6″ (I) **(**formed due to the correlation between the methyl protons at δ_H 2.04 and the ester carbonyl carbon atom at δc 173.0 ppm in unit (I)**)** was established via the correlations between the diastereotopic protons (H-6″ at δ_H 4.34 and 4.32) of the galactopyranoside moiety and the ester carbonyl carbon atom at δc 173.0 (I) in unit (I).

Besides, the HMBC spectrum indicated the junction (C-3(I)-C-3′(II)) joining both units which was identified due to the correlation between H-2′ (δ_H 6.95) in unit II and C-3 (δc 116.2) in unit I. In addition, the singlet characteristic resonance of flavone unit II at δ_H 6.68 (1H, s, H-3(II)), confirms the interflavonoid linkage to be of type [I-(3)-II-(3′)].

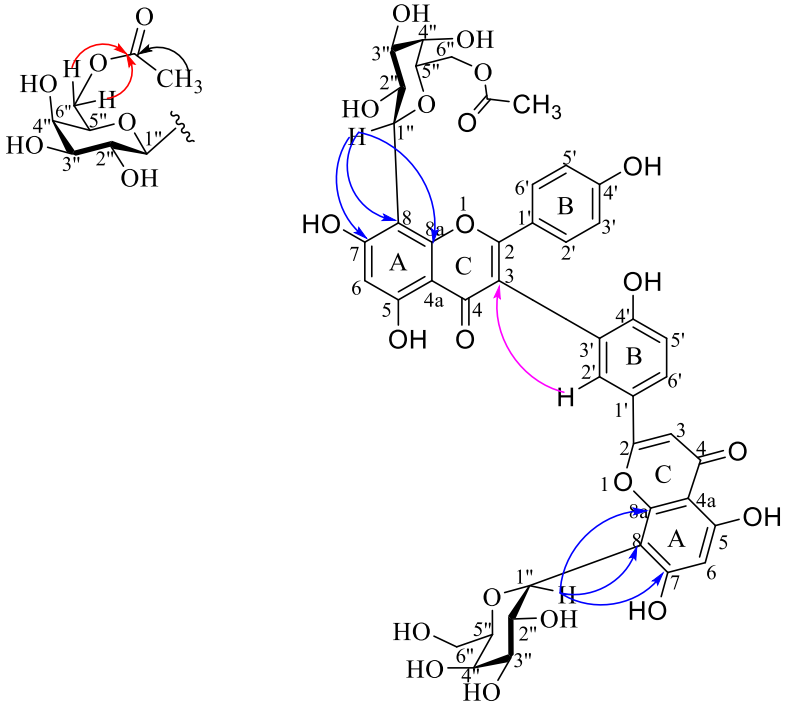
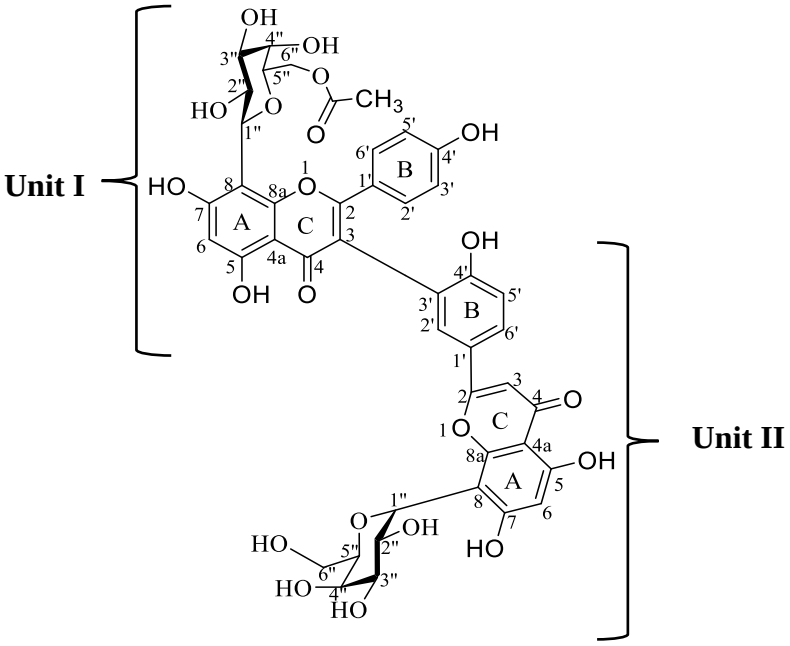


Figure 35: Some HMBC correlations observed in GGF

According to the nature of ring B(I) and B(II) of their respective unit, the above data suggested a vitexin moiety (8-C-galactoside apigenin) attributed to unit I and an orientin moiety (8-C-galactoside luteolin) attributed to unit II. On the basis of the above information, GGF was identified as: **6"-(I)-*O*-acetyl vitexin-[I-(3)-II-(3')]-orientin** (Compound **88**).



88

The ^{1}H (δ_H in ppm and *J* values in Hz) and ^{13}C (δc in ppm) assignments of compound **88** are summarized on **Table 18**.

Table 18: ^{1}H-NMR (600MHz, MeOH-d_4) and ^{13}C-NMR (150MHz, MeOH-d_4) data of GGF

Position	δ_H (*J* values in Hz)		δ_C (ppm)	
Unit I/II	Unit I	Unit II	Unit I	Unit II
2	-	-	166.8, C	166.8, C
3	-	6.66 (1H) s	111.0, CH	103.4, CH
4	-	-	184.2, C	184.2, C
4a	-	-	103.2, C	103.2, C
5	-	-	162.7, C	162.7, C
6	6.29 (1H) s	6.29 (1H) s	99.4, CH	99.4, CH
7	-	-	164.8, C	164.8, C
8	-	-	104.9, C	104.9, C
8a	-	-	158.2, C	158.2, C
1′	-	-	122.4, C	132.9, C
2′	8.24 (2H) d (8.3)	6.95 (1H) d (1.7)	130.8, CH	129.8, CH
3′	6.91 (2H) d (8.3)	-	123.0, CH	116.2, C
4′	-	-	161.3, C	163.4, C
5′	6.91 (2H) d (8.3)	6.96 (1H) d (8.0)	123.0, CH	117.2, CH
6′	8.24 (2H) d (8.3)	(7.91 (H) dd (8.0; 1.7)	130.8, CH	129.8, CH
1"	5.04 (1H) d (8.0)	5.04 (1H) d (8.0)	75.7, CH	75.7, CH
2"	4.39 (1H) m	4.39 (1H) m	71.1, CH	71.1, CH
3"	4.01 (1H) m	4.00 (1H) m	76.8, CH	76.8, CH
4"	3.86 (1H) m	3.83 (1H) m	70.1, CH	70.1, CH
5"	4.37 (1H) m	4.31 (1H) m	78.9, CH	78.9, CH
6" (A)	4.34 (1H) m	3.67 (1H) m	66.0, CH	66.0, CH
6" (B)	4.32 (1H) m	3.66 (1H) m	66.0, CH	66.0, CH
H_3**C**OCO-6"	2.04 (3H) s	-	20.8, CH_3	-
H_3C**OC**O-6"		-	173.0, C	-

II.3. EVALUATION OF THE INHIBITORY ACTIVITY OF GGB (COMPOUND 87 AND GGF (COMPOUND 88) AGAINST INTEGRASE ENZYME IN VITRO

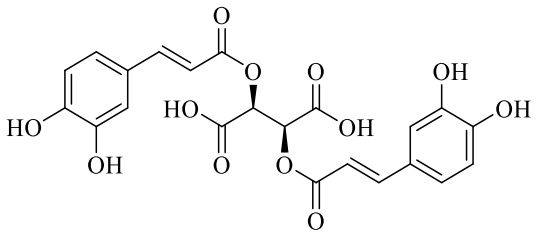
The inhibitory activity of GGB and GGF against integrase enzyme with their respective cytotoxicity were determined as described in **III.8.**

6"-*O*-acetyl-3′-*O*-methylorientin-[I-(3)-II-(6)]-6"-O-acetyl-3'-O-methylorientin(**87**) and 6"-(I)-*O*-acetyl vitexin-[I-(3)-II-(3')]-orientin (**88**) were evaluated for their inhibitory activity against integrase (catalyzes the integration of HIV-I's viral DNA into the host cell's DNA). **Table 19** displayed the inhibition constant (**IC_{50}**) and cytotoxicity concentration (**CC_{50}**) of active Chicoric acid and GGF.

Table 19: Inhibition constant and cytotoxicity concentration of Chicoric acid and GGF

Compound	IC_{50} (µg/mL)	CC_{50} (µg/mL)
Chicoric acid (89)	0.006	-
GGF (88)	3.1	5.2

From the table above, the prominent inhibitory effect on HIV-I integrase enzyme was shown by 6"-(I)-*O*-acetyl vitexin-[I-(3)-II-(3')]-orientin (**88**) (IC_{50}= 3.1 µg/mL). Its IC_{50} value was compared to that of the control compound, Chicoric acid (**89**) (IC_{50}= 0.006 µM), a pure competitive inhibitor of HIV-I integrase used clinically in the early stages of viral replication. Unfortunately, 6"-(I)-*O*-acetyl vitexin-[I-(3)-II-(3')]-orientin (**88**) proved to be non-selective as it demonstrated a CC_{50}= 5.2 µg/mL (selectivity index of 1.7). The prominent inhibitory effect on HIV-I integrase that showed GGF (IC_{50}= 3.1 µg/ml) could be explained by the presence of free hydroxyl groups all over the molecule as similarly demonstrated in the article **(Messi *et al.*, 2022)**. However, 6"-*O*-acetyl-3′-*O*-methylorientin-[I-(3)-II-(6)]-6"-O-acetyl-3'-O-methylorientin (**87**) was shown inactive on HIV-I integrase enzyme and this may be due to the methoxy groups found in its structure.



89

II.4. MOLECULAR DOCKING

The aim of molecular docking was to predict the binding interactions of GGF and GGB with integrase active site respectively. The results of this docking analysis unequivocally revealed that, GGB and GGF exhibited considerably stronger binding energies with HIV-CCD when compared to Chicoric acid, the positive control.

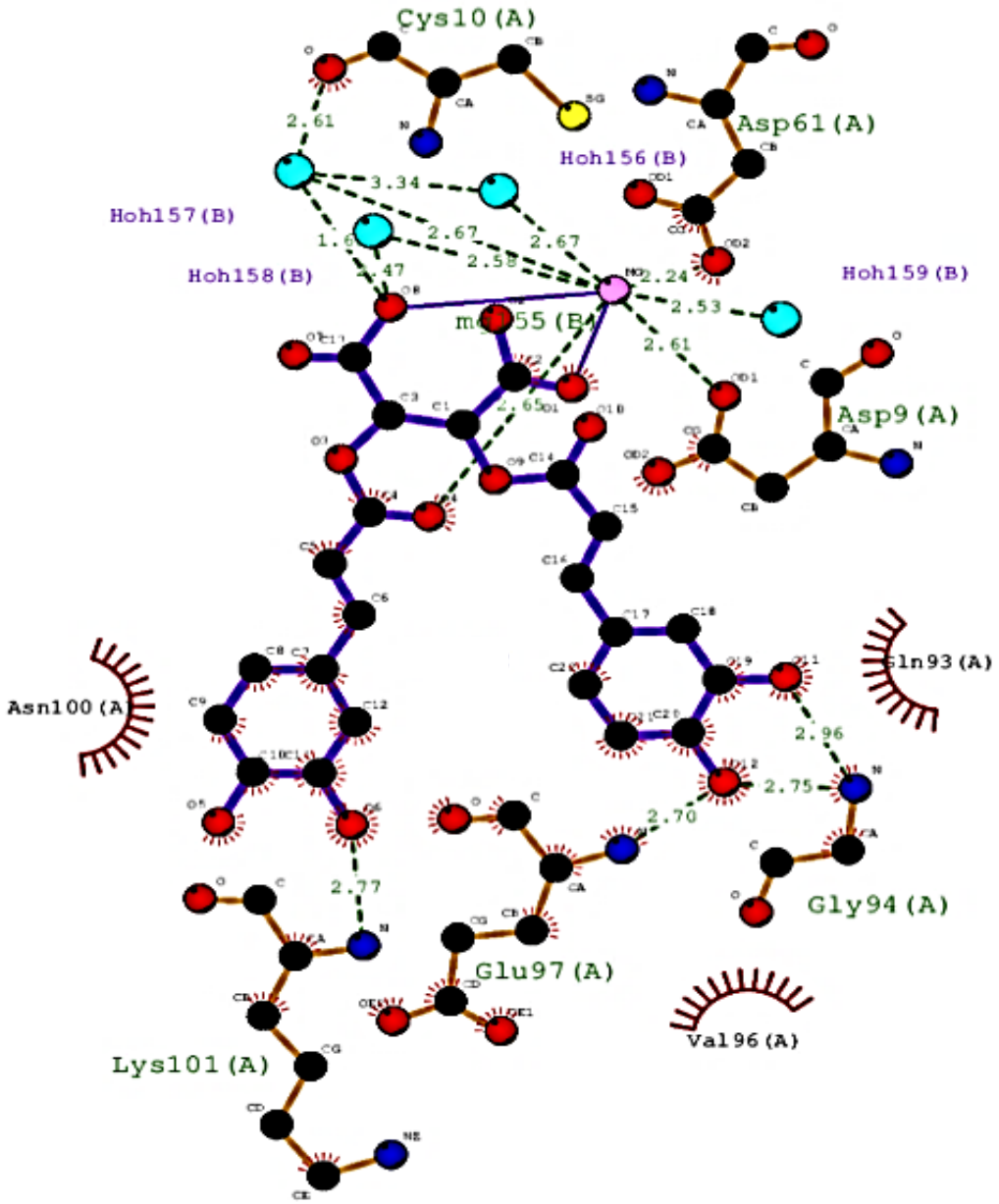


Figure 36: HIV-I integrase-Chicoric acid binding pose

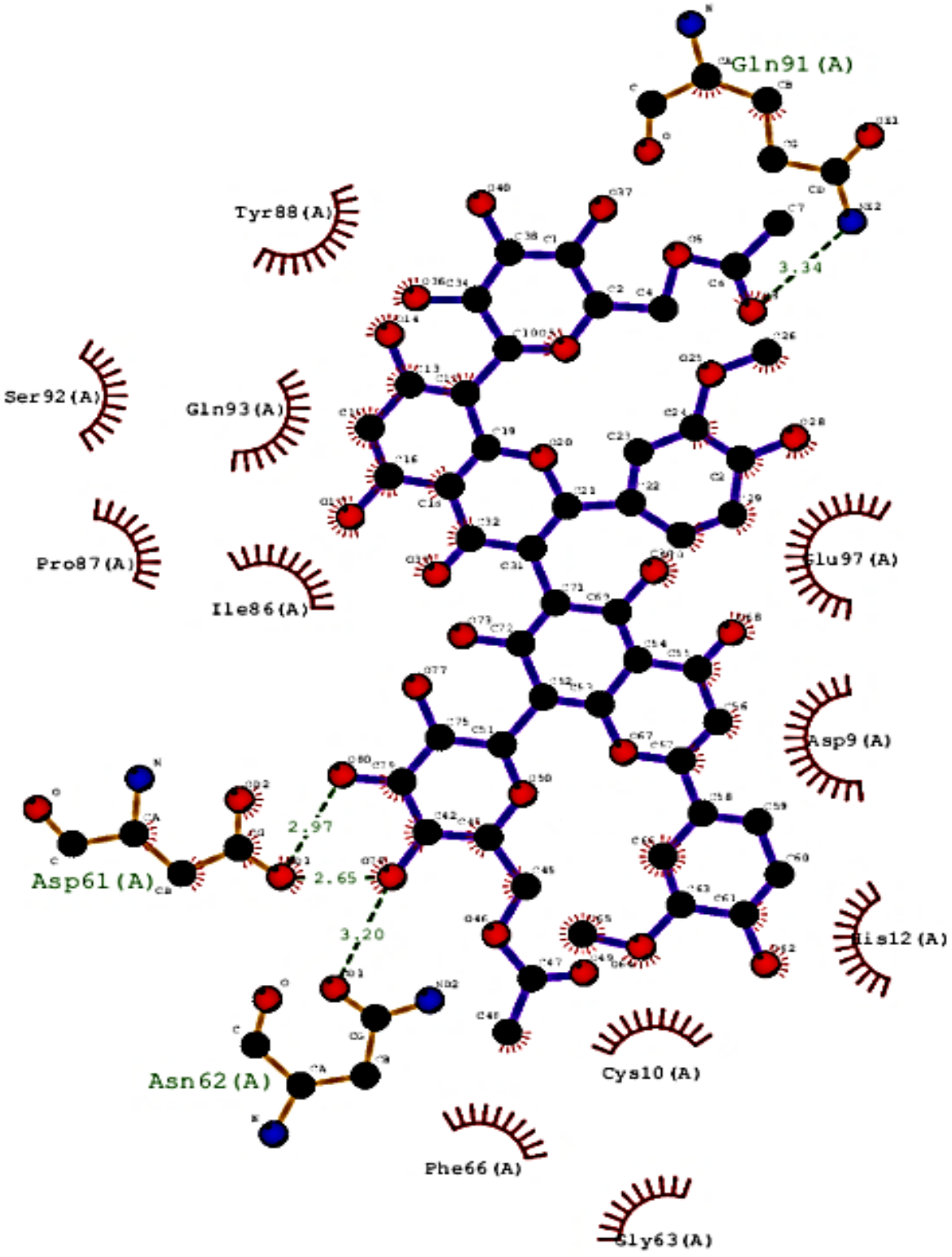


Figure 37: HIV-I integrase-GGB binding pose

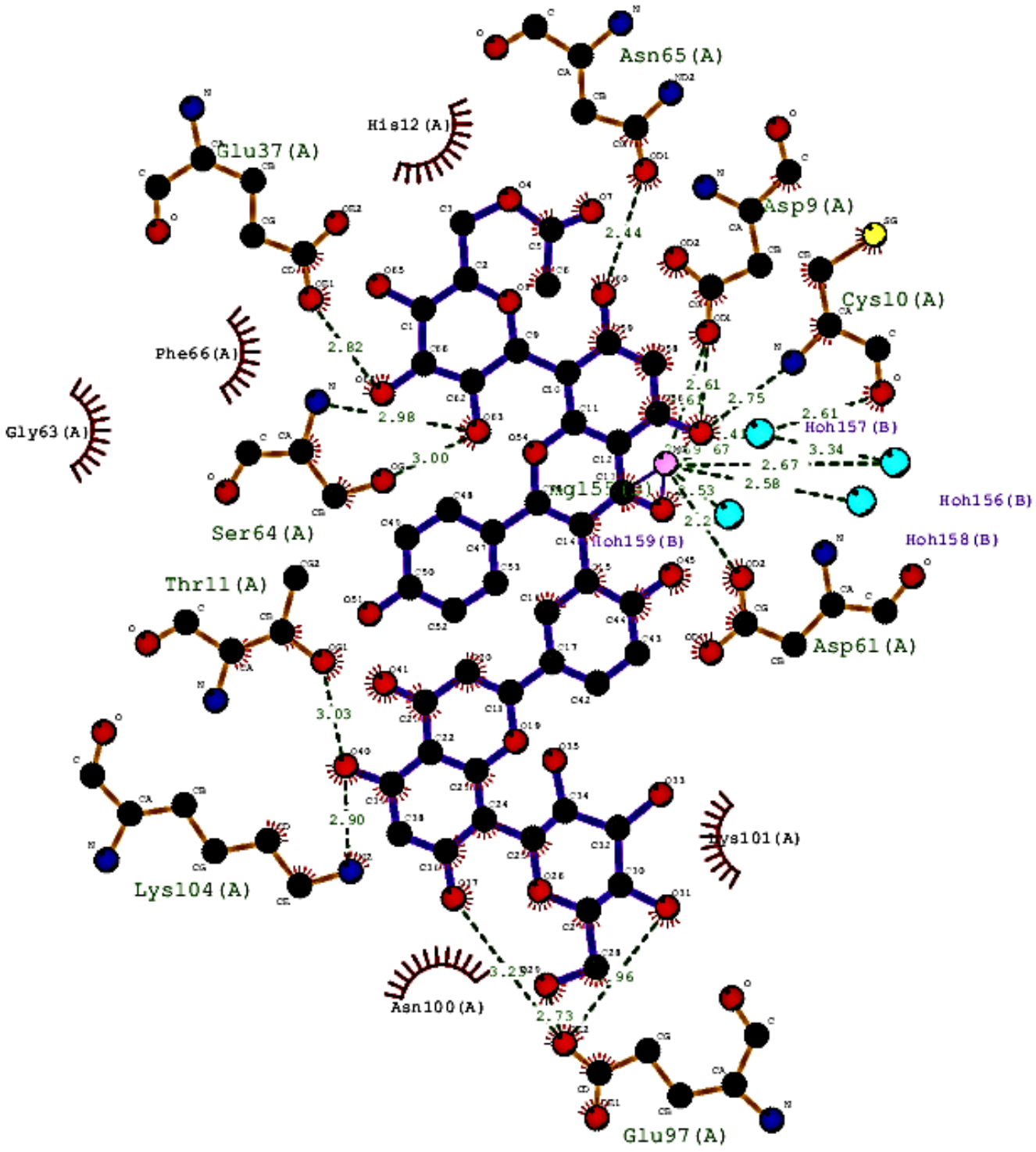


Figure 38: HIV-I integrase-GGF binding pose

The tables below show the predicted binding interactions of GGB and GGF with HIV-IN CCD **(Table 20)** and the receptor ligand interaction fingerprints **(Table 21)**

Table 20: Predicted binding energies of Chicoric acid, GGB and GGF with HIV-IN CCD

Compound	Docking energy (Kcal/mol)	Inhibition constant	Cluster occupancy
Chicoric acid **89**	-5.56	12.87	96
GGB **87**	-6.4	9.03	89
GGF **88**	-8.92	5.92	98

Table 21: Receptor-ligand interaction fingerprints.

Ligand	Receptor's amino acids
Chicoric acid **89**	**D64, C65**, D116, Q148, **G149**, V151, **E52,** N155, **K156**
GGB **87**	D64, **C65**, H67, E92, **D116**, **N117**, N120, I141, P142, Y143, **Q146**, S147, **Q148**, E152
GGF **88**	**D64**, **C65**, **T66**, H67, **E92**, **D116**, G118, **S119**, N120, F121, **E152**, N155, K156, **K159**

The inhibition mechanism of Chicoric acid is potentially linked to the displacement of the cofactor as a result of interactions with specific chemical groups within the compound. Interaction fingerprint analysis unveiled that, GGF also engaged directly with the Mg^{2+} cofactor, as well as through coordinating water molecules. This suggested a mode of action similar to that of Chicoric acid.

Intriguingly, all the compounds under scrutiny established interactions with the D64D116E152 motif within the binding pocket. Notably, both Chicoric acid and GGF demonstrated a robust hydrogen bonding network with the catalytic triad. Conversely, for GGB, interactions were predominantly facilitated by van der Waals forces. Furthermore, it's noteworthy that GGB, akin to Chicoric acid, engaged in conventional hydrogen bond linkages with residue Q148 situated in the 140 s loop, facing the active site. Additional common residues within the 140 s loop formed interactions with all the compounds, excluding GGF. These included S147 and G149. Moreover, all the compounds demonstrated interactions with the C65 thiol group, a highly efficient nucleophile during catalysis. Given the documented emergence of CCD mutants (G118H, N155H, and Q148H/G140S) linked to the development of drug resistance against Elvitegravir and Dolutegravir, the further development of the reported inhibitors as a novel class of anti-HIV-IN inhibitors holds substantial promise. In summary, the distinctive interaction profiles of these compounds with critical residues within the binding pocket underscore their potential as valuable candidates for further exploration and development in the realm of anti-HIV-Integrase inhibitors.

CONCLUSION AND PERSPECTIVES

The aim of this study was to isolate and characterize biflavonoids from the roots of *Garcinia gnetoides* capable of inhibiting HIV-I integrase enzyme thus preventing viral replication. The phytochemical investigations on the acetone extract of the roots of *Garcinia gnetoides* led to the isolation of six compounds (**GGB**, **GGD**, **GGF**, **GGH$_1$**, **GGH$_2$** and **GGI**) from which only two (**GGB** and **GGF**) were fully characterized. Structural analyses were carried out using HR-ESI mass spectrometry in the positive mode, 1D and 2D nuclear magnetic resonance techniques. This resulted to the identification of GGB and GGF as **6"-*O*-acetyl-3′-*O*-methylorientin-[I-(3)-II-(6)]-6"-O-acetyl-3'-O-methylorientin** (87) and **6"-(I)-*O*-acetyl vitexin-[I-(3)-II-(3')]-orientin** (88) respectively.

The evaluation of the inhibitory activity of GGB (87) and GGF (88) *in vitro* showed that, GGF (88) exhibits a noteworthy inhibition of HIV-I integrase **(IC$_{50}$= 3.1 µM)** but is cytotoxic **(CC$_{50}$= 5.2 µM)**. The presence and number of free hydroxyl groups seem responsible for this inhibitory activity meanwhile, the methoxy groups appear to have the opposite effect since GGB (87) was shown inactive. Docking analysis revealed the binding interactions of GGB (87) and GGF (88) with integrase receptor respectively. GGF (88) and GGB (87) (**-8.92 Kcal/mol** and **-6.4 Kcal/mol** correspondingly) presented lower binding scores than Chicoric acid (89) (**-5.56 Kcal/mol**). The distinctive interaction profiles of these compounds with critical residues within the binding pocket underscore the potential as valuable candidates for further exploration and development in the realm of anti-HIV-I integrase inhibitors. Also, these upshots indicate the necessity to confirm our observations *in vivo*, which will be the direction of our future research.

As an extension of this work, we intend to:

- Characterize and elucidate the remaining isolates (GGD, GGH$_1$, GGH$_2$ and GGI).
- Evaluate their inhibitory activity against integrase enzyme *in vitro*.
- Evaluate the inhibitory activity of all isolates against integrase enzyme *in vivo*.
- Purify and explore the remaining fractions (A, C, E and G).
- Explore the other solvent extracts (Hexane and EtOAc extract).
- Explore other plant parts (fruits, leaves and tree trunk)

CHAPTER III: EXPERIMENTAL PART

III.1. MATERIALS

III.1.1. Chemical reagent

The organic solvents used for extraction are: MeOH, MeOH/H_2O, Hexane, EtOAc, Acetone and eluents: CH_2Cl_2/MeOH and MeOH. They were all of commercial grade and distilled.

III.1.2. Plant material

The roots of *Garcinia gnetoides* was collected in the Center region of Cameroon precisely at the Mefou National Park in September 2023. The identification was done by the Botanist and a voucher specimen **(N° VN1312)** was deposited at the national herbarium in Yaounde (Cameroon).

III.2. EXTRACTION, ISOLATION AND PURIFICATION OF PLANT MATERIAL

III.2.1. Extraction

The roots of *Garcinia gnetoides* were cut, air dried and crushed into a yellowish fine powder of mass 794 g then subjected to maceration for 72 hours at room temperature using 3 L of MeOH. It was then concentrated using a rotary vapor (BUCHI 461 water bath) and dried in an oven resulting into a crude extract of mass 452 g. This crude extract was mixed with MeOH/H_2O in the ratio 80 :20 then successively partitioned with solvents in increasing polarity (that is: Hexane, EtOAc and Acetone) which were concentrated to obtain their respective extract (that is: 248 g, 176 g and 74 g respectively).

III.2.2. Isolation and purification

10 g were removed from the Acetone extract for biological tests. The remaining 64 g of the Acetone extract of the roots of *Garcinia gnetoides* was chromatographed in silica gel with CH_2Cl_2 / MeOH as eluent. The elution process was carried out in increasing polarity from 50 :1 to the ratio 1:1.

A total of 80 fractions of 200 ml each were collected and concentrated under rotary vapor. They were then grouped together into 9 major fractions on the basis of their TLC profile and named as: A (1.83 g), B (1.04 g), C (0.97 g), D (1.69 g), E (0.83 g), F (1.91 g), G (1.72 g), H (2.53 g) and I (1.23 g).

Table 22: Chromatogram of Acetone extract of the roots of *Garcinia gnetoides*

Eluent ratio (DCM/MeOH)	Major Fraction	Mass	Coloration	Solubility	Observations
50 :1	A (1-8)	1.83 g	Greenish	DCM	Oily form and mixture of compounds.White precipitates were observed insoluble in MeOH, DCM and MeOH/DCM.
30 :1	B (9-13)	1.04 g	Greenish	MeOH	Oily form with green pure precipitate observed insoluble in DCM but soluble in DMSO.
30 :1	C (14-25)	0.97 g	Whitish	MeOH	Pasty, sticky appearance, with a mixture of compounds. No precipitate observed.
20 :1	D (26-36)	1.69 g	Pale orange	MeOH	Mixture of three spots as presented by the TLC plate. No precipitate observed.
10 :1	E (37-46)	0.83 g	Pale orange	DMSO	Sticky appearance showing one spot with trials on the TLC plate. It is insoluble in MeOH, DCM, DCM/MeOH. No precipitate observed.
5 :1	F (47-51)	1.91 g	Yellow	DCM	Mixture of one colorless, three pale orange and one brownish spot as presented by the TLC plate. No precipitate observed.
2 :1	G (52-61)	1.72 g	Yellowish	MeOH	Pasty and sticky appearance with a mixture of compounds. No precipitate observed.
2 :1	H (62-76)	2.53 g	Pale brown	MeOH	Mixture of pale brown spots with trails as revealed by TLC plate after calcination. Yellow precipitate observed.
1 :1 100% MeOH	I (77-80)	1.23 g	Black	DMSO	A large brownish spot with trails was revealed on TLC plate after calcination. Black precipitates were observed.

Once the roughing complete, we proceeded to the purification of these different fractions. In view of **Table 22** above, some fractions could not be purified because they were neither in crystal form nor powder but instead in oily, pasty and sticky form.

- **Fraction A, B, C, E and G**

Fraction A (1.83 g, CH_2Cl_2/MeOH, 50 :1), fraction B (1.04g, CH_2Cl_2/MeOH, 30 :1) were not purified because of their oily form. Fraction C (0.97 g, CH_2Cl_2/MeOH, 30 :1), fraction E (0.83 g, CH_2Cl_2/MeOH, 10:1) and fraction G (1.72 g, CH_2Cl_2/MeOH, 2 :1) showed a pasty and sticky aspect hence could not be purified.

However, from fraction B, amorphous yellow precipitates were observed. They were separated from the oily content by siphoning and were washed with DCM. The TLC plate revealed that they were pure and named GGB (0.34 g).

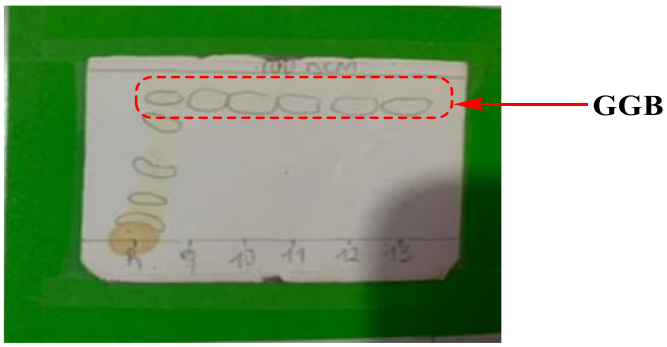


Figure 39: TLC plate of GGB

- **Fraction D**

Fraction D (1.69 g, CH_2Cl_2/MeOH, 20 :1) was purified using silica gel chromatography from which whitish pure precipitates (precipitates observed in bottle 3', 4, 5' and 6') were obtained at 30 :1 and named GGD (0.53 g).

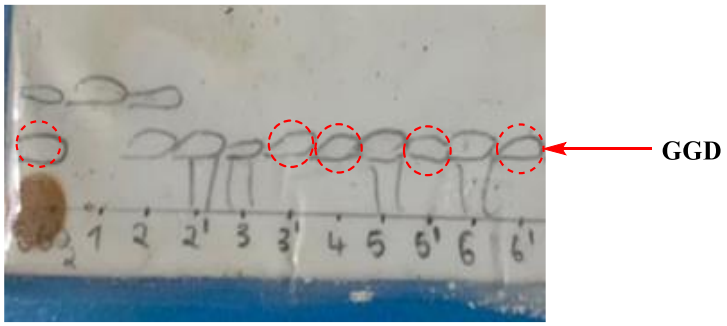


Figure 40: TLC plate of GGD

- **Fraction F**

Fraction F (0.91 g, CH_2Cl_2/MeOH, 5 :1) was purified using silica gel chromatography with CH_2Cl_2/MeOH as eluent, following an increasing polarity gradient (20 :1, 15 :1, 10 :1, 5 :1, 2 :1, 1 :1 and 100% MeOH). The TLC plate after calcination revealed pure yellow powder. These precipitates were obtained at 10 :1 and named GGF (0.48 g).

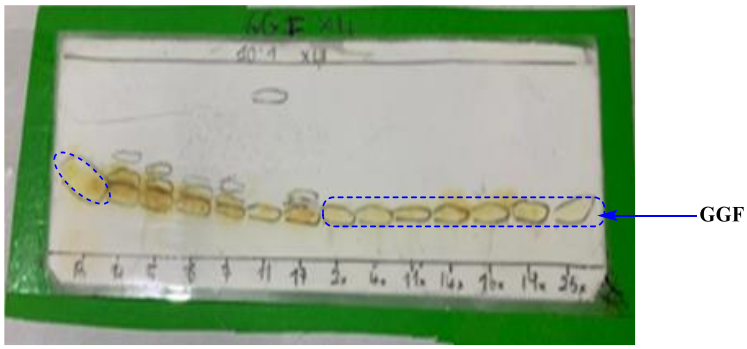


Figure 41: TLC plate of GGF

- **Fraction H**

Fraction H (2.96 g, CH_2Cl_2/MeOH, 2 :1) was purified using sephadex LH-20 from which, two pure compounds were obtained: one yellowish powder in small quantity indexed GGH_1 (0.37 g) and one brownish powder GGH_2 (0.78 g) as revealed by the TLC plate.

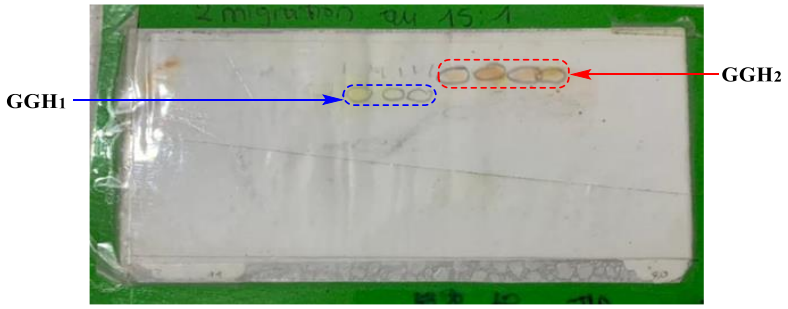


Figure 42: TLC plate of GGH_1 and GGH_2

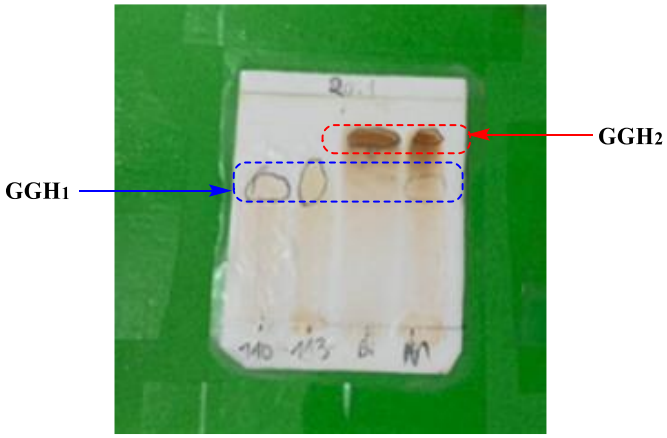


Figure 43: Comparative TLC plate of GGH_1 and GGH_2

- **Fraction I**

Fraction I (1.23 g CH_2Cl_2/MeOH: 1 :1) could not be purified neither with sephadex LH-20 nor with silica gel chromatography simply because it did not dissolve in any available polar systems at our disposal. It was washed with acetone and indexed GGI (0.63 g). The purification of GGI was verified using a TLC plate.

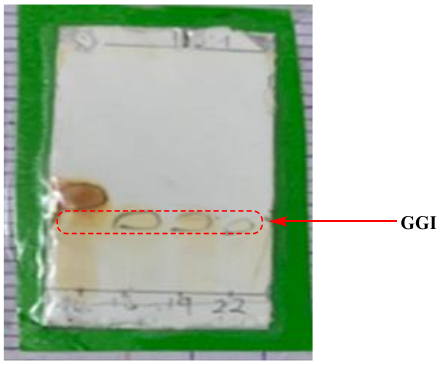


Figure 44: TLC plate GGI

III.3. FUNCTIONAL GROUP TEST WITH CRUDE EXTRACT

Phytochemical screening made the identification of different families of compounds present in the acetone extract of *Garcinia gnetoides* possible. It was carried out by the method described by **(Juniad and Patil, 2020).**

III.3.1. Shinoda's test

- **Aim:** Identification of flavonoids.
- **Procedure:** Plant extract is dissolved in 5 mL alcohol + fragments of magnesium ribbon + few drops of concentrated HCl.
- **Result:** A pink to crimson colored solution indicates the presence of flavonoid.

III.3.2. Molish's test

- **Aim:** Identification of sugars in a solution.
- **Procedure:** 2 mL filtrate + 2 drops of alcoholic α-naphthol + 1 mL concentrated H_2SO_4 (along sides of the test tube).
- **Result:** A violet ring formed indicates the presence of sugars.

III.4. THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) was achieved with precoated silica gel plates 60 F_{254} (Merck, 0.25 mm thick) supported on aluminum plates as stationary phase. These plates are then immersed into a chromatographic vacuum. The solvent is elevated up to 0.5 cm, constituting the mobile phase, which is generally a binary or tertiary mixture of solvents.

The spots revelation on the TLC plate was done by using:

- A UV lamp of type Universal lamp model Nu-4KL of wavelength at 254 nm and 366nm.
- Iodine vapour.
- Pulverization using 10% diluted H_2SO_4 and the plate dried on a hot plate at a temperature of about $60^{O}C$.

III.5. COLUMN CHROMATOGRAPHY

Column chromatography is used to separate small quantities of each component of a mixture. Silica gel-60 was used as the stationary phase. There are two elution modes in column chromatography:

- **The isocratic mode**: The polarity of the mobile phase is maintained from the beginning to the end of the elution process.
- **The polarity gradient mode**: The eluent system varies with increasing solvent polarity.

In view of all the above, the preparation of the column is done in several steps, including:

- **Silica activation**

Silica gel is activated by dropping it for 24h in the solvent at room temperature. Also, silica gel can be activated by heating in an oven at 110°C for 20 minutes.

- **Column preparation**

Silica gel is introduced into the column with an organic solvent. To prevent breakage of the stationary phase, the solvent must be poured continuously or gradually into the chromatographic column.

- **Deposit of the extract to be splitted**

The extract is mixed with a volume of methylene chloride (DCM) and then a little silica gel is added. The solvent is evaporated in the oven until a fine homogenous powder is obtained. The deposit is prepared with 2 g of extract and 50 g of silica gel (30 times to the mass of the deposit).

III.6. PURIFICATION AND SEPARATION METHODS

III.6.1. Silica gel column

The roughing of the Acetone extract was realized by adsorption chromatography on a stationary phase silica gel 60 (63-200 µm, Merck). The different colon heights (for roughing and purification with silica gel colon) were chosen according to the quantity of the extract. The elution system was a binary solvent composed of CH_2Cl_2/MeOH gradients in determined proportions on TLC before separation. The extract to be fractioned was adsorb on a quantity of corresponding stationary phase at 5 to 10 times its mass. The insertion of the extract in the silica gel colon was done in its solid form after activation of the silica gel.

III.6.2. Filtration gel

This technique is mainly used for the filtration and separation of mixtures according to their sizes. The insertion of the extract is done in liquid form and the solvent use is mostly MeOH. Hence the stationary phase was Sephadex LH-20 (Pharmacia) and the mobile phase was MeOH. The rate, generally slow, did not exceed 1mL/min. The colon height was selected according to the quantity of sample.

III.6.3. Lavation

Lavation consists of pouring a few mL of organic solvent into a bottle containing the precipitate (an organic solvent in which the precipitate is insoluble) and the liquid after mixing is siphoned off using a Pasteur pipette. The experiment is repeated several times before rechecking the purity of the precipitate.

III.7. PHYSICAL AND CHEMICAL METHODS

III.7.1. Mass Spectrum

- The mass spectrum of different pure compounds isolated were obtained by electrospray ionization (ESI).
- ESI High resolution mass spectrometer (HR-ESI-MS).

III.7.2. Nuclear Magnetic Resonance spectrum (NMR)

NMR spectra were recorded at 25°C using a Bruker 600 spectrometer (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C NMR. Deuterated MeOH (CD_3OD) and Dimethyl sulfoxide (DMSO-*d6*) were used as solvents. Chemical shift (δ) is reported in part per million (ppm)

and the spectra were referenced relative to Me_4Si internal standard at 0ppm. Coupling constants (*J*) were reported in Hz. The 2D NMR spectra: ^1H-^1H COSY, HMBC, HSQC, ROESY and NOESY were used for appropriate elucidation.

III.8. BIOLOGICAL ACTIVITY

The inhibitory activities of compound GGB and GGF against HIV-I integrase was achieved using the following protocol: These isolates were dissolved in dimethyl sulfoxide (99.7%, Molecular Biology grade, Sigma-Aldrich, USA) to a stock concentration of 10mg/mL and stored at -20^{o}C prior to biological evaluation. The anti-HIV replication of GGB and GGF was assessed against the prototypic subtype B clone of HIV-I, NL4-3, *in vitro* using the previously described dual enhancement of the cell infection to phenotypic resistance (deCIPHER) assay as described by the article **Messi et *al.*, 2024**. All the compounds were screened across four concentration points 0.025, 0.25, 2.5 and 10 mg/mL), in technical duplicates and with one independent biological repeat. Chicoric acid served as a positive inhibition control of viral replication while dilution buffer containing the same concentration of DMSO as the extract was used as negative control.

Cytotoxicity of the GGB and GGF was assessed parallel to the anti-HIV replication studies similar cell culture conditions but without addition of the virus. Plates were read out following 96 hours of incubation, using Alamar Blue reagent (Sigma-Aldrich, USA, 98%).

III.9. MOLECULAR DOCKING

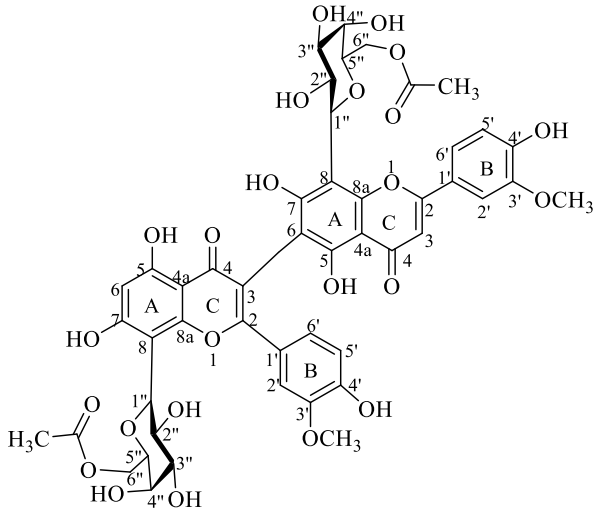
A rigorous investigation involving the catalytic core domain (CCD) of HIV-IN was carried out. The objective was to elucidate the potential interactions between this domain and the two biflavonoid isolates (GGB and GGF). To ensure methodological consistency, a preparatory procedure for both receptor and ligand outlined in the study by **Musyoka *et al.*, 2018** was adopted. Charge assignments for all atoms within the receptor and various ligands were meticulously executed following the Gasteiger-Huckel method. Additionally, we integrated a charge of 1.7 for the Mg^{2+} cofactor within the receptor's AutoDock file, aligning with established precedent. For the docking process, a strategically positioned grid box, spanning 60 points along the x, y and z axe, with its center coordinates set at (-18.6, 30.1, 66.6), was defined around the receptor molecule. Employing the Lamarckian Genetic Algorithm (LGA), 100 docked poses for each ligand were systematically generated. Subsequent to this extensive docking simulation, cluster analysis was applied to discern the

most favorable docked pose, distinguished by the lowest docking energy score, for further in-depth scrutiny. To scrutinize and interpret the intricate binding interactions between the CCD of HIV-IN and the two isolates (GGB and GGF), we harnessed LigPlot to derive comprehensive interaction fingerprint for each receptor-ligand complex. This rigorous investigating approach underpins our ability to draw substantive conclusions regarding the receptor engagement with the various ligands and serves as a robust foundation for subsequent analytical pursuits.

III.10. PHYSICAL AND CHEMICAL CHARACTERISTICS OF GGB AND GGF

Only two compounds out of the six isolated were fully characterized and elucidated.

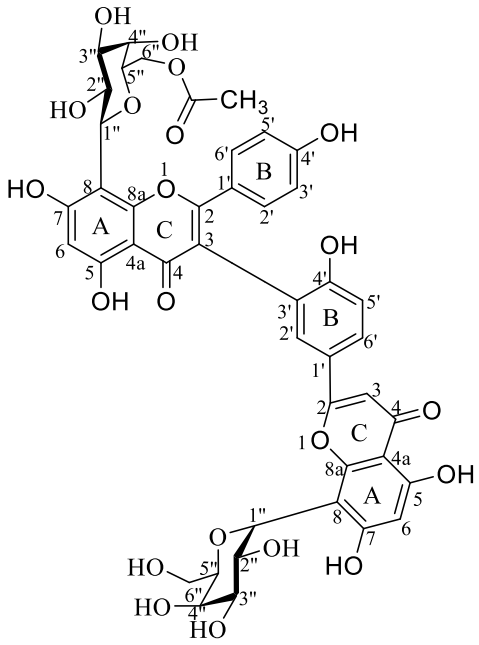
- **Compound GGB:**



- Positive to Shinoda's and Molish tests.
- Amorphous yellow powder.
- Soluble in DMSO
- IC- (HR-ESI MS) **$[M+H]^+$ m/z**
- Molecular formula: $C_{48}H_{47}O_{24}$
- Molecular mass: 1007.2463 amu
- NMR ^{1}H Spectrum (600 MHz, DMSO-*d_6*): 7.00 (1H, s, H-3 **(II)**), 13.35 (1H, s, 5-OH **(I/II)**), 6.28 (1H, s, H-6 **(I)**), 7.56 (1H, d, 2 Hz **(I/II)**), 6.89 (1H, d, 8.2 Hz, H-5′ **(I/II)**), 8.27 (1H, dd, 8.2; 2 Hz, H-6′ **(I/II)**), 4.68 (1H, d, 9.8 Hz, H-1" **(I)**), 4.66 (1H, d, 9.8 Hz, H-1" **(II)**), 4.05 (1H, m, H-2" **(I)**), 4.04 (1H, m, H-2" **(I)**), 4.23 (1H, m, H-3" **(I)**), 4.22 (1H, m, H-3" **(II)**), 4.94 (1H, m, H-4" **(I/II)**), 3.79 (1H, m, H-5" **(I)**), 3.78 (1H, m, H-5" **(II)**), 4.21 (1H, m, H-6_A" **(I)**), 4.09 (1H, m, H-6_B" **(I)**), 4.20 (1H, m, H-6_A" **(II)**), 4.07 (1H, m, H-6_B" **(II)**), 3.88 (3H, s, 3′-OC**H**$_3$), 1.98 (3H, s, **H**$_3$COCO-6" **(I/II)**.

- ^{13}C NMR Spectrum (150 MHz, DMSO-d_6): 164.7 (C-2 **(I)**), 164.5 (C-2 **(II)**), 110.8 (C-3 **(I)**), 104.5 (C-3 **(II)**), 182.7 (C-4 **(I)**), 182.4 (C-4 **(II)**), 102.9 (C-4a **(I/II)**), 161.0 (C-5 **(I/II)**), 98.5 (C-6 **(I/II)**, 110.4 (C-6 **(II)**), 163.1 (C-7 **(I)**), 162.6 (C-7 **(II)**), 104.6 (C-8 **(I/II)**), 156.9 (C-8a **(I)**), 156.6 (C-8a **(II)**), 121.7 (C-1′ **(I)**), 121.1 (C-1′ **(II)**), 121.1 (C-2′ **(I)**), 120.9 (C-2′ **(II)**), 148.3 (C-3′ **(I)**), 148.2 (C-3′ **(II)**), 151.2 (C-4′ **(I/II)**), 116.4 (C-5′ **(I/II)**), 123.3 (C-6′ **(I/II)**), 75.5 (C-1" **(I/II)**), 74.2 (C-2" **(I/II)**), 76.3 (C-3" **(I/II)**), 69.9 (C-4" **(I/II)**), 77.2 (C-5" **(I/II)**), 65.4 (C-6_A" **(I/II)**),64.7 (C-6_B" **(I/II)**), 56.5 (3′-OMe **(I)**), 56.3 (3′-OMe **(II)**), 21.2 (**H$_3$C**OCO-6" **(I/II)**), 171.0 (H$_3$C**OC**O-6" **(I)**), 170.9 (H$_3$C**OC**O-6" **(II)**).

➢ **Compound GGF:**



- Positive to Shinoda's and Molish’s tests.
- Yellow powder
- Soluble in MeOH
- IC-(HR-ESI MS) **[M + H]$^+$ m/z**
- Molecular formula: $C_{44}H_{43}O_{21}$
- Molecular mass: 907.2302 amu
- ^{1}H NMR Spectrum (600 MHz, MeOH-d_4): 6.66 (1H, s, H-3 **(II)**), 6.29 (1H, s, H-6 **(I/II)**), 8.24 (2H, d, 8.3 Hz, H-2′/H-6′ **(I)**), 6.91 (2H, d, 8.3 Hz, H-3′/H-5′ **(II)**), 6.95 (1H, d, 1.7 Hz, H-2′ **(II)**), 6.96 (1H, d, 8.0 Hz, H-5′ **(II)**), 7.91 (1H, d, 8.0 ;1.7 Hz, H-6′ **(II)**), 5.04 (1H, d, 8.7 Hz, H-1" **(I/II)**), 4.00 (1H, m, H-2"**(I/II)**), 3.86 (1H, m, H-

3"**(I/II)**), 4.39 (1H, m, H-4"**(I)**), 4.38 (1H, m, H-4"**(II)**), 4.37 (1H, m, H-5"**(I)**), 4.31 (1H, m, H-5"**(II)**), 4.34 (1H, m, H-6_A"**(I)**),4.28 (1H, m, H-6_B"**(I)**), 3.67 (1H, m, H-6_A"**(II)**), 2.04 (3H, s, 3′-OC**H**$_3$).

- ^{13}C NMR Spectrum (150 MHz, MeOH-d_4): 166.8 (C-2 **(I/II)**), 111.1 (C-3 **(I)**), 103.4 (C-3 **(II)**), 184.2 (C-4 **(I/II)**), 103.2 (C-4a **(I/II)**), 162.7 (C-5 **(I/II)**), 99.4 (C-6 **(I/II)**), 164.8 (C-7 **(I/II)**), 104.9 (C-8 **(I/II)**), 158.2 (C-8a **(I/II)**),122.4 (C-1′ **(I)**), 132.9 (C-1′ **(II)**), 130.8 (C-2′/C-6′**(I)**), 123.0 (C-3′/C-5′ **(I)**), 129.8 (C-2′ **(II)**), 117.2 (C-3′ **(II)**), 161.3 (C-4′ **(I)**), 163.4 (C-4′ **(II)**), 116.2 (C-5′ **(I)**), 117.0 (C-5′ **(II)**), 125.3 (C-6′ **(II)**), 75.7 (C-1" **(I/II)**), 71.1 (C-2" **(I/II)**), 76.8 (C-3" **(I/II)**), 70.1 (C-4" **(I/II)**), 78.9 (C-5" **(I/II)**), 66.0 (C-6" **(I/II)**), 20.8 (**H**$_3$**C**OCO-6" **(I)**), 173.0 (H_3C**OC**O-6" **(I)**).

REFERENCES

Adjapmoh M. F., Toze F. A., Songue J. L., Langat M. K., Kapche G. D., Hameed A., Lateef M., Shaiq M. A., Mbaze L. M. and Wansi J. D. (2016). A New Ceramide and Biflavonoid from the Leaves of *Parinari hypochrysea* (Chrysobalanaceae). *Natural Products Communications,*11, 615–620.

Ahmed A., Adonis B., Osmany C., Anna L. and Luca R. (2013). Biflavonoids, Main Constituents from *Garcinia bakeriana* Leave. *Natural Products Communication*, 8(9), 1237-1240.

Akongwi M., Tih A. E., Nyongbela K. D., Samje M., Ghogomou T. R. and Bodo B. (2019). Brevipedicelones D and E, Two C–O–C Flavonoid Dimmers from the Leaves of *Garcinia brevipedicellata* and Anti-onchocercal. *Natural Products and Bioprospecting*, 9, 61-68.

Alain M. L., Denis K. S., Simplice J. N., Tsague T. V., Hycienth F. T., Jean D. W., Yoshihito S. and Norbert S. (2017). A new depsidone derivative from the leaves of *Garcinia polyantha*. *Natural Product Research*, 32(9), 1033-1038.

Alok K. V. and Ram P. (2010). Biological potential of Flavones. *Natural products report,* 27, 1571–1593.

Anu A. P., Lekshmi N. M. and Rameshkumar K. B. (2016). Structural diversity of secondary metabolites in *Garcinia* species. *Jawaharal Nehru Tropical Botanic Garden and Research Institute*, 19-88.

Bai H., Li W., Koike K., Dou D., Pei Y., Chen Y. and Nikaido T. A. (2003). novel biflavonoid from roots of *Glycyrrhiza uralensis* cultivated in China. *Chemical and Pharmaceutical Bulletin*, 51, 1095–1097.

Bernadette B., Raimana H., Alain M., Delphine C., Karl P., Augustin E., Pierre-Alain C., Kurt H. and Muriel C. (2014). Isolation and biological activity of compounds from *Garcinia preussii*. *Pharmaceutical Biology*, 52, 704-711.

Bhargav S., Shivalingaiah J., Chandan D., Siddesh V., Sumanth R., Anisha S., Bhavana H., Abhigna N., Akash P., Chandrashekar S., Ashwini P., Shiva P. and Chandan S.

(2023). Computational studies applied to flavonoids of *Garcinia morella* against parkinson's diseases. *European chemical bulletin*, 12 (S3), 5559 – 5569.

Bruna L., Lidiani F., Wilson H., Felipe O., Danielle B., Karine C., Rita C., Priscila A., Arnildo P., Wander F., Marcel A., Patricia O. and Paul R. (2020). Medicinal Potential of *Garcinia* species and their compounds. *Molecules*, 25, 4513.

Botanical Releam: *Garcinia gnetoides* [on line]. Available on ''https://www.botanicalrealm.com/plant-identification/garcinia-gnetoides/'' Consulted on 27/09/2024 at 05 :20 am.

Burkill H.M. (1997). The useful plants of West tropical Africa. *Royal botanic Gardens, Kew (K)*, vol 2, 1-969.

Carini J. P., Kaiser S., Ortega G. G. and Bassani L. (2013). Development, optimisation and validation of a stability-indicating HPLC method of achyrobichalcone quantification using experimental designs. *Phytochemical Analysis*, 24, 193–200.

Carolina F. and Goran W. (2023). Primary structure of Glycans by NMR Spectroscopy. *Chemical Reviews*, 123, 1040-1102.

Chaoquin L., Paul C., Fang C. W., Gautam S., Ling Z. W. and Boon C. G. (2017). Garcinol: Current status of its anti-oxidative, inflammatory and anti-cancer effects. *Cancer letters*, 1-5.

Choo C. Y., Sulong N. Y., Man F. and Wong T. W. (2012). Vitexin and isovitexin from the leaves of *Ficus deltoidea* with in-vivo alpha-glucosidase inhibition. *Journal of Ethnopharmacology*, 142, 776–781.

Chutichot M., Supotchana S., Anumart B. and Rutt S. (2013). A new biphenyl and other constituents from the wood of *Garcinia schomburgkiana*, *Natural Product Research*, 27(23), 1-5.

Cushine T. P. and Lamb A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26, 343-356.

Cuyckens F., Ma Y., Pocsfalvi G. and Claeys M. (2000). Tandem mass spectral strategies for structural characterization of flavonoid glycosides. *Analysis*, 28(10), 888-895.

David S. (2021). Scalar coupling scales with bonding. *Nature Reviews Chemistry*, 5, 598.

Diptesh B., Avijit C., Swapna M. and Biswajit G. (2023). Root culture. A potent method for improve secondary metabolite production of Solanaceous plant. *Frontiers in Plant Science*, 1-12.

Edwin R., Pongpun S., Suttira K. and Santi T. (2016). New depsidones and xanthone from the roots of *Garcinia schomburgkiana*. *Fitoterapia*, 111, 73-77.

Elfita E., Muharni M., Latief M., Darwati D., Widiyantoro A., Supriyatna S., Bahti H., Dachriyanus D., Cos P. and Maes L. (2009). Antiplasmodial and other constituents from four Indonesian *Garcinia* species. *Phytochemistry*, 70, 907-912.

Eva R., Pieter O., Niessen W., Freek A., Cees G. and Udo A. (2006). Analytical separation and detection methods for flavonoids. *Journal of Chromatography*, 1112, 31-63.

Fang Q. C. (2006). Research of Natural Pharmaceutical Chemistry. Pecking Union Medical College Press, Beijing, China.

Felipe T. M., Antonoi C. D., Thiago C., Kamila R. D., Marcelo H., Maria E. C. and Luiz C. A. (2008). Composition and anti-inflammatory and antioxidant activities of the volatile oil from the fruit peel of *Garcinia brasiliensis*. *Chemisty and biodiversity*, 5, 251-257.

Fengke L., Binsheng L., Zhuo C., Ping L. and Chunlin L. (2021) Ethnobotanical Study on *Garcinia* (Clusiaceae) in China. *Acta Societatis Botanicorum Poloniae*, 90, 1-15.

Filip C. and Magda C. (2003). Mass spectrometry in the structural analysis of flavonoids. *Journal of mass spectrometry*, 39, 1-15.

Gontijo V. S., Dos Santos M. H. and Viegas C. J. (2017). Biological and Chemical Aspects of Natural Biflavonoids from Plants: A Brief Review. *Mini-Reviews in Medicinal Chemistry*, 17, 834–862.

Guedje N. M., Roger F. and Bernard A. N. (2000). Le Genre *Garcinia* (Guttiferae) au Cameroun, diversité et utilisations traditionnelle, 2-14.

Guedje N. M. and Fankap R. (2001). Utilisations traditionnelles de *Garcinia lucida* et *Garcinia kola* (Clusiaceae) au Cameroun. *Nationale Plantentuin van Belgie*, 71, 747-758.

Guedje N. M. (2002). La gestion des populations d'arbres comme outil pour une exploitation durable des produits forestiers non-ligneux : l'exemple de *Garcinia lucida* (Sud Cameroun). Tropenbos-Cameroun programme, Kribi, Cameroun; 1-29.

Guedje N. M., Pieter A. Z., Heinjo D., Bernard F. and Jean L. (2007). Tree bark a non - timber forest product: The effect of bark collection on population structure and dynamics of *Garcinia lucida* Vesque. *Forest Ecology and Management*, 240, 1-12.

Guedje N. M. (2020). The genus *Garcinia* L. genetic resources diversity and utilization. *CABI Reviews*, 9, 1-25.

Harbone J. B. and Williams C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, 55, 481-504.

Hawa P., Nurdin S., Mustanir Y., Darusman D., Erlidawati E., Safrida S. and Musri M. (2020). Macrophylloflavone: A New Biflavonoid from *Garcinia macrophylla* Mart. (Clusiaceae) for antibacterial, antioxidant and anti-Type 2 Diabetes Mellitus Activities. *Research Article*, 14, 30-39.

Heller W. and Forkman G. (1988). "The flavonoids:Advances in Research since 1980", Editor Harbone J. B. Chapman and Hall, London, 399-425.

Hemshekhar M., Sunitha K., Sebastin M., Devaraja S., Kemparaju K., Vishwanath B., Niranjana S. R. and Girish K. S. (2011). An overview on genus *Garcinia:* phytochemical and therapeutical aspects. *Phytochem Review*, 10, 325–351.

Hosakatte N. M., Vijayalaxmi S. D., Dayanand D., So-Young P. and Kee Y. P. (2018). Bioactive Compounds from *Garcinia* fruits of high economic value for food and health. *Springer link*, 1–28.

Hosakatte N. M., Dayanand D., Yaser H. D. and Abdullah I. (2020). Phytochemicals and Biological Activities of *Garcinia morella* (Gaertn.) Desr.: A Review. *Molecules*, 25(23), 5690.

Hui Y., Mario F., Satoshi T., Scott B., Bei J., Margaret J., Basile I., Bernard W., Edward J. and Kennelly P. (2010). Benzophenones and Biflavonoids from *Garcinia livingstonei* Fruits. *Journal of Agricultural and Food chemistry*, 58(8), 4749–4755.

Hussain M., Zahra D., Malik A., Ejaz A., Siddiqui H. and Choudhary M. (2008). Flavonoid C-glycosides from *Abutilon pakistanicum*. *Heterocycles*, 75, 645–653.

Inder P. S., Jasmeen S., Parikshit B. and William J. F. (2009). Phloroglucinol compounds of therapeutic interest: global patent and technology status. *Expert Opinions*, 19(6), 847-866.

Irene S., Gwendoline C., Soek S. T., Siau H. M., Roghayeh A. K., Shaari D. and Vivien Y. M. (2015). A new Benzophenone from *Garcinia bethamiana. Records of Natural Products*, 10(3), 355-361.

Jackson B., Locksley H. D., Scheinmann F. and Wolstenholme W. A. (1971). Extractives from Guttiferae: The Isolation and Structure of Four Novel Biflavones from the Heartwoods of *Garcinia buchananii* Baker and *G.eugeniifolia* Waal. *Journal of the Chemical Society.Series. C.*, 3791-3804.

Jane P. A., David M. and Andale A. R. (1981). ^{13} C-N.M.R. studies of D-glucose and D-galactose monosulphates. *Carbohydrate research*, 93, 177-190.

Journal storage (JSTOR) [on line]. Available on ‘‘https://www.plants.jstor.org/’’ [Consulted on 24.08.2024 at 02 :18 am].

Juniad R. S. and Patil M. K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603-608.

Kaufman P. B., Duke J. A., Brielmann H., Boik J. and Hoyt J. E. (1997). A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: implications for human nutrition and health. *Journal of Alternative and Complementary Medicine*, 3(1), 7-12.

Karak P. (2019). Biological activities of flavonoids: an overview. *International journal of pharmaceutical sciences and research*, 10(4), 1567-1574.

Kreft S., Knapp M. and Kreft I. (1999). Extraction of rutin from buck wheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis. *Journal of Agricultural and Food Chemistry*, 47(11), 4649.

Liangqin X., Zeyuan D., Jie Z., Huanhuan D., Wei W., Banghuai X. and Xiaoru L. (2022). Comparison of Flavonoid O-glycoside, C-glycoside and their aglycones on antioxidant capacity and metabolis, during *in vitro* digestion and *in vivo*. *Foods*, 11, 882.

Lih-Geeng C., Ling-Ling Y. and Ching-Chiung W. (2007). Anti-inflammatory activity of mangostins from *Garcinia mangostana*. *Food and Chemical Toxicology*, 1-5.

Maan T. K., Kholoud F. G., Waad A. S., Aisha A. A., Gamal A. M. and Sabrin R. M. (2023). Recent advances on natural depsidones: sources, biosynthesis, structure-activity relationship, and bioactivities. *Peer J*, 11, 15394.

Mabry T. J., Markham K. R. and Thomas M. (1970). The systematic identification of flavonoids.

Magadula J. J. and Suleimani H. O. (2010). Cytotoxic and anti-HIV activities of some Tanzanian *Garcinia* species. *Tanzania Journal of Health Research*, 12(2), 2-6.

Marc S. M. and Gilles D. (2012). Contribution to the taxonomy of *Garcinia* (Clusiaceae) in Africa, including two new species from Gabon and a key to the Lower Guinean species. *Phytokeys*, 17, 41–62.

Mattias U. R., Karel D. K., Reko L. L., Petri T., Reijo S. and Reko L. (2003). Conformation of Galactose Ring Adopted in Solution and in Crystalline Form as Determined by Experimental and DFT ^{1}H NMR and Single-Crystal X-ray Analysis. *The Journal of Organic Chemistry*, 69, 18-25.

Marina L. and Robert F. (2016). Nuclear landscape of HIV-1 infection and integration. *Nature Reviews Microbiology*, 15, 69–82.

Mariana V., Sorana I. and Florentina G. (2013). Effect of pH on the fluorescence characteristics of some flavone's probes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 123, 303-308.

McGhie T. K. and Markham K. R. (1994). Separation of flavonols on electrophoretic mobility. *Phytochemical analysis*, 5, 121.

Messi A. N., Susan L. B., Owona B. A., Anke W., Kamto L. D., Ndongo J. T., Siwe-Noundou X., Poka M., Demana P. H., Rui W. M., Ngo M. J., Pegnyemb D. E. and Bochet C. G. (2022). *In vitro* and *in silico* potential inhibitory effects of new biflavonoids from *Ochna rizomatosa* on HIV-1 Integrase and *Plasmodium falciparium*. *Pharmaceutics*, 14, 1701.

Messi A. N., Tsakem B., Akongwi M., Bodede O., Moyo P., Baleba R.M.M., Urda L., Klimkait T., Noundou S. X., Ngo M. J. and Pegnyemb D. E. (2024). Rhizomatoflavonoid D and other Flavonoids from the Twigs of *Ochna rhizomatosa* as potential inhibitor of HIV-1. *Chemistry Africa*, 1-8.

Miyake Y., Shimoi K., Kumazawa S., Yamamoto K., Kinae N. and Osawa T. (2000). Identification and antioxidant activity of flavonoid metabolites in plasma and urine of eriocitrin treated rats. *Journal of Agricultural and Food* Chemistry, 48(8), 3217-3224.

Middleton E. J. (1998). Effect of plant flavonoids on immune and inflammatory cell function. *Advances in Experimental Medicine and Biology*, 439, 175-182.

Munekazu I., Shin M. and Kouduke K. (1980). ^{13}C Nuclear Magnetic Reasonance (NMR) spectral studies on polysubstituted Flavonoids. I. ^{13}C NMR spectra of flavones. *Chemical and Pharmaceutical Bulletin*, 28(3), 708-716.

Munmee D., Bishnu P. S., Giasuddin A., Cakkalur B. N. and Monjoy K. C. (2012). *In vitro* anti-oxidant and total phenolic content of *Dillenia india* and *Garcinia penducalata,* commonly used fruits in Assamese cuisine. *Basic Research Article*, 2(2), 31-35.

Murray M. T. (1998). Quercetin: Nature's Antihistamine. *Better Nutrition*, 60, 1-10.

Mohd L. B. (2005). Bioassay-guided isolation and identification of bioactive compounds from *Garcmza penangzana* leaves. Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science.

Mohd. I., Mohd K., Mehtab P. and Mohd S. K. (1994). Isoflavones from *Garcinia nervosa. Phytochemistry*, 36, 807-809.

Nazre M., Newman M. F., Pennington R. T. and Middleton D. J. (2018). Taxonomic Revision of *Garcinia* Section *Garcinia* (Clusiaceae). *Phytotaxa*, 373, 1, 001–052.

Nguyen T. T., Pham V. C., Le T. T., Nguyen T. T., Ba T. C. and Ninh T. S. (2019). Antimicrobacterial xanthones from *Garcinia mackeaniana* leaves. *Vietnam Journal of Chemistry*, 58(3), 343-348.

Nonaka G., Kawahara O. and Nishioka I. (1983). Tannins and Related Compounds. XV. A New Class of Dimeric Flavan-3-ol Gallates, Theasinensins A and B, and Proanthocyanidin Gallates from Green Tea Leaf. (1). *Chemical and Pharmaceutical Bulletin*, 31, 3906–3914.

Panche A. N., Diwan A. D. and Chandra S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, 5(47), 1-15.

Panda S. S., Chanda M., Sakhujab R. and Jain S. C. (2013). Xanthones as Potential Antioxidants. *Bentham Science Publishers*, 20, 4481-4507.

Panthong K., Pongcharoen W., Phongpaichit S. and Taylor W. C. (2006). Tetraoxygenated xanthones from the fruits of *Garcinia cowa*. *Phytochemistry*, *67*, 999-1004.

Peter G. and Raouf A. (1982). Systematic significance of xanthones, benzophenones and biflavonoids in *Garcinia*. *Biochemical Systematics and Ecology*, 10, 9-20.

Phanruethai P., Chutima K., Chanyapat S., Sakchai H., Pawinee P., Kanoknetr S., Surawat J., Radeekorn A., Jitra L., Chanita N., Palangpon K., Samran P., Thaworn J., Manat P., Patoomratana T. and Vichai R. (2018). Anti-HIV and cytotoxic biphenyls, benzophenones and xanthones from stems, leaves and twigs of *Garcinia speciosa*. *Phytochemistry*, 147, 68-79.

Plants of the World Online (POWO). Available on ‘‘ https://powo.science.kew.org/’’ [Consulted on 25.10.2024 at 03 :09 pm].

Priya C. and Hari N. (2018). A study on leaf and petiole anatomy of endemic and vulnerable species of *Garcinia*. *Journal of Emerging Technologies and Innovative Research*, 5(12), 509-511.

Regional data platform of West and Central African herbaria (riha.african-herbaria) [on line]. Available on ‘‘http://riha.african-herbaria.org/’’ [Consulted on 24.08.2024 at 10 :08 am].

Ren Y., Blanco E. J., Fuchs, J. R., Soejarto D. D., Burdette J. E., Swanson S. M. and Kinghorn A. D. (2019). Potential Anticancer Agents Characterized from Selected Tropical Plants. *Journal of Natural Products*, 82, 657–679.

Rolly G. F., Kirk C. P., Yongle D., Andriamalala R., Ana L. V., Maria B. C., Vincent E. R., Daniel T. C. and David G. I. (2018). Phloroglucinols from the Roots of *Garcinia dauphinensis* and their antiproliferative and antiplasmodial Activities. *Journal of Natural Products*, 82(3), 431–439.

Sadaquat A., Renee G., Subramaniam S., Christian B. and Claude S. (2020). Benzophenones of *Garcinia pseudoguttifera* (Clusiaceae). *Photochemistry*, 53(2), 281-284.

Saelee A., Souwalak P. and Wilawan M. (2015). A new prenylated biflavonoid from the leaves of *Garcinia dulcis*. *Formerly Natural Product Letters*, 29(20), 1884-1888.

Sarita K., Amol B., Smita D., Kavita S. and Mukta A. (2009). Flavonoids: An Overview. *Journal of Pharmacy Research*, 2(6),1148-1154.

Savannah P., Michael E., Braman N., Roan W., Hill A., Magolan J., Hofmann T., Timo S. and Onesmo B. (2023). *Garcinia buchananii* stem bark extract and its bioactive constituents manniflavanone, GB-2 and buchananiflavanone attenuate intestinal inhibitory neuromuscular transmission. *Journal of Smooth Muscle Resea*, 59, 34-57.

Schieber A., Keller P., Streker P., Klaiber I. and Carle R. (2002). Detection isorhamnetin glycosides in extracts of apples (*Malus domestica* cv. 'Brettacher') by HPLCPDA and HPLC-APCI-MS/MS. *Phytochemical analysis*, 13, 87.

Shameer P. S., Rameshkumar K. B. and Mohanan N. (2016). Diversity of *Garcinia* species in the Western Ghats: Phytochemical Perspective. *Jawaharlal Nehru Tropical Botanic Garden and Research Institute*, 2-16.

Shamsul O. N., Wan W. S., Farediah A. and Hasnah M. S. (2020). Cytotoxicity of triterpenes from the leaves of *Garcinia prainiana* King (Guttiferae). *Marmara Pharmaceutical Journal*, 21,127-131.

Shao-Xing C., Min W. and Boon-Nee L. (1996). Active Constituents against HIV-1 Protease from *Garcinia mangostana*. *Planta medica*, 62(4), 381-382.

Stewart A. J., Bozonnet S., Mullen W., Jenkins G. I., Lean M. E. and Crozier A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *Journal of Agricultural and Food Chemistry*, 48(7), 2663-2669.

Suppisak C., Chutima K., Panida S., Samran P., Palangpon K., Thaworn J., Pawinee P., Surawat J., Radeekorn A., Kanoknetr S., Jitra L., Chanita N., Narong N. and Vichai R. (2018). Polycyclic polyprenylated acylphloroglucinols and biphenyl derivatives from the roots of *Garcinia nuntasaenii* Ngerns. & Suddee. *Phytochemistry*, 146, 63-74.

Sweeny P. W. (2008). Phylogeny and floral diversity in the genus *Garcinia* (Clusiaceae) and relatives. *International Journal of Plant Science*, 169(9), 1288-1303.

Tessa C. C., Nishad M. S. and Marsden D. M. (2024). HIV Persistence, Latency, and Cure Approaches: Where Are We Now? *Molecule*, 16(7), 1163.

The World checklist of vascular plants (WCVP) [on line]. Available on ‘‘https://www.gbif.org/’’ [Consulted on 28.09.2024 at 01 :13 pm].

Thai Q. B., An T. B., Kien T. N., Vy T. N., Binh T. D. and Lien-Hoa D. N. (2016). *Tetrahedron Letters*, 57(23), 2524-2529.

Thi T., Van T., Pham V., Tra N., Tu A., Doan T., Bich N. and Marc L. (2021). A new flavonoid from the leaves of *Garcinia mckeaniana* Craib and α-glucosidase and acetylcholinesterase inhibitory activities. *Natural Product Research*, 36, 5074-5080.

Usesful tropical plants [On line]. Available on ‘‘https://tropical.theferns.info/’’ [Consulted on 25.10.2024 at 11 :10 pm].

Vatcharin R., Somsak S., Pueksa K. and Souwala P. (2005). Friedolanostanes and Lanostanes from the Leaves of *Garcinia hombroniana*. *Journal of Natural Products*, 68, 1222-1225.

Wang C., Maier U., Keil M., Zenk M., Bacher A., Rohdich F. and Eisenneich W. (2003). Phenylalanine independent biosynthesis of 1, 3, 5, 8-tetraxanthine: A retrosynthetic NMR study with soot culture of Sweita china. *European Journal of Biochemistry*, 270, 2950-2958.

Waterman P. G. and Crichton E. G. (1980). Xanthones and bioflavonoids from *Garcinia densivenia* stem bark. *Phytochemistry*, 19, 2723-2726.

Weisheng F., Zhiyou H. and Meng L. (2017). Isolation and Structure Identification of Flavonoids. InTech., London.

Weniger B., Vonthron-Sénécheau C., Kaiser M., Brun R. and Anton R. (2006). Comparative antiplasmodial, leishmanicidal and antitrypanosomal activities of several biflavonoids. *Phtyomedicine***,** 13, 176-180.

Wen-Nee T., Melati K., Keng-Chong W., Woei-Yenn T. and Darah I. (2016). Antioxidant compounds from the stem bark of *Garcinia atroviridis*. *Journal of Asian Natural Products Research*, 18, 804-811.

Wenqiang Y., Xu C., Yanli L., Shaofen G., Zhen W. and Xiuling Y. (2020). Advances in Pharmacological Activities of Terpenoid. *Natural Products Communication*, 15(3), 1-13.

West African Plants: A photo guide [on line]. Available on ‘‘http://www.westafricanplants.senckenberg.de/’’ [Consulted on 24.09.2024 at 12 :01 am].

William A. (2003). NMR Spectroscopy in the Study of Carbohydrates: Characterizing the Structural Complexity. *Wiley Periodicals*, 19(A), 1-19.

World Health Organization (WHO) [on line]. Available on ‘‘https://www.who.int/teams/global-HIV’’ [Consulted on 29.08.2024]

Xu W., Chang-Qiang K., Yi-Ping Y. and Yang Y. (2008). New Biphenyl Constituents from *Garcinia oblongifolia*. *Wiley Online Library*, 91(5), 938-943.

Xue T., Fangfang Z., Hongli T., Qingqing L., Yitong L., Zhinan M., Yu C. and Guangzhong Y. (2020). Acylphloroglucinol and tocotrienol derivatives from the fruits of *Garcinia paucinervis. Fitoterapia*, 146, 2-6.

Yapwattanaphun C., Subhadrabandhu S., Sugiura A., Yonemori K. and Utsunomiya N. (2002). Utilization of some *Garcinia* species in Thailand. *Acta Horticulturae*, 575, 563–570.

Yuh-Meei L., Herbert A., Michael T., Yeah-Huei S., Mata-Greenwood E., Thitima P., Pezzuto J., Raymond F., Stephen H. and Fa-Ching C. (1997). *In Vitro* Anti-HIV Activity of Biflavonoids Isolated from *Rhus succedanea* and *Garcinia multiflora*. *Journal of Natural Products*, 60(9).

Yun-Yun Y., Lu H., Ping W., Guo-Zhu S., Tian-Tian S. and Chang-Cai B. (2015). Advances on chemical constituents and bioactivities of genus *Stellera*. *China Journal of Chinese Medicinal Materials*, 40, 4324–4332.

Zhang L., Chun-Tang C., Jing-Jy C., Hui-Chi H., Li-Ming Y., Chia-Chin L., Kenneth F., Kuo-Hsiung L. and Yao-Haur K. (2010). Cytotoxic Polyisoprenyl Benzophenonoids from *Garcinia subelliptica***.** *Journal of Natural Products*, 73(4), 557–562.

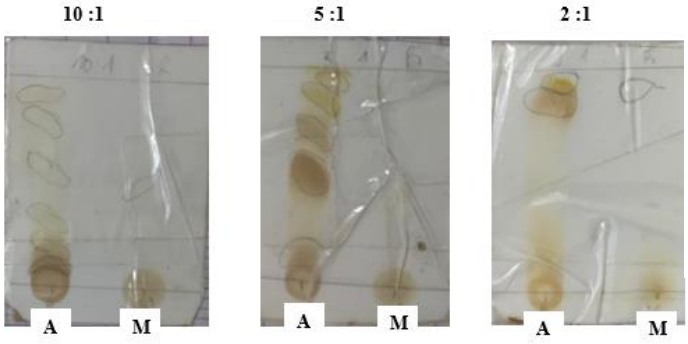
Zenish J. J., Paraag S. G. and Rani S. K. (2017). Biphenyls and their derivatives as synthetically and pharmacologically important aromatic structural moieties. *Arabian Journal of Chemistry*, 10(2), S2051-S2066.

Zixi W., Fankai S., Sanping X., Jidong W., Yijie L., Junling D., Mingxuan S. and Bainian S. (2018). A new species of *Garcinia* (Clusiaceae) from the middle Miocene of Fujian, China and a phytogeographic analysis. *Geological Journal*, 1-14.

Appendix 1 : Roots of *Garcinia gnetoides* **(Photo MEWOLO, 2023)**

Appendix 2 : Yellowish powder of the roots of *Garcinia gnetoides* **(Photo MEWOLO, 2023)**

Appendix 3 : TLC profile of Acetone and Methanol extract of *Garcinia gnetoides* **(Photo MEWOLO, 2023)**



Legend

A: Acetone extract

M: Methanol extract

Elution system: DCM/MeOH

Appendix 4 : Silica gel Chromatographic column of Acetone extract of *Garcinia gnetoides* **(Photo MEWOLO, 2023)**