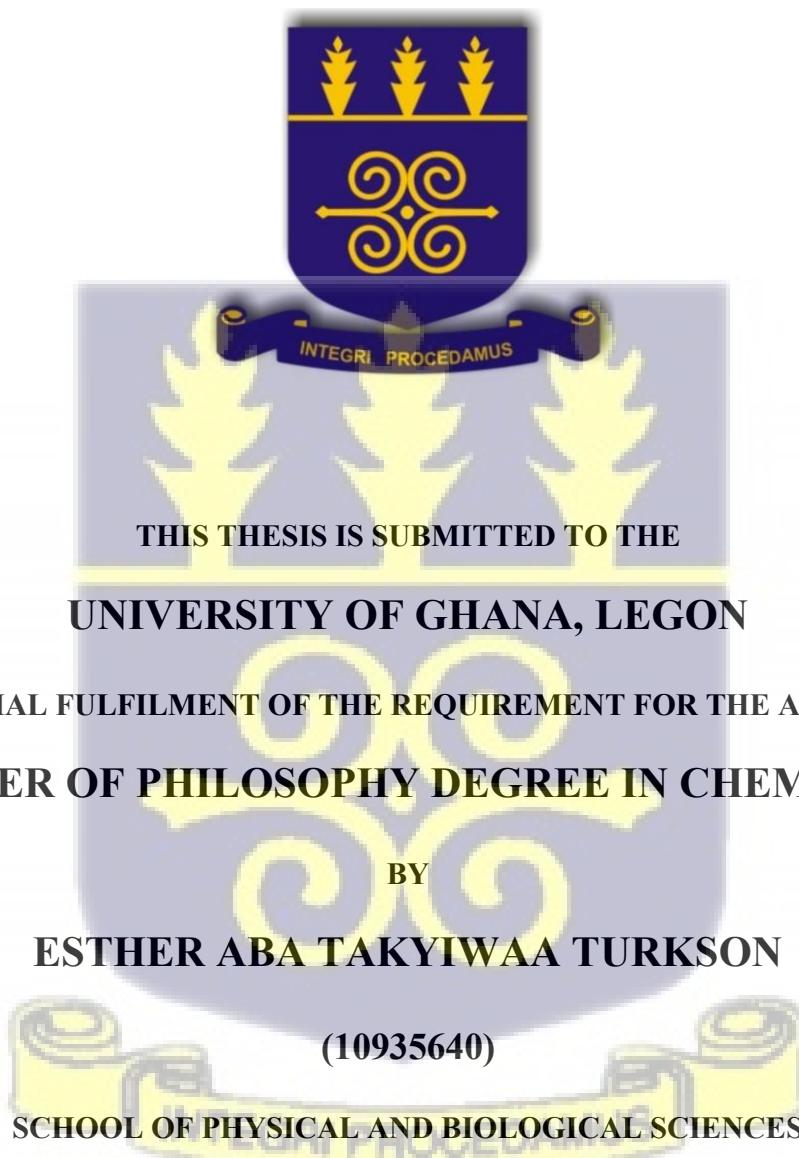


***IN VITRO* ANTITRYPANOSOMAL ACTIVITY AND MOLECULAR DOCKING
STUDIES OF THE CONSTITUENTS OF THE DICHLOROMETHANE FRACTION OF
TALBOTIELLA GENTII STEM BARK**



**THIS THESIS IS SUBMITTED TO THE
UNIVERSITY OF GHANA, LEGON**

**IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF
MASTER OF PHILOSOPHY DEGREE IN CHEMISTRY**

BY

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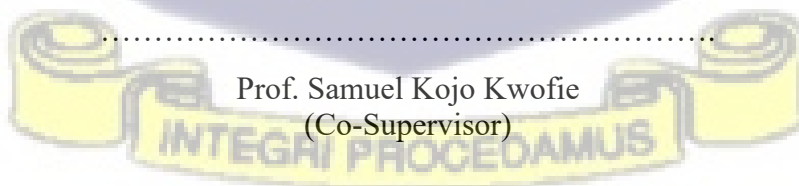
DECLARATION

I hereby declare that this thesis is the result of my own original research conducted under the supervision of Prof. Mary Anti Chama toward the award of a Master of Philosophy Degree in Chemistry at the Department of Chemistry, University of Ghana. It has not been presented for any other degree at this University or elsewhere.

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ABSTRACT

Trypanosomiasis remains a major neglected tropical disease, with existing therapies limited by toxicity, drug resistance, and poor accessibility in endemic regions, emphasizing the need for safer chemotherapeutic agents from natural sources. This study investigated *Talbotiella gentii* Hutch. & Greenway (Fabaceae-Detarioideae), a critically endangered Ghanaian plant traditionally used to manage stomach disorders, to determine whether it contains phytoconstituents with antitrypanosomal potential. The research was guided by the hypothesis that some of its bioactive compounds possess antiparasitic properties capable of inhibiting key *Trypanosoma brucei* enzymes. The dichloromethane (DCM) stem bark extract was subjected to chromatographic separation and spectroscopic analysis, yielding three known compounds, friedelan-3-one (friedelin), lup-20(29)-en-3 β -ol (lupeol), and a sterol mixture containing stigmast-5-en-3 β -ol (β -sitosterol) and (22E)-stigmasta-5,22-dien-3 β -ol (stigmasterol), as well as a semi-synthetic derivative, lup-20(29)-en-3 β -yl acetate (lupeol acetate). *In vitro* assays on the isolates against *Trypanosoma brucei brucei* using the Alamar Blue method revealed that lupeol acetate exhibited the highest potency (IC_{50} = 2.9 μ g/mL), followed by lupeol (IC_{50} = 3.5 μ g/mL), the sterol mixture (IC_{50} = 13.9 μ g/mL), and friedelin (IC_{50} = 45.5 μ g/mL), relative to diminazene aceturate (IC_{50} = 0.05 μ g/mL). The docking results showed that all compounds demonstrated significant binding to *Tb*DHFR, with binding energies ranging from -10.8 to -8.7 kcal/mol, surpassing those of reference inhibitors cycloguanil and pyrimethamine (-8.0 and -7.2 kcal/mol, respectively). Additionally, all compounds displayed strong binding affinities for *Tb*PTR1, with binding scores ranging from -11.2 to -9.0 kcal/mol, which were superior to the standard inhibitor. These results suggest that the compounds may inhibit both enzymes, thereby contributing to their observed antitrypanosomal activity by potential mechanisms of action through DHFR and PTR1 inhibition. These findings

provide a scientific basis for the traditional use of *T. gentii* and constitute the first report on its phytochemical and antitrypanosomal profile, highlighting lupeol and lupeol acetate as a promising lead scaffolds. The results enhance understanding of triterpenoid and sterol structure–activity relationships and underscore the potential of Fabaceae-derived metabolites as templates for developing safer and affordable antitrypanosomal drugs.

Keywords: *Talbotiella gentii*, Phytochemical analysis, Triterpenoids, Steroidal compounds, Molecular docking, Dihydrofolate Reductase (*Tb*-DHFR), Pteridine Reductase 1 (*Tb*-PTR1), *In vitro* Antitrypanosomal activity



DEDICATION

This thesis is dedicated to God Almighty for His guidance, to my supervisor, Prof. Mary Anti Chama, for her unwavering support, and to my beloved parents, Mr Israel Ekow Turkson and Mad. Salome Obeng Badu, for their love and encouragement.



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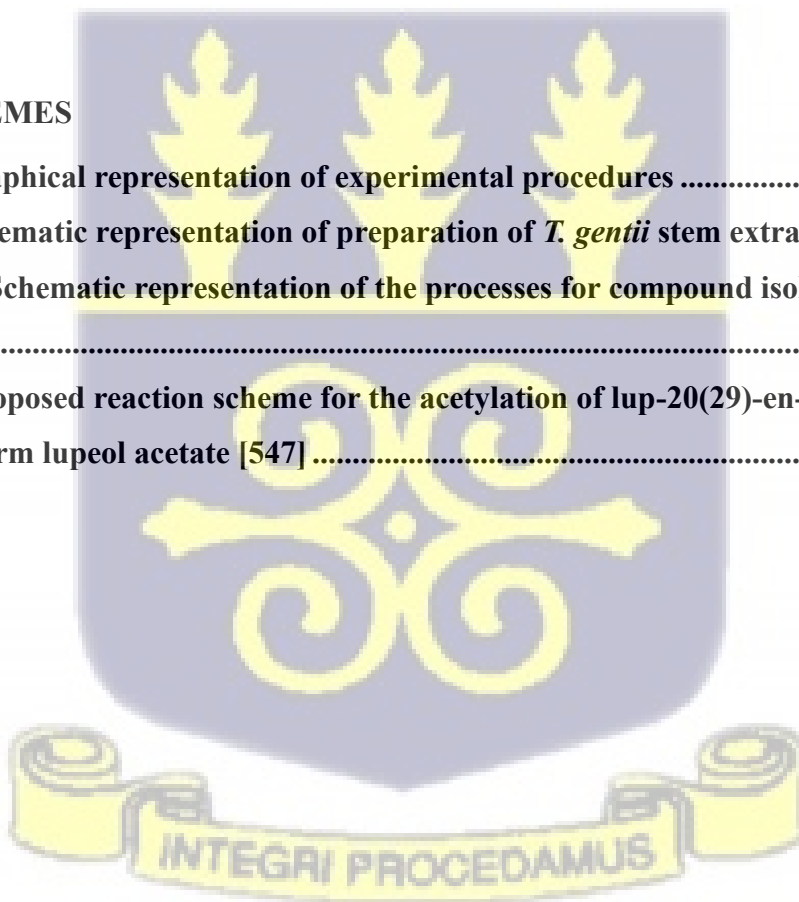
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CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Throughout history, nature has been humanity's primary source of medicine, with plants providing 80% of treatments even before the advent of synthetic drugs in the early 1900s, highlighting a significant reliance on plant-based remedies¹. Natural products remain vital in pharmaceutical research, playing a key role in chemotaxonomic and phytochemical studies that drive drug discovery¹. Notably, they account for around 60% of anticancer drugs and 75% of treatments for infectious diseases, either in their natural form or as derivatives². Over 90% of modern therapeutics originate from natural product prototypes, and approximately 66% to 75% of the global population still rely on medicinal plants as their primary healthcare source³. While the focus in developed countries has fluctuated over time, the reliance on medicinal plants continues to drive research in this field. Notably, about 25% of all prescribed medications still come from higher plants⁴. Medicinal plants can be administered as isolated bioactive compounds or formulated into preparations such as infusions, essential oils, tinctures, and extracts, depending on the plant's properties. Isolated compounds are selected for their potent, targeted effects, while extracts and tinctures are preferred for plants with milder actions or when their active ingredients have not yet been fully characterized⁵. In countries such as Germany, France, and Switzerland, where herbal medicine is deeply rooted and regulated, these preparations are widely used⁵. Globally, medicinal plants have been the foundation for some of the most significant therapeutic discoveries. For instance, *Serenoa repens* (Saw palmetto) was evaluated for its efficacy in treating lower urinary tract symptoms associated with benign prostatic hyperplasia^{6,7}. Several well-known modern plant-derived drugs such as vincristine from *Catharanthus roseus*, pilocarpine from

Pilocarpus jaborandi, and digoxin from *Digitalis lanata* remain clinically significant, treating cancers, glaucoma, and cardiovascular disorders, respectively^{8,9}. In Ghana, the use of plants in traditional medicine also remains extensive, with a wealth of ethnobotanical knowledge recorded over time. Plants such as, *Dichapetalum madagascariense* (Dichapetalaceae), known as *Ekumnkura* in Fante and *Kumnkura* in Twi, treats whitlow¹⁰. Numerous plant species are used across Ghana for ailments ranging from headaches to infections, forming a rich foundation for ethnopharmacological studies¹⁰. Given the urgent need for new therapies, especially for parasitic diseases, the ethnomedicinal use of plants is increasingly seen as a promising avenue for drug discovery. The Fabaceae family, in particular, is recognized for its pharmacological potential. Specifically, the bark of *Acacia catechu*, *Bauhinia variegata*, *Albizia lebbek*, and *Acacia nilotica*, has been utilized to alleviate symptoms of diarrhoea, ulcers, and inflammation¹¹.

Within the Fabaceae family, one plant with notable potential is *Talbotiella gentii* Hutch. & Greenway, a critically endangered and endemic species in Ghana¹². Despite its use in traditional medicine to treat stomach ulcers¹³, this plant has not yet been extensively studied for its phytochemical properties. Given its ethnomedicinal significance and the global need for more effective treatments against parasitic infections, *Talbotiella gentii* holds promise for discovering anti-parasitic or antimicrobial compounds worthy of further investigation.

Trypanosomiasis remains a significant health and economic burden in endemic regions, particularly sub-Saharan Africa. Globally, over 65 million people are at risk, mainly across 36 African countries, though human cases have declined due to sustained control efforts¹⁴. However, Animal African Trypanosomiasis (AAT) continues to cause over US\$4.5 billion in livestock-related losses annually, affecting both agricultural productivity and food security¹⁵. Africa bears over 95% of the global burden of trypanosomiasis, with the Democratic Republic of Congo alone

accounting for over 70% of the total global human cases¹⁶. In West Africa, Ghana was declared free from Human African Trypanosomiasis transmission in humans as of 2020, with the WHO confirming fewer than 4 cases between 2018 and 2020¹⁷. However, animal trypanosomiasis remains endemic in the northern and forest-savannah zones, thereby sustaining a zoonotic reservoir and the risk of human spillover where veterinary care is limited¹⁸. Current treatments are chemotherapeutic, and until recently were administered only intravenously, with drugs like eflornithine, which requires a 14-day hospitalization for intravenous infusion by trained medical personnel¹⁹. However, there is now an oral formulation, fexinidazole, introduced into clinical use in 2019, which has transformed treatment by allowing outpatient care without the need for intravenous access²⁰. The oral route is preferable because it is less complicated and does not require trained medical personnel for administration²¹. These chemotherapeutic treatments are limited by several challenges: resistance to drugs like diminazene aceturate and isometamidium has been widely reported²², high toxicity of medications such as melarsoprol can cause fatal encephalopathy²³, cost and poor accessibility in rural communities²⁴, lack of field-adapted therapies²⁵, and absence of effective vaccines due to antigenic variation of the parasite²⁶. These limitations highlight the urgent need for alternative therapies.

Due to the limitations of existing treatments, attention has turned toward natural product drug discovery. Medicinal plants offer a rich source of bioactive compounds with potential anti-trypanosomal activity²⁷. Plant-derived compounds stand out in drug discovery because they're naturally bioavailable, often safer than synthetic drugs, and offer unique structures with the ability to act on multiple targets, making them both effective and sustainable options²⁸⁻³⁰. In response to these challenges, natural products have gained renewed attention as promising sources of novel antitrypanosomal scaffolds^{31,32}. Several recent studies have reported plant-derived compounds

showing *in vitro* and *in vivo* efficacy against *T. brucei* and *T. congolense*³³. This contextual foundation underlines the scientific rationale for investigating *Talbotiella gentii*, an unstudied Ghanaian Fabaceae species with recognized ethnomedicinal relevance, as a potential source of novel antitrypanosomal agents contributing to the search for safer, affordable, and effective therapeutic alternatives.

1.2 General Description of Plant

Talbotiella gentii, an evergreen leguminous tree and one of the few endemic forest trees in Ghana, is classified as Critically Endangered according to International Union for Conservation of Nature (IUCN) criteria¹². It can grow up to 18 meters tall, with a diameter of 25 to 60 cm at breast height (DBH) with several straight and steeply rising branches supporting a thin crown³⁴. The species' bark is dark brown, longitudinally fissured, and varies between smooth and rough textures. Its stems are covered with tiny hairs, and its buds are neatly arranged with protective scales. The leaves are composed of rhomboid-shaped leaflets, typically arranged in 5-7 pairs, with the lower surface of each leaflet containing small hairs and glands³⁴. The leaves begin as red and gradually turn green as they mature. The lower leaf surface bears small hairs and glands, with a distinct diagonal midvein. The tree produces small, fragrant flowers arranged in racemes, usually containing 6-12 blossoms with a red axis, 21-38 mm long, a 2-13 mm peduncle, and hairy floral bracts that are caducous, narrowly oblong, glabrous, and ciliate. The partially open, petaloid sepals of *T. gentii* are white or pink, fragrant, concave, and oval-shaped, with a few long hairs on their inner surfaces that surround the nectar³⁴. The hypanthium, measuring 2.5–3 mm deep, is sparsely hairy on the outside and smooth on the inside. The flowers typically have no petals, and the sepals are white to pink in colour. Each flower contains 10 glabrous stamens and a densely hairy ovary,

topped with a style that is 6–8 mm long. The fruit pod (Figure 1-1) is flat and triangular, ranging in size from 3.5 to 5.5 cm in length, and it typically contains one to three seeds³⁴.



A



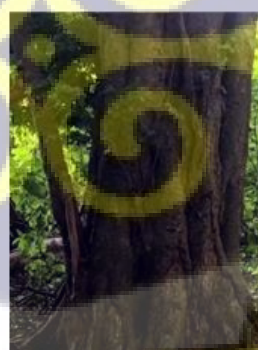
B

Figure 0-1 The flowers and leaves (A); and fruit pods (B) of *T. gentii*¹³

The matured seeds are elliptical, dark brown, and measure between 14 and 17 mm in length. Seed dispersal is explosive³⁵. *T. gentii* produces prolific seeds annually from October to December with abundant germination occurring beneath the parent trees' canopy³⁵. Young trees can grow in shaded areas, as well as gaps. *T. gentii* can also regenerate abundantly through new shoots arising from fallen adult trees, thus ensuring a diverse range of tree sizes.

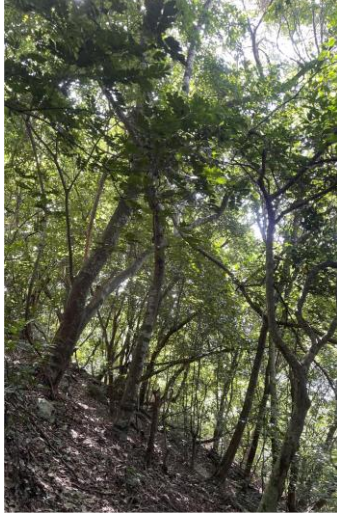


C



D

Figure 0-2 *T. gentii* seeds (C) and tree trunk (D) [adapted from <https://youtu.be/stE-Rq6cZtw>]

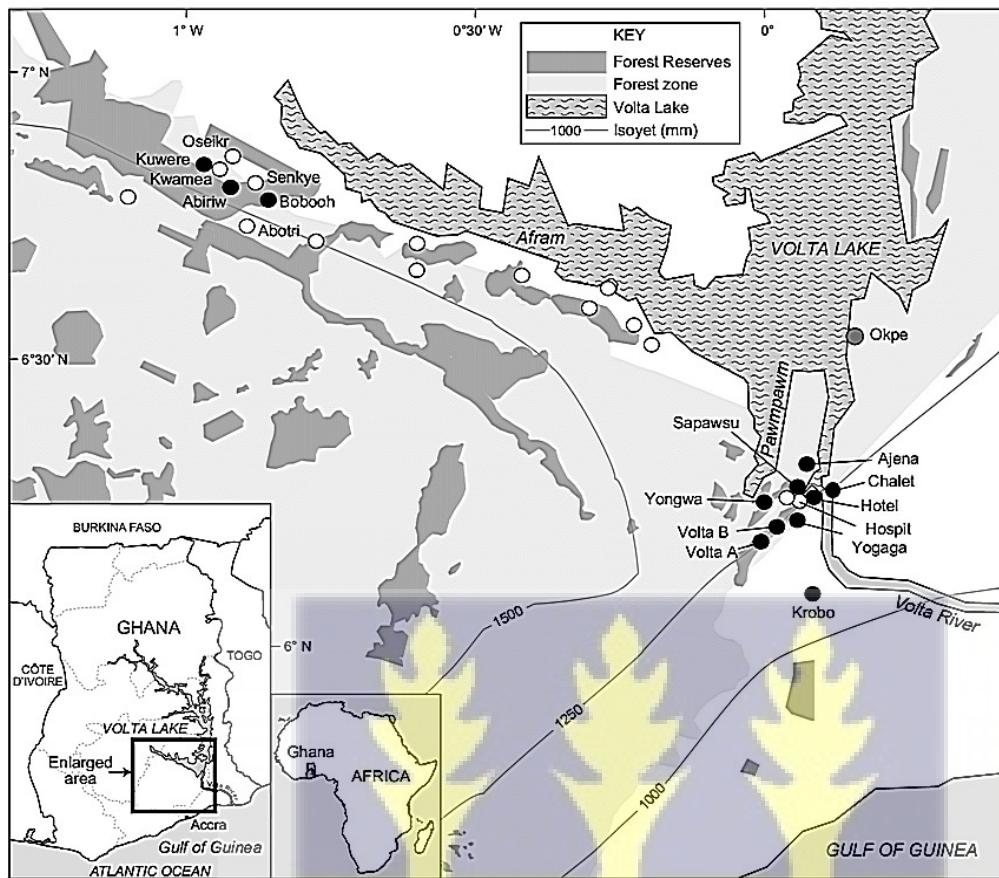


E

Figure 0-3 A colony of *T. gentii* tree (E)³⁶

1.3 Geographical Location and Distribution of *Talbotiella gentii*

Talbotiella gentii Hutch. & Greenway, endemic to Ghana has no known occurrences outside of this region. This species prospers in arid semi-deciduous woodlands, particularly on rocky landscapes. It frequently develops in isolation or forms a stretch between savannas and deciduous rainforests. It is typically situated at elevations of 100 m to 350 m^{18,22}. *T. gentii* is found in rocky and seasonally dry forest areas within Ghana's Eastern and Ashanti regions, particularly in the Afram and Volta basins. It is primarily found in significant biodiversity areas like the Bandai Hills, Sapawusu, and Yongwa forest reserves, which are home to rare plant species^{13,37,38}. Additionally, this species is documented in several towns including Kwahu, Abetifi, Huhunya, Mpraeso, Agogo, and Akatsi. Specimens have been collected from various locations such as Krobo Mt, Akosombo, and Afram Mankrong Forest Reserve, spanning the period from 1908 to 1994^{34,38-40}. These towns mentioned and other documented towns are represented in Figure 1-3, the southeastern map of Ghana⁴⁰.



Legends: ● = surviving populations ○ = extinct populations

Map 0-1: Map of the Distribution of *T. gentii* in Ghana³⁸.

1.4 Taxonomy of *Talbotiella gentii*

The scientific classification of *T. gentii* are^{41–43};

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Fabales

Family: Fabaceae (Leguminosae)

Subfamily: Detarioideae

Tribe: Amherstieae

Genus: *Talbotiella* Baker f.

Species: *Talbotiella gentii* Hutch. & Greenway

1.4.1 Synonyms and Local Names

There are no synonyms recorded for this name. However, there are a few documented local names.

In Ghana, the plant is called *gbletfo* or *kpeteple* by the Adangme^{39,44}, and *Takurowanua* by the Ashantis^{39,44,45}.

1.5 Local Uses of *T. gentii*

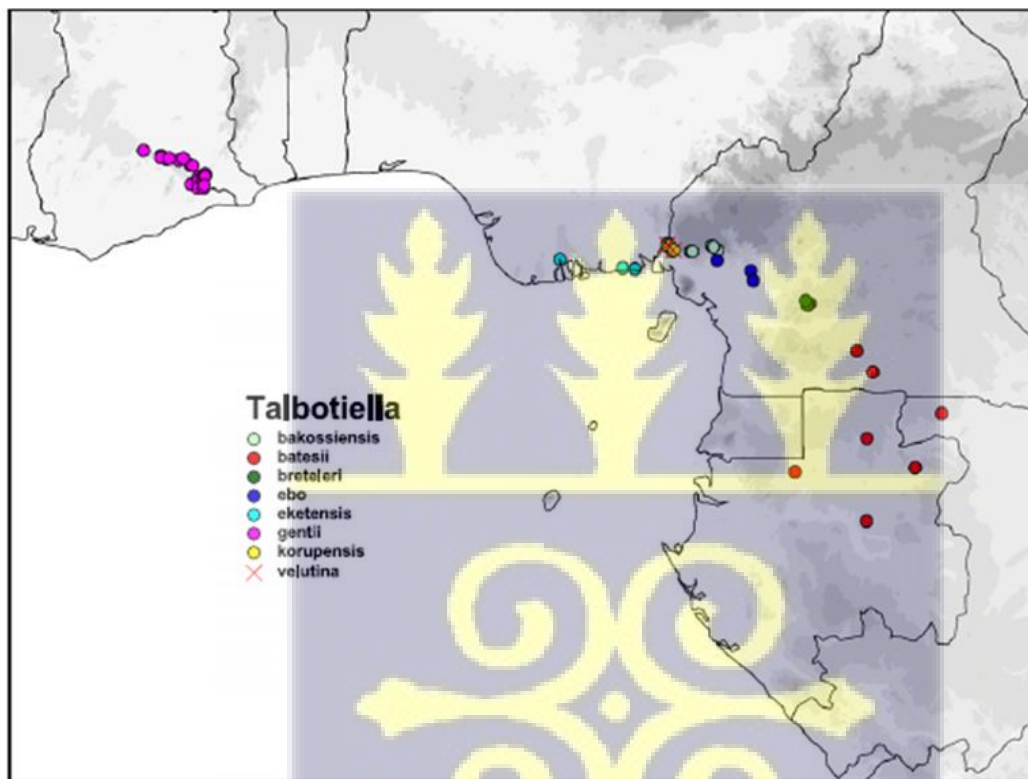
Traditionally, the stem of *T. gentii* is used for treating stomach ulcers³⁷ while its roots are utilized for cancer treatment⁴⁵.

The population of *T. gentii* is declining primarily due to habitat destruction. Its dense wood, prized for charcoal production, has led to its local name, 'Takorowanua' which translates to 'charcoal tree.' In addition, the wood is also used in constructing pillars and bridges, as well as in crafting furniture³⁷.

1.6 Other Species of The Genus *Talbotiella* and Their Geographical Locations

There are a total of nine species of *Talbotiella*⁴⁶. The eight species of *Talbotiella* that had been identified before 2010 are *T. gentii* Hutch. & Greenway, *T. bakossiensis* Cheek, *T. batesii* Baker f., *T. breteleri* (Aubrév.) Mackinder & Wieringa, *T. ebo* Mackinder & Wieringa, *T. eketensis* Baker f., *T. korupensis* Mackinder & Wieringa and *T. velutina* Burgt & Wieringa³⁴. A new species of *Talbotiella*, *T. cheekii*, was recently discovered in Guinea in 2017⁴⁷.

T. gentii is the only endemic to Ghana (Upper Guinea), while seven species belong to the Lower Guinea region. *T. eketensis* is exclusively found in the southeastern part of Nigeria. *T. batesii* inhabits southern Cameroon, Gabon, and Congo Brazzaville, and five species are unique to Cameroon. Among the recently discovered Cameroonian species, *T. korupensis* and *T. velutina* are uniquely situated in the Caesalpinioideae-abundant forest of south Korup, in the southwest region of Cameroon³⁴¹⁸. This geographical distribution is represented in Map 1-2 below:



Map 0-2: Map of the Distribution of *Talbotiella* species in West Africa and Central Africa (Adapted from³⁴)

1.7 Problem Statement, Hypothesis, and Justification

Trypanosomiasis remains a major neglected tropical disease, with existing chemotherapeutic options constrained by toxicity, drug resistance, and poor accessibility to these drugs⁴⁸. These limitations highlight the urgent need for safer, affordable, and more effective therapeutic alternatives, particularly from natural products. In tropical and subtropical regions, traditional medicine relies extensively on plant-based remedies for the treatment of parasitic diseases such as trypanosomiasis, and several of these species have exhibited promising activity against *Trypanosoma* spp.^{48–51}. Although medicinal plants have long served as sources of bioactive compounds for drug discovery², most species remain chemically unexamined⁵², leaving a vast reservoir of potentially bioactive metabolites unexplored⁵³. Indeed, only about 10% of higher plants have been investigated for their potential medicinal value⁵⁴, underscoring the need for continued natural product research.

Talbotiella gentii (Fabaceae) is an ethnomedicinal plant in Ghana, traditionally used for the treatment of cancer⁴⁵, and stomach ulcer³⁷, the latter is often associated with *Helicobacter pylori* infection⁵⁵. Human African trypanosomiasis is known to lead to intestinal damage^{56,57,58}. Moreover, one of the traditional uses of *T. gentii* is in the treatment of stomach ulcers caused by *Helicobacter pylori*, which leads to gastric ulceration⁵⁹. This association suggests that the plant may also be effective in managing intestinal ulceration, including the intestinal damage observed in trypanosomiasis. For this reason, the isolated compounds are to be tested against *Trypanosoma* species. Additionally, despite its ethnomedicinal relevance, no documented research exists on the chemical constituents, and only one pharmacological study on the cancer activity of the extracts has been documented⁶⁰. The plant, therefore, needs to be investigated for its bioactive compounds.

The ethnomedicinal use and unexplored chemistry of *T. gentii*, therefore, justify its scientific investigation. Its traditional use in treating stomach ulcers suggests the presence of bioactive compounds with potential antiparasitic activity. There are some instances where plants traditionally used in treating a particular ailment were found to be active against other ailments. For example, *Tephrosia vogelii* was used traditionally as a fish poison⁶¹ without any parasitic use. Scientific studies, however, indicated that the extracts are active against Leishmania parasites⁶². Similarly, extracts of *Piliostigma thonningii*, which is used traditionally for treating stomach-ache, coughs and snakebites⁶³, have been reported to exhibit antitrypanosomal activity⁶⁴. Likewise, *Azadirachta indica* (Neem), traditionally used for treating skin infections and fever⁶⁵, was later found to possess antiplasmodial and antitrypanosomal activity⁶⁶. Moreover, *Anthonotha macrophylla*, a related species within the same tribe (Amherstieae) as *T. gentii*, traditionally used to treat intestinal-related discomfort and sexually transmitted diseases⁶⁷, has shown promising antitrypanosomal activity with an IC₅₀ value of $13.6 \pm 0.21 \mu\text{g/mL}$ ⁶⁸. These findings support the rationale that plants not traditionally used for treating parasitic infections may harbour metabolites with diverse activities, including antiparasitic potential. Therefore, *T. gentii*, which is not traditionally used for treating parasitic infections, may also show parasitic activity against trypanosomiasis.

The study is therefore guided by the hypothesis that *T. gentii* contains diverse phytoconstituents, some of which may possess antiparasitic properties, including potential antitrypanosomal agents. To test this hypothesis, *in vitro* assays will be used to determine the antitrypanosomal activity of extracts and isolates from the stem bark of *T. gentii*. Although molecular docking studies have identified inhibitors of *T. brucei* enzymes such as dihydrofolate reductase (*TbDHFR*) and pteridine reductase 1 (*TbPTR1*), most investigations have centred on synthetic compounds or a limited

number of known phytochemicals^{69,70}. The molecular targets and binding interactions of understudied natural products, particularly those derived from *T. gentii*, remain unexplored, and only a few existing antitrypanosomal studies have integrated *in vitro* bioassays with *in silico* docking to elucidate mechanisms of action^{71–73}. This study, therefore, seeks to bridge this knowledge gap by evaluating the *in vitro* antitrypanosomal activity of the constituents of the dichloromethane fraction of *T. gentii* stem bark, complemented by molecular docking studies to predict potential protein targets, assess binding affinities, and elucidate possible modes of action. Since natural products have long served as a foundation for antiparasitic drug discovery⁷⁴, this integrated approach will provide valuable insights into its potential as a novel source of antitrypanosomal compounds and lay the groundwork for further pharmacological development.

The outcomes of this study are therefore expected to contribute to drug discovery research by identifying lead compounds for the treatment of trypanosomiasis caused by the *T. b. brucei*, and to provide baseline data for further phytochemical and pharmacological studies. Beyond its scientific contribution, the findings will also benefit livestock farmers, as trypanosomiasis, a zoonotic disease, affects cattle and other domestic animals, causing productivity and economic losses⁷⁵. Improving treatment options could enhance animal health and productivity, thereby supporting rural livelihoods and strengthening food security. This study, therefore, seeks to address this knowledge gap by evaluating the *in vitro* antitrypanosomal activity of the constituents of the dichloromethane fraction of *T. gentii* stem bark, alongside molecular docking studies to predict possible protein targets and assess binding affinities and to suggest the mode of action. Given *T. gentii*'s traditional relevance in treating similar conditions, its evaluation for antitrypanosomal activity is scientifically warranted.

1.8 Overall Aim

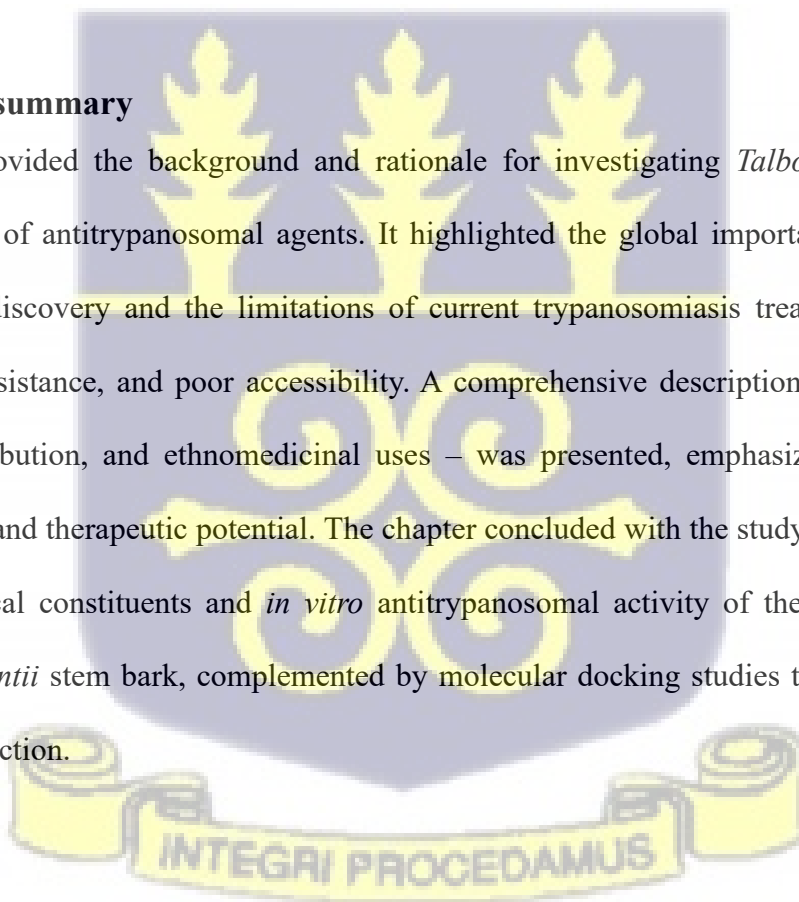
This current project seeks to evaluate the antitrypanosomal constituents of the dichloromethane (DCM) extract of the stem bark of *Talbotiella gentii* through *in vitro* and *in silico* studies.

1.9 Main Objectives of the Project

- Evaluate the phytochemical constituents of the stem bark of *Talbotiella gentii*.
- Assess the antitrypanosomal activity of the isolates of the stem bark of *Talbotiella gentii*
- Computationally assess the mechanism of binding of the identified compounds from the plant.

1.10 Chapter summary

This chapter provided the background and rationale for investigating *Talbotiella gentii* as a potential source of antitrypanosomal agents. It highlighted the global importance of medicinal plants in drug discovery and the limitations of current trypanosomiasis treatments, including toxicity, drug resistance, and poor accessibility. A comprehensive description of *T. gentii* – its taxonomy, distribution, and ethnomedicinal uses – was presented, emphasizing its unstudied phytochemistry and therapeutic potential. The chapter concluded with the study's aim to evaluate the phytochemical constituents and *in vitro* antitrypanosomal activity of the dichloromethane fraction of *T. gentii* stem bark, complemented by molecular docking studies to predict possible mechanisms of action.



2.1 Summary of Chapter

This chapter reviewed the literature on human African trypanosomiasis (HAT), highlighting its transmission, disease stages, and the major challenges associated with current chemotherapeutic options. Despite progress in control measures, treatment remains constrained by drug toxicity, resistance, and limited accessibility, underscoring the urgent need for safer and more affordable therapeutic alternatives from natural sources.

Since this study focuses on *Talbotiella gentii*, a plant species that has not yet been explored for its phytochemical or pharmacological properties, the literature review extended to related genera within the Amherstieae tribe of the Fabaceae family to infer potential chemical and biological profiles. Species within these genera are known to contain triterpenoids, sterols, flavonoids, and phenolic compounds that exhibit diverse biological activities, including antimicrobial and antiparasitic effects. The absence of studies on *Talbotiella*, therefore, represents a significant chemotaxonomic and pharmacological gap that this research seeks to address. Understanding the chemical diversity within the Amherstieae tribe is therefore essential for identifying new natural scaffolds with potential antitrypanosomal activity.

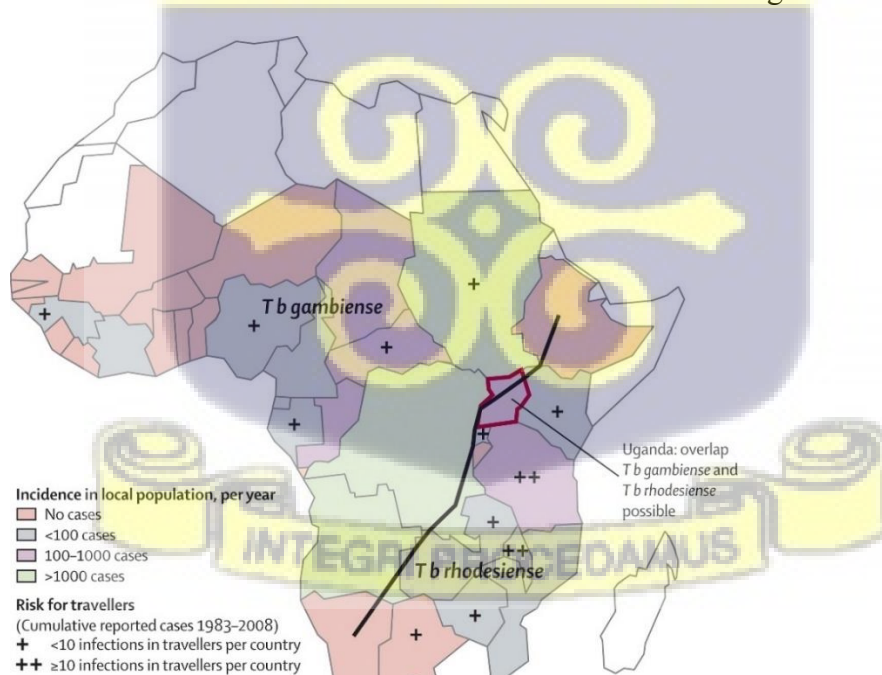
Building on the phytochemical insights above, this chapter also examined, this chapter also examined relevant *in vitro* and *in silico* studies on *Trypanosoma brucei* enzymes, particularly pteridine reductase 1 (*TbPTR1*) and dihydrofolate reductase (*TbDHFR*), which are validated molecular targets for drug discovery. These insights provide a theoretical foundation for integrating phytochemical isolation, biological screening, and molecular docking in the present study.

2.2 Trypanosomiasis

Trypanosomiasis is a tropical disease caused by protozoa of the genus *Trypanosoma*. It has two primary forms: African trypanosomiasis, caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, and Chagas disease, caused by *Trypanosoma cruzi*^{76,77}. Chagas disease, or American trypanosomiasis, affects around 6 to 7 million people, mainly in 21 Latin American countries. It is transmitted by kissing bugs through their faeces or urine⁷⁸. *Trypanosoma evansi* (which causes surra) and *Trypanosoma brucei brucei* affect animals in Asia, Africa, and South America⁷⁹.

2.3 The Human African Trypanosomiasis

Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is a vector-borne disease caused by *Trypanosoma* protozoans. It is transmitted to humans *via* tsetse fly bites after the flies acquire the parasites from infected hosts^{80,81}. It is predominantly found in sub-Saharan Africa where the disease affects rural communities involved in agriculture, fishing, animal



Map 2-1: A map showing the endemic regions of *T. b. gambiense* and *T. b. rhodesiense*

husbandry, or hunting. Its distribution is focal, varying from single villages to larger regions, with incidence differing across areas^{81–83}. HAT has two forms: *Trypanosoma brucei rhodesiense* (8% of cases) causes rapid central nervous system invasion and can be fatal within weeks, while *Trypanosoma brucei gambiense* (92% of cases) progresses more slowly, affecting the central nervous system over several years⁸⁴.

2.3.1 Mechanism of cellular invasion by Trypanosoma parasite

The entire life cycle of the African trypanosome happens outside the cells of the host. The fly becomes infected with bloodstream promastigotes and transforms into the procyclic trypanosome parasites in the midgut of the fly. These parasites then leave and transform into epimastigotes. These epimastigotes then multiply and transform into metacyclic trypomastigotes in the salivary glands of the fly. During a blood meal on a human, these infected tsetse flies introduce metacyclic

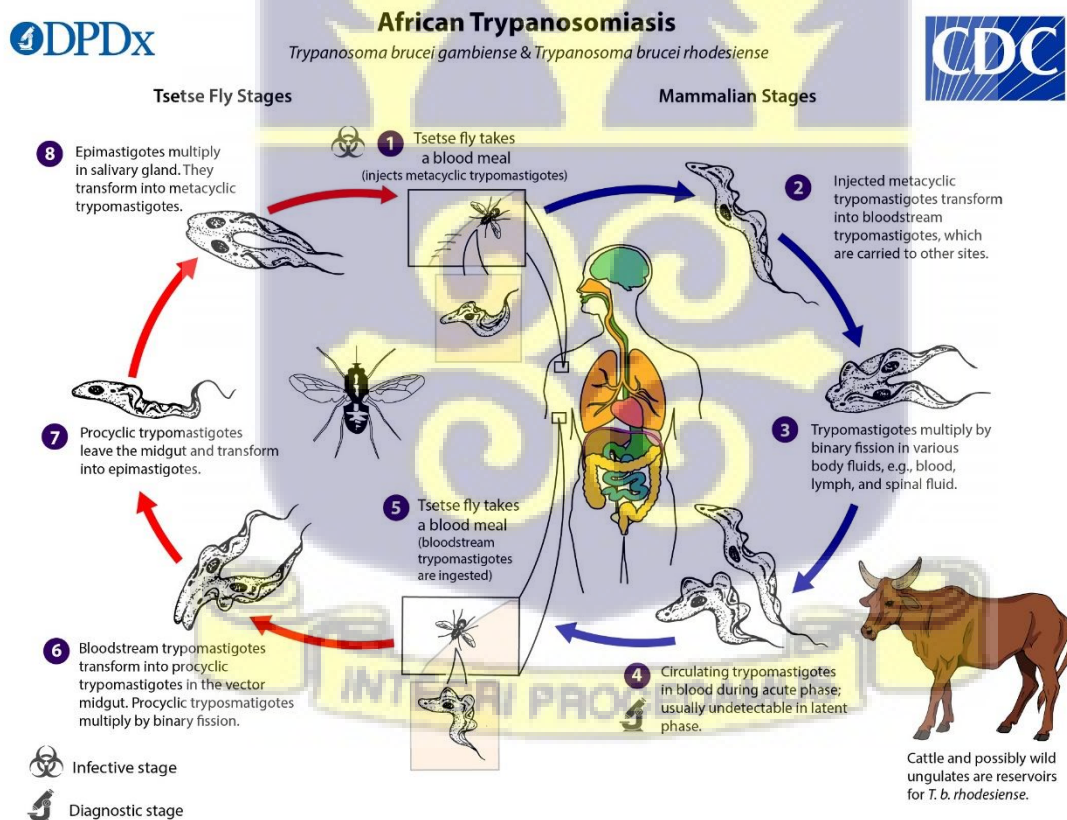


Figure 2-1 The life cycle and stages of *T. b. gambiense* and *T. b. rhodesiense* adapted from CDC (Adapted from the CDC)

trypomastigotes into the skin tissue of the host. These parasites move into the lymphatic system, then they progress to the bloodstream, where they transmogrify to bloodstream trypomastigotes. They then replicate throughout the lymph and spinal fluid^{80,85}.

2.3.2 The Stages of HAT and their symptoms

African trypanosomiasis progresses from a cutaneous stage to the hemolymphatic phase, and finally to the central nervous system (CNS). After a tsetse fly bite, parasites cause inflammation and a painful chancre that usually heals in 3-4 weeks, though *Trypanosoma brucei gambiense* often lacks a chancre. The initial phase includes symptoms like fever, headaches, and peripheral swelling, with possible pulmonary and cardiac complications. Winterbottom's sign, swollen neck lymph nodes, is a key diagnostic feature^{86,87}.

In the following stage, parasites invade the brain and spinal cord. This occurs within a month for *T. b. rhodesiense* but may take years for *T. b. gambiense*. The CNS invasion leads to headaches, sleep disturbances, cognitive decline, and neurological issues such as tremors, ataxia, and psychoses, along with weight loss and endocrine problems^{25,86-89}.

2.3.3 Treatments for the Different Stages of HAT Infection

Without treatment, HAT is generally fatal. Over the past two decades, ongoing control measures have resulted in a remarkable 97% decrease in the number of new cases⁹⁰. Diagnosing and treating HAT is intricate and demands specialized skills. Treatment follows standard protocols, with the choice of drug therapy depending on the parasite subspecies and infection stage^{25,91-95}. Due to the cost and availability of these drugs, many locals turn to phytotherapy.

+

Table 2-1 Table containing the drugs used to treat HAT during different stages of infection

	Stage	Mode of Application	Dosage per body weight (mg/kg)	Adverse effect
<i>Trypanosoma brucei gambiense</i>				
Pentamidine	First	Intramuscular	4 mg/kg once daily for a week	Hypoglycaemia, diarrhoea, injection site pain, nausea, vomiting
Eflornithine	Second	Intravenous Infusion of >30 min	100 mg/kg every 6 h for 2 weeks	Thrombocytopenia, anaemia, diarrhoea, nausea, vomiting, convulsions; leucopenia
Melarsoprol	Second	Intravenous	2.2 mg/kg once daily for 10 days	Encephalopathic syndromes, pruritus, maculopapular eruptions, thrombophlebitis, peripheral motoric or sensorial neuropathies
<i>Trypanosoma brucei rhodesiense</i>				
Suramin	First	Intravenous	Test dose of 4–5 mg/kg on day 1, then five injections of 20 mg/kg every 7 days maximum dose per injection 1 g	Hypersensitivity reactions (acute, late); albuminuria, cylinduria, haematuria, peripheral neuropathy
Melarsoprol	Second	Intravenous	Three series of 3.6, 3.6, 3.6 mg/kg bodyweight, the series spaced by intervals of 7 days: maximum dose per day 180 mg	Encephalopathic syndromes, pruritus, maculopapular eruptions, thrombophlebitis, peripheral motoric or sensorial neuropathies

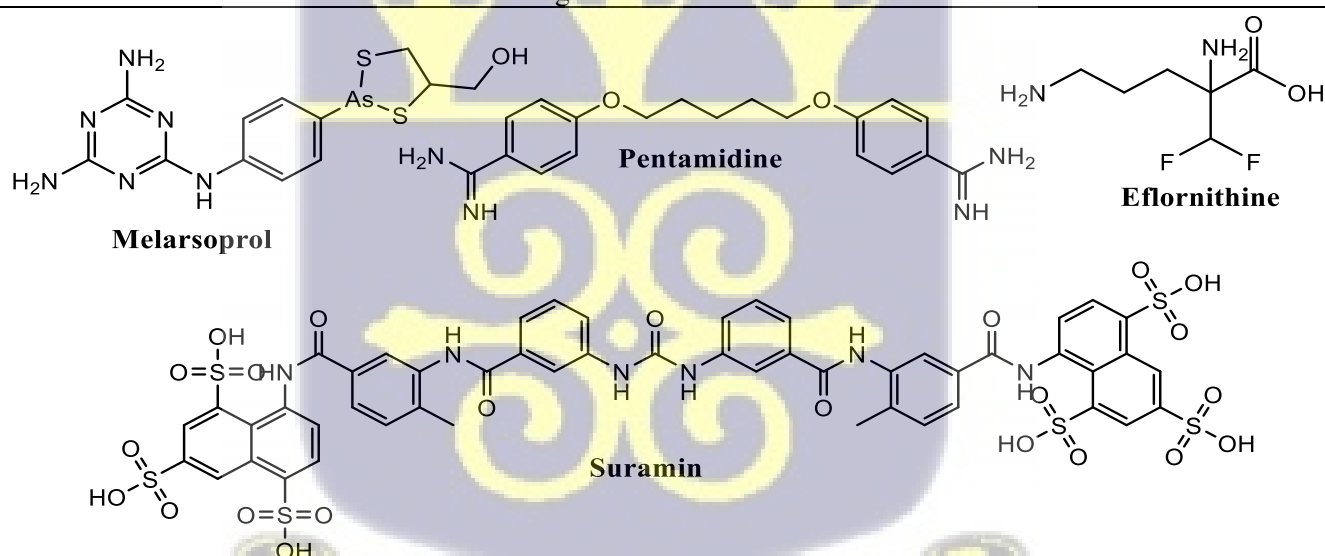


Figure 2-2 Chemical structure of drugs used to treat Trypanosomiasis at distinct stages of infection

2.3.4 Summary of Trypanosomiasis

Trypanosomiasis remains a significant parasitic disease of global concern, caused by various *Trypanosoma* species that affect both humans and animals. The human form, African trypanosomiasis, persists mainly in sub-Saharan Africa and manifests as either the chronic *T. brucei gambiense* or the acute *T. brucei rhodesiense* infection, each progressing through distinct hemolymphatic and neurological stages. The parasite's complex extracellular life cycle and ability to evade immune responses through antigenic variation make treatment and vaccine development particularly challenging. Although drugs such as pentamidine, suramin, eflornithine, and melarsoprol remain the mainstay of therapy, their administration is constrained by toxicity, cost, and limited accessibility, especially in endemic rural regions. These challenges underscore the urgent need for safer, affordable, and more effective therapeutic alternatives, strengthening the rationale for exploring natural products with potential antitrypanosomal activity.

2.4 Medicinal Plants as a Foundation for Modern Therapeutics: Global and Ghanaian Perspectives

Globally, medicinal plants have been the foundation for some of the most significant therapeutic discoveries. For instance, *Serenoa repens* (Saw palmetto) was evaluated for its efficacy in treating lower urinary tract symptoms associated with benign prostatic hyperplasia^{6,7}. *Pilocarpus jaborandi* is the sole known source of pilocarpine, a compound used to treat glaucoma and dry mouth^{96,97}. Similarly, *Catharanthus roseus* (Madagascar periwinkle) has given us the potent anticancer drugs vinblastine and vincristine, which are widely used in chemotherapy^{98,99}. Other plant-derived compounds like silymarin from *Silybum marianum* (milk thistle) and digoxin from *Digitalis lanata* (woolly foxglove) continue to play essential roles in treating liver disorders and heart conditions, respectively^{100,101}.

In Ghana, the use of plants in traditional medicine also remains extensive, with a wealth of ethnobotanical knowledge recorded over time. Plants such as, *Dichapetalum madagascariense* (Dichapetalaceae), known as *Ekumnkura* in Fante and *Kumnkura* in Twi, treats whitlow; *Blumea viscosa* (Compositae), or *Afun nena* in Fante, alleviates headaches; *Lantana camara* L. (Verbenaceae), called *Ananse dokono* in Twi, serves as an analgesic and anti-rheumatic; and *Tiliacora warneckeii* (Menispermaceae), known as *Dsidse* in Fante, is used to treat typhoid, hernia, and menstrual irregularities are few examples of plants commonly employed in Ghanaian traditional medicine¹⁰. Given the urgent need for new therapies, especially for parasitic diseases, the ethnomedicinal use of plants is increasingly seen as a promising avenue for drug discovery.

2.4.1 Phytotherapy for Trypanosomiasis

2.4.1.1 Some Plants in the Fabaceae Family Used for Treatment of Trypanosomiasis

Several plants within the Fabaceae family have been traditionally used by local communities for the treatment of sleeping sickness. For instance, *Entada abyssinica* A. Rich (Nsofi) is administered through a decoction or maceration of the roots, which are administered anally, as a clyster, or orally as a drink¹⁰². A decoction of the trunk of *Dianiella alsteeniana* P.A. Duvign (Nlomba) is administered as a drink and used as a facial wash to treat trypanosomiasis. An infusion of the roots of *Senna occidentalis* (L.) Link (Dinioka nioka or Kikunde Kim) is also taken as a drink to combat sleeping sickness¹⁰². *Parkia clappertoniana* Keay roots are boiled with red potash and given to animals to drink. For *Lonchocarpus laxiflorus* Guill. & Perr., the stem bark is mixed with guinea corn flour and potash and administered to animals. *Parkinsonia aculeata* L. stem bark, combined with the stem bark of *Erythrina senegalensis* and leaves of *Striga* spp., is mixed with the animal's feed¹⁰³. The stem barks of *Afzelia africana* Sm. ex Pers. and *Prosopis Africana* (Guill. & Perr.) Taub. are boiled together, and the resulting extract is mixed with potash and given to animals to

drink. *Tamarindus indica* L. leaves are boiled and given to animals to drink. The ground Tamarind is mixed with raw beans to feed animals¹⁰³. The roots, bark and leaves of plants such as, *Ehretia amoena*, *Capparis elaeagnoides*, *Vernonia auriculifera*, *Vernonia subuligera*, *Securinega virosa*, *Entada abyssinica*, *Albizia gummifera*, *Lantana camara*, and *Garcinia huillensis* have been used by Ugandans to treat Trypanosomiasis in the form of infusion¹⁰⁴. These highlight the potential antitrypanosomal activity and overall biological activity of species in the Fabaceae family.

2.5 The phytotherapy The Fabaceae Family and its Ethnomedicinal Importance

2.5.1 Overview of the Fabaceae Family

Fabaceae, also known as Leguminosae, is a significant group of flowering plants. The name "Fabaceae" originates from the genus *Faba*, which has since been incorporated into the genus *Vicia*. Its diverse characteristics make it vital to humans, serving as crops, green manures, forages, and sources for various natural products like flavors, dyes, and medicinal substances¹⁰⁵. Fabaceae is the third-largest flowering plant family which comprises of more than 750 genera and over 19,000 species, spanning herbs to towering canopy trees. These plants thrive in diverse climates, from temperate and humid to arid, highland, and savannah regions¹⁰⁶. The Leguminosae family was originally subdivided into three groups: papilionodae, Caesalpinioideae, and Mimosoideae, primarily based on their flower characteristics. However, a more recent classification, utilizing chloroplast gene matK phylogeny, identifies six well-supported subfamilies: Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae, and Papilionoideae⁴¹. Within these subfamilies, the genus *Talbotiella* is grouped under the subfamily Detarioideae.

The Detarioideae subfamily consists of 84 genera and 760 species⁴¹, almost exclusively tropical with genera present in Central and South America, Africa and Southeast Asia; and the genus *Schotia* in sub-tropical South Africa^{107,108}. The Detarioideae subfamily is currently

classified into six tribes: Amherstieae, Afzelieae, Barnebydendreae, Detarieae, Saraceae, and Schotieae. It is particularly notable for having a large percentage of unispecific genera (35%) and only nine genera with more than 20 species¹⁰⁹. Having established a foundational understanding of the Fabaceae family, the traditional uses of its plants in various cultures and healing practices warrant examination.

2.5.2 The Traditional Uses of Plants of the Fabaceae Family:

The Fabaceae family, renowned for its wellness and longevity benefits, stands out among medicinal plants due to its unique phytochemicals and significant ethnobotanical and ethnomedicinal use. Specifically, the bark of *Acacia catechu*, *Bauhinia variegata* *Albizia lebbek*, and *Acacia nilotica*, has been utilized to alleviate symptoms of diarrhoea, ulcers, and inflammation¹¹. The foliage from plants such as *Mimosa diplotricha*, *Acacia nilotica*, *Bauhinia acuminata*, *Cassia fistula*, *Desmodium triflorum*, *Erythrina variegata*, and *Cajanus cajan* has been employed in treating a range of conditions including skin ailments, menstrual issues, respiratory troubles, and gastric disorders¹¹⁰. Exploring the traditional uses of Fabaceae plants offers valuable insights into the phytochemicals they harbor and their biological effects. This understanding naturally leads to a closer examination of the Amherstieae tribe, known for its large number of species and unique contributions to the chemical diversity of the family, with the genus *Talbotiella* being a notable member of this tribe.

Several plant species have been documented for their ethnomedicinal applications (Table 2-2). For instance, the bark of *Acacia catechu* is used for treating diarrhoea¹¹ and has other medicinal properties, including astringent, anthelmintic, antidysentery, and antipyretic effects, as well as treatments for ulcers, boils, and skin conditions such as leucoderma and psoriasis¹¹⁰. Similarly, *Albizia lebbek* bark is used for inflammation¹¹, while its leaves and seeds are utilized for

ophthalmia, asthma, and as a tonic for the brain health, and among other ailments¹¹⁰. *Acacia nilotica* bark powder is applied externally for ulcers¹¹, and its leaves, pods, and flowers are known for their astringent, tonic, and antipyretic properties, addressing conditions like gonorrhoea, dysentery, and cough¹¹⁰. *Butea monosperma* flower lotion is used for eye diseases¹¹, whereas its bark is recognized for its aphrodisiac, laxative, and astringent properties, treating conditions like dysentery and stomatitis¹¹⁰. *Cassia fistula* leaf paste is applied to ringworm lesions¹¹, while its leaves and roots are used for leprosy, syphilis, and malaria¹¹⁰.

Table 2-2 Some Ethnomedicinal Plants of the Fabaceae family

Plant	Medicinal uses	Plant part	Reference
<i>Acacia catechu</i> (L. f.) Willd.	diarrhoea	bark	11
	astringent, anthelmintic, antidysentery, antipyretic, cures itching, inflammations, sore throat, bronchitis, indigestion, ulcers, boils, leucoderma, psoriasis, leprosy and elephantiasis, strengthens the teeth.	bark	110
<i>Albizia lebbek</i> (L.) Benth	inflammation	bark	11
	ophthalmia, asthma, astringent, tonic to the brain, gonorrhoea, tubercular glands, leucoderma and leprosy.	leaf, seed	110
<i>Acacia nilotica</i> (L.) Willd. ex Delile	ulcer	bark	11
	astringent, tonic to the liver and brain, antipyretic, leucoderma, gonorrhoea, strangury, diarrhea, cystitis, vaginitis, dysentery, ophthalmia, cough and insanity.	leaf, pods, flower	110
<i>Butea monosperma</i> (Lam.) Taub.	eye diseases	flowers	11
	aphrodisiac, laxative, anthelmintic, dysentery, piles, cold, cough, astringent, diarrhea and stomatitis.	bark	110
<i>Cassia fistula</i> L	ringworm lesions	leaf	11
	burning sensation, leprosy, syphilis and malaria.	leaf, root	110

2.6 The Amherstieae tribe

There are 50 recognized genera within the Amherstieae tribe, which includes the *Talbotiella* genus¹⁰⁹. The Amherstieae tribe features leaflets that are opposite or alternate without translucent gland dots and flowers that are bilaterally to radially symmetrical, with bracteoles often larger than

the sepals in the bud. The number of sepals and petals varies, and stamens can range from 3 to 80, with some genera having staminodia. The tribe is pantropical, primarily found in wet tropical evergreen forests, with 34 genera in Africa and nine in Central and South America¹¹¹. Plants within this tribe have been documented for their ethnomedicinal uses.

2.6.1 Traditional Uses of Plants in Amherstieae Tribe

Species within the Amherstieae tribe have been documented for their use in treating certain ailments in Asia and Africa. Examples of these plants include *Amherstia nobilis*, *Anthonotha macrophylla* (P. Beauv.), and *Berlinia grandiflora* (Vahl). *Amherstia nobilis* is used traditionally to treat arthritis, fever, and digestive issues¹¹². In western Ghats, south India, a decoction of the leaves of *Amherstia nobilis* is used to treat diabetes¹¹³. *Anthonotha macrophylla* (P. Beauv.) is used ethnomedicinally to treat dysentery, yellow fever, gonorrhea and diarrhea in the Oyo state of Nigeria¹¹⁴. For *Berlinia grandiflora* (Vahl), the bark sap is used for wound care, while a bark decoction treats hemorrhoids, liver issues, helps ease labor pains, and manages gastrointestinal disorders¹¹⁵. Its leaves prepared as decoction relieves fever, vomiting, and improves circulations¹¹⁶. Additionally, in southwestern Nigeria, leaf extract is used to treat diabetes, cancer, diarrhea, and worms¹¹⁷.

2.7 The Phytochemistry of the Amherstieae tribe

Among the 50 genera in this tribe, only 11 have species that have undergone phytochemical analysis, leading to the identification of phytocompounds: *Amherstia*, *Anthonotha*, *Berlinia*, *Brachystegia*, *Brownea*, *Cynometra*, *Humboldtia*, *Julbernardia*, *Macrolobium*, *Tamarindus*, and *Scorodophloeus*. Phytochemical studies on species from the various genera within the Amherstieae tribe have identified a diverse range of phytochemicals, including lectins, saponins, alkaloids, terpenoids, steroids, anthraquinones, glycosides, flavonoids, phenolic acids, and tannins. These

species serve as valuable reservoirs of phytochemicals, with research leading to the isolation and identification of over 600 phytoconstituents, many of which exhibit antimicrobial, antioxidants, anti-inflammatory, anticancer, and antidiabetic properties. It is also worth noting that studies on the antitrypanosomal activity of extracts from species within this tribe are limited. The only documented example is the phytochemical investigation of the ethanolic extract of *Anthonotha macrophylla* leaves, which revealed the presence of saponins, phenolic compounds, free-reducing sugars, flavonoids, and phytosterols, and demonstrated some antitrypanosomal activity with an IC_{50} value of $13.6 \pm 0.21 \mu\text{g/mL}$ ⁶⁸. Despite this progress, the number of species with published phytochemical studies remains limited. To address this gap, the following sections provide a detailed discussion of the phytoconstituents identified in these genera within the Amherstieae tribe and their species. Having established the general phytochemistry of the tribe, we now shift our focus to the specific chemical constituents of each genus, beginning with *Talbotiella*, to explore their unique contributions to its chemical diversity.

Collectively, these studies reveal an uneven distribution of research attention across the tribe—while certain genera such as *Berlinia* and *Tamarindus* are chemically well characterized, others, including *Talbotiella*, remain virtually unexamined. This imbalance highlights a significant knowledge gap in the chemotaxonomic mapping of the Amherstieae tribe and provides a strong justification for investigating *T. gentii* to uncover potentially novel or bioactive secondary metabolites.

2.7.1 Phytochemical Studies on the Genus *Talbotiella*

Talbotiella gentii Baker Hutch. & Greenway(year) is a small forest tree in the Fabaceae, specifically in the Detarioideae subfamily⁴¹. The family Fabaceae has six (6) subfamilies⁴¹. The genus *Talbotiella* has nine species including *Talbotiella gentii* Hutch & Greenway *Talbotiella*

bakossiensis Cheek, *Talbotiella batesii* Baker f, *Talbotiella breteleri* (Aubrev) Mackinder & Wieringa, *Talbotiella cheekii* Burgt, *Talbotiella ebo* Mackinder & Wieringa, *Talbotiella eketensis* Baker f., *Talbotiella korupensis* Mackinder & Wieringa, and *Talbotiella velutina* Burgt & Wieringa¹¹⁸. *Talbotiella* species are mainly found in West and West-Central African forests. All of the nine species within the *Talbotiella* genus are classified as threatened by the International Union for Conservation of Nature (IUCN) since 2001¹¹⁹..

The existing literature on species of the *Talbotiella* genus primarily focuses on their taxonomic and ecological characteristics, with minimal attention to phytochemical or pharmacological profiling. A recently published paper, which is the only pharmacological investigation of *Talbotiella gentii* to date, examines its anti-proliferative properties. It was found that the IC₅₀ values of the leaf extract against cancer cell lines ranged from 129.9 to 201.5 µg/mL, while the bark extract exhibited IC₅₀ values between 105 and 300 µg/mL⁶⁰. Although related genera within the Amherstieae tribe have yielded flavonoids, triterpenes, and phenolic derivatives with notable bioactivities, comparable investigations on *T. gentii* are entirely lacking. This absence of chemical and biological data represents a clear gap in the phytochemical literature, positioning *T. gentii* as an unexamined source of potentially novel or bioactive secondary metabolites, highlighting the need for this thesis.

Furthermore, given the chemical diversity already reported within related genera of the Amherstieae tribe, it is plausible that *T. gentii* may produce structurally related triterpenoids and sterols with untested pharmacological potential, especially against parasitic diseases such as trypanosomiasis.

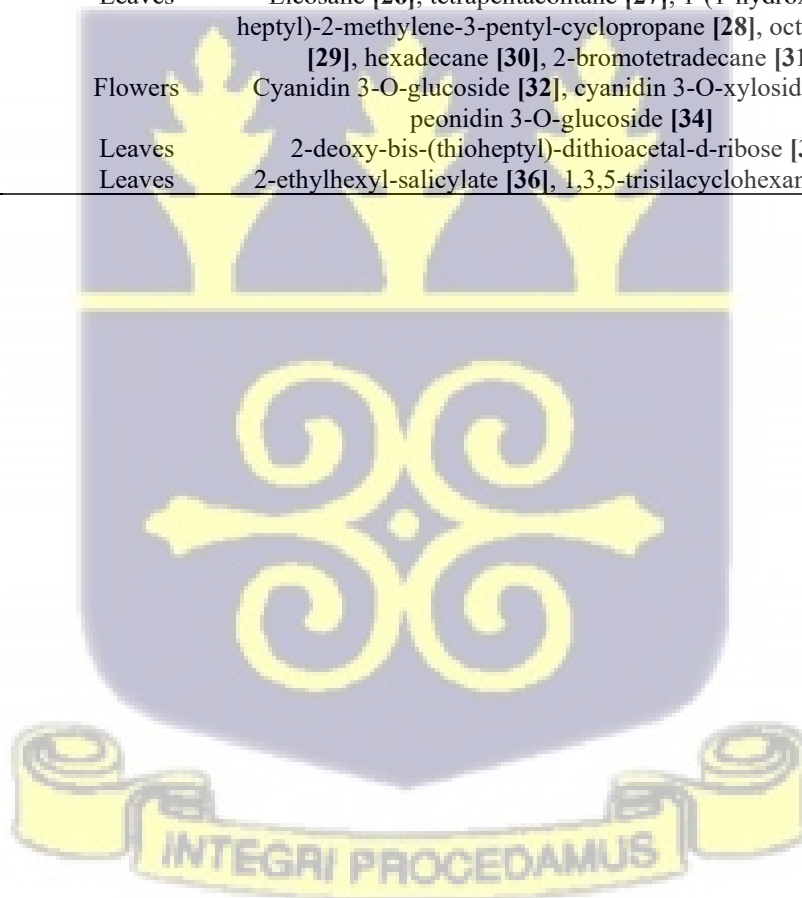
2.7.2 The *Amherstia* Genus

The *Amherstia* genus, which is monotypic, has been documented to contain anthocyanins, flavonoids and fatty acids^{120,121}. *Amherstia* is a phenotypic genus comprising only one species, *Amherstia nobilis*. The phytochemical analysis done on this plant is relatively non-exhaustive. The studies done on the flowers of this species have been found to contain anthocyanins and flavonoid glycosides⁸⁵, whereas the leaves contain phenolic compounds, aliphatic aldehyde, fatty alcohol, sugars, silicon-containing compound, saturated and unsaturated fatty acids¹²¹. The anthocyanins cyanidin 3-O-glucoside [32], cyanidin 3-O-xyloside [33], peonidin 3-O-glucoside [34] and the flavonoids isorhamnetin 3-O-glucoside [1], isorhamnetin 3-O-rutinoside [2], isorhamnetin 3,7-diglucoside [3] isorhamnetin 7-O-glucoside [4], kaempferol 3-O-glucoside [5], kaempferol-3-O-rutinoside [6], quercetin 3-O-rutinoside [7], were isolated from the flowers of *A. nobilis*¹²⁰. Phytochemical analysis carried out on the leaves using GC-MS indicated the presence of other compounds such as, triterpenoids (squalene [19], β -tocopherol [20]), acyclic diterpenoids (3,7,11,15-tetramethyl-2-hexadecen-1-ol [9], phytol acetate [10], neophytadiene [8]), phenolic compounds (2,4-bis-(1,1-dimethylethyl)phenol [21], 2-ethylhexyl-salicylate [36]), aldehyde((Z)-7-tetradecenal [22]), (alcohols 3-heptadecanol [23], 4-methyl-4-heptanol [24], 1-butyl-cyclohexanol [25]), hydrocarbons (eicosane [26], tetrapentacontane [27], 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-cyclopropane [28], octadecane [29], hexadecane [30]), 2-bromotetradecane [31], sugar derivative (2-deoxy-bis-(thioheptyl)-dithioacetal-d-ribose [35]), and 1,3,5-trisilacyclohexane [37]¹²¹. Table 2.2 below summarizes the various compounds that have been identified in the leaves and flowers of *A. nobilis*.

Table 2-3 Phytochemicals Identified from the *Amherstia* Genus

Phytochemical	Plant parts	Compounds	reference
Flavonoids	Flowers	isorhamnetin 3-o-glucoside [1], isorhamnetin 3-o-rutinoside [2], isorhamnetin 3,7-diglucoside [3] isorhamnetin 7-O-	¹²⁰

Diterpenoids	Leaves	glucoside [4], kaempferol 3-O-glucoside [5], kaempferol-3-O-rutinoside [6], quercetin 3-O-rutinoside [7], neophytadiene [8]	121
		3,7,11,15-tetramethyl-2-hexadecen-1-ol [9], phytol acetate [10]	121
Unsaturated fatty acid	Leaves	9,12-octadecadienoic acid (linoelaidic acid) [11]	121
Saturated fatty acid ester	Leaves	octadecenoic acid, 2-hydroxy-1-(hydroxymethyl)-ethyl ester [12]	121
Saturated fatty acid	Leaves	tetradecanoic (myristic) acid [13], octadecanoic acid [14], hexadecanoic (palmitic) acid [15], octadecanoic acid ethyl ester [16], hexadecanoic acid ethyl ester [17], isopropyl-myristate [18]	121
Non-steroidal triterpenoids	Leaves	Squalene [19], β -tocopherol [20]	121
Phenolic compounds	Leaves	2,4-bis-(1,1-dimethylethyl)phenol [21]	121
	Leaves	(Z)-7-tetradecenal [22]	121
Alcohols and hydrocarbons	Leaves	3-heptadecanol [23], 4-methyl-4-heptanol [24], 1-butyl-cyclohexanol [25]	121
Anthocyanins	Leaves	Eicosane [26], tetrapentacontane [27], 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-cyclopropane [28], octadecane [29], hexadecane [30], 2-bromotetradecane [31]	121
	Flowers	Cyanidin 3-O-glucoside [32], cyanidin 3-O-xyloside [33], peonidin 3-O-glucoside [34]	120
Sugar silane	Leaves	2-deoxy-bis-(thioheptyl)-dithioacetal-d-ribose [35]	121
	Leaves	2-ethylhexyl-salicylate [36], 1,3,5-trisilacyclohexane [37]	121



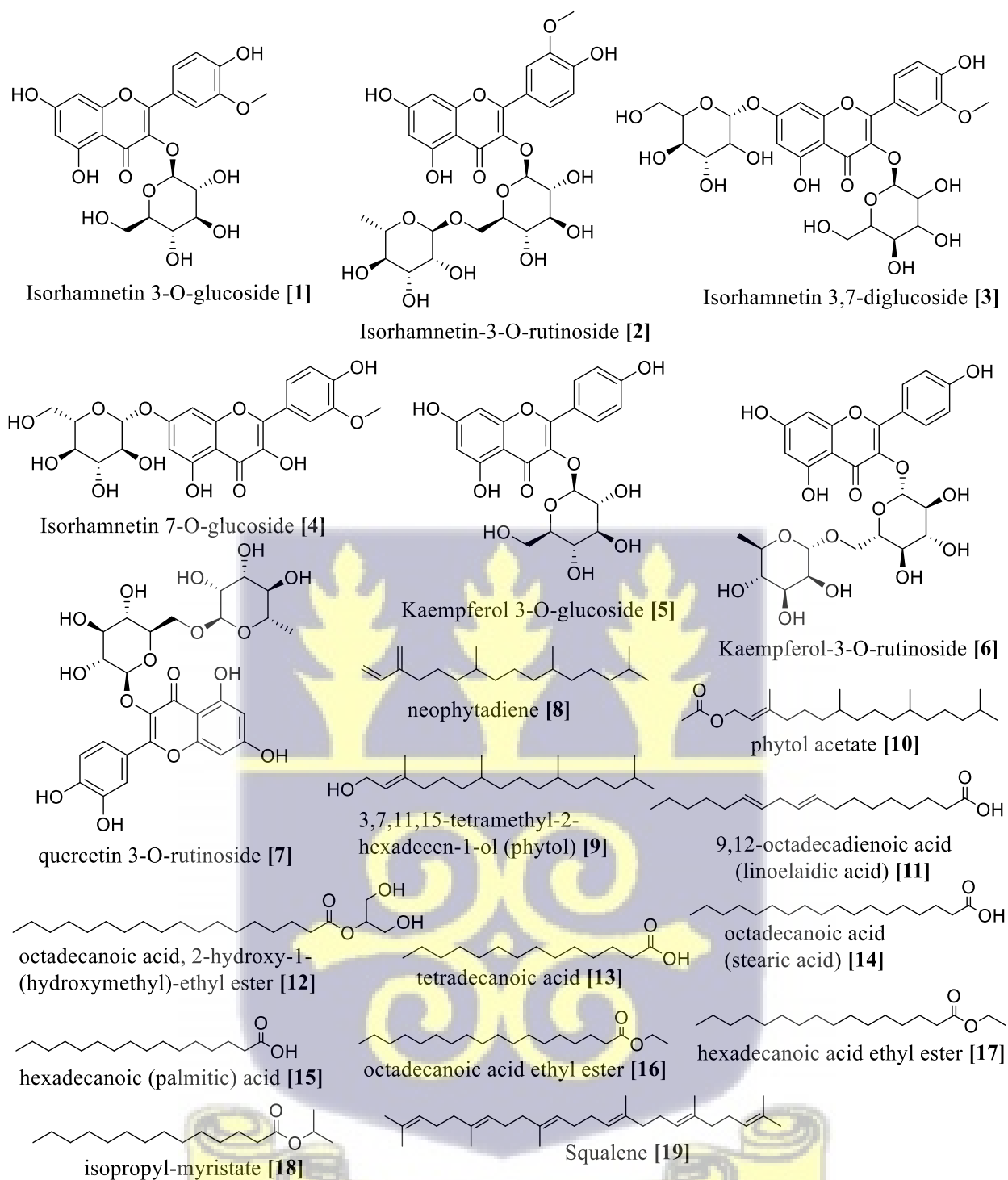


Figure 2-3 Structures of phytochemicals identified in *Amherstia nobilis*

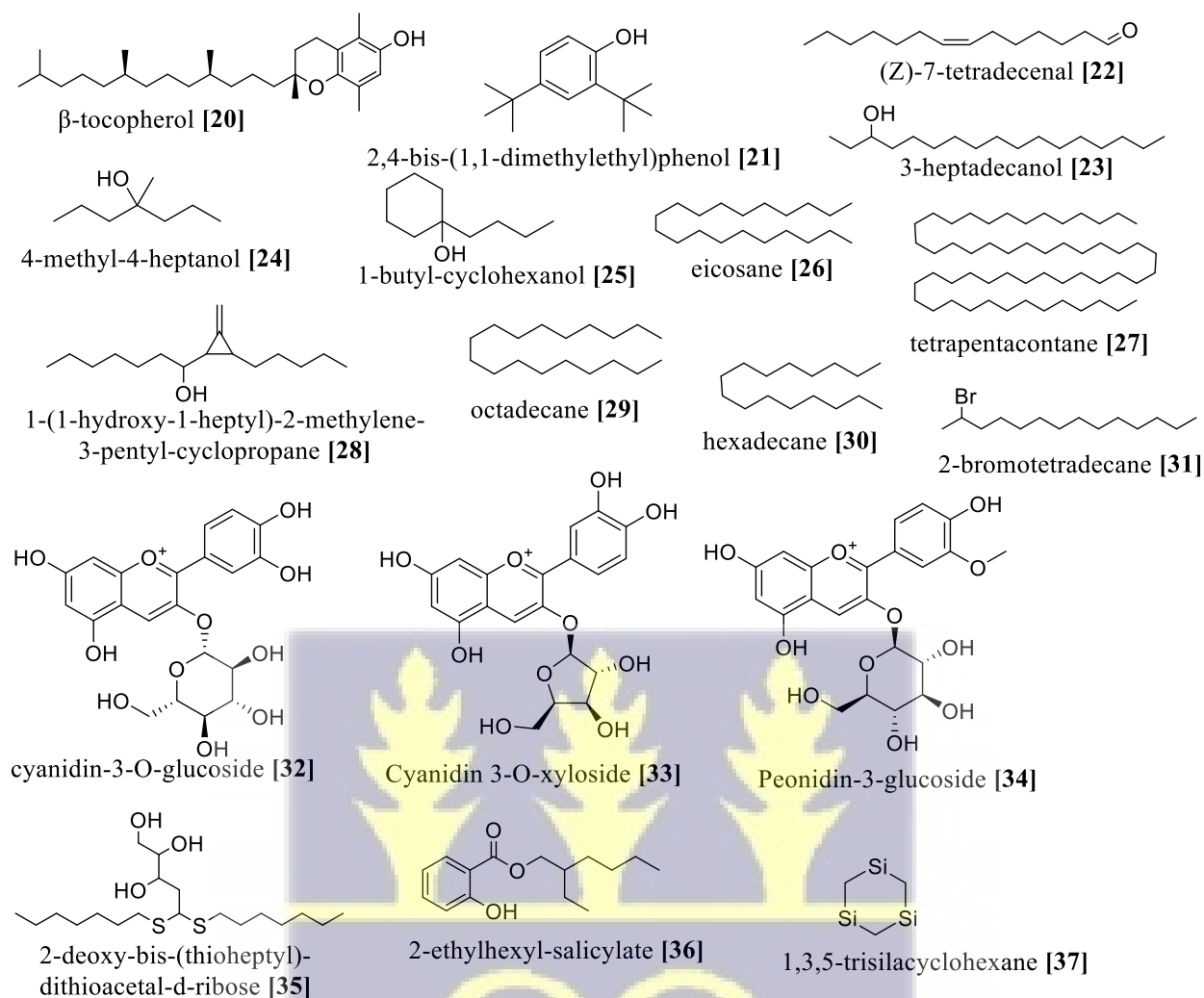


Figure 2-4 Structures of phytochemicals identified in *Amherstia nobilis* contd.

2.7.3 The *Anthonotha* Genus

The genus *Anthonotha* P.Beauv. (Fabaceae) comprises 17 species exclusively native to Cameroon and Democratic Republic of Congo. It is represented by 10 species, including *Anthonotha macrophylla* P. Beauv, and *Anthonotha cladantha* (Harms) J.Léonard¹²². Out of the 17 species under the *Anthonotha* genus, only two species, *Anthonotha macrophylla* P. Beauv, and *Anthonotha cladantha* (Harms) J. Léonard have undergone phytochemical analysis. *Anthonotha* contains fatty acids, phytosterols, terpenoids, and phenolic compounds. Table 2-3, figure 2-5, and figure 2-6

below summarizes the different classes of phytochemicals identified in various parts of *A. cladantha* and *A. macrophylla*, providing a detailed account of its chemical diversity. The phytochemical analysis of *Anthonotha* species revealed the presence of diverse classes of compounds across various plant parts. Voufack et al. conducted a phytochemical analysis of the crude methanolic extracts from the roots and stem bark of *A. cladantha* (Harms) J. Léonard, resulting in the isolation of twelve compounds, which were identified using their physical and spectral properties¹²³. Two triterpenoids, lupeol [38] and hederagenin [39], as well as two phytosteroids, stigmasterol [42] and stigmasterol glucoside [43], were isolated from the stems¹²³. The roots also yielded two triterpenoids, 2 α -hydroxylupeol [40] and betulinic acid [41]; three phytosteroids, β -sitosterol [44], cycloeucalenol [45], and stigmasterol-4-en-3-one [46]; two saturated fatty acids and their esters, including triacontanoic acid [51] and dihydroxypropyl hexacosanoate [52]; and the ketone heptacosan-2-one [60]¹²³.

For *A. macrophylla*, analysis of the seed oils by Ugoeze et al. using GC/MS identified seven compounds, including three phytosteroids: stigmasterol [42], campesterol [47], and γ -sitosterol [48]; one unsaturated fatty acid: cis-vaccenic acid [50]; and three saturated fatty acids: hexadecanoic (palmitic) acid [15], octadecanoic acid [14], and hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl)ethyl ester [53]⁶⁷. Kinyok et al. conducted a study focused on isolating and characterizing chemical constituents from the leaves of *A. macrophylla*¹²². By applying various chromatographic techniques (TLC, CC, HPLC), the methanolic extract of the leaves yielded one novel alkaloid, 4-C- β -D-glucosyl(1,1-bis(N-(E),N'-(E)-diferuloyl)ethanamide)buta-1,3-diene [55], along with six known compounds. These included an alkane, 7-methyltetradecane [59], reported for the first time as a natural product, a fatty acid ester, 2,3-dihydroxypropyl heptacosanoate [54], two isocoumarins, bergenine [57] and tri-O-methylnorbergenin [58], a sterol, sitosterol glucoside

[49], and a disaccharide, α -D-glucopyranosyl-(1 $\rightarrow$ 2')- β -D-sorbopyranoside (digobiose) [56]. The structures of these compounds were elucidated using spectroscopic techniques, including extensive 1D and 2D NMR and HR-MS experiments¹²². This comprehensive profiling highlights the phytochemical diversity within the *Anthonotha* genus, demonstrating its potential for further exploration in pharmacological and therapeutic studies.

Table 2-4 Phytochemicals Identified in *Anthonotha cladantha* and *Anthonotha macrophylla*

Phytochemical	Plants	Plant part	Compounds	reference
Non-steroidal triterpenoids	<i>A. cladantha</i>	stems	lupeol [38], hederagenine [39]	123
		roots	2 α -hydroxylupeol [40], betulinic acid [41]	123
	<i>A. cladantha</i>	stems	stigmaterol [42], stigmaterol glucoside [43]	123
Phytosterols		roots	β -sitosterol [44], cycloeucalenol [45], stigmast-4-en-3-one [46]	123
Unsaturated fatty acid	<i>A. macrophylla</i>	seeds	campesterol [47], [42], γ -sitosterol [48]	67
		leaves	sitosterol glucoside [49]	122
	<i>A. macrophylla</i>	seeds	cis-vaccenic acid [50]	67
Saturated fatty acid and ester	<i>A. cladantha</i>	roots	triacontanoic acid [51], dihydroxypropyl hexacosanoate [52]	123
	<i>A. macrophylla</i>	seeds	hexadecanoic (palmitic) acid [15], octadecanoic acid [14], hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl)ethyl ester [53]	67
		leaves	2,3-dihydroxypropyl heptacosanoate [54]	122
Alkaloid	<i>A. macrophylla</i>	leaves	4-C- β -D-glucosyl(1,1-bis(N-(E),N'-(E)-diferuloyl)ethanamide)buta-1,3-diene [55]	122
		leaves	α -D-glucopyranosyl- (1 $\rightarrow$ 2')- β -D-sorbopyranoside or digobiose [56]	122
Sugar derivatives	<i>A. macrophylla</i>	leaves	bergenine [57]; tri-o-methylnorbergenin [58]	122
Isocoumarins	<i>A. macrophylla</i>	leaves	7-methyltetradecane [59]	122
Aliphatic hydrocarbon	<i>A. cladantha</i>	root	heptacosan-2-one [60]	123
Ketone				



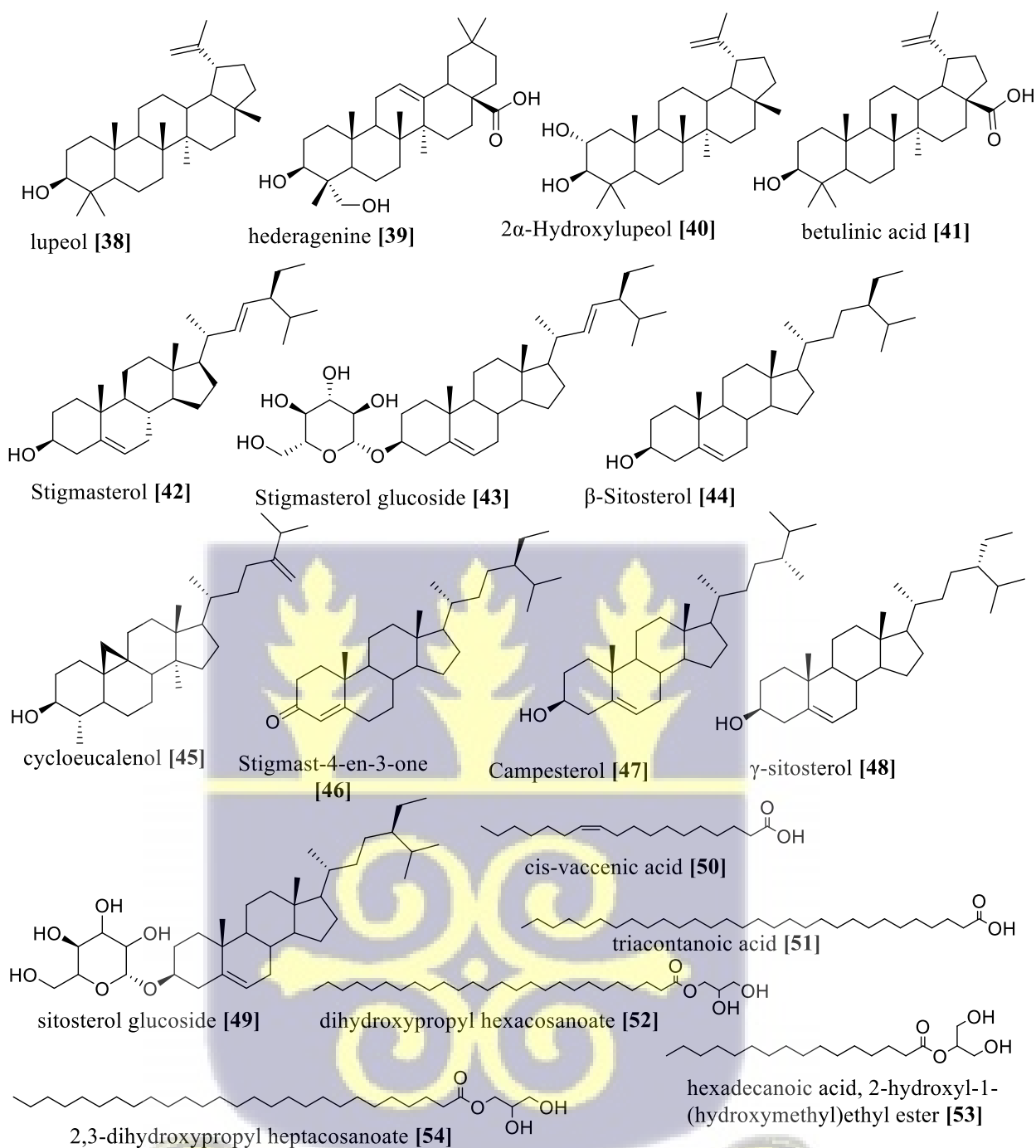


Figure 2-5 Structures of compounds identified in *Anthonotha* species

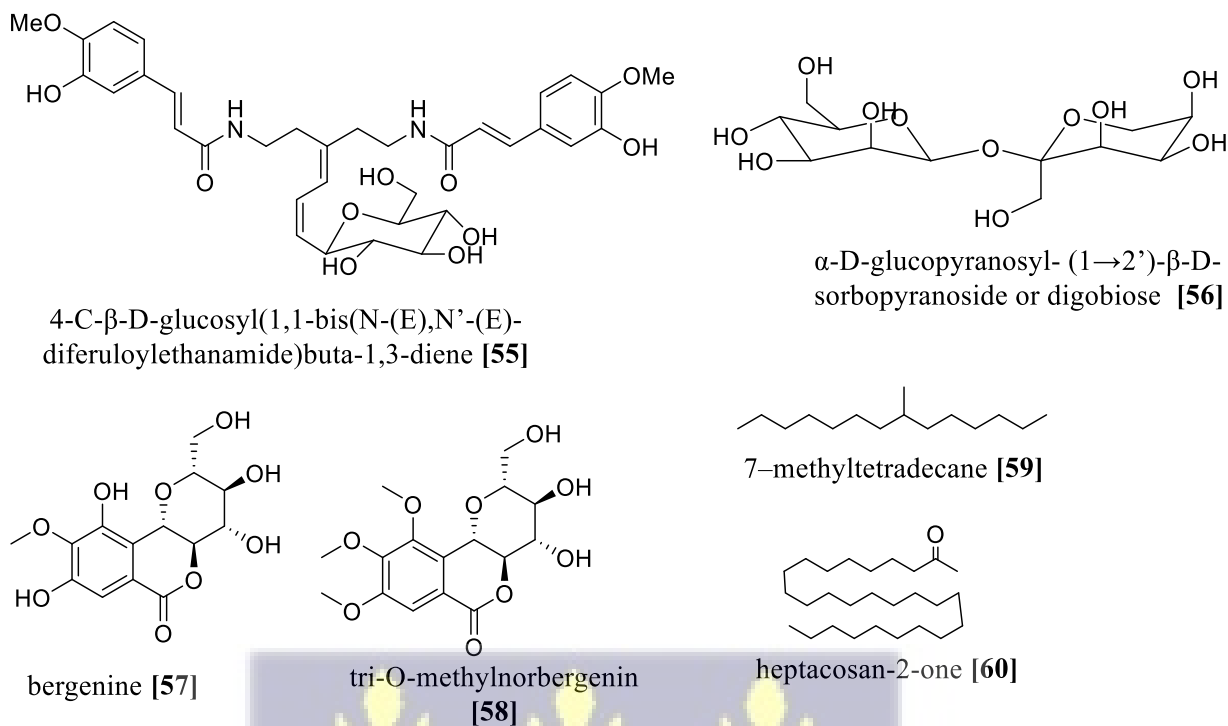


Figure 2-6 Structures of compounds identified in *Anthonotha species* contd.

2.7.4 The *Berlinia* Genus

Berlinia Sol. ex Hook.f. & Benth. (also known as *Macroberlinia* (Harms) Hauman or *Westia* Vahl) is a genus of flowering plants in the family Fabaceae comprising of 21 species of trees that are native to sub-Saharan Africa, ranging from Guinea to Chad, Tanzania, Mozambique, and Angola¹²⁴. Only two species have gone under constituent analysis; *Berlinia confusa* and *Berlinia grandiflora*. For the *B. confusa*, the only phytochemical constituent analysis was done on the stem bark, which led to the isolation of the non-steroidal triterpenoid betulinic acid [41]¹²⁵, sterol glucoside sitosteryl-β-d-glucoside [49]^{125,126} and the glycerides: 1-O-docosanoyl-sn-glyceride [61], 1-O-Pentadecanoyl-sn-glycerol [62]; 1-O-(13-methyltetradecanoyl)-sn-glycerol [63]¹²⁵ and 1-O-tetracosanoyl-sn-1-glycerol [64]¹²⁵.

Phytochemical analysis of *B. grandiflora*, using GC-MS, revealed diverse bioactive compounds across various parts. The alkaloid 9-octadecenamide [65] was identified in the seeds¹²⁷, while monoterpeneoid carvacrol [66] was isolated from the stem bark¹²⁸. The leaves contained norisoprenoids (α -ionone [67], E- β -ionone [68]), sesquiterpenoids (geosmin [69]), and diterpenoids (phytol [9] and kaur-16-ene [71])¹²⁸. Unsaturated fatty acid, 9-octadecenoic acid (oleic acid) [72] was found in the leaves and seeds, while the stem bark contained (E)-9-octadecenoic acid [73] and related esters^{127,128}. Aliphatic hydrocarbons (tetracosane [77]) and aldehydes (4-oxononanal [83]) were detected in the stem bark, alongside alcohols like cis-9-tetradecen-1-ol [92] and the ketone, 3,5-octadien-2-one [94]¹²⁸. The sugar (α -methylmannofuranoside [95]) was present in the seeds⁹³, while naphthalene derivatives (6-butyl-7-hexyl-1,2,3,4-tetrahydronaphthalene [96]) was detected from the leaves¹²⁸. Saturated fatty acids, octadecanoic acid [14], hexadecanoic (palmitic) acid [15] and pentadecanoic acid [97], were also detected in seeds and stem bark^{127,128}. Table 2-4 lists the phytoconstituents of the leaves, seeds and stem bark of *B. grandiflora*.

Table 2-5 Phytochemicals Identified in the various part of *B. grandiflora*

Phytochemicals class	Plant Part	Specific Compounds Identified	Reference
Alkaloid	seeds	9-octadecenamide [65]	127
Monoterpenoid	Stem bark	Carvacrol [66]	128
Norisoprenoid	Leaves	α -Ionone [67], E- β -Ionone [68]	128
Sesquiterpenoid	Leaves	Geosmin [69]	128
	stem	hexahydrofarnesyl acetone [70]	128
Diterpenoid	Leaves	Kaur-16-ene [71]; phytol [9]	128
Unsaturated fatty acids and ester	Leaves, seeds	9-octadecenoic acid (Oleic acid) [72]	127,128
	Stem	9,12-octadecadienoic acid (linoelaidic acid) [11], (E)-9-octadecenoic acid [73]; (Z)-9-octadecen-4-olide [74]; Z-8-pentadecen-1-ol acetate [75]	128
Aliphatic hydrocarbon	Leaves	2,2,4,5,7,7-hexamethyl- (E, Z)- 3,5-octadiene [76]; tetracosane [77]	128
	Stem bark	E-1,9-tetradecadiene [78]; 7-hexadecyne [79]; heptadecane [80]; 2-methylbicyclo [2.2.2] octane [81]	128
Aldehydes	Leaves	2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde [82]	128
	Stem bark	4-oxononanal [83]; E-2-decenal [84]; E, E-2,4-dodecadial [85]; (E, E)-2,4-decadial [86]; E-	128

		tetradec-2-enal [87]; 4-heptenal [88]; cis, cis-7,10-hexadecadienal [89]; cis-9-hexadecenal [90]; (Z)-7-tetradecenal [22]	
Alcohols	Leaves	2,6,6-trimethyl-1-cyclohexene-1-ethanol [91]	128
	Stem bark	cis-9-tetradecen-1-ol [92]	128
Ketones	Leaves	2-pentadecanone [93]	128
	Stem bark	3,5-octadien-2-one [94]	128
Sugar	seeds	α -methyl-mannofuranoside [95]	127
Naphthalene	Leaves	6-butyl-7-hexyl-1,2,3,4-tetrahydronaphthalene [96]	128
Saturated fatty acids	Seed	pentadecanoic acid [97], octadecanoic acid [14]	127
	Stem bark	hexadecanoic (palmitic) acid [15]	128

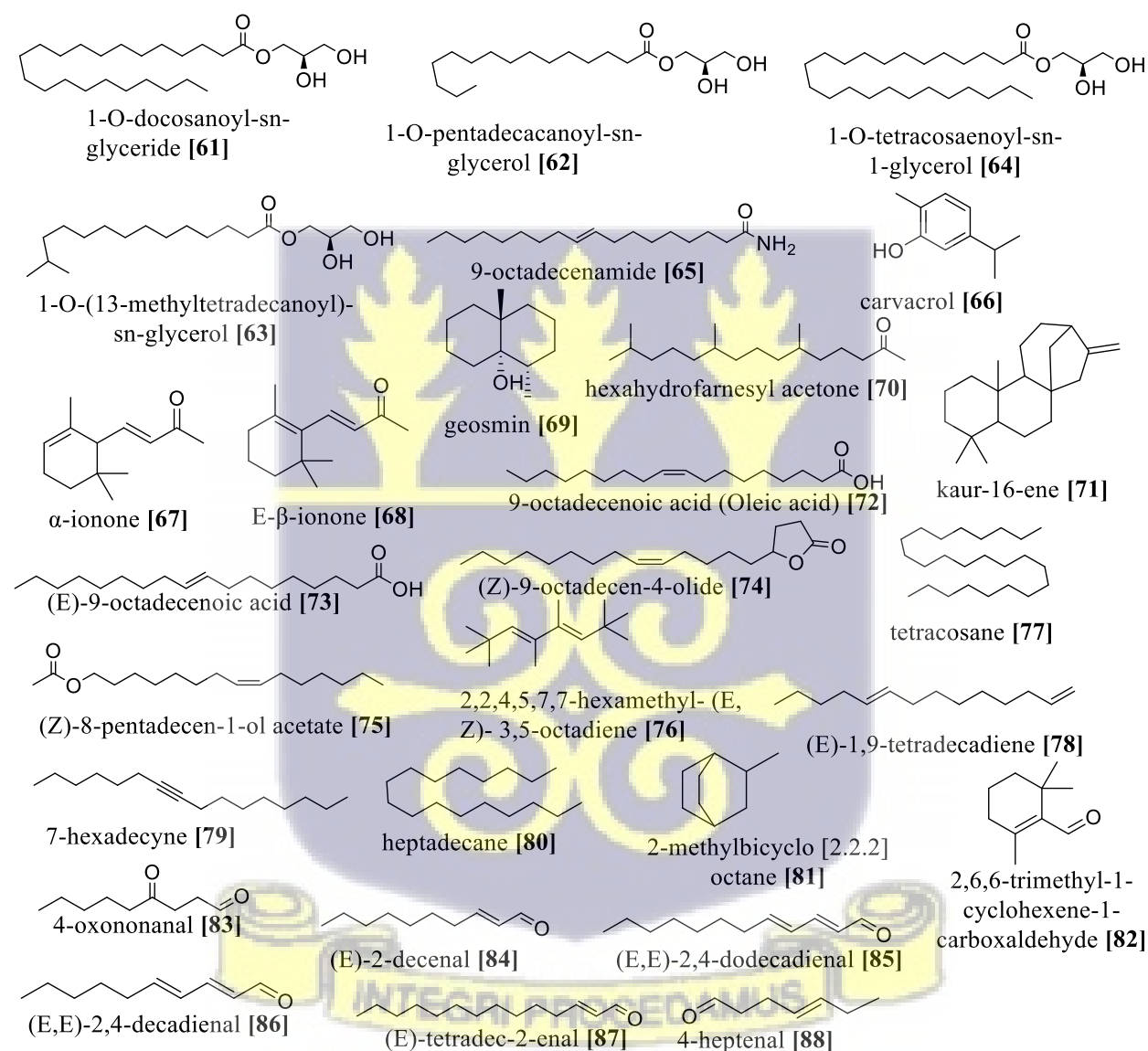


Figure 2-7 Structures of compounds identified in *Berlinia* species

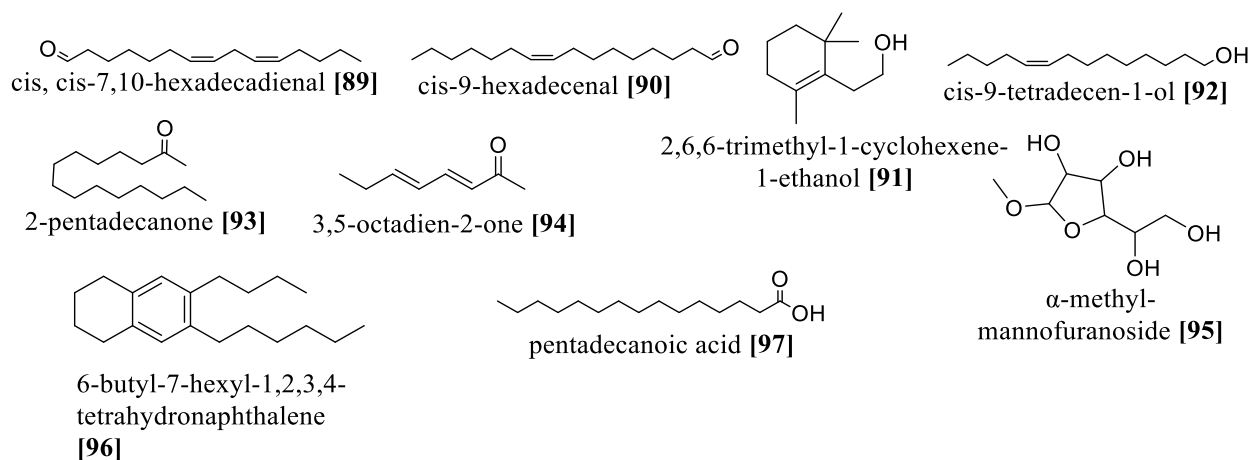


Figure 2-8 Structures of compounds identified in *Berlinia* species, contd.

2.7.5 The *Brachystegia* Genus

Currently, 32 species of the *Brachystegia* Benth. genus are recognized¹²⁹, with phytochemical analyses conducted on *Brachystegia eurycoma*, *Brachystegia nigerica*, and *Brachystegia spiciformis*. These studies have identified various classes of phytochemicals. *B. eurycoma* contains flavonoids, alkaloids, terpenoids, fatty acids, phytosterols, phenolics, benzoids, aliphatic compounds, furans, and sugar derivatives. *B. nigerica* leaves contain terpenoids, hydrocarbons, and benzoids, while its seeds are rich in fatty acids. In *B. spiciformis* seeds, alkaloids and fatty acids have been identified.

2.7.5.1 Flavonoids in *Brachystegia*

A total of eleven (11) flavonoids (Table 2-5 and Figure 2-9) have been identified in *B. eurycoma*. Three compounds, genistein [98], prunetin (5,4'-dihydroxy-7-methoxyisoflavone) [99] were isolated¹³⁰ whereas, 4',5-Dihydroxy-7-methoxyflavone [100] was identified using GC-MS¹³¹ in the stem bark. One flavonoid 3-(5-hydroxy-2-(4-hydroxyphenyl)-4-methyl-chromen-3-yl) propanoic acid [108] was isolated from the stem¹³², and seven others in the seeds; quercitrin [105],

rutin [101], catechin [104], luteolin [103], 4',5-dihydroxy-7-methoxyflavone [100], kaempferol [106] were detected with high-performance liquid chromatography (HPLC-DAD)^{133,134}, and the isolate 2-(4-ethylphenyl)-5-hydroxy-3-methyl-6,7-dihydrofurochromen-4-one [107]¹³⁵.

Table 2-6 Flavonoids in *Brachystegia* genus

Phytochemicals	Species of Plant	Plant Part	Compounds Identified	References
Flavonoid	<i>Brachystegia eurycoma</i> Harms	Stem bark	genistein [98], prunetin (5,4'-dihydroxy-7-methoxyisoflavone) [99]	130
		Stem bark	4',5-dihydroxy-7-methoxyflavone [100]	1
		seeds	rutin [101], quercetin [102], luteolin [103]	134
		seed	catechin [104], [101], quercitrin [105], [102], kaempferol [106]	133
		seeds	2-(4-ethylphenyl)-5-hydroxy-3-methyl-6,7-dihydrofurochromen-4-one [107]	135
		stem	3-(5-hydroxy-2-(4-hydroxyphenyl)-4-methyl-chromen-3-yl) propanoic acid [108]	132

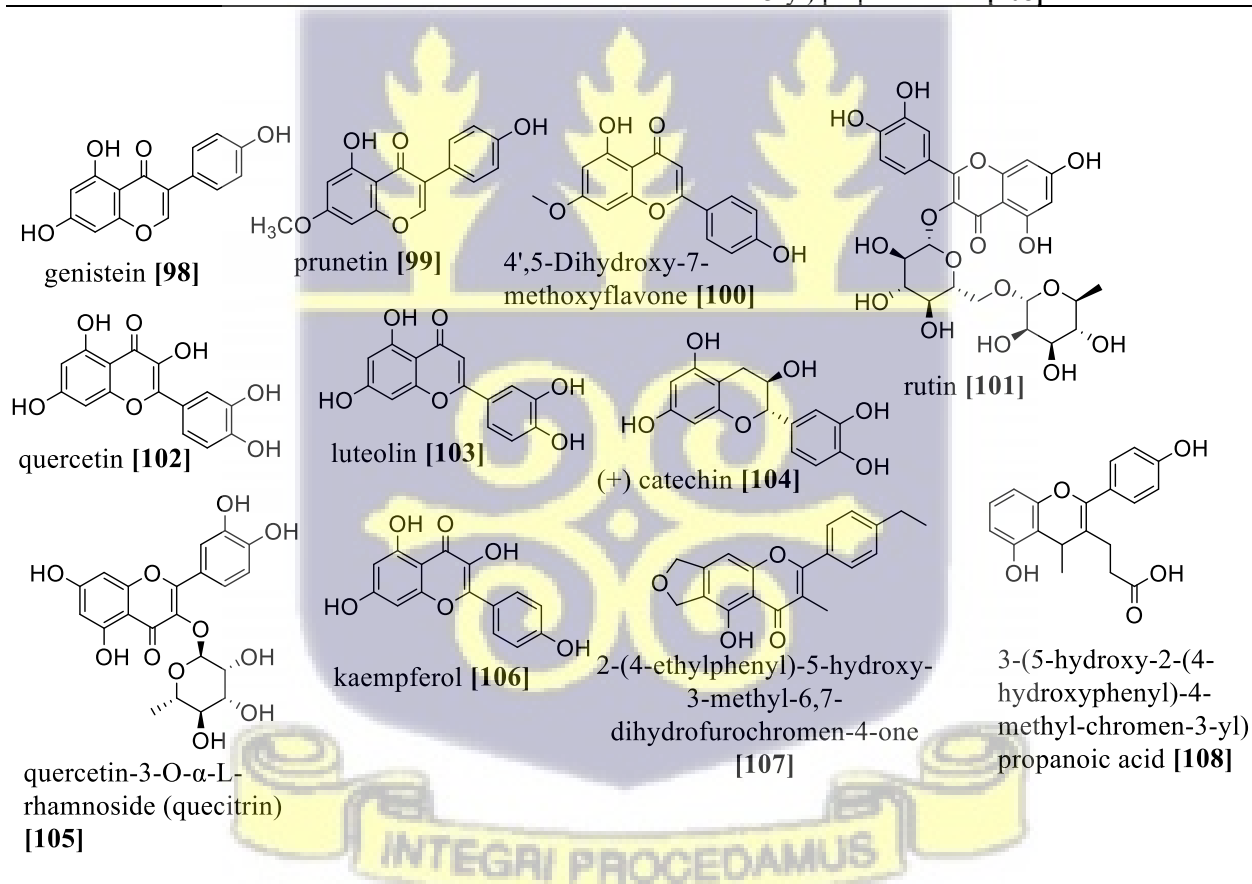


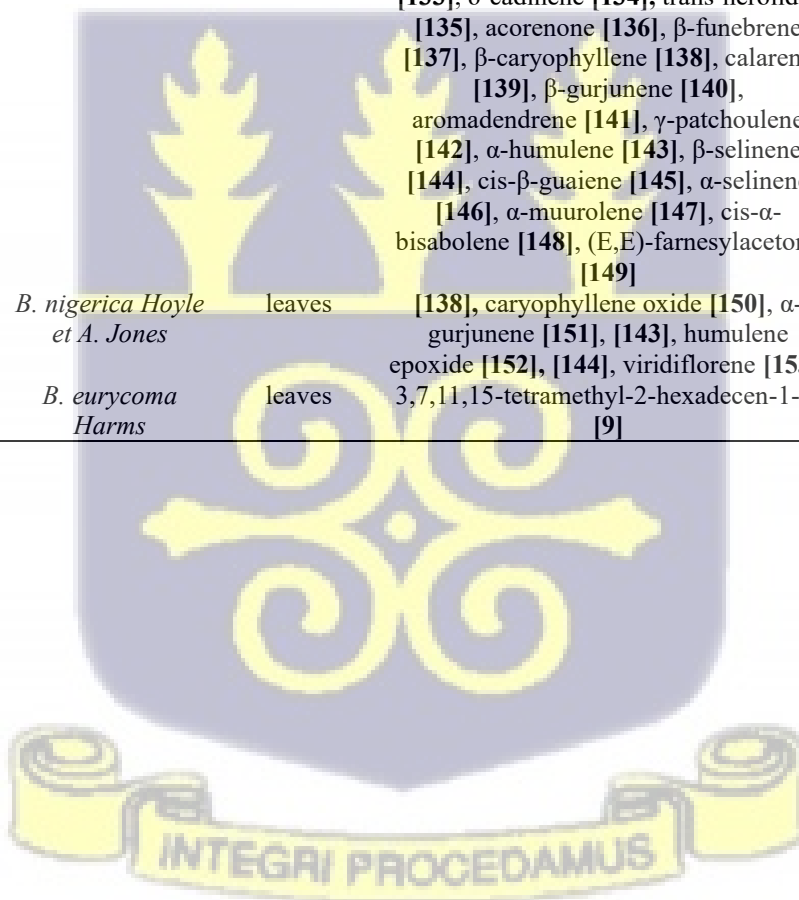
Figure 2-9 Structures of flavonoids identified in *Brachystegia eurycoma*

2.7.5.2 Terpenoids in *Brachystegia*

Different classes of triterpenoids; monoterpenes, norisoprenoid, sesquiterpenoid, and diterpenoids were identified in the leaves of *Brachystegia eurycoma* and *Brachystegia nigerica* as indicated in the Table 2-6. Ogunbinu et al. analyzed the leaf oil of *B. eurycoma* using GC/MS. The analysis revealed the presence of seven monoterpenoids: α -pinene [109], α -phellandrene [110], p-cymene [111], 1,8-cineole [112], terpinen-4-ol [113], α -terpineol [114], geranyl acetone [115]¹³⁶; four norisoprenoids: α -ionone [67], E- β -ionone [68], geijerene [121], 6,10-dimethyl-2-undecanone [122]; twenty-nine sesquiterpenoids, including geosmin [69], hexahydrofarnesyl acetone [70], δ -elemene [123], α -copaene [124], elemene [125], alloaromadendrene [126], β -acoradiene [127], γ -gurjunene [128], β -chamigrene [129], γ -muurolene [130], germacrene d [131], γ -cadinene [132], 7-epi- α -selinene [133], δ -cadinene [134], trans-nerolidol [135], acorenone [136], β -funebrene [137], β -caryophyllene [138], calarene [139], β -gurjunene [140], aromadendrene [141], γ -patchoulene [142], α -humulene [143], β -selinene [144], cis- β -guaiene [145], α -selinene [146], α -muurolene [147], cis- α -bisabolene [148], (E,E)-farnesylacetone [149]; and the only diterpenoid, 3,7,11,15-tetramethyl-2-hexadecen-1-ol [9]¹³⁶. In similar study using GC/MS, the leaves of *B. nigerica* were also found to contain seven monoterpenoids: terpinolene [116], α -terpinene [117], myrcene [118], α -pinene [109], δ -3-carene [119], limonene [120], and p-cymene [111]; six sesquiterpenoids: β -caryophyllene [138], caryophyllene oxide [150], α -gurjunene [151], α -humulene [143], humulene epoxide [152], β -selinene [144], and viridiflorene [153]. However, neither triterpenoids nor phytosteroids were identified¹³⁷.

Table 2-7 Terpenoids Identified from *Brachystegia* genus

Phytochemicals	Plant species	Plant Part	Compounds Identified	References
Monoterpenes	<i>B. eurycoma</i> Harms	leaves	α -pinene [109], α -phellandrene [110], p-cymene [111], 1,8-cineole [112], terpinen-4-ol [113], α -terpineol [114], geranyl acetone [115]	136
	<i>B. nigerica</i> Hoyle et A. Jones	leaves	terpinolene [116], α -terpinene [117], myrcene [118], [109], δ -3-carene [119], limonene [120], [111]	137
Norisoprenoid	<i>B. eurycoma</i> Harms	leaves	α -Ionone [67], E- β -Ionone [68], geijerene [121], 6,10-dimethyl-2-undecanone [122]	1
Sesquiterpenoid	<i>B. eurycoma</i> Harms	leaves	geosmin [69], hexahydrofarnesyl acetone [70], δ -elemene [123], α -copaene [124], elemene [125], alloaromadendrene [126], β -acordiadiene [127], γ -gurjunene [128], β -chamigrene [129], γ -muurolene [130], germacrene D [131], γ -cadinene [132], 7-epi- α -selinene [133], δ -cadinene [134], trans-nerolidol [135], acorenone [136], β -funebrene [137], β -caryophyllene [138], calarene [139], β -gurjunene [140], aromadendrene [141], γ -patchoulene [142], α -humulene [143], β -selinene [144], cis- β -guaiene [145], α -selinene [146], α -muurolene [147], cis- α -bisabolene [148], (E,E)-farnesylacetone [149]	136
Diterpenoids	<i>B. nigerica</i> Hoyle et A. Jones	leaves	[138], caryophyllene oxide [150], α -gurjunene [151], [143], humulene epoxide [152], [144], viridiflorene [153],	137
	<i>B. eurycoma</i> Harms	leaves	3,7,11,15-tetramethyl-2-hexadecen-1-ol [9]	136



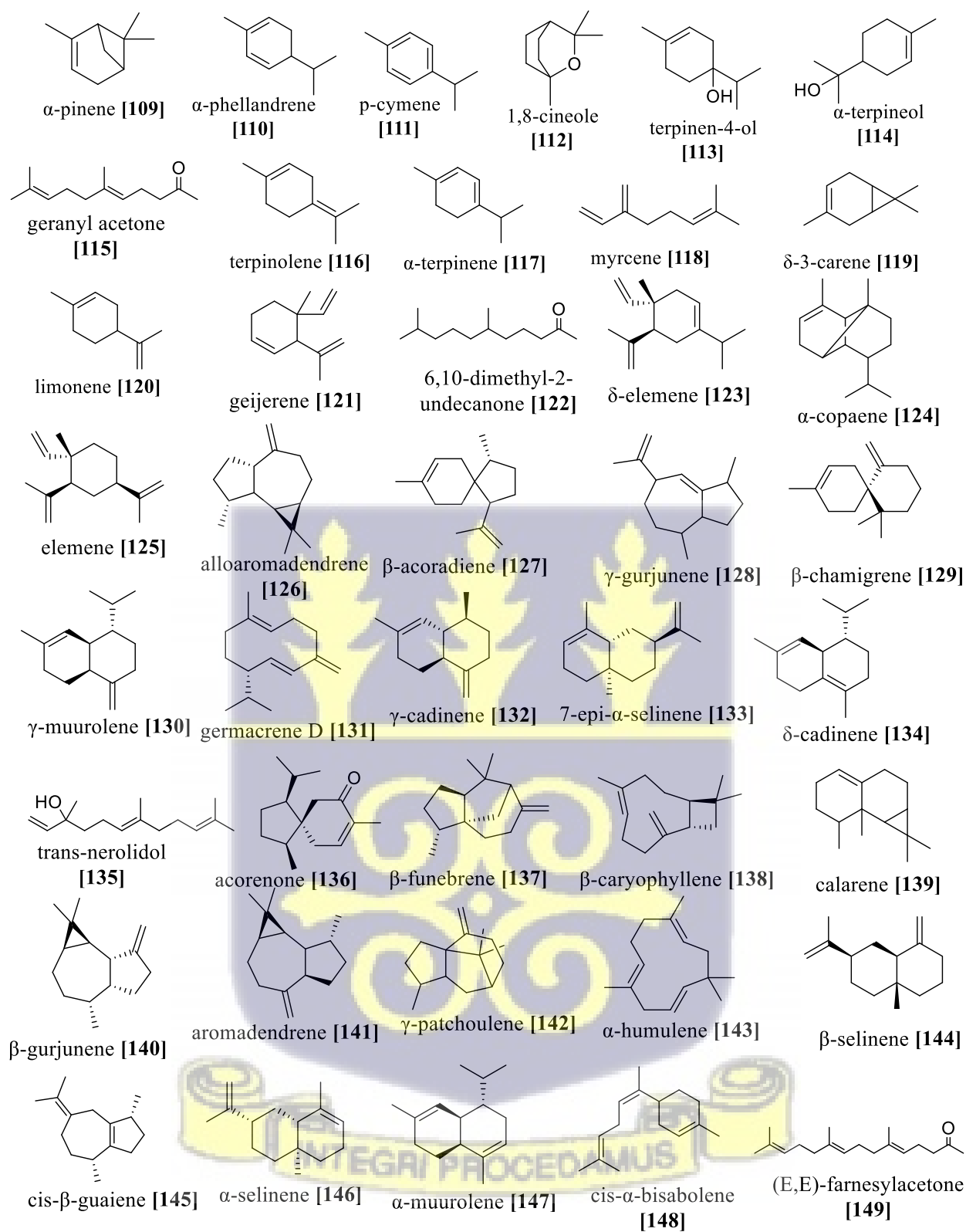


Figure 2-10 Structures of terpenoids identified in *Brachystegia species*

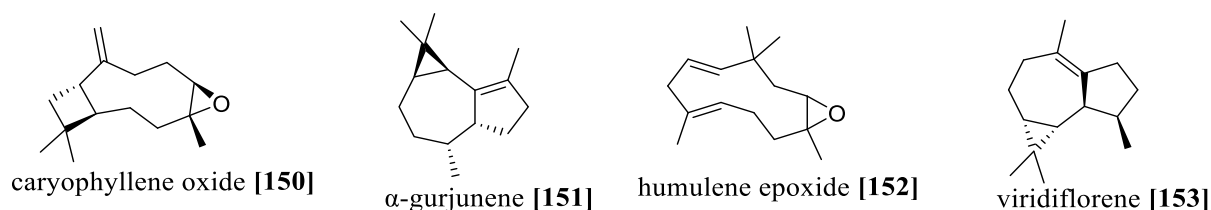


Figure 2-11 Terpenoids in *Brachystegia* species contd.

2.7.5.3 Unsaturated and Saturated Fatty acids in *Brachystegia*

The identification of compounds in *B. eurycoma* was performed using GC-MS, whereas the analysis of *B. nigerica* utilized gas-liquid chromatography. Table 2-7 and figure 2-12 summarizes the fatty acids identified in both *B. eurycoma* and *B. nigerica*. The seeds of *B. eurycoma* were found to contain several unsaturated fatty acids, including 9,12-octadecadienoic acid ethyl ester [154], 9,12-octadecadienoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester [155], and 9-octadecenoic acid ethyl ester [156]¹³⁸. Further analysis of the seeds revealed additional compounds such as 9,12-octadecadienoic acid (linoelaidic acid) [11], 9-octadecenoic acid (Oleic acid) [72], linolenic acid [158], and erucic acid [159]¹⁰⁵. In the stem extract, four unsaturated fatty acids, 9,12-octadecadienoic acid (linoelaidic acid) [11], 9-octadecenoic acid (Oleic acid) [72], [156], and 7-hexadecenoic acid methyl ester [157], were identified, along with two saturated fatty acids, hexadecanoic (palmitic) acid [15] and hexadecanoic acid ethyl ester [17]^{131,138}. The seeds of *B. eurycoma* also contained several saturated fatty acids and their derivatives, including octadecanoic acid [14], [15], [17], docosanoic (behenic) acid [161], tetracosanoic acid [162], ethyl tetracosanoate [163], eicosanoic (arachidic) acid [164], and docosanoic acid ethyl ester [165]¹³⁸. Additional analyses confirmed the presence of saturated fatty acids such as [15], [14], [164], octanoic (caprylic) acid [166], decanoic (capric) acid [167], margaric acid [168], tetradecanoic

(myristic) acid [13], [15], [14], methyl tetradecanoate [169], and hexadecanoic acid methyl ester [170]¹³⁹. The stem also contained [15] and [17]¹³⁸. In *B. nigerica*, the fatty acid composition has only been documented in the seeds. These analyses identified three unsaturated fatty acids, 9,12-octadecadienoic acid (linoelaidic acid) [11], 9-octadecenoic acid (Oleic acid) [72], and arachidonic acid [160], along with five saturated fatty acids, including [13], [14], [15], [164], and dodecanoic (lauric) acid [171]¹⁴⁰.

Table 2-8 Fatty acids Identified in *Brachystegia* Genus

Phytochemicals	Plant species	Plant Part	Compounds Identified	References
Unsaturated fatty acids and esters	<i>B. eurycoma</i>	Seeds	9,12-Octadecadienoic acid ethyl ester [154]; 9,12-Octadecadienoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester [155]; 9-Octadecenoic acid ethyl ester [156]	138
		Stem bark	9,12-octadecadienoic acid (linoelaidic acid) [11], 9-octadecenoic acid (oleic acid) [72], [156], 7-hexadecenoic acid, methyl ester [157]	131
		seed	[11], [72], linolenic acid [158], erucic acid [159]	139
Saturated fatty acids and esters	<i>B. nigerica</i>	Seed	[11], [72], arachidonic acid [160]	140
	<i>B. eurycoma</i>	Seeds	octadecanoic acid [14], hexadecanoic (palmitic) acid [15], hexadecanoic acid ethyl ester [17], docosanoic (behenic) acid [161], tetracosanoic acid [162], ethyl tetracosanoate [163], eicosanoic (arachidic) acid [164], docosanoic acid ethyl ester [165]	138
		Stem	[15], [17]	131,138
		Seed	[15], [14], [164], octanoic (caprylic acid) [166], decanoic (capric) acid [167], margaric acid [168]	139
		seed	tetradecanoic (myristic) acid [13]; [15], [14], methyl tetradecanoate [169]; hexadecanoic acid, methyl ester [170];	141
	<i>B.nigerica</i>	Seed	[13], [14], [15], [164], dodecanoic (lauric) acid [171]	140

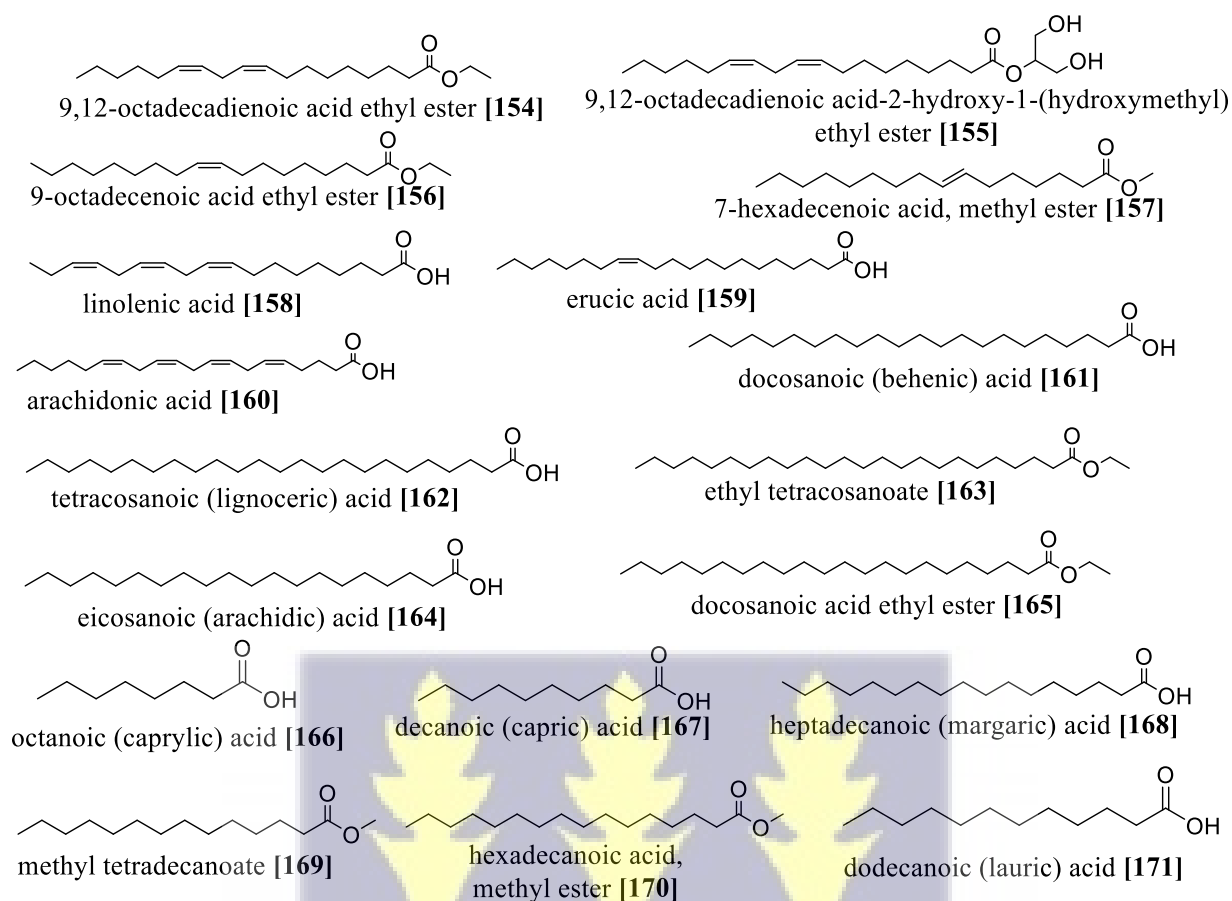


Figure 2-12 Structures of fatty acids identified in *Brachystegia* species.

2.7.5.4 Phenolic and Benzoid compounds in *Brachystegia*

Four natural products, gallic acid [172], caffeic acid [173], ellagic acid [174], chlorogenic [175]^{133,134}, and three O-hydroxyphenol [176], 1,2,3-trihydroxybenzene [177], 2-hydroxy-5-methylisophthalaldehyde [178]¹³¹ phenolic compounds were identified using reverse-phase high-performance liquid chromatography (HPLC-DAD) on the seed extract and GC-MS on the stem bark extract of *B. eurycoma* respectively. In another study by Igwe et al., the compound bis-6,6-methylene bis (4-tert-butyl-2-methylphenol [179] was isolated and characterized from the its stem¹⁴². Other benzene derivative compounds, (benzoids), were also identified in both *B. eurycoma* and *B. Nigerica* as shown Table 2.8. Only two benzoids, cumene [184], and mesitylene

[185] were identified in the latter. The former showed three compounds; 1,2-benzenedicarboxylic acid [180], and 4- (4-phenyl-1,4-dihydronaphthalen-1-yl) pentenoic acid [181] in the stem bark, and one 1,3-dimethylnaphthalene [182] in its leaves as shown in Table 2-8.

Table 2-9 Phenolic and Benzoid compounds in the *Brachystegia* genus

Phytochemicals	Plant species	Plant Part	Compounds Identified	References
Phenolic compounds	<i>B. eurycoma</i>	Seeds	Gallic acid [172], Caffeic acid [173], Ellagic acid [174]	134
		seeds	[172], [173], [174], chlorogenic [175]	133
		Stem bark	O-Hydroxyphenol [176], 1,2,3-Trihydroxybenzene [177], 2-Hydroxy-5-methylisophthalaldehyde [178]	131
		stem	bis-6,6-methylenebis(4-tert-butyl-2-methylphenol [179]	142
Benzene derivatives (Benzoid compounds)	<i>B. eurycoma</i>	Stem bark	1,2-benzenedicarboxylic acid [180]	1
		Stem bark	4- (4-phenyl-1,4-dihydronaphthalen-1-yl) pentenoic acid [181]	143
		leaves	1,3-dimethylnaphthalene [182]	136
		Stem bark	ethyl 2-hydroxybenzyl sulfone [183]	138
		leaves	cumene [184], mesitylene [185]	137

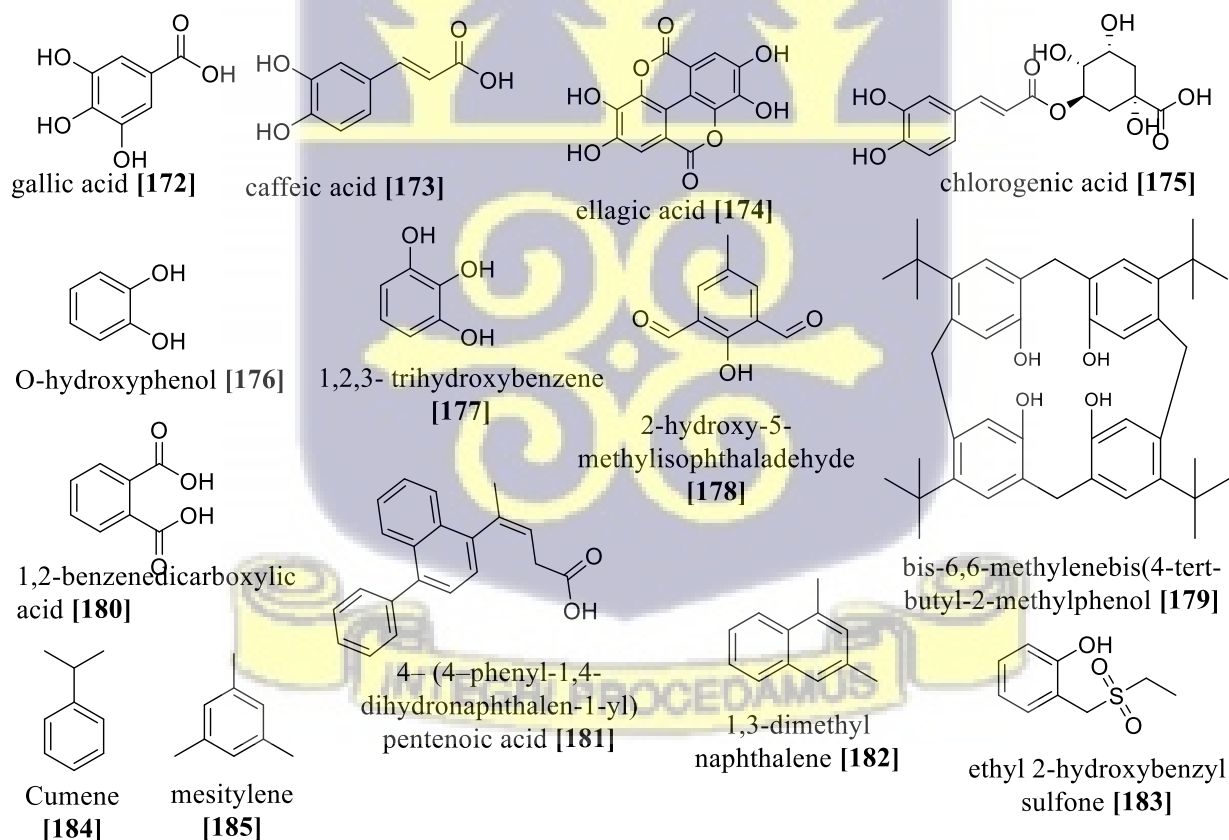


Figure 2-13 Structures of phenolic and benzoid compounds identified in the *Brachystegia* species.

2.7.5.5 Other Phytochemicals in *Brachystegia*

A total of eight alkaloids have been identified in *B. eurycoma*: five in the stems and three in the seeds, whereas one alkaloid has been found in *B. spiciformis*. The crude extract of *B. eurycoma* stem bark was analyzed and confirmed to contain the alkaloid caffeine [187] through mass spectrometric (MS) analysis¹³⁰. GC/MS of the same plant part also confirmed the presence of 9-octadecenamide [186], (4-(2-methylpiperidine-1-sulfonyl)-phenyl)-(2-methylpiperidin-1-ynl)-methanone [188], N,N-diethyl-4-(morpholine-4-sulfonyl)-benzenesulfanamide [189] 1,2,4-trimethyl-3-nitro-3,3,1-nonan-9-one [190]¹³¹. A subsequent GC/MS study on the seeds by Ahamefula et al. revealed the presence of the compounds 1-hexanamine, 3,5,5-trimethyl- [191]; 1-dodecanamine [192]; and (5R, 8aR)-5-propyloctahydroindolizine [193]¹⁴¹, while the seeds of *B. spiciformis* contain dihydroxy-2,2-bis(6-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl)propanal [195] in the stem¹⁴⁵ and 4-hydroxy-2-(1-p-tolyethylidene)-5,6-dihydrofuro-3-one [196] in the seeds¹⁴⁶. Sugars such as α -methyl-4-O-methyl-D-mannoside [197]¹⁴¹ have been identified from the seeds, as well as, 1,6-anhydro- β -D-glucopyranose [198] and 2-O-methyl-D-mannopyranose [199] from the stem using GC/MS¹³⁸. Similar studies revealed the presence of the aliphatic hydrocarbons nonane [200] from the leaves of *B. nigerica*¹³⁷ and tetradecane [201] from the leaves of *B. eurycoma* Harms¹³⁶. The aldehyde butanal-4-hydroxy-3-methyl- [202] has been detected in the stem bark of *B. eurycoma*¹³¹, while the alcohol 2-penten-1-ol, (Z)- [203] was identified in its seeds¹⁴¹.

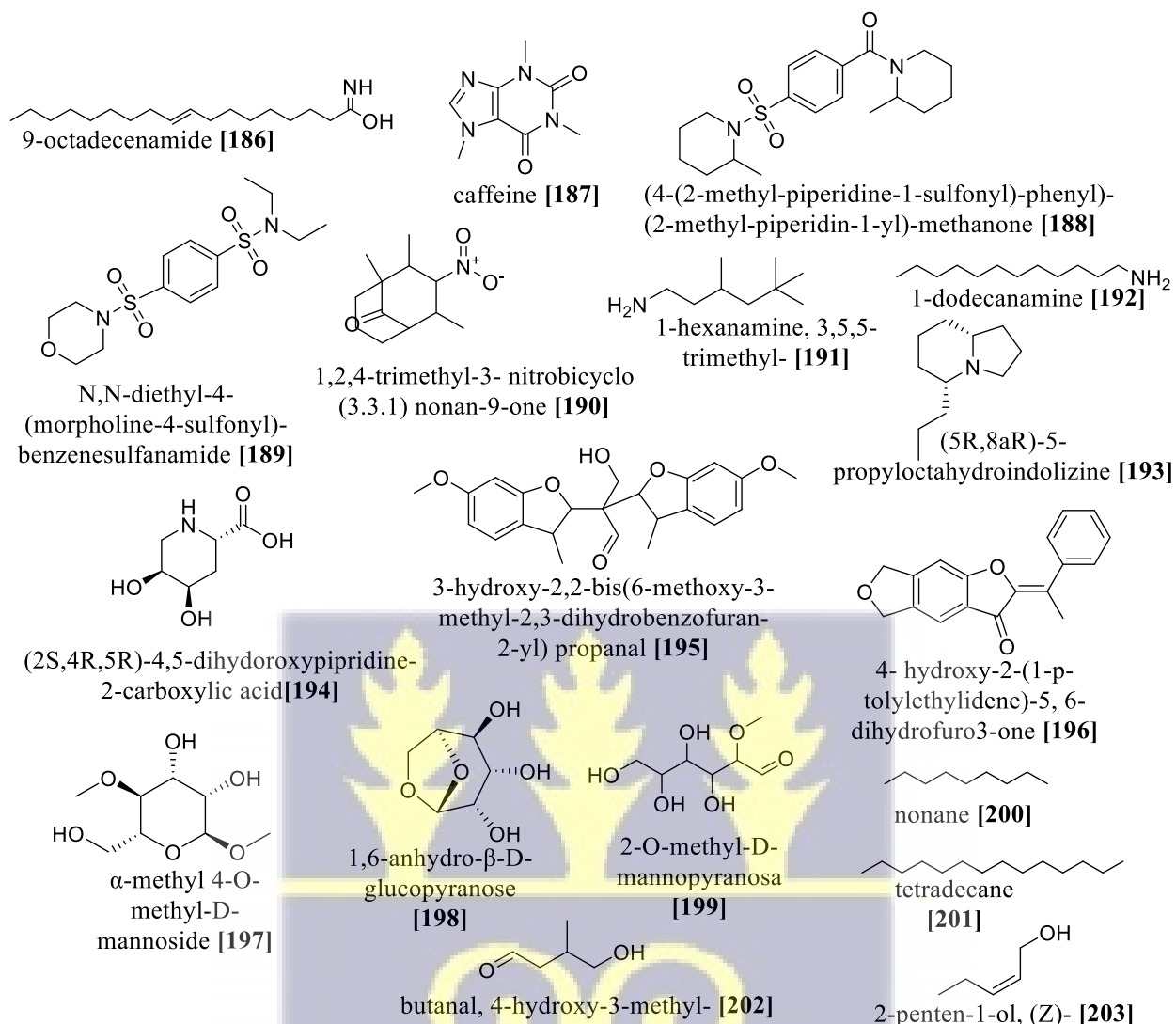


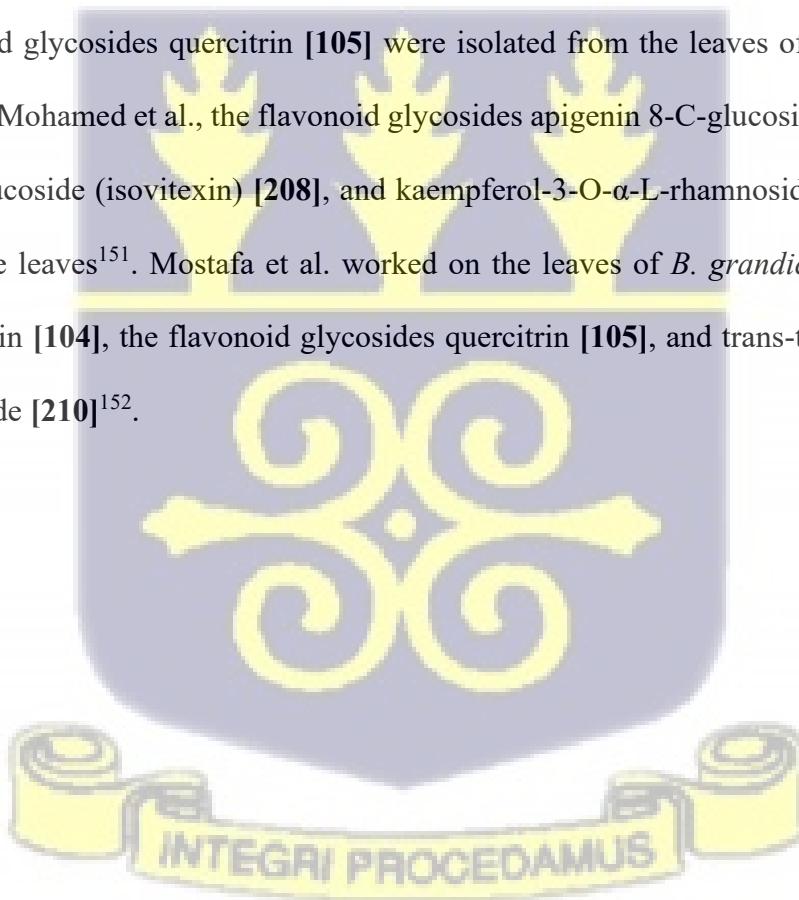
Figure 2-14 Structures of other phytocompounds in phenolic and benzoid compounds identified the *Brachystegia* species.

2.7.6 The Brownea Genus

The *Brownea* Jacq. genus (also known as *Hermesias* Loefl.) comprises 22 accepted species with its native range in Tropical America¹⁴⁷. Of the 22 species under the *Brownea* genus, only three species have undergone phytochemical analysis: *Brownea ariza*, *Brownea grandiceps* and *Brownea macrophylla*.

2.7.6.1 Anthocyanins, Tannins and Flavonoids in *Brownea* Genus

Stashenko et al. performed phytochemical studies on the flowers of *Brownea ariza*, which led to the identification of three anthocyanins using Liquid Chromatography-Mass Spectrometer- Time of Flight (LC-MS-TOF). These included pelargonidin-3,5-diglucoside [204], pelargonidin-3-glucoside [205], and peonidin [206]¹⁴⁸. The only documented tannin in this genus is browplasminin, which consists predominantly of heteroflavan-3-ols of catechin with B-type linkages, extending up to heptadecamers (~5000 Da). It was isolated using HPLC from the flowers of *B. grandiceps* and characterized by MALDI-ToF MS¹⁴⁹. One flavonoid and four flavonoid glycosides have been isolated from the leaves of *Brownea ariza*. The flavonoid quercetin [102] and the flavonoid glycosides quercitrin [105] were isolated from the leaves of *B. ariza*¹⁵⁰. In a similar study by Mohamed et al., the flavonoid glycosides apigenin 8-C-glucoside (vitexin) [207], apigenin 6-C-glucoside (isovitexin) [208], and kaempferol-3-O- α -L-rhamnoside [209] were also isolated from the leaves¹⁵¹. Mostafa et al. worked on the leaves of *B. grandiceps* to isolate the flavonoid catechin [104], the flavonoid glycosides quercitrin [105], and trans-taxifolin-3-O- α -L-rhamnopyranoside [210]¹⁵².



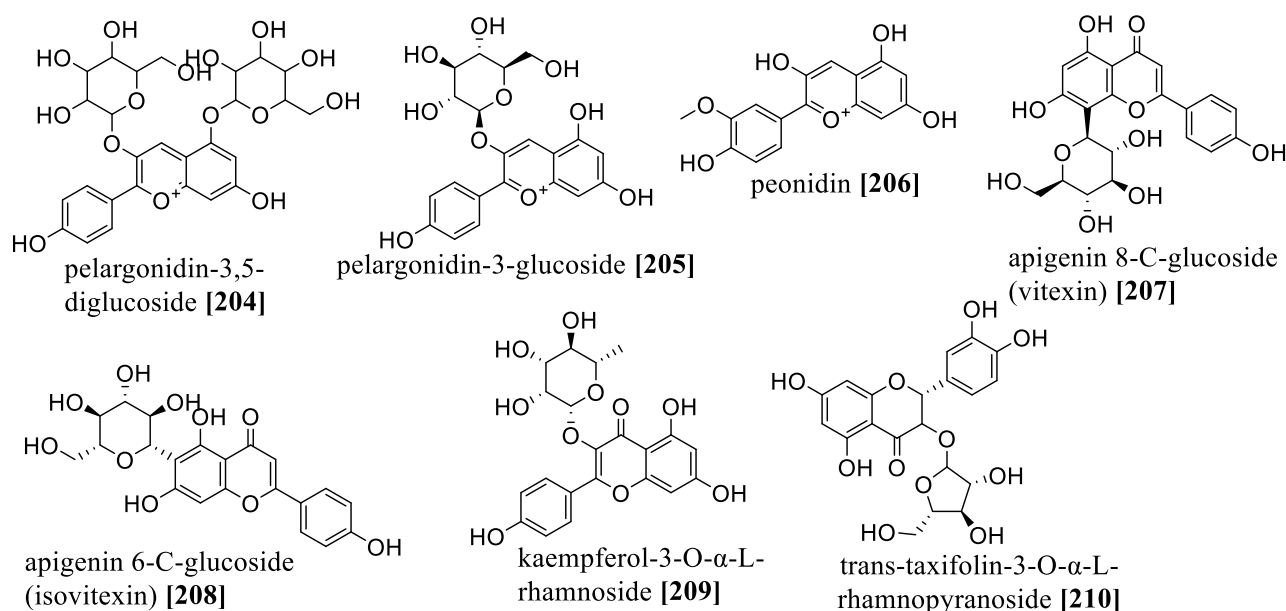


Figure 2-15 Structures of Anthocyanins, Tannins and Flavonoids in *Brownea* Genus

2.7.6.2 Terpenoids identified in *Brownea* genus

The GC/MS analyses of the stem and leaves of *Brownea grandiceps* by Korany et al. revealed the presence of six monoterpenoids in the stems; three monoterpenoids, two norisoprenoids, a sesquiterpenoid, and a diterpenoid in the leaves; as well as a triterpenoid found in both plant parts. These monoterpenoids include terpinen-4-ol [113], α -terpineol [114], β -linalool [211], linalool oxide [212], nerol [213], and anethole [214] in the stem¹⁵³, and compounds such as limonene [120], β -linalool [211], and geranyl acetone [115] in the leaves¹⁵³. The norisoprenoids α -ionone [215], and E- β -ionone [216], the sesquiterpenoid (E,E)-farnesylacetone [149], and the diterpenoid 3,7,11,15-tetramethyl-2-hexadecen-1-ol [9] have also been found in the leaves. At the same time, triterpenoid squalene [19] was found to be present in both the leaves and stems¹⁵³.

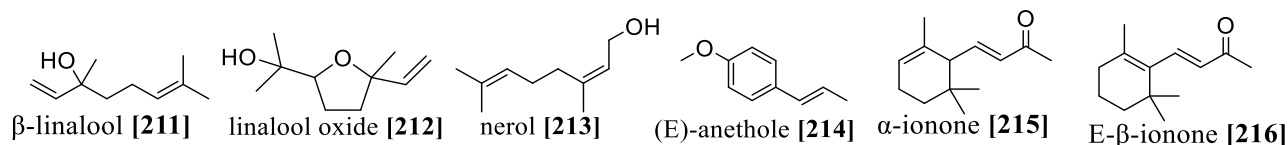


Figure 2-16 Structures of terpenoids in *Brownea* species.

2.7.6.3 Aliphatic Compounds in *B. grandiceps*

GC-MS analyses done on the leaves and stems of *B. grandiceps*, by Korany et al., detected various aliphatic compounds such as hydrocarbons, aldehydes, alcohols and ketones¹⁵³. Table 2-9 below illustrates the different phytoconstituents. The phytochemical analysis of *Brownea grandiceps* revealed a diverse array of compounds, including aliphatic hydrocarbons, aldehydes, alcohols, and ketones, distributed across different plant parts. In the leaves, the aliphatic hydrocarbons identified were 2-methylheptane [217], (Z)-1,3-dimethylcyclohexane [218], (E)-1,4-dimethylcyclohexane [219], 5-tert-butyl-1,3-cyclopentadiene [220], n-octane [221], (Z)-1,4-dimethylcyclohexane [222], n-heneicosane [223], n-tricosane [224], Tetracosane [77], n-pentacosane [225], n-hexacosane [226], n-heptacosane [227], n-octacosane [228], n-nonacosane [229], 2-methyltriacontane [230], and 3-methylnonacosane [231]¹⁵³. In the stems, the hydrocarbons included (Z)-1,3-dimethylcyclohexane [218], (E)-1,4-dimethylcyclohexane [219], 2-methylheptane [217], 5-tert-butyl-1,3-cyclopentadiene [220], n-octane [221], (Z)-1,4-dimethylcyclohexane [222], n-tricosane [224], n-pentacosane [225], n-hexacosane [226], n-heptacosane [227], and n-nonacosane [229]¹⁵³. Aliphatic aldehydes identified in the leaves included 2-hexenal, (E)- [232], hexanal [233], n-nonanal [234], 2,6-nonadienal, (E,Z)- [235], 2-nonenal, (E)- [236], decanal [237], (E, E)-2,4-Decadienal [86], 4-Heptenal [88], heptanal [238], 2,4-hexadienal, (E,E)- [239], 2,4-heptadienal, (E,E)- [240], n-pentadecanal [241], (Z,Z)-7,10,-hexadecadienal [242], and (Z,Z,Z)-7,10,13-hexadecatrienal [243]¹⁵³. The stems contained some overlapping aldehydes, including 2-hexenal,

(E)- [232], hexanal [233], n-octanal [244], n-nonanal [234], decanal [237], [88], heptanal [238], E-2-Decenal [84], and n-pentadecanal [241]¹⁵³. Aliphatic alcohols were also detected, with the leaves containing 2-penten-1-ol, (Z)- [203], 2-hexyn-1-ol [245], 3-hexen-1-ol, (Z) [246], 2-hexen-1-ol, (Z)- [247], and 1-hexanol [248]¹⁵³. The stems contained 2-hexyn-1-ol [245], 3-hexen-1-ol, (Z) [246], 2-hexen-1-ol, (Z)- [247], 1-hexanol [248], and 1-pentanol [249]¹⁵³. Ketones were identified in both leaves and stems. In the leaves, the ketones included 2-hexanone [250] and 2-pentadecanone, 6,10,14-trimethyl [251], while the stems contained 2-hexanone [250], 2-heptanone [252], and 6-methyl-5-heptene-2-one [253]¹⁵³. These findings highlight the phytochemical diversity of *Brownea grandiceps*, emphasizing its potential for further investigation into its chemical and biological significance.

Table 2-10 Aliphatic compounds identified in *Brownea grandiceps*

Phytochemicals	Plant Part	Compounds Identified	References
Aliphatic hydrocarbons	Leaves	2-methylheptane [217]; (Z)-1,3-dimethylcyclohexane [218]; (E)-1,4-dimethylcyclohexane [219]; 5-tert-butyl-1, 3-cyclopentadiene [220]; n-octane [221]; (Z)-1,4-dimethylcyclohexane [222]; n-heneicosane [223]; n-tricosane [224]; tetracosane [77]; n-pentacosane [225]; n-hexacosane [226]; n-heptacosane [227]; n-octacosane [228]; n-nonacosane [229]; 2-methyl triacontane [230]; 3-methyl nonacosane [231]	153
	Stems	[218]; [219]; [217]; [220]; [221]; [222]; [224]; [225]; [226]; [227]; [229]	153
Aliphatic aldehydes	leaves	2-hexenal, (E)- [232]; hexanal [233]; n-nonanal [234]; 2,6-nonadienal, (E, Z)- [235]; 2-nonenal, (E)- [236]; decanal [237]; (E, E)-2,4-Decadienal [86]; 4-Heptenal [88]; heptanal [238]; 2,4-hexadienal, (E,E)- [239]; 2,4-heptadienal, (E,E)- [240]; n-pentadecanal [241]; (Z,Z)-7,10,-hexadecadienal [242]; (Z,Z,Z)-7,10,13-hexadecatrienal [243]	153
	stems	[232]; [233]; n-octanal [244]; [234]; [237]; [88]; [238]; E-2-Decenal [84]; [241]	153
Aliphatic alcohol	Leaves	2-penten-1-ol, (Z)- [203]; 2-hexyn-1-ol [245]; 3-hexen-1-ol, (Z) [246]; 2-hexen-1-ol, (Z)- [247]; 1-hexanol [248]	153
	Stem	[245]; [246]; [247]; [248], 1-pentanol [249]	153
Ketones	Leaves	2-hexanone [250]; 2-pentadecanone, 6,10,14-trimethyl [251]	153
	Sems	[250]; 2-heptanone [252]; 6-methyl-5-heptene-2-one [253]	153

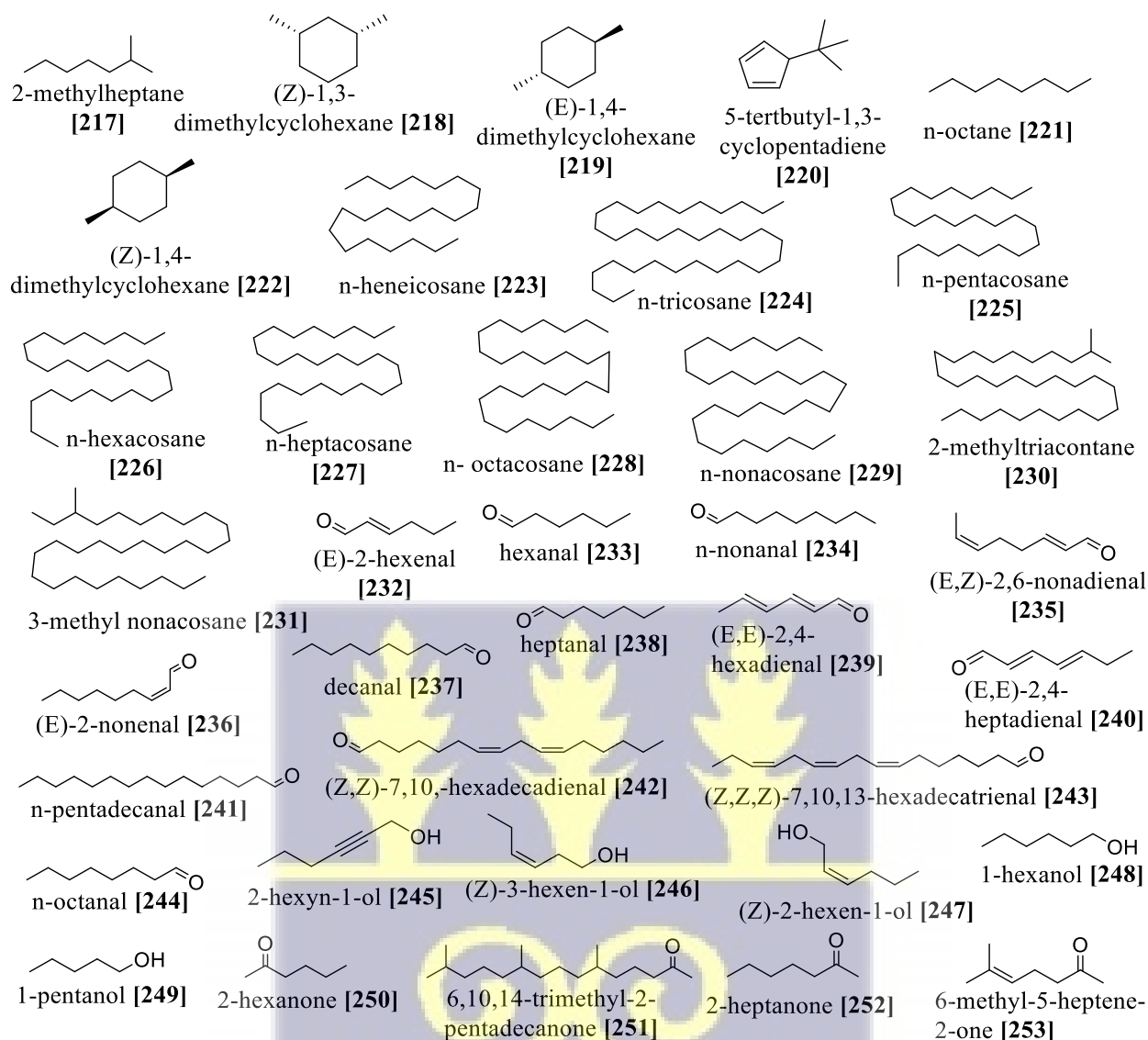


Figure 2-17 Structures of aliphatic compounds identified from the *Brownea grandiceps*

2.7.6.4 Other Compounds in *Brownea* Genus

In *Brownea grandiceps*, furans such as (E)-2-(2-pentenyl)-furan [254] and 2-pentyl-furan [255] were detected using GC-MS in the leaves, with the latter, 2-pentyl-furan [255], also present in the stems¹⁵³. Additionally, carotenoids in the flowers of *B. macrophylla*, including luteoxanthin [256], β -cryptoxanthin [257], and β -carotene [258] were determined by liquid chromatography (Rapid

Resolution Liquid Chromatography (RRLC) and Ultra-High Performance Liquid Chromatography (UHPLC)¹⁵⁴. In *Brownea ariza*, the unsaturated fatty acid 3,7,9,10-tetramethylundec-2-enyl palmitate [259] was isolated in the leaves, alongside the phenolic compound 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)propan-1-one [260]¹⁵¹. In *Brownea grandiceps*, the leaves yielded the unsaturated fatty acids 13R-hydroxy-9Z,11E-octadecadienoic acid [261] and 13R-hydroxy-9Z,11E,15Z-octadecatrienoic acid [262]¹⁵², while both leaves and stems contained butyl ethanoate [263], methyl salicylate [264], and the benzene derivative toluene [265] when analyzed with GC-MS¹⁵³. These findings underscore the diverse chemical profiles within the *Brownea* genus.

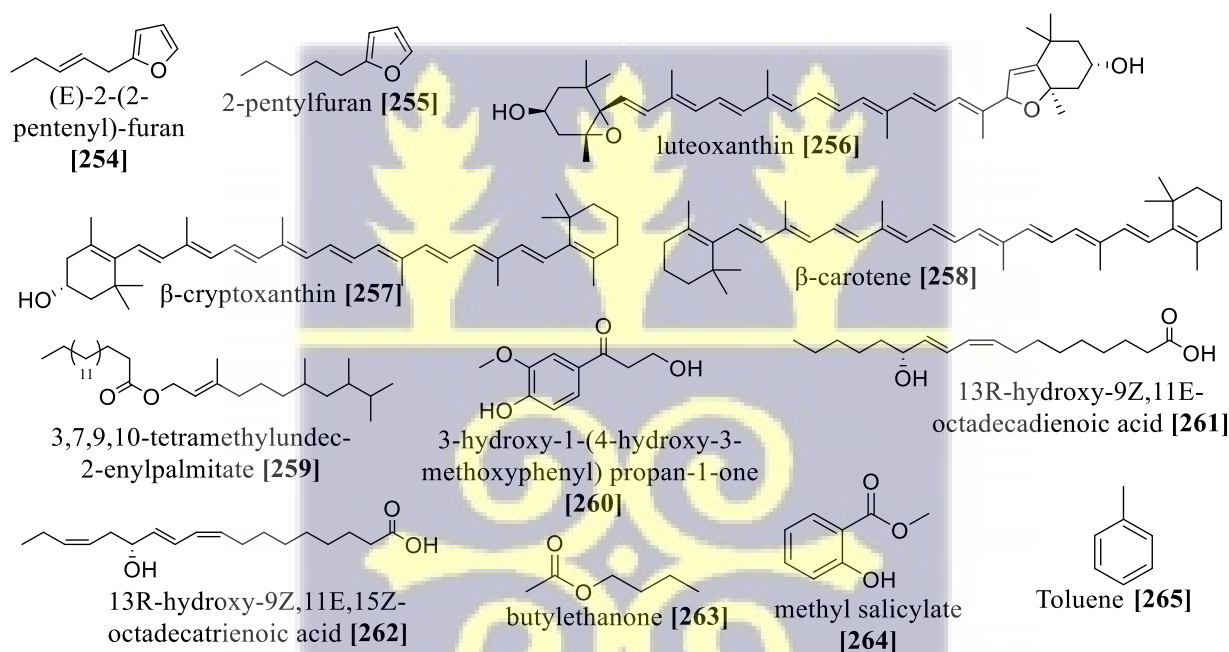


Figure 2-18 Structures of other compounds in *Brownea* species

2.7.7 The *Cynometra* Genus

The *cynometra* L. genus (also known as *Iripa* Adans., *Maniltoa* Scheff., *Metrocynia* Thouars, *Pseudocynometra* Kuntze, or *Schizosiphon* K.Schum.) contains 113 species¹⁵⁵. Out of these, only six species have gone under phytochemical analysis to identify compounds in the various part of

the plants. These species are; *Cynometra cauliflora*, *Cynometra hankei*, *Cynometra vogelii*, *Cynometra iripa*, *Cynometra megalophylla* Harms, and *Cynometra Ramiflora* Linn.

2.7.7.1 Tannins in the *Cynometra* Genus

The tannins procyanidin trimer [266], procyanidin tetramer [267], procyanidin pentamer [268], procyanidin hexamer [269], procyanidin B2 [270] were detected in the leaves of *C. cauliflora* and *C. iripa* using liquid chromatography-diode array detection-electrospray ionization-tandem mass spectrometry and chemical LC-UV/DAD-ESI/MS respectively¹⁵⁶. The compounds are illustrated in figure 2-19 below. The bark of *C. iripa* was also found to contain a similar tannin profile to *C. cauliflora*. The main tannins identified in *C. iripa* leaf extracts using LC-UV/DAD-ESI/MS were characterized as B-type proanthocyanidins, detected through RDA fragmentation. The chromatograms showed 11 major peaks, which included four peaks for monomeric catechin units formed through retro-Diels–Alder (RDA) fragmentation, consistent with B-type proanthocyanidin tetramers, trimers, and pentamers (two isomers)¹⁵⁷. Additional B-type proanthocyanidins were observed in the bark extracts, including tetrameric (two isomers), trimeric, and dimeric forms (two isomers). These findings highlight the presence of diverse tannin structures, particularly dimeric and oligomeric proanthocyanidins, in both leaf and bark extracts¹⁵⁷.



Tannins

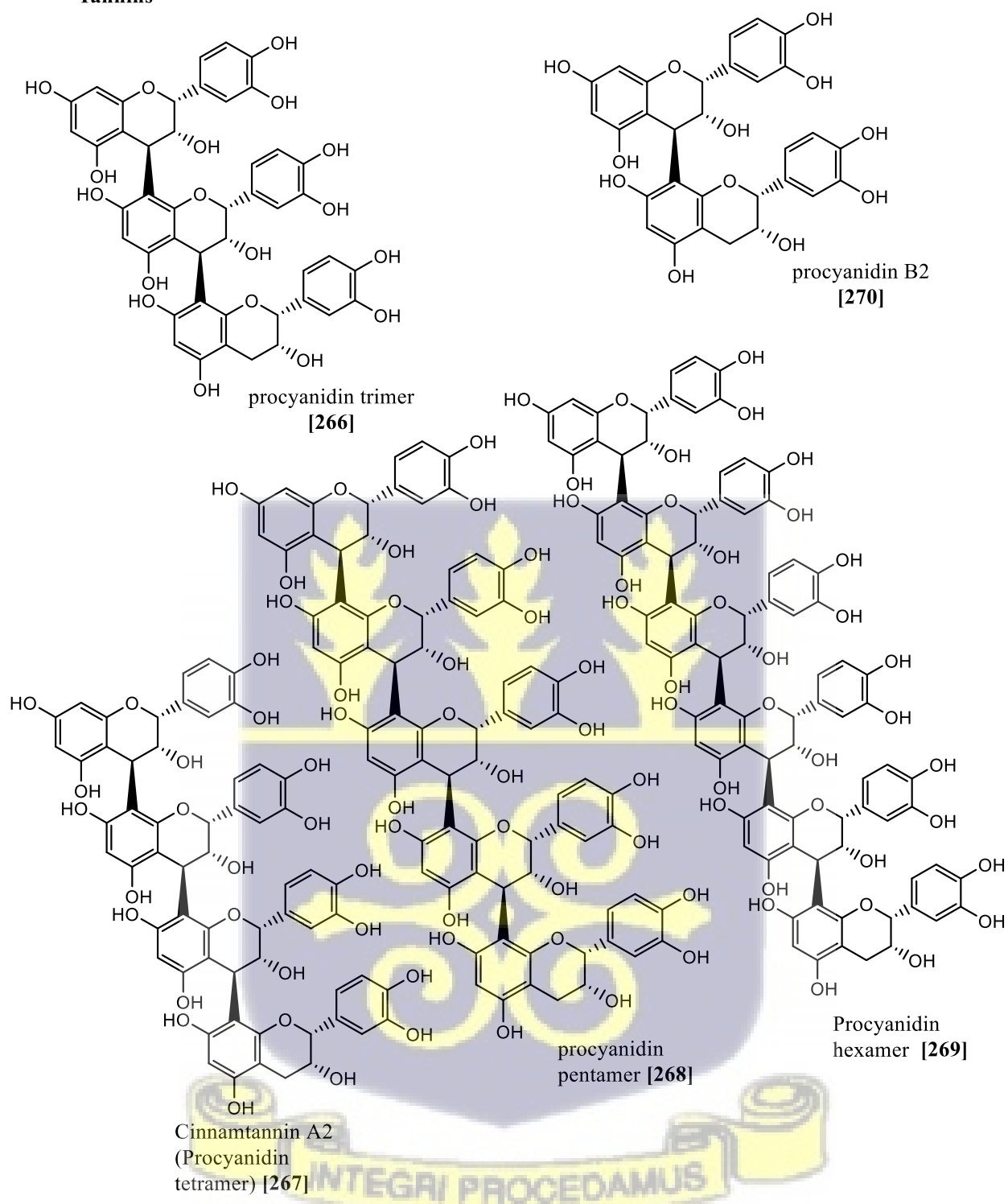


Figure 2-19 Structures of tannins in *Cynometra* species.

2.7.7.2 Flavonoids in *Cynometra*

Using LC-DAD (liquid chromatography with a diode array detector) and LC-MS/MS (liquid chromatography with tandem mass spectrometry), *C. cauliflora* leaves were found to contain four flavonoid compounds; apigenin 8-C-glucoside (vitexin) [207], apigenin 6-C-glucoside (isovitexin) [208], apigenin-6-C-glucoside-8-C-glucoside [271], and catechin [104]¹⁵⁶, in addition to, two pentosides (taxifolin pentoside [272] and quercetin pentoside [273]), and four hexosides (kaempferol hexoside [274], quercetin hexoside [275], kaempferol-coumaroyl hexoside [276], and isorhamnetin hexoside [277])¹⁵⁶. Another phytochemical analysis done on its leaves by Sumarlin et. al in 2020 led to the isolation of other flavonoids; apigenin [278], naringenin [282], amorphenin [283], nobiletin [284], isorhamnetin [285], and epigallocatechin gallate [286]¹⁵⁸. The flavonoid luteolin [103]¹⁵⁹⁻¹⁶¹ and apigenin [278]^{159,161}, eriodictyol [279]^{160,161}, naringenin [282]¹⁶¹ were isolated from the twigs have also been documented to contain the flavonoids acacetin [287], 3',4',7-trihydroxyflavone [288], luteolin 3',5 dimethyl ether [289], 4',7-dihydroxyflavone [290]^{160,161} whereas eriodictyol [279] and apigenin [278] were only isolated from the stems¹⁶². Another phytochemical analysis done on the plant led to the isolation of taxifolin 3-O-arabinofuranoside [280], apigenin 8-C-glucoside (vitexin) [207], apigenin 6-C-glucoside (isovitexin) [208], and acacetin-7-O- β -glucoside [281]¹⁶³.

In *Cynometra iripa*, the analysis by high-resolution liquid chromatography coupled to a photodiode array and a mass spectrometry detector using electrospray ionization (LC-UV/DAD-ESI/MS) as well as a tentative co-chromatography with authentic standards revealed the presence of taxifolin pentoside isomers 1, 2, and 3, quercetin 7-O-pentoside/apioside, kaempferol 7-O-glucoside [292], quercetin 3-O-glucoside [293], and apigenin [278] in the leaves¹⁵⁷, while the bark extract gave confirmatory signals for quercetin-3-O-pentosyl-pentoside [294], taxifolin 3-O-

rhamnosides 1, 2, and 3, quercitrin 3''-O-gallate [296], and apigenin [278]¹⁵⁷. The bark of *C. ramiflora* Linn yielded the isolate apigenin [278]¹⁶⁴ as summarized in Table 2-11.

The flavonoids identified in *C. iripa* extracts included taxifolin pentoside isomers (1, 2, and 3), quercetin 3-O-glucoside, quercetin 7-O-pentoside/apioside, kaempferol 7-O-glucoside, and apigenin in the leaf extracts. In the bark extracts, additional flavonoids were observed, such as quercetin-3-O-pentosyl-pentoside [294], taxifolin 3-O-rhamnoside (three isomers), quercitrin 3''-O-gallate, and apigenin. Among these, quercetin-3-O-glucoside and taxifolin pentoside were the major flavonoids in the leaf extracts, while taxifolin 3-O-rhamnoside was predominant in the bark extracts.

Table 2-11 Flavonoids from the *Cynometra* genus

Phytochemicals	Plant Species	Plant Part	Compounds Identified	References
Flavonoid and Flavonoid glycosides	<i>C. cauliflora</i>	leaves	apigenin 8-C-glucoside (vitexin) [207], apigenin 6-C-glucoside (isovitexin) [208], apigenin-6-C-glucoside-8-C-glucoside [271], taxifolin pentoside [272], catechin [104], kaempferol hexoside [274], quercetin pentoside [273], quercetin hexoside [275], kaempferol-coumaroyl hexoside [276] isorhamnetin hexoside [277]	156
		stem plant	apigenin [278], eriodictyol [279] taxifolin 3-O-arabinofuranoside [280], apigenin 8-C-glucoside (vitexin) [207], apigenin 6-C-glucoside (isovitexin) [208], acacetin 7-O-β-glucoside [281]	162 163
		leaves	[278], naringenin [282], amorphenin [283], nobiletin [284], isorhamnetin [285], epigallocatechin gallate [286]	158
	<i>C. iripa</i>	twigs	[278], luteolin [103] [103], [279], acacetin [287], 3',4',7- trihydroxyflavone [288], [103], [279], [278], [282], [287], luteolin 3',5 dimethyl ether [289], 4',7-dihydroxyflavone [290]	159 160 161
		leaves	taxifolin pentoside isomer 1,2, 3 [272], quercetin 7-O-pentoside/apioside [291], kaempferol 7-O-glucoside [292], quercetin 3-O-glucoside [293], [278], quercetin-3-O-pentosyl-pentoside [294], taxifolin 3-O-rhamnoside 1,2,3 [astilbin] [295], quercitrin 3''-O-gallate [296], [278]	157
		bark		157
	<i>C. Ramiflora</i>	bark	[278]	164

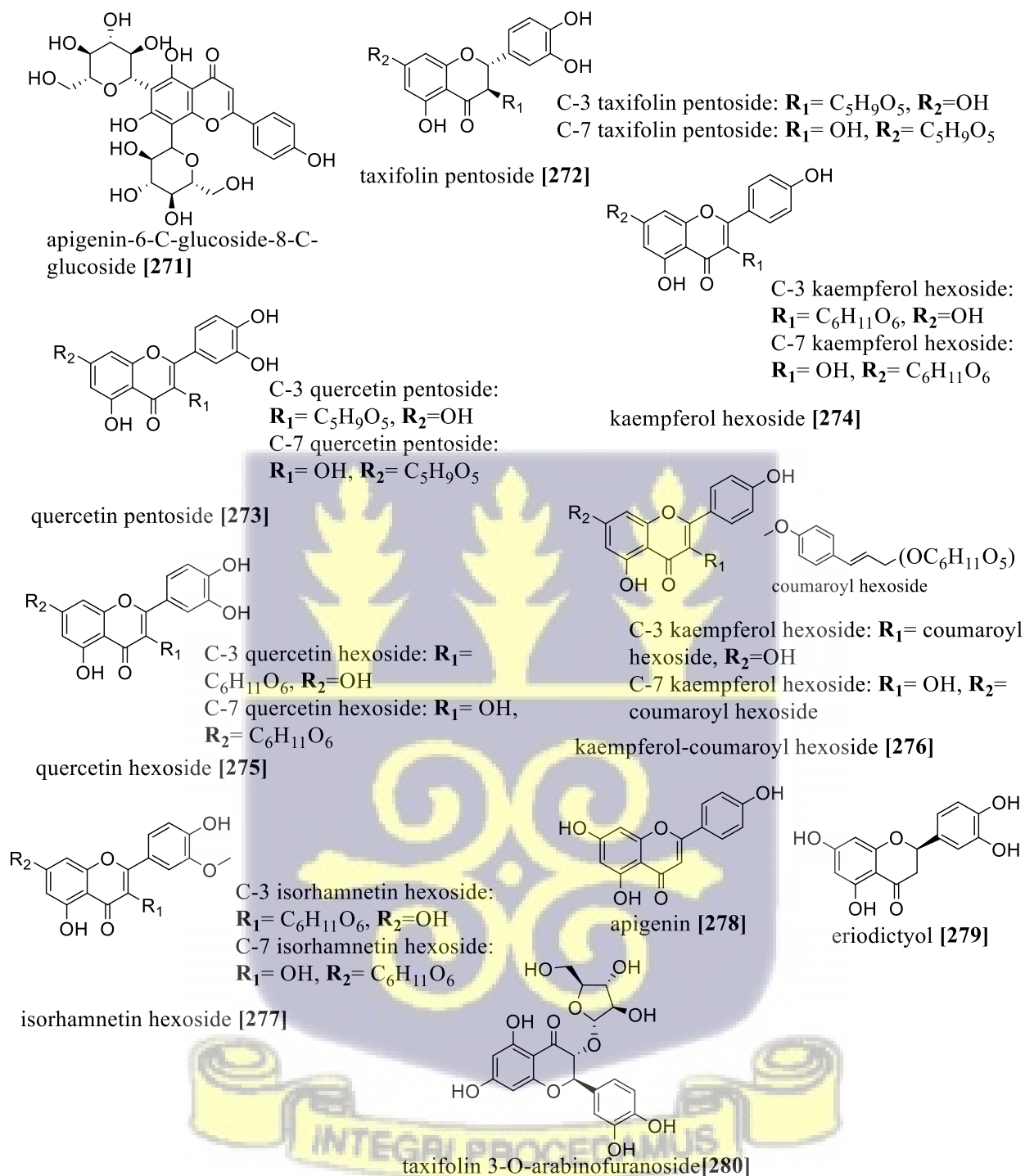


Figure 2-20 Structures of flavonoids in *Cynometra* species

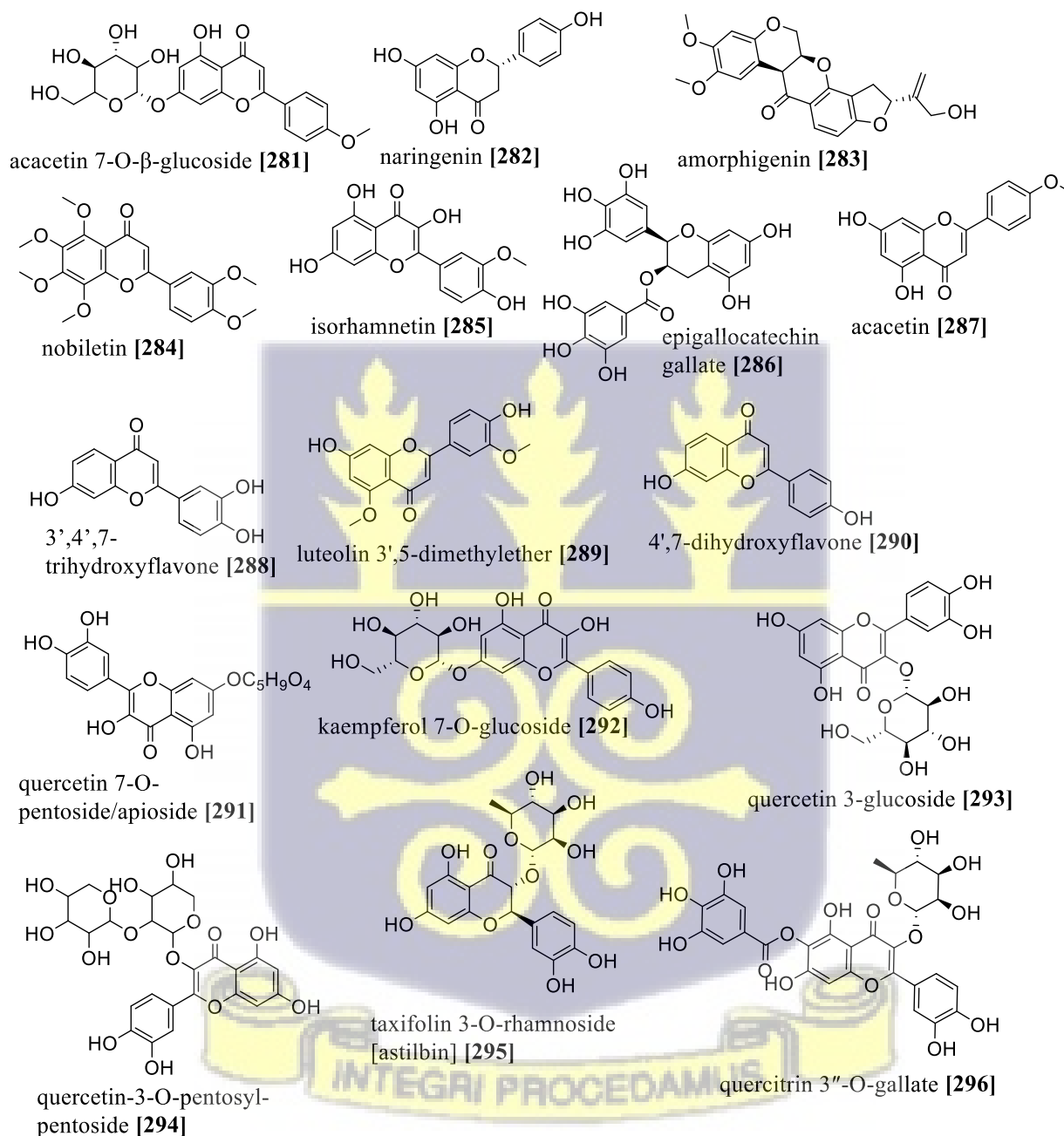


Figure 2-21 Structures of flavonoids in *Cynometra* species contd.

2.7.7.3 Terpenoids from the *Cynometra* Genus

Several monoterpenoids, sesquiterpenoids, diterpenoids and triterpenoids have been documented to be present in the cynometra genus, particularly *C. cauliflora*. Table 2-11 summarizes the various classes of terpenoids in this genus. In the plant *Cynometra cauliflora*, various phytochemicals were identified across different plant parts. From the leaves, monoterpenoids such as α -Thujene [297], β -linalool [211], α -pinene [109], α -terpineol [114], neo-dihydrocarveol [298], 6-camphenol acetate [299], β -pinene [300], myrcene [118], trans-dihydro- α -terpinyl acetate [301], δ -3-carene [119], α -terpinene [117], verbanol acetate [302], p-cymene [111], limonene [120], (Z)- β -ocimene [303], γ -terpinene [304], terpinolene [116], and cis-carvone oxide [305] were found¹⁶⁵. Other identified compounds from the twigs include (E)- β -ocimene [306], santolina alcohol [307], dihydromyrcenol [308], β -linalool [211], trans-sabinene hydrate [309], cis-verbenol [310], cis-4-caranone [311], fragranol [312], geraniol [313], and (E)-cis-jasmonol [314]¹⁶⁵. In the fruits of *Cynometra cauliflora*, trans-pulegol [315] and cis-thujone [173], limonene [120] were identified¹⁶⁵.

For *Cynometra vogelii*, one monoterpenoids that was detected in the leaves is myrcene [118]¹⁶⁶. In *Cynometra megalophylla* Harms, the roots contained a range of compounds, including p-cymen-8-ol [317], (E)- β -ocimene [306], β -pinene [300], sabinene [318], terpinen-4-ol [113], terpinen-4-yl acetate [319], terpinolene [116], 1,8-cineole [112], carvacrol [66], trans-piperitol [320], borneol [321], cis-p-menth-2-en-1-ol [322], trans-p-menth-2-en-1-ol [323], α -Phellandrene [110], and β -phellandrene [324], among others¹⁶⁷.

Table 2-12 Monoterpenoids Identified in *Cynometra* spp.

Phytochemicals		Plant Species	Plant Part	Compounds Identified	References
Monoterpenoids	<i>C. cauliflora</i>	leaves		α -thujene [297], β -linalool [211], α -pinene [109], α -terpineol [114], neo-dihydrocarveol [298], 6-camphenol acetate [299], β -pinene [300], myrcene [118], trans-dihydro- α -terpinyl acetate [301], δ -3-carene [119], α -terpinene [117], verbanol acetate [302], p-cymene [111], limonene [120], (Z)- β -ocimene [303], γ -terpinene [304], terpinolene [116], cis-carvone oxide [305]	165
		Twigs		(E)- β -ocimene [306], santolina alcohol [307], dihydromyrcenol [308], [211], trans-sabinene hydrate [309], cis-verbenol [310], cis-4-caranone [311], fragranol [312], geraniol [313], (E)-cis-jasmonol [314]	165
		Fruits		trans-pulegol [315], cis-thujone [316], [120]	165
	<i>C. vogelii</i>	leaves		[118]	166
	<i>C. megalophylla</i>	roots		anethole [214], [109], [118], α -terpinene [117], [111], [120], [304], p-cymen-8-ol [317], [306], [300], sabinene [318], terpinen-4-ol [113], terpinen-4-yl acetate [319], terpinolene [116], 1,8-cineole [112], carvacrol [66], trans-piperitol [320], borneol [321], cis-p-menth-2-en-1-ol [322], trans-p-menth-2-en-1-ol [323], α -phellandrene [110], β -phellandrene [324]	167



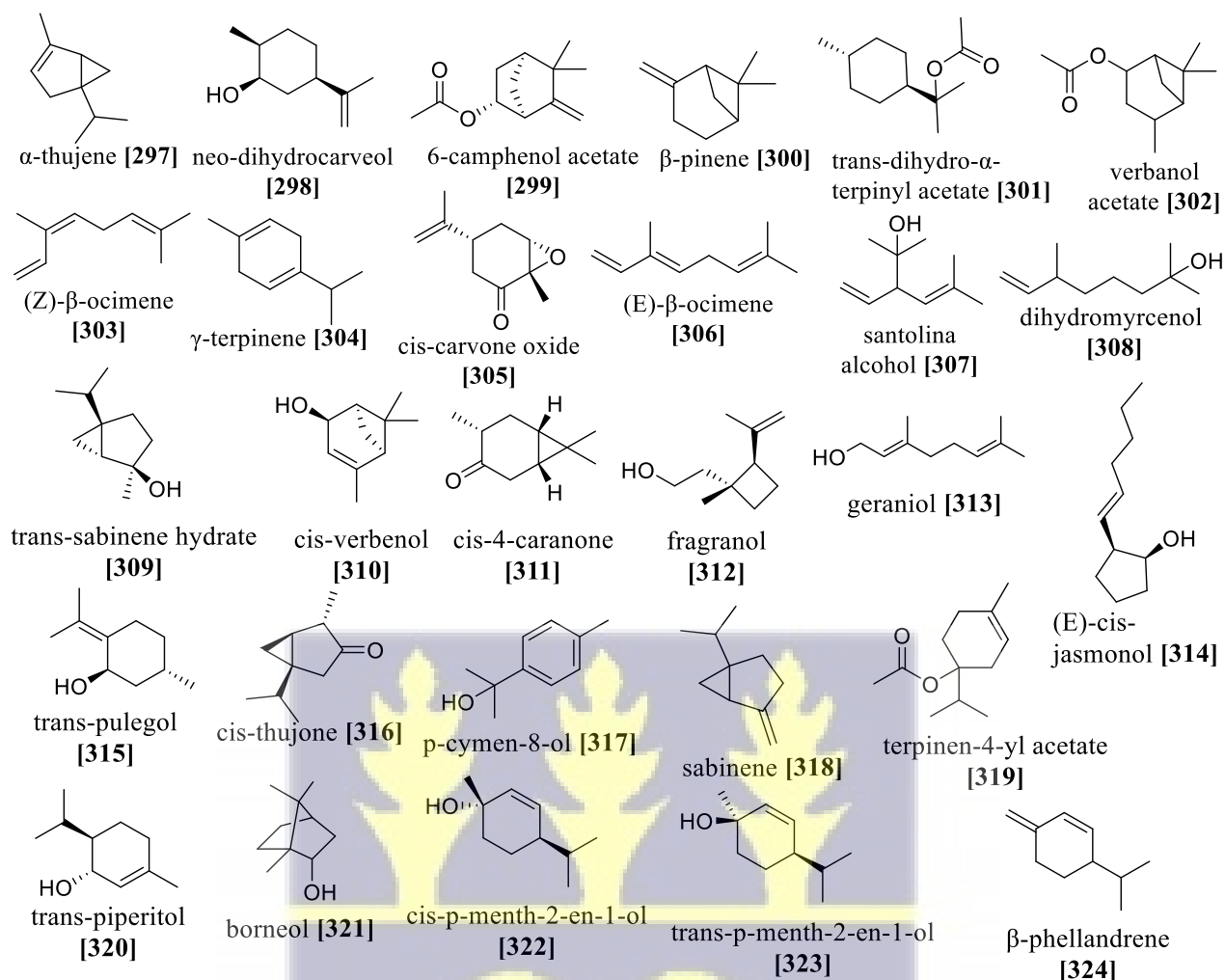


Figure 2-22 Structures of monoterpoids identified in *Cynometra* species

In terms of sesquiterpenoids, *cynometra cauliflora* exhibited compounds like α -himachalene [325], β -chamigrene [129], α -bulnesene [326], and trans-cadina-1,4-diene [327] in its leaves, while twigs contained squamulosone [328], α -eudesmol acetate [329], and occidol acetate [330]¹⁶⁵. The fruits of *Cynometra cauliflora* also revealed a rich variety of sesquiterpenoids, including β -bisabolene [331], (E)- γ -bisabolene [332], α -bulnesene [326], γ -cadinene [132], sesquisabinene [333], germacrene D [131], β -chamigrene [129], α -muurolene [147], ishwarane [334], 9-epi-(E)-caryophyllene [335], prezizaene [336], cis- β -farnesene [337], α -guaiene [338], elemene [125], β -cubebene [339], cubebol [340], γ -cuprenene [341], agarospirol [342], valerianol [343],

allohimachalol [344], trans-cadina-1,4-diene [327], epi- β -bisabolol [345], occidenol [346], selina-3,7(11)-diene [347], elemol [348], longiborneol acetate [349], β -calacorene [350], 11- α H-himachal-4-en-1 β -ol [351], longipinanol [352], nootkatol [353], globulol [354], cryptomerione [355], longiborneol [356], curcumenol [357], trans- β -elemenone [358], (2E,6E)-farnesol [359], 1,10-di-epi-cubenol [360], (E)-nuciferol [361], eremoligenol [362], (Z)-lanceol [363], γ -eudesmol [364], α -chenopodiol [365], α -acorenol [366], occidol acetate [330], hinesol [367], and α -muurolol [368]¹⁶⁵. For *Cynometra vogelii*, additional sesquiterpenoids were detected in the leaves β -caryophyllene [138], α -humulene [143], β -selinene [144], α -selinene [146], caryophyllene oxide [150]¹⁶⁶. In *Cynometra megalophylla* Harms roots, additional sesquiterpenoids such as α -eudesmol [369], hinesol [367], and β -eudesmol [370] were identified. *Cynometra ramiflora* leaves contained Spiro[4.5]decan-7-one and 1,8-dimethyl-8,9-epoxy-4-isopropyl [371]¹⁶⁸.

Table 2-13 Structures of sesquiterpenoids identified in *Cynometra* species

Phytochemicals		Plant Species	Plant Part	Compounds Identified	References
Sesquiterpenoids	<i>C. cauliflora</i>	leaves		aromadendrene [141], α -himachalene [325], β -chamigrene [129], α -bulnesene [326], trans-cadina-1,4-diene [327]	165
		twigs		squamulosone [328], α -eudesmol acetate [329], occidol acetate [330]	165
		fruits		β -bisabolene [331], (E)- γ -bisabolene [332], [326], γ -cadinene [132], sesquisabinene [333], germacrene D [131], [129], α -muurolene [147], ishwarane [334], 9-epi-(E)-caryophyllene [335], prezizaene [336], cis- β -farnesene [337], α -guaiene [338], elemene [125], β -cubebene [339], cubebol [340], γ -cuprenene [341], agarospirol [342], valerianol [343], allohimachalol [344], [327], epi- β -bisabolol [345], occidenol [346], selina-	165

		3,7(11)-diene [347], elemol [348], longiborneol acetate [349], β -calacorene [350], 11- α H-himachal-4-en-1 β -ol [351], longipinanol [352], nootkatol [353], globulol [354], cryptomerione [355], longiborneol [356], curcumenol [357], trans- β -elemenone [358], (2E,6E)-farnesol [359], 1,10-di-epi-cubenol [360], (E)-nuciferol [361], eremoligenol [362], (Z)-lanceol [363], γ -eudesmol [364], α -chenopodiol [365], α -acorenol [366], [330], hinesol [367], α -muurolol [368]	
<i>C. vogelii</i>	leaves	β -caryophyllene [138], α -humulene [143], β -selinene [144], α -selinene [146], caryophyllene oxide [150]	166
<i>C. megalophylla</i>	plant roots	[138], trans-nerolidol [135] [138], [143], alloanomadendrene [126], germacrene D [131], [144], [150], [338], [354], α -eudesmol [369], [367], β -eudesmol [370]	169 167
<i>C. ramiflora</i>	leaves	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl [371]	168



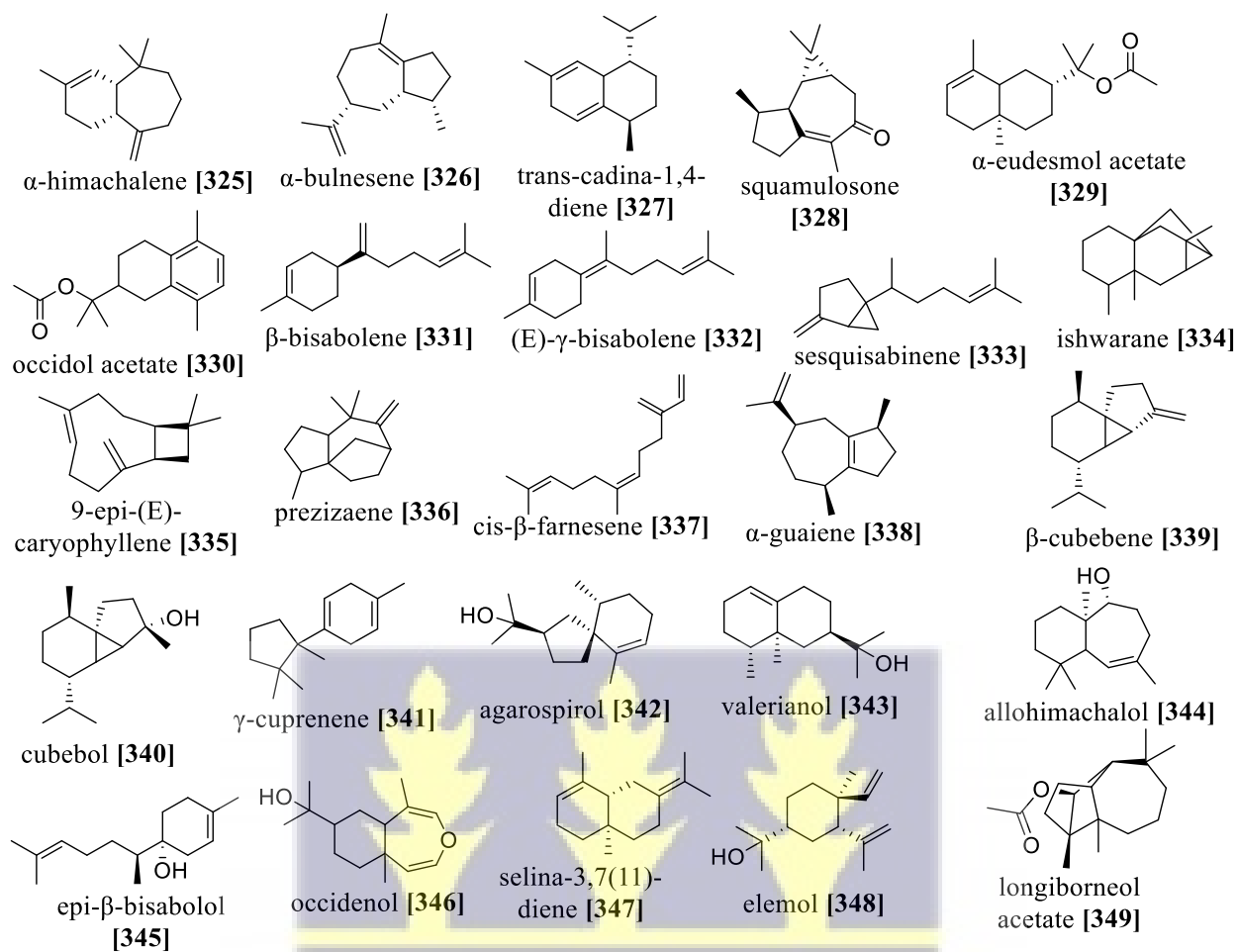


Figure 2-23 Structures of sesquiterpenoids identified in *Cynometra* species

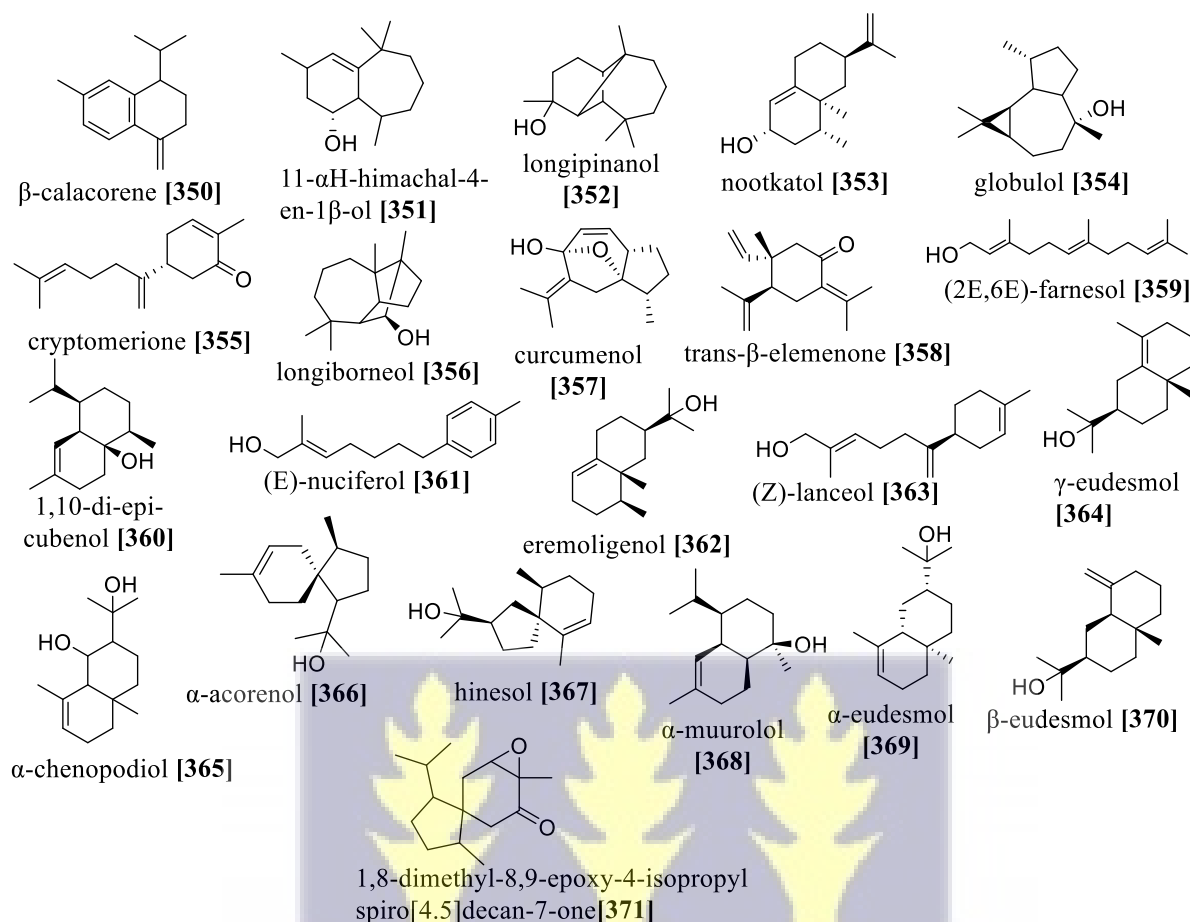


Figure 2-24 Structures of sesquiterpenoids identified in *Cynometra* species contd.

Diterpenoid 3,7,11,15-tetramethyl-2-hexadecen-1-ol [9] was found in *Cynometra vogelii*¹⁶⁹ and *Cynometra Ramiflora* Linn¹⁶⁸. *Cynometra Ramiflora* Linn leaves revealed the triterpenoids 17 α (H), 21 β (H)-Homohopane [372] and Docosa-2,6,10,14,18-pentaen-22-al, 2,6,10,15,18-pentamethyl-, all-trans [373]¹⁶⁸.

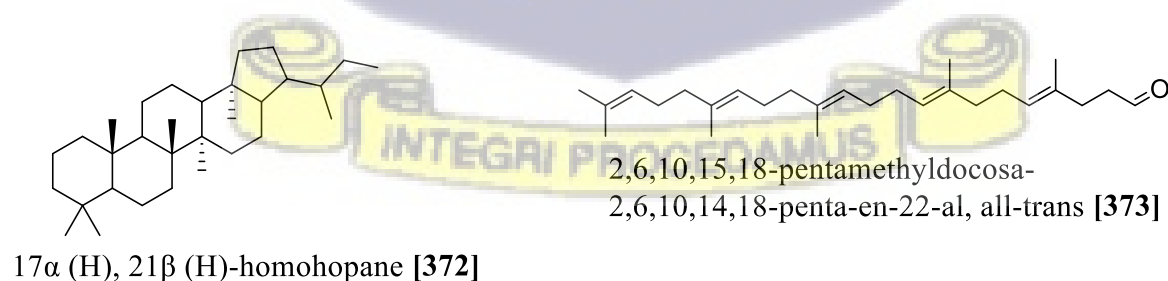


Figure 2-25 Structures of triterpenoids identified in *Cynometra* species.

2.7.7.4 Unsaturated and Saturated Fatty acid derivatives from *Cynometra* Species

Unsaturated fatty acids identified in *Cynometra* species include 9,12-octadecadienoic acid (linoelaidic acid) [11] in twigs and fruits of *Cynometra cauliflora*¹⁶⁵. *C. iripa* leaf oil was found to contain 9-octadecenoic acid (oleic acid) [72], cis-11-eicosenoic acid [374], and linolenic acid [158] when analysed with GC-FID¹⁷⁰, while its seed oil, using GC-FID and GC-MS showed the presence of tetracosanoic acid [162], 9-octadecenoic acid (oleic acid) [72], 9,12-octadecadienoic acid (linoelaidic acid) [11], [158], cis-8,11,14-eicosatrienoic acid [375], and cis-13,16-docosadienoic acid [376]^{170,171}. When Samadd et al. analysed *C. ramiflora* by GC-MS, the leaves contained 9,12-octadecadienoic acid methyl ester [377], 11-octadecenoic acid methyl ester [378], and carbonic acid, eicosyl vinyl ester [379] whilst the seeds of had 9-octadecenoic acid ethyl ester [156] and 9,12-octadecadienoic acid (linoelaidic acid) [11]¹⁶⁸.

Saturated fatty acids identified in *Cynometra* species include hexadecanoic acid, methyl ester [170], glycidyl palmitate [380], hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl)ethyl ester [53], 3-chloropropionic acid, heptadecyl ester [381], triacontyl heptafluorobutyrate [382], tetratriacontyl pentafluoropropionate [383], nonahexacontanoic acid [384], and dodecyl cis-9,10-epoxyoctadecanoate [385] in *C. ramiflora* leaves¹⁶⁸. *C. megalophylla* roots contained hexadecanoic (palmitic) acid [15] and tetradecanoic (myristic) acid [13]¹⁶⁷, while *C. cauliflora* leaves had ethyl 2-methyl pentanoate [386], and its twigs contained octanoic (caprylic acid) [166], decanoic (capric) acid [167], and [171]¹⁶⁵. *C. vogelii* leaves had Isopropyl palmitate [387]¹⁶⁶. *C. iripa* leaf oil contained hexanoic (caproic) acid [388], dodecanoic (lauric) acid [171], [13], [15], octadecanoic acid [14], eicosanoic (arachidic) acid [164], docosanoic (behenic) acid [161], and pentadecanoic acid [97], while its seed oils contained hexanoic (caproic) acid [388], [171], [13],

[15], [14], [164], [161], and tricosanoic acid [389]¹⁷⁰. Seeds of *Cynometra iripa* also had butyric acid 2-pentadecyl ester [390]¹⁷¹.

Table 2-14 Fatty acids identified n *Cynometra* spp.

Phytochemicals	Plant Species	Plant Part	Compounds Identified	References
Unsaturated fatty acid	<i>C. cauliflora</i>	twigs and fruits	9,12-octadecadienoic acid (linoelaidic acid) [11]	165
	<i>C. iripa</i>	leaf oil	9-octadecenoic acid (oleic acid) [72], cis-11-eicosenoic acid [374], linolenic acid [158]	170
		seed oil	tetracosanoic acid [162], [72], [11], [158], cis-8, 11, 14-eicosatrienoic acid [375], cis-13,16-docosadienoic acid [376]	170 171
	<i>C. ramiflora</i>	leaves	9,12-octadecadienoic acid methyl ester [377]; 11-octadecenoic acid, methyl ester [378]; carbonic acid, eicosyl vinyl ester [379]	168 168
		seed	9-octadecenoic acid ethyl ester [156], [11]	168
	<i>C. ramiflora</i>	leaves	hexadecanoic acid, methyl ester [170]; glycidyl palmitate [380]; hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl)ethyl ester [53]; 3-chloropropionic acid, heptadecyl ester [381]; triacontyl heptafluorobutyrate [382]; tetratriacontyl pentafluoropropionate [383]; nonahexacontanoic acid [384]; dodecyl cis-9,10-epoxyoctadecanoate [385]	168
	<i>C. megalophylla</i>	roots	hexadecanoic (palmitic) acid [15], tetradecanoic (myristic) acid [13]	167
	<i>C. cauliflora</i>	leaves	ethyl 2-methyl pentanoate [386]	165
		twigs	octanoic (caprylic acid) [166], decanoic (capric) acid [167], Dodecanoic (lauric) acid [171]	165
		seed coat	methyl tetradecanoate [169]	171
Saturated fatty acids	<i>C. vogelii</i>	leaves	isopropyl palmitate [387]	169
		-	[15]	169
	<i>Cynometra iripa</i>	Leaf oil	hexanoic (caproic) acid [388], [171], [13], [15], octadecanoic acid [14], eicosanoic (arachidic) acid [164], docosanoic (behenic) acid [161], pentadecanoic acid [97]	170

Seed oils	[388], [171], [13], [15], [14], [164], [161], tricosanoic acid [389]	170
Seeds	butyric acid 2-pentadecyl ester [390]	171

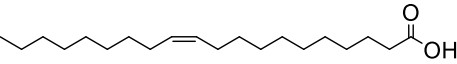
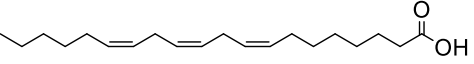
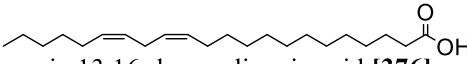
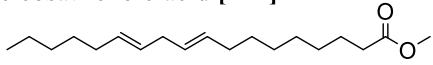
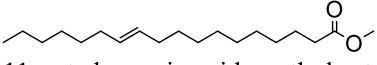
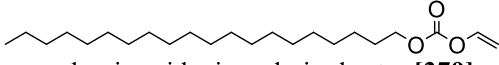
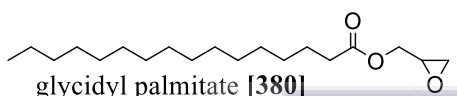
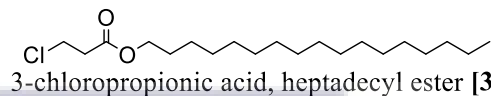
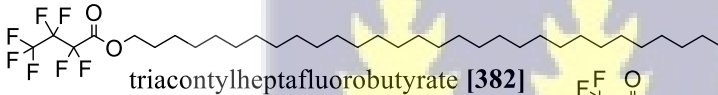
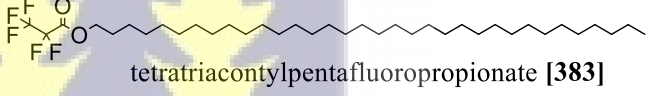
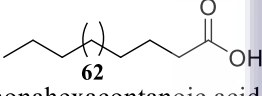
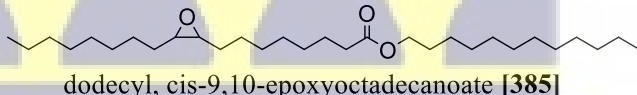
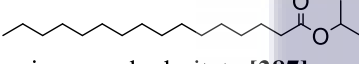
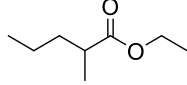
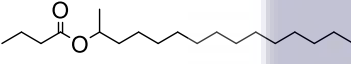
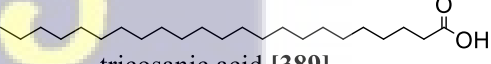
	
11-eicosenoic acid [374]	cis-8,11,14-eicosatrienoic acid [375]
	
cis-13,16-docosadienoic acid [376]	9,12-octadecadienoic acid methyl ester [377]
	
11-octadecenoic acid, methyl ester [378]	carbonic acid, eicosyl vinyl ester [379]
	
glycidyl palmitate [380]	3-chloropropionic acid, heptadecyl ester [381]
	
triacontylheptafluorobutyrate [382]	tetratriacontylpentafluoropropionate [383]
	
nonahexacontanoic acid [384]	dodecyl, cis-9,10-epoxyoctadecanoate [385]
	
isopropylpalmitate [387]	ethyl-2-methylpentanoate [386]
	
butyric acid, 2-pentadecyl ester [390]	tricosanoic acid [389]

Figure 2-26 Structures of fatty acids identified in *Cynometra* species.

2.7.7.5 Phytosteroids

Phytosterols identified in *Cynometra* species include compounds Stigmast-4-en-3-one [46] and β -sitosterol [44] in the seeds of *Cynometra iripa* whereas the seed coat of *C. iripa* contained 3β -cholesta-4,6-diene-3-ol [391] and progesterone [392]¹⁷¹. Additionally, *C. ramiflora* had phytosterol compounds campesterol [47] and γ -sitosterol [48]¹⁶⁸.

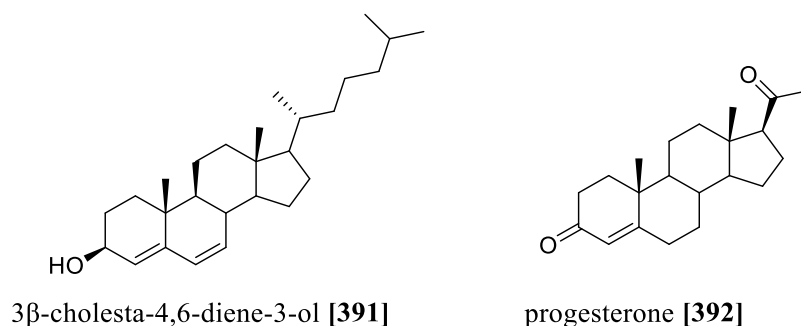


Figure 2-27 Structures of sterols identified in *Cynometra iripa*.

2.7.7.6 Phenolic and Benzoid compounds from *Cynometra spp*

Phenolic compounds identified in *Cynometra* species include p-vinyl guaiacol [393] in the leaves of *C. cauliflora*¹⁶⁵, and 5,7-dihydroxychromone [394] found in the twigs of *C. cauliflora*^{161,172}. Additionally, *C. vogelii* leaves contained phenolic compounds methyl salicylate [264]¹⁶⁶, while the seed coat of *C. iripa* was found to have 3,5-di-tert-butyl-4-hydroxybenzaldehyde [395]¹⁷¹, and the seed contained vitamin E¹⁷¹. The stem and leaves of *C. ramiflora* had phenolic compounds Caffeic acid [173]¹⁶⁴, with 2,6-dimethoxy-4-(2-propenyl)-phenol [396] detected in the leaves¹⁶⁸.

Benzene derivatives identified in *Cynometra* species include 1,2-benzenedicarboxylic acid butyl 2-ethylhexyl ester [397] and 1,2-benzenedicarboxylic acid mono (2-ethylhexyl) ester [398] in the seed coat of *C. iripa*¹⁷¹, and bis(2-ethylhexyl) phthalate [399] in the leaves of *C. ramiflora*¹⁶⁸. These compounds are grouped in the Table 2-14 below

Table 2-15 Phenolic and Benzoid compounds Identified from the *Cynometra* Genus

Phytochemicals	Plant Species	Plant Part	Compounds Identified	References
Phenolic compounds	<i>C. cauliflora</i>	Leaves	p-vinyl guaiacol [393]	165
	<i>C. cauliflora</i>	Twigs	5,7-dihydroxychromone [394]	161,172
	<i>C. vogelii</i>	Leaves	methyl salicylate [264]	166
	<i>C. iripa</i>	Seed coat	3,5-di-tert-butyl-4-hydroxybenzaldehyde [395]	171
		Seed	vitamin E	171

	<i>C. ramiflora</i>	Stem and leaves	caffeic acid [173]	164
		leaves	phenol, 2,6-dimethoxy-4-(2-propenyl)- [396]	168
Benzene derivatives	<i>C. iripa</i>	Seed coat	1,2-benzenedicarboxylic acid butyl 2-ethylhexyl ester [397]; 1,2-benzenedicarboxylic acid mono (2-ethylhexyl) ester [398]	171
	<i>C. ramiflora</i>	leaves	bis(2-ethylhexyl) phthalate [399]	168

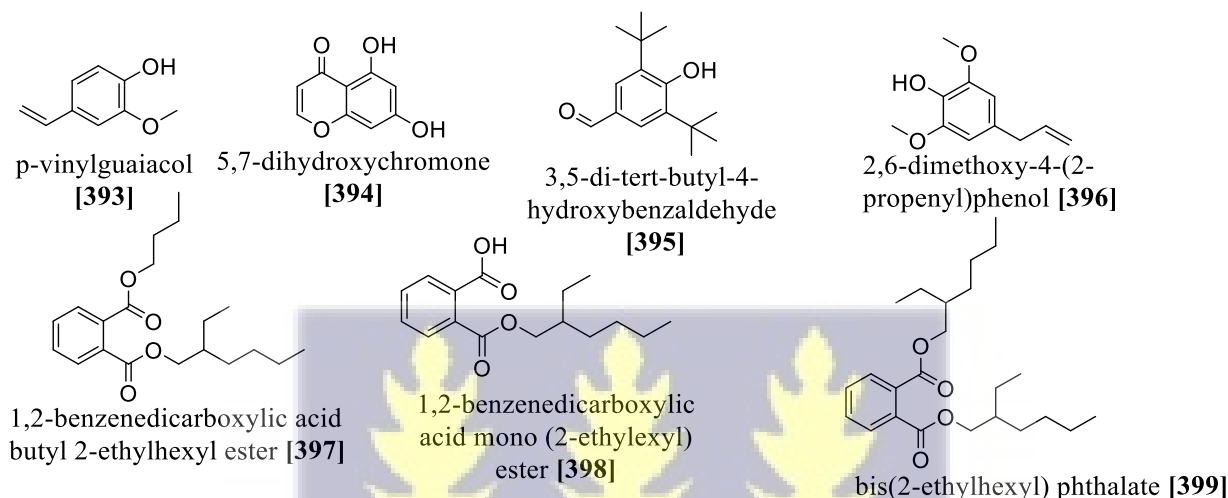


Figure 2-28 Structures of phenolic and benzoid compounds identified from the *Cynometra* genus

Coumarins have also been identified in various *Cynometra* species. In the leaves of *C. cauliflora*, the following coumarins were found: xanthotoxin [400], fraxetin [401], and capensine [402]¹⁵⁸. Additionally, in *C. ramiflora*, 3-(2,3,4-trihydroxyphenyl)-7-hydroxycoumarin [403] was identified in both the leaves and stem bark¹⁶⁴.

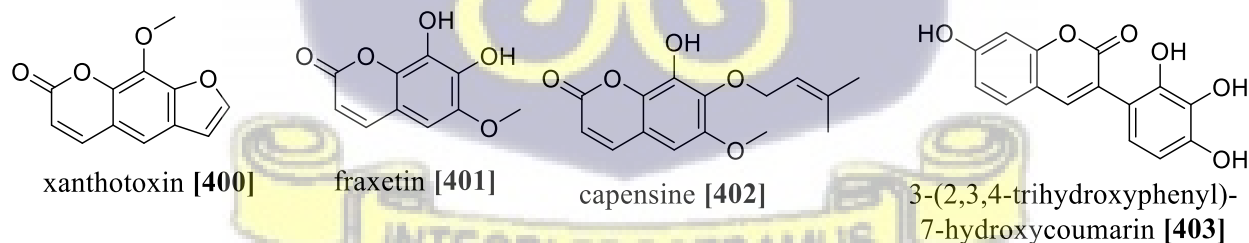


Figure 2-29 Structures of coumarins identified from the *Cynometra* genus

Aliphatic hydrocarbons have been identified in various *Cynometra* species. For instance, the leaves of *C. cauliflora* contain (3E)-2-methyl-octen-5-yne [404]¹⁶⁵. In the roots of *C. megalophylla* Harms, the following compounds were identified: decane [405], undecane [406], dodecane [407], n-tridecane [408], tetradecane [201], pentadecane [409], hexadecane [30], 2-methylbicyclo [2.2.2] octane [81], and octadecane [29]¹⁶⁷. Additionally, the leaves of *C. ramiflora* contain octadecane 5-methyl [410], tetradecane 4-methyl [411], pentatriacontane [412], 5,5-diethylheptadecane [413], tetracontane,3,5,24-trimethyl- [414], 2-methylhexacosane [415], decane,5,6-bis(2,2-dimethylpropylidene)(E,Z) [416], cyclotetradecane,1,7,11-trimethyl-4-(1-methylethyl) [417], cyclononane 1,1,4,4,7,7-hexamethyl [418], and 1,8,11-heptadecatriene,(Z,Z)- [419]¹⁶⁸.

Aliphatic aldehydes were also reported in *C. ramiflora* Linn, where its leaves contain 2-butenal,2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- [420] and (Z)-7-hexadecenal [421]¹⁶⁸. Additionally, in *C. megalophylla* Harms, aliphatic aldehyde n-nonanal [234] was identified in the roots¹⁶⁷. Fatty alcohols were identified in various plant parts of *Cynometra*. The seed of *C. iripa* contains falcarinol [422], while its seed coat contains 1-eicosanol [423]¹⁷¹. Furthermore, the leaves of *C. ramiflora* Linn were found to contain (E,E,Z)-1,3,12-nonadecatriene-5,14-diol [424]¹⁶⁸. Ketones have been found in *C. ramiflora* Linn, where its leaves contain 1-cyclohexyl-2-methyl-prop-2-en-1-one [425] and 9-undecene-4,6-dione,3,5,7,10-tetramethyl [426]¹⁶⁸. Sulphur compounds were identified in *C. ramiflora*, with its leaves containing thiophene tetrahydro 1 1-dioxide [427]¹⁶⁸.

Table 2-16 Aliphatic compounds isolated from the *Cynometra* Genus

Phytochemicals	Plant Species	Plant Part	Compounds Identified	References
Aliphatic hydrocarbons	<i>C. cauliflora</i>	leaves	(3E)-2-methyl-octen-5-yne [404]	¹⁶⁵
	<i>C. megalophylla</i>	roots	decane [405], undecane [406], dodecane [407], n-tridecane [408], tetradecane [201], pentadecane [409],	¹⁶⁷

	<i>C. ramiflora</i>	leaves	hexadecane [30], 2-methylbicyclo [2.2.2] octane [81], octadecane [29] octadecane, 5-methyl- [410]; tetradecane, 4-methyl- [411]; pentatriacontane [412]; 5,5-diethylheptadecane [413]; tetracontane, 3,5,24-trimethyl- [414]; 2-methylhexacosane [415]; decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)- [416]; cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)- [417]; cyclononane, 1,1,4,4,7,7-hexamethyl- [418]; 1,8,11-heptadecatriene, (Z,Z)- [419] 2-butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- [420]; 7-hexadecenal, (Z)- [421]	168
Aliphatic aldehydes	<i>C. ramiflora</i>	leaves		168
	<i>C. megalophylla</i>	roots	n-nonanal [234]	167
Fatty alcohols	<i>C. iripa</i>	Seed	falcarinol [422]	171
		Seed coat	1-eicosanol [423]	171
	<i>C. ramiflora</i>	Leaves	E,E,Z-1,3,12-nonadecatriene-5,14-diol [424]	168
Ketones	<i>C. ramiflora</i>	leaves	1-cyclohexyl-2-methyl-prop-2-en-1-one [425]; 9-undecene-4,6-dione, 3,5,7,10-tetramethyl- [426]	168
sulphur	<i>C. ramiflora</i>	leaves	thiophene, tetrahydro-, 1,1-dioxide [427]	



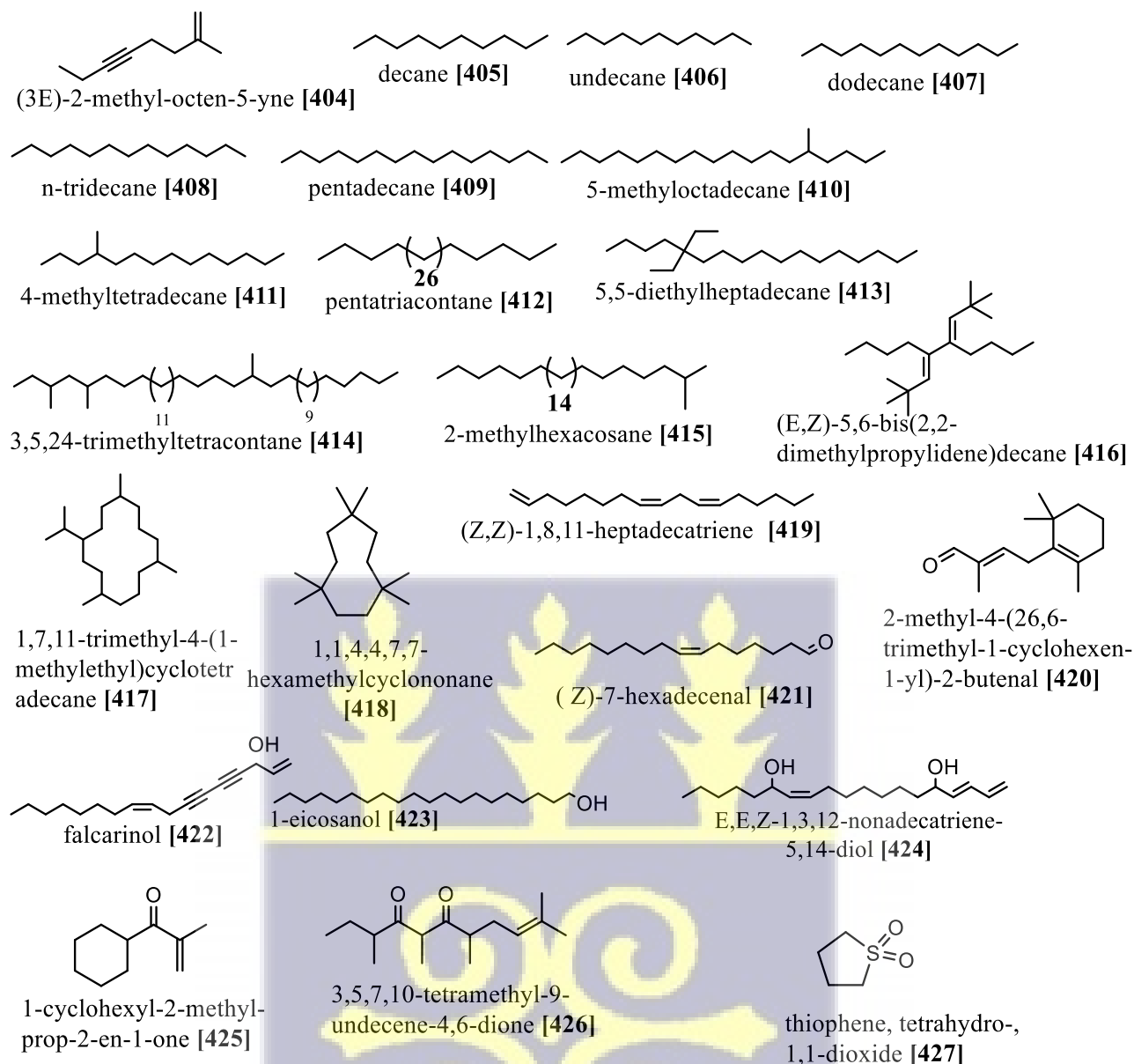


Figure 2-30 Structures of aliphatic compounds identified from the *Cynometra* Genus

2.7.7.7 Alkaloids and Other Compounds in *Cynometra* spp.

For *C. hankei*, the imidazole alkaloids cynometriline [428], N₁-demethylcynometriline [429], and cynodine [430] were isolated from both the stem bark and seeds, while N₁-demethylcynodine [431] was isolated exclusively from the stem bark¹⁷³. The aliphatic alkaloids were indicated to be present in the leaves of *C. ramiflora*¹⁶⁸. Under the same analyses, the furan 2(5H)-Furanone, 5-(7-hydroxy-

6-methyloctyl)- [434] was identified in the leave extract¹⁶⁸. Using LC-MS, Sumarlin et al., identified the anthocyanins malvidin [435], cyanidin [436], oenin [437] from the leave extracts¹⁵⁸.

Table 2-17 Alkaloids and Other Compounds in *Cynometra* species

Phytochemicals	Plant Species	Plant Part	Compounds Identified	References
Alkaloids	<i>C. hankei</i>	Stem bark	cynometrine [428], N ₁ -demethyl cynometrine [429], cynodine [430], N ₁ -demethyl cynodine [431]	173
	<i>C. ramiflora</i>	seeds	[428], [429], [430]	173
		leaves	tetradecanamide [432]; palmitoleamide [433]	168
Furans	<i>C. ramiflora</i>	leaves	2(5H)-Furanone, 5-(7-hydroxy-6-methyloctyl)- [434]	168
anthocyanin	<i>C. cauliflora</i>	Leaves	malvidin [435], cyanidin [436], oenin [437]	158
Triterpenoid/ carotenes	<i>C. iripa</i>	Seed coat	2,5-di-ter-butyl-1,4-benzoquinone [438] Squalene [19] β-carotene [258]	171

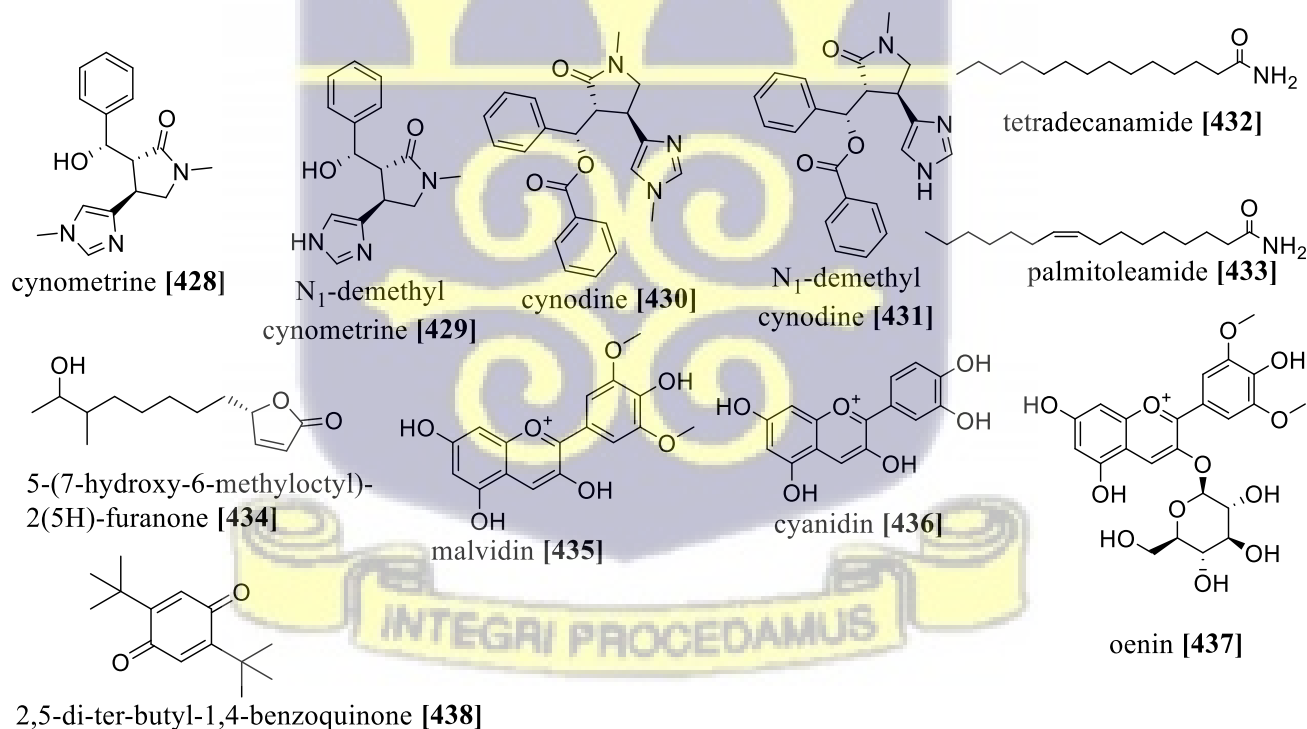


Figure 2-31 Structures of alkaloids and other compounds identified in *Cynometra* species.

2.7.8 The *Humboldtia* Genus

There are eight species grouped under the *Humboldtia* Vahl (also known as *Batschia* Vahl) genus¹⁷⁴. Out of the eight accepted species, five species, *Humboldtia unijuga* Bedd., *Humboldtia laurifolia*, *Humboldtia sanjappae* Sasidh. & Sujanapal, and *Humboldtia vahliana* Wight. have undergone phytochemical analysis involving the identification of compounds (Table 2-17). Phytochemical investigations into the *Humboldtia* genus revealed the presence of various flavonoids across different plant parts and species. In *H. unijuga*, Nair et al. isolated the flavonoids (-)-epicatechin [439], naringenin 7-O- β -glucopyranoside [440], and additional compounds apigenin [278], naringenin [282], luteolin [103], and eriodictyol [279] the roots, as well as, (-)-epicatechin [439] from the stems¹⁷⁵. The bark of *H. laurifolia* yielded (2R,3R)-3,5,7,3',5'-pentahydroxyflavone [441] and 5,7,4'-trihydroxyflavone (apigenin [278])¹⁷⁶. The presence of compounds of flavonoids (-)-epicatechin [439], procyanidin B7 [442], pomiferin [443], (+)-sophorol [444], formononetin [445], and 2'',6''-di-O-acetylononin [446] in both leaf and stem extracts, along with compounds luteolin [103] and naringenin [282] from the stem extracts of *H. sanjappae* were verified by LC-MS¹⁷⁷. Lastly, the aerial parts of *H. vahliana* Wight. contained compound apigenin [278]¹⁷⁸.

Table 2-18 Flavonoids in *Humboldtia* species

Phytochemicals	Species of Plant	Plant Part	Compounds Identified	References
Flavonoids	<i>H. unijuga</i>	roots	apigenin [278], naringenin [282], (-)-epicatechin [439], naringenin 7-O- β -glucopyranoside [440], eriodictyol [279], luteolin [103]	175
		stem	[439]	175
	<i>H. laurifolia</i>	bark	(2R,3R)- 3,5,7,3',5'-pentahydroxyflavone [441], 5,7,4'- trihydroxyflavone (apigenin [278]),	176

<i>H. sanjappae</i>	leaf and stem extract	[439], procyanidin B7 [442], pomiferin [443], [103], [282], (+)-sophorol [444], formononetin [445], 2'',6''-di-O-acetylononin [446]	177
<i>Humboldtia vahliana</i> Wight.	Aerial parts	[278]	178

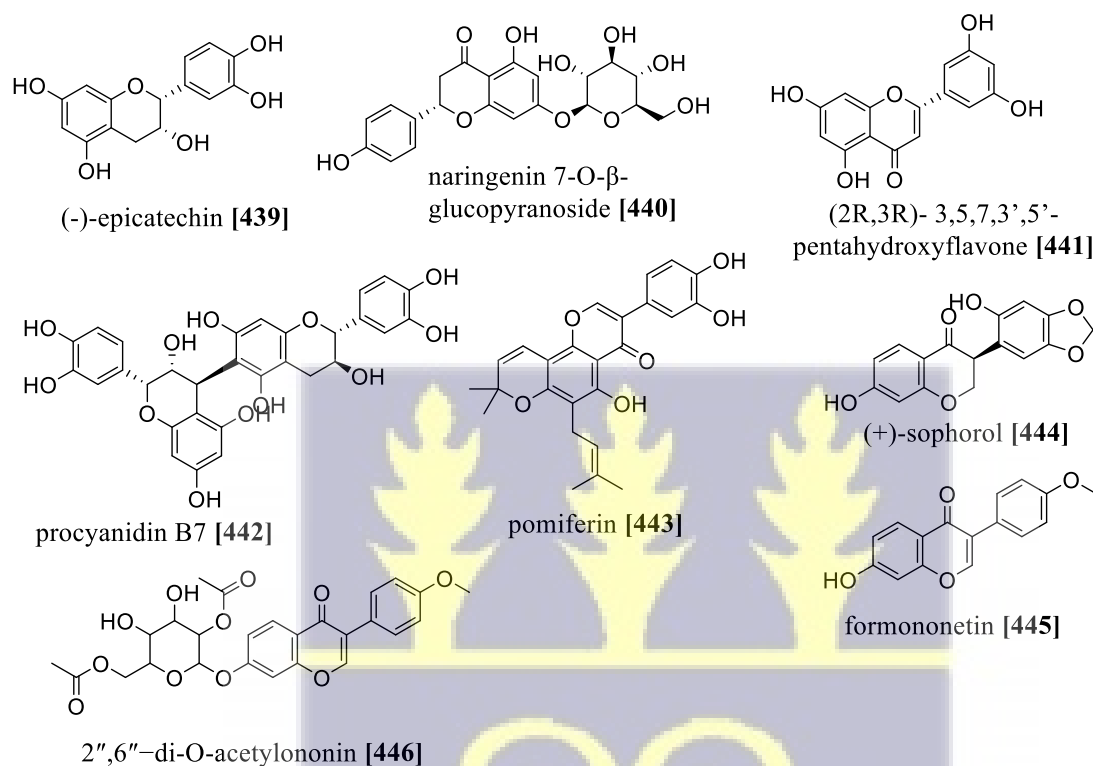


Figure 2-32 Structures of flavonoids identified in *Humboldtia* species.

2.7.8.1 Unsaturated and saturated fatty acids in *Humboldtia* Genus

The LC-MS analysis done on the leaves and stems of *H. sanjappae* Sasidh. & Sujanapal indicated the presence of 17-hydroxylinolenic acid [447], an unsaturated fatty acid, as reported by Sidhic et al¹⁷⁷. Saturated fatty acids Dodecanoic (lauric) acid [171] and hexadecanoic (palmitic) acid [15] have also been isolated from in the roots of *Humboldtia unijuga*¹⁷⁵.

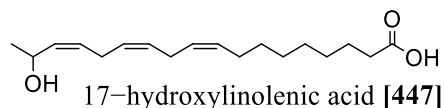


Figure 2-33 Structures of fatty acids in *Humboldtia* spp.

2.7.8.2 Terpenoids in *Humboldtia* Genus

The phytochemical content of various plant species has been explored in several studies. For instance, *H. unijuga* (roots) contains the sesquiterpenoid glycoside pisumionoside [448]¹⁷⁵. In *H. sanjappae* (leaf and stem), the diterpenoid lactapiperanol C [449] was identified using LC-MS¹⁷⁷. Non-steroidal triterpenoids and esters were isolated from *H. unijuga* (roots), including compounds such as erythrodiol-3-acetate [450]¹⁷⁹, oleanolic aldehyde acetate [451], oleanolic acid-3-acetate [452], oleanolic acid [453], maslinic acid [454], and methyl-3-(tetradecanoyloxy)olean-12-en-28-oate [455]¹⁷⁵. Also in the stems of *H. unijuga*, compounds like lup-20(29)-en-3-yl 3-hydroxyheptadecanoate [456], methyl-3-(dodecanoyloxy)olean-12-en-28-oate [457], and 28-oxoolean-12-en-3-yl myristate [458] were also isolated, along with erythrodiol-3-acetate [450], lupeol [38], and oleanolic aldehyde acetate [451]¹⁷⁵. The leaves of *Humboldtia laurifolia* afforded 3 α -methoxyfriedelan [459], and its timber and bark also gave [451] and [38] as isolates¹⁷⁶. In *H. sanjappae* (leaf and stem), additional compounds such as maslinic acid [454] and ganoderic acid F [460] were identified in the LC-MS¹⁷⁷. Lastly, the aerial part of *H. vahliana* Wight gave 3 α -methoxyfriedelan [459] as an isolate. Table 2-18 shows the various phytocompounds.

Table 2-19 Terpenoids identified in the *Humboldtia* genus

Phytochemicals	Species of Plant	Plant Part	Compounds Identified	References
Sesquiterpenoid glycoside	<i>H. unijuga</i>	roots	pisumionoside [448]	175
Diterpenoids	<i>H. sanjappae</i>	leaf, stem	lactapiperanol C [449]	177
Non-steroidal triterpenoids and esters	<i>H. unijuga</i>	roots	erythrodiol-3-acetate [450] oleanolic aldehyde acetate [451], lupeol [38], oleanolic acid-3-acetate [452],	179 175

		[450], oleanolic acid [453], maslinic acid [454], methyl 3-(tetradecanoyloxy)olean-12-en-28-oate [455]	
	stems	lup-20(29)-en-3-yl 3-hydroxyheptadecanoate [456], methyl 3-(dodecanoyloxy)olean-12-en-28-oate [457], 28-oxoolean-12-en-3-yl myristate [458], [450], [38]; [451] 3 α -methoxyfriedelan [459]	175 176 176
<i>H. laurifolia</i>	leaves		
	timber,		
	bark		
<i>H. sanjappae</i>	leaf, stem	[454], ganoderic acid F [460]	177
<i>H. vahliana</i>	aerial part	[459]	178

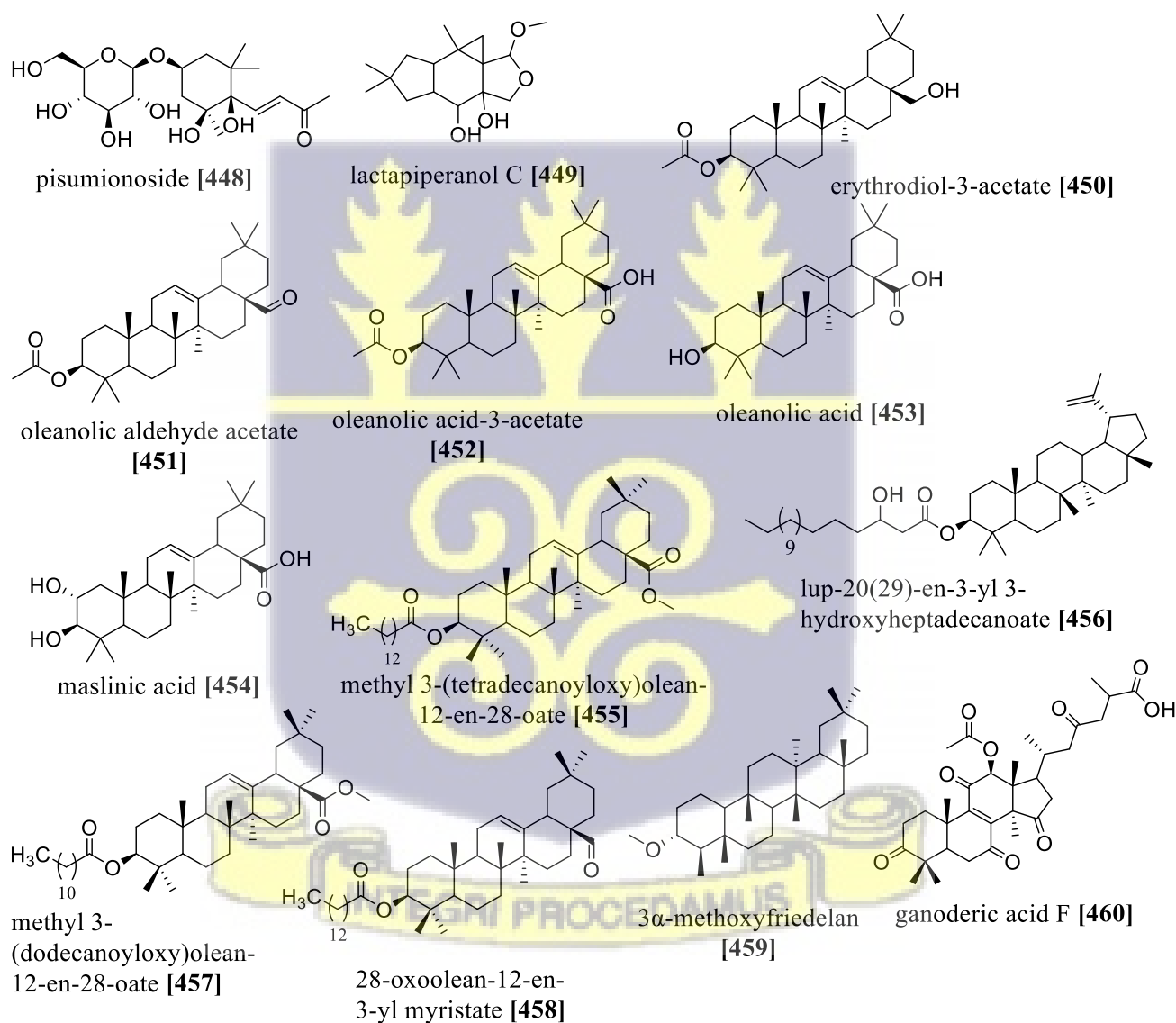


Figure 2-34 Structures of terpenoids identified in *Humboldtia* spp.

2.7.8.3 Phytosterols in *Humboldtia* Genus

Phytochemical investigations of the *Humboldtia* genus highlighted the presence of steroids triterpenoids across three species. In *H. unijuga*, sitosterol palmitate [461] and other sterols, β -sitosterol [44] and sitosterol glucoside [49] were isolated from the roots and stems¹⁷⁵. *H. laurifolia* bark yielded an unidentified sitosteryl ester and related sterol β -Sitosterol [44]¹⁷⁶, while *H. vahliana* aerial parts yielded sitosterol-3-O- β -D-glucopyranoside (sitosterol glucoside [49]) alongside additional sterols β -Sitosterol [44]¹⁷⁸.

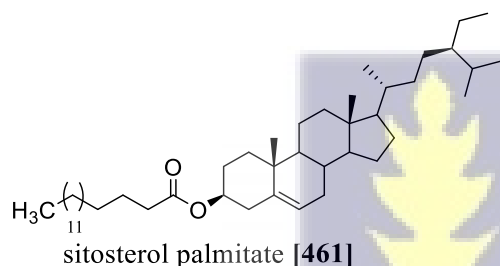


Figure 2-35 Structure of phytosteroid identified in *Humboldtia* species.

2.7.8.4 Phenolic, Glycerides and Alkaloid Compounds in *Humboldtia* Genus

In *H. unijuga*, the roots were found to contain compounds such as 3,4,5-trimethoxyphenyl glucoside [462], khaephuoside A [463], and other phenolics 2,4-bis-(1,1-dimethylethyl)phenol [21], while the stem yielded 3,4,5-trimethoxyphenyl glucoside [462]^{175,179}. For *H. sanjappae*, leaves and stems exhibited maritimetin [464], cymorcin diglucoside [465], and D8'-merulinic acid A [466] in LC-MS analysis¹⁷⁷. Additionally, the chromene, 5,7-dihydroxy-4H-chromen-4-one [467] was among the isolated compounds from the roots of *H. unijuga*¹⁷⁵. The only alkaloids,

ryanodine [468] and richotomine [469] however were identified in the leaves and stems extracts via LC-MS by Sidhic et al¹⁷⁷.

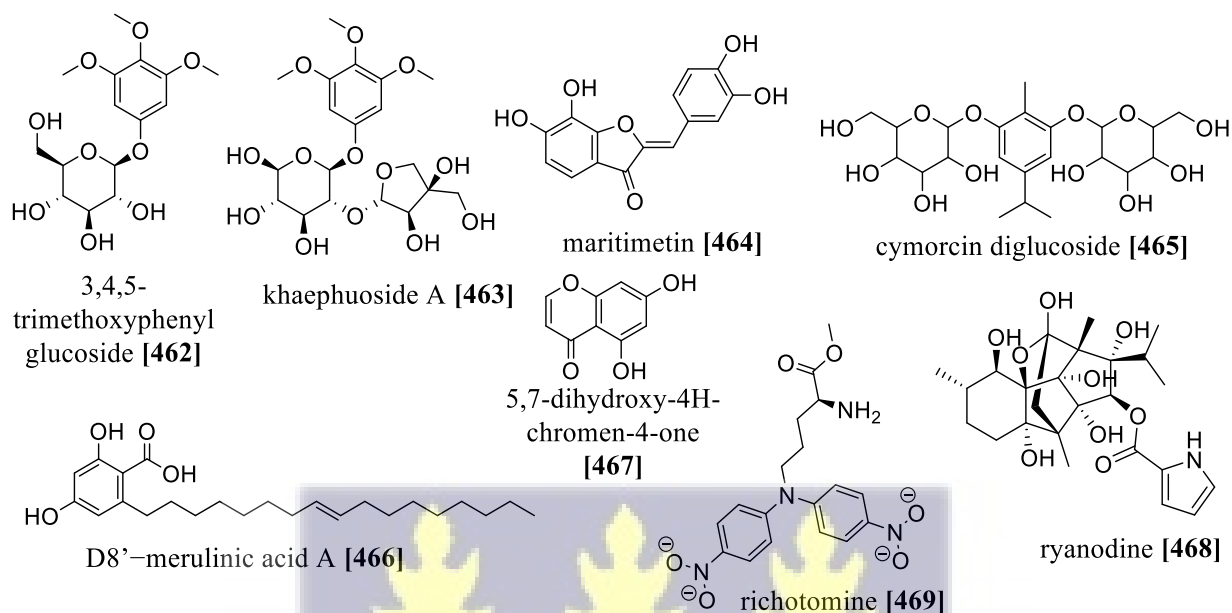


Figure 2-36 Structures of phytochemicals identified in *Humboldtia* species

The phytochemical studies on glycerides in the *H. unijuga* proved the presence of glycerides when Nair et al performed phytochemical analyses on the roots and stems. This study isolated and yielded, (2S)-1-O-palmitoyl-2-O-(9Z-octadecenoyl)-3-O-(6'-sulfo- α -D-quinovopyranosyl)-glycerol [470] and (2S)-1-O-palmitoyl-2-O-(13Z-octadecenoyl)-3-O-(α -D-galactopyranosyl)-1-6-O- β -D-galactopyranosyl-glycerol [471]¹⁷⁵ from the roots. Whereas the stems gave the isolate 1-O-palmitoyl-3-O-(6-sulfo- α -D-glucopyranosyl) glycerol [472]^{175,179141,145}.

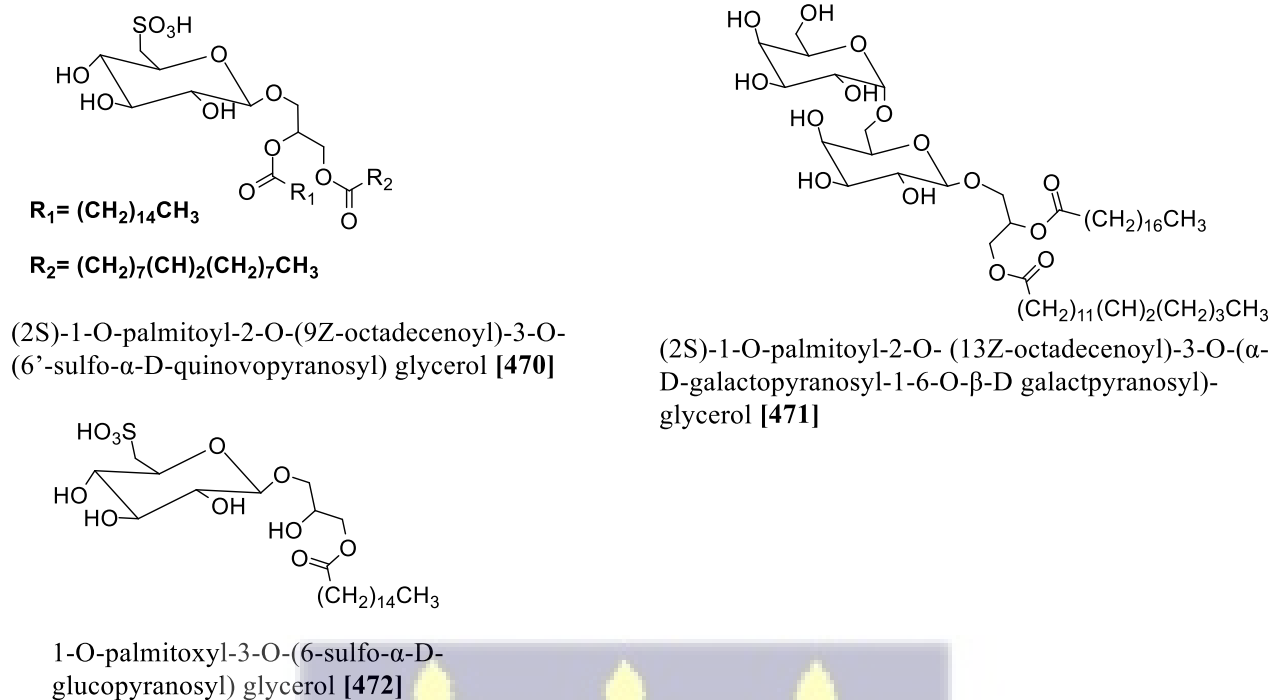


Figure 2-37 Structures of glycerides identified in *Humboldtia* species

2.7.9 The *Julbernardia* Genus

There are ten species recognized under the *Julbernardia* Pellegr. (also known as *Paraberlinia* Pellegr., *Pseudoberlinia* P.A.Duvign., *Seretoberlinia* P.A.Duvign., or *Thylacanthus* Tul.) genus¹⁸⁰. However, phytoconstituent analysis has only been done on *J. paniculata*. Through high-performance liquid chromatography coupled to diode-array detector (HPLC-DAD) and GC-MS it was possible to determine the phytochemical profile of *J. paniculata* which reveals various bioactive compounds distributed across different plant parts. Flavonoids such as rutin [101], taxifolin [473], and quercetin [102] are present in the bark, while the leaves contain [473], [102], and medicarpin [474]¹⁸¹. Monoterpenoids like linalool oxide [212] and menthol [475], as well as the diterpenoids 3,7,11,15-tetramethyl-2-hexadecen-1-ol [9] and neophytadiene [8] were identified in the leaves¹⁸¹. Additionally, triterpenoids, including alnulin (taraxerol) [476], lupeol

[38], D:C-friedoolean-8-en-3-one [477], β -Amyrin [478] and 3-keto-urs-12-ene [479], are prevalent in the leaves¹⁸¹. Unsaturated fatty acid esters, particularly linolenic acid methyl ester [480] and 9,12-octadecadienoic acid (linoelaidic acid) [11], along with saturated fatty acids like hexadecanoic (palmitic) acid [15], have been detected in the leaves¹⁸¹. The phytosterols identified include 23S-ethylcholest-5-en-3 β -ol [381] and stigmasterol [42]¹⁸¹.

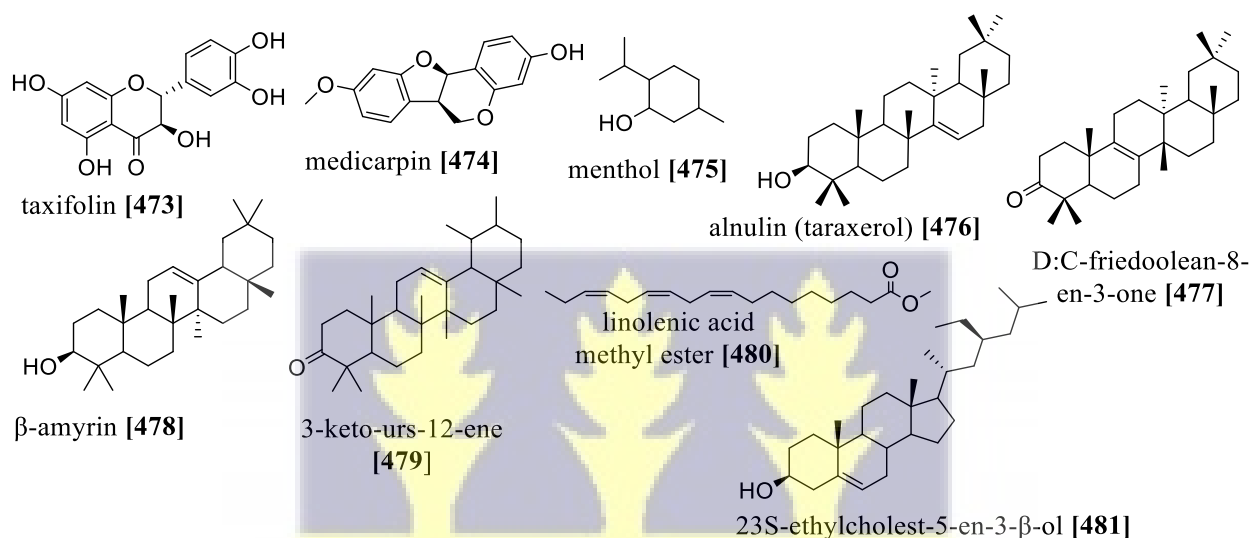


Figure 2-38 Structures of phytochemicals identified in *Julbernardia* species

J. paniculata was found to contain a variety of phenolic compounds and benzene derivatives. The bark is rich in phenolic compounds such as ellagic acid [174], chlorogenic [175], syringic acid [482], ferulic acid [483], p-coumaric acid [484], and rosmarinic acid [485]¹⁸¹. In the leaves, phenolic compounds include phenol [486], gallic acid [172] caffeic acid [173], ellagic acid [174], vanillic acid, [482], [483], [484], [485], 1-(P-methoxyphenyl)-2-methoxyprop-1-ene [487], and the compound megastigma-5,7-diene-3,4,9-triol [488]¹⁸¹. Additionally, benzene derivatives found in the leaves include 1,2-benzenedicarboxylic acid [180] and 1,4-dihydrophenanthrene [489]¹⁸¹. The leaves of *Julbernardia paniculata* exhibit diverse phytochemical constituents,

including aliphatic hydrocarbons such as 1-docosene [490] and 1-hexadecene [491]¹⁸¹. Ketones identified in the leaves include 2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadiene-1-one [492]¹⁸¹. Alkaloids present in the leaves are 9-octadecenamide [65], atropine [493], dodecanamide [494], and 4-oxazolecarboxylic acid [495]¹⁸¹. Furthermore, furan derivatives, including 6H-benzofuro [3,2-c][1]benzopyran-6a(11aH)-ol [496] and 2(4H)-benzofuranone [497], have also been identified in the leaves¹⁸¹.

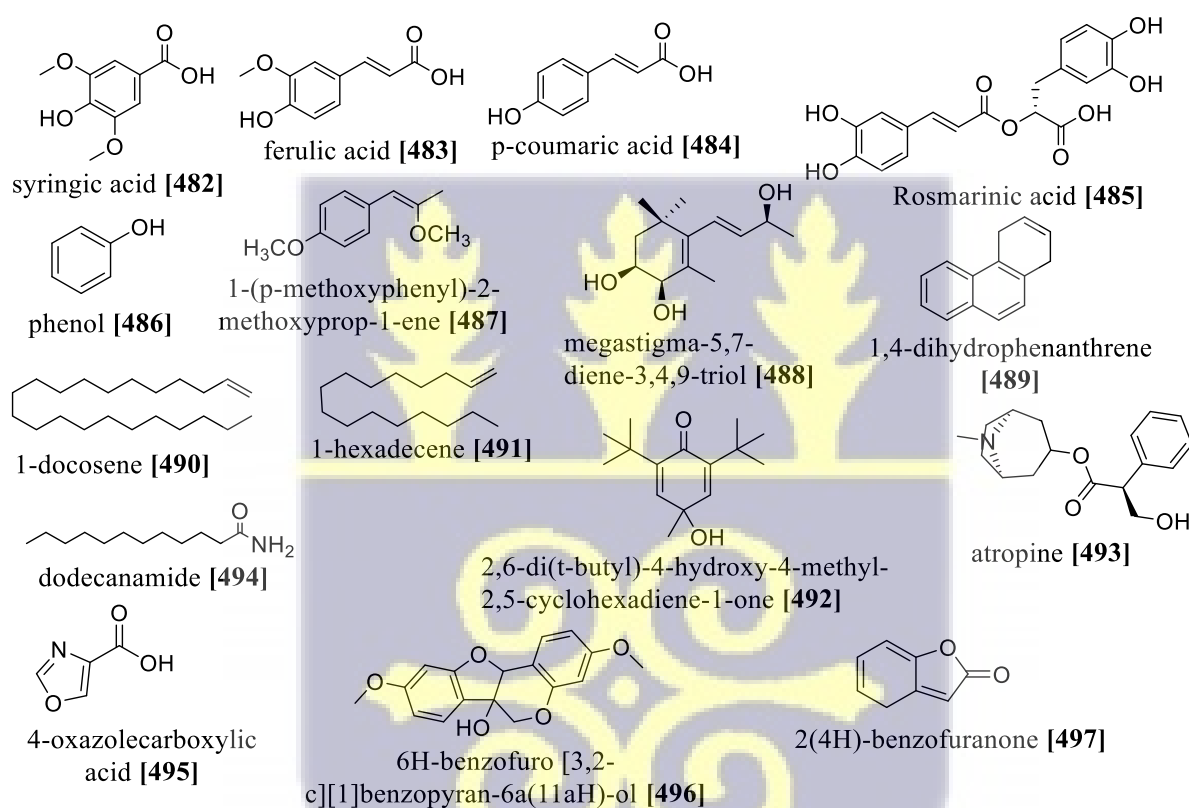


Figure 2-39 Structures of phytochemicals identified in *Julbernardia* species Contd.

2.7.10 *Macrolobium* Genus

There Includes 78 Accepted Species¹⁸² *Macrolobium acaciifolium* and *Macrolobium latifolium*

Vogel are the species that have been worked on. Extensive phytochemical studies involving flavonoid composition analysis of *Macrolobium* species lead to the isolation of a variety

flavonoids, including kaempferol-3-O-rutinoside [6], quercetin 3-O-rutinoside [7], quercetin 3-O-glucoside [293], quercitrin [105], luteolin-3'-O- α -L-rhamnoside [498], kaempferol-7-O-rutinoside [499], astilbin [295], neoastilbin [500], neoisoastilbin [501], and isoastilbin [502], and were isolated from the leaves of *M. acaciifolium*¹⁸³. The branches of this species are rich in flavonoids such as luteolin [103], eriodictyol [279], apigenin [278], 4',7-dihydroxyflavone [290], and chrysoeriol [503]¹⁸³. Additionally, *M. latifolium* is reported to contain apigenin [278] in its stem¹⁸⁴.

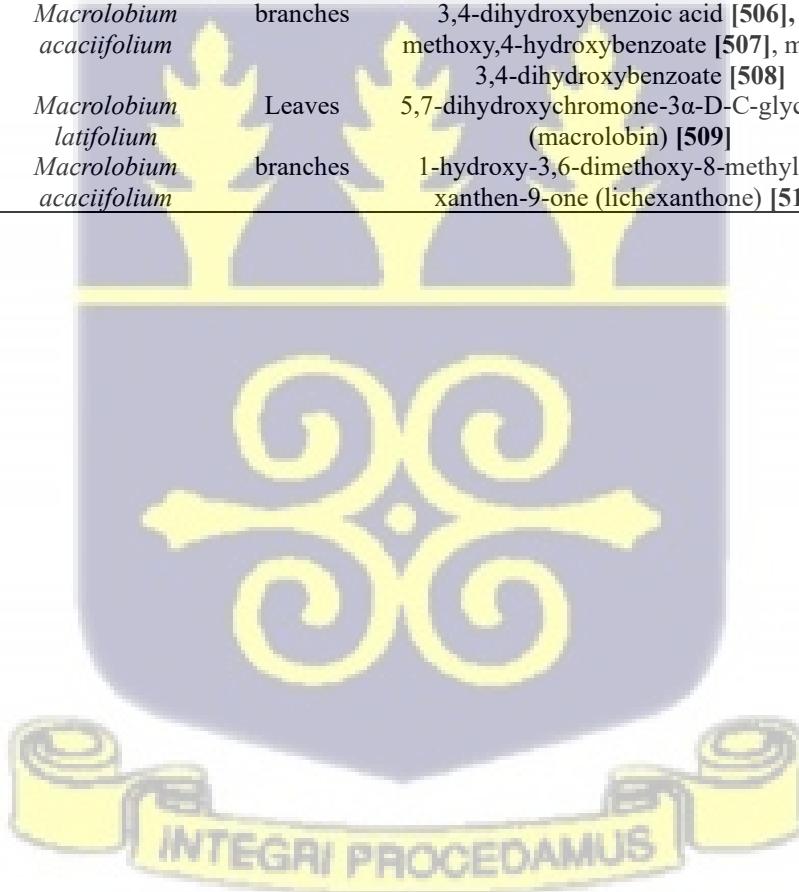
Macrolobium species have been documented to contain non-steroidal triterpenoids. In *M. acaciifolium*, the leaves are reported to have lupeol [38], β -amyrin [478], and 24-methylenecycloartanol [504], while the branches contain friedelin [505]¹⁸⁵. Similarly, the leaves of *M. latifolium* yielded friedelin [505] and alnulin (taraxerol) [477] in another phytochemical investigation¹⁸⁴. For the phytosterol content of the genus, both leaves and branches of *M. latifolium*, contain β -sitosterol [44] and stigmasterol [42] as isolated by Silva et al.¹⁸⁵. Additionally, Nascimento et al., have reported to have isolated β -sitosterol [44], stigmasterol [42], and campesterol [47] from the leaves of *M. latifolium*¹⁸⁴.

Phenolic compounds 3,4-dihydroxybenzoic acid [506], 3-methoxy,4-hydroxybenzoate [507], methyl 3,4-dihydroxybenzoate [508] were also isolated from the branches of *M. acaciifolium*¹⁸³.

Chromenes have been identified in species of the *Macrolobium* genus, with notable findings including 5,7-dihydroxychromone-3 α -D-C-glycoside (macrolobin) [509] isolated from the leaves of *Macrolobium latifolium*¹⁸⁴. Additionally, the branches of *Macrolobium acaciifolium* yielded 1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one (lichexanthone) [510]¹⁸⁵. These findings have been summarized in Table 2-19.

Table 2-20 Phytochemicals From Species of *Macrolobium* Genus

Phytochemicals	Plant Species	Plant Part	Compounds Identified	References
Flavonoids	<i>Macrolobium acaciifolium</i>	leaves		183
		branches	chrysoeriol [503], apigenin [278], eriodictyol [279], luteolin [103]	183
	<i>Macrolobium latifolium</i> Vogel	stem	[278]	184
Non-steroidal triterpenoids	<i>Macrolobium acaciifolium</i>	Leaves	lupeol [38], β -amyrin [478], 24-methylenecycloartanol [504]	185
		Branches	friedelin [505]	185
	<i>Macrolobium latifolium</i>	Leaves	[505], alnulin (taraxerol) [477]	184
Phytosterols	<i>Macrolobium latifolium</i>	Leaves and branches	β -sitosterol [44], stigmasterol [42]	185
		leaves	[42], [44], campesterol [47]	184
	<i>Macrolobium latifolium</i> Vogel			
Phenolic compounds	<i>Macrolobium acaciifolium</i>	branches	3,4-dihydroxybenzoic acid [506], 3-methoxy,4-hydroxybenzoate [507], methyl 3,4-dihydroxybenzoate [508]	183
Chromenes	<i>Macrolobium latifolium</i>	Leaves	5,7-dihydroxychromone-3 α -D-C-glycoside (macrolobin) [509]	184
	<i>Macrolobium acaciifolium</i>	branches	1-hydroxy-3,6-dimethoxy-8-methyl-9h-xanthen-9-one (lichexanthone) [510]	185



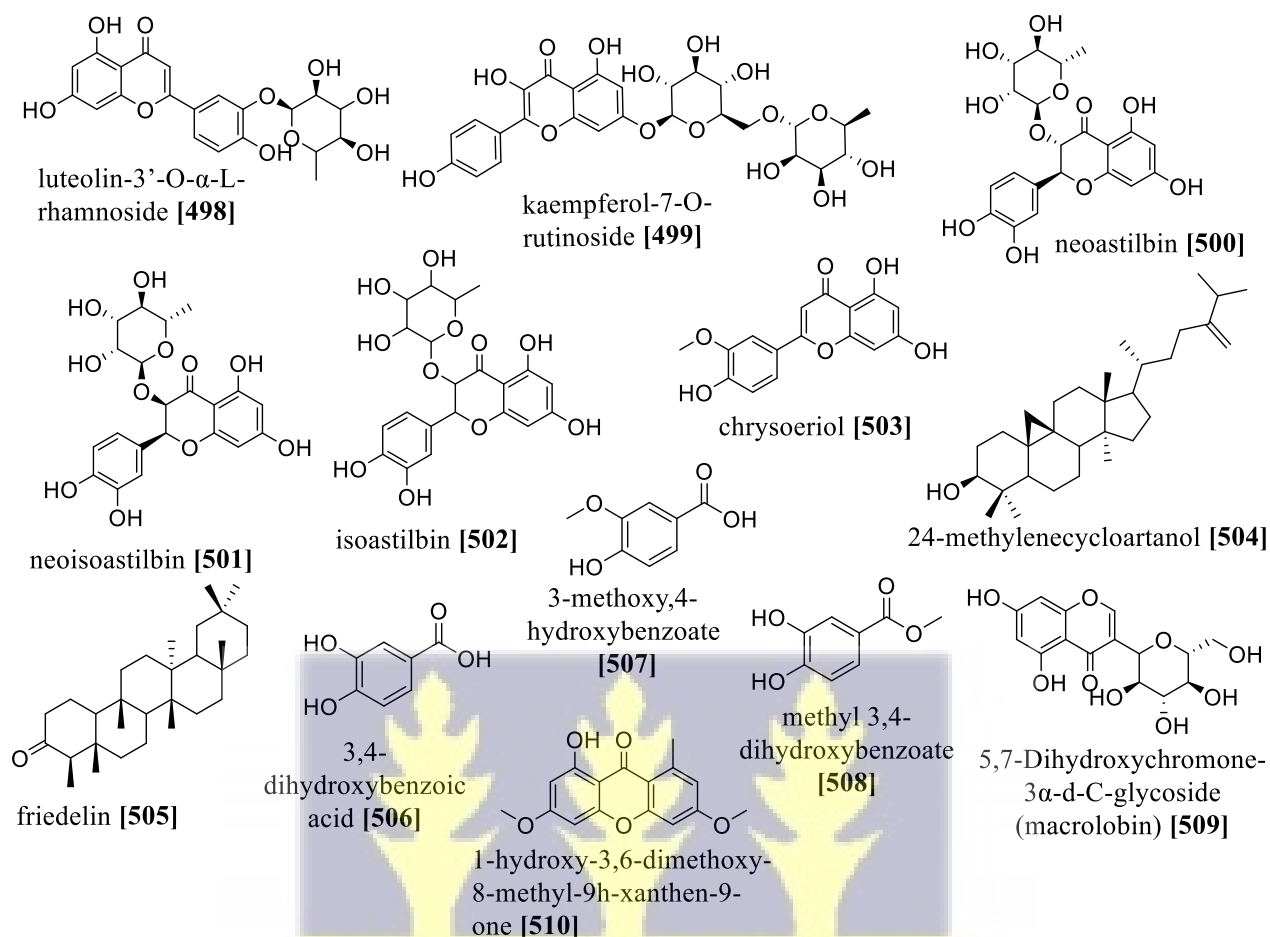


Figure 2-40 Structures of phytochemicals identified in *Macrolobium* species.

2.7.11 The *Scorodophloeus* Genus

The *Scorodophloeus* genus comprises three species: *S. fischeri*, *S. torrei*, and *S. zenkeri*¹⁸⁶. Among these, phytochemical studies have focused solely on *S. zenkeri*, revealing the presence of terpenoids, phytosterols, phenolic compounds, tannins, saponins, alkaloids, fatty acids, and sulfur-containing compounds. Monoterpenoids, phenolic compounds, non-steroidal triterpenoids, and phytosterols have been identified in the seeds of *Scorodophloeus zenkeri*, showcasing the chemical diversity of this plant. The monoterpenoids identified in the seeds using GC-FID and GC/MS analyses include compounds such as α -Pinene [109], limonene [120], (Z)- β -ocimene [303], and

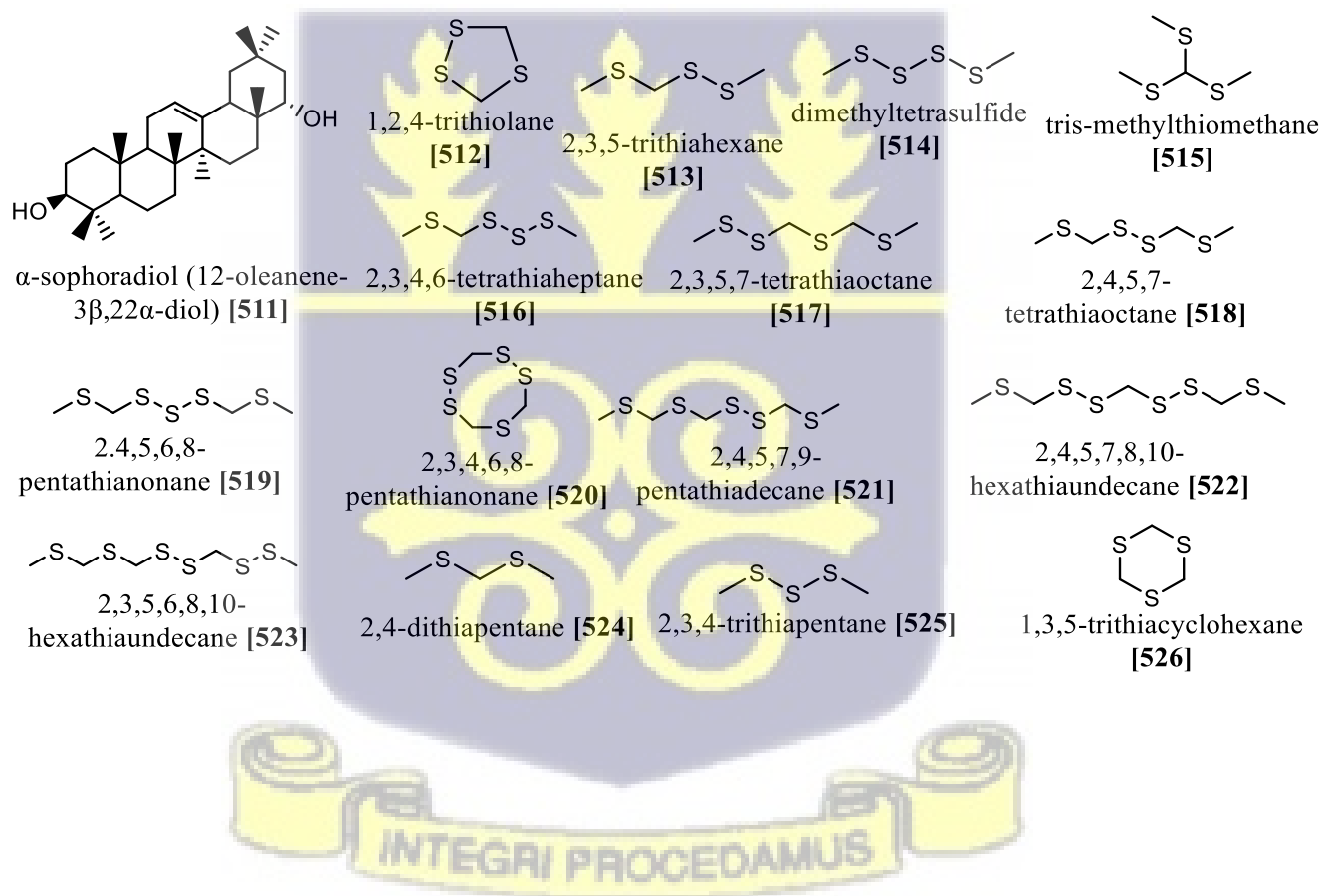
(E)- β -ocimene [306]¹⁸⁷. A phenolic compound, [190], was also identified from the seeds¹⁵³. Non-steroidal triterpenoids, such as lupeol [38] and α -sophoradiol (12-oleanene-3 β ,22 α -diol) [511], in addition to the phytosteroid β -sitosterol [44] were isolated from the seeds as well¹⁸⁸.

Phytochemical analysis revealed a variety of sulfur compounds in the bark and seeds. The bark contains 1,2,4-trithiolane [512], 2,3,5-trithiahexane [513], dimethyltetrasulfide [514], tris-methylthiomethane [515], 2,3,4,6-tetrathiaheptane [516], 2,3,5,7-tetrathiaoctane [517], 2,4,5,7-tetrathiaoctane [518], 2,4,5,6,8-pentathianonane [519], 2,3,4,6,8-pentathianonane [520], 2,4,5,7,9-pentathiadecane [521], 2,4,5,7,8,10-hexathiaundecane [522], and 2,3,5,6,8,10-hexathiaundecane [523]¹⁸⁹. The seeds were found to contain 2,4-dithiapentane [524], 2,3,4-trithiapentane [525], 1,3,5-trithiacyclohexane [526], 3-methyl-2,4,5-trithiahexane [527], 3,6-dimethyl-2,4,5,7-tetrathiaoctane [528], and 6-(methylsulfonyl)-1,2,3-dithiazinan-4-one [529] alongside other sulfur-rich compounds, including 1,2,4-trithiolane [512] and 2,3,5-trithiahexane [513]^{187,188}. Additionally, oxidized derivatives identified in the bark include 2,3,5-trithiahexane 5-oxide [530], 2,4,5,7-tetrathiaoctane-2-oxide [531], S-(methylthiomethyl)methanesulfonylthioate [532], methylthio(methylthiomethyl)sulfone [533], 2,3,5,7-tetrathiaoctane-3,3-dioxide [534], methylsulphonylmethylthiomethane [535], methylmethanethiosulfonate [536], bis-methylsulphonylmethane [537], and bis-(methylthiomethyl)sulfone [538]¹⁹⁰. Hydrocarbons identified in the seeds include tetradec-1-ene [539]¹⁸⁷. The major phytoconstituents in the various parts of *S. zenkeri* that have been documented are sulfur-containing compound as illustrated in Table 2-20.

Table 2-21 Sulfur-containing compounds and Hydrocarbons Identified in *Scorodophloeus zenkeri*

Phytochemicals	Plant Part	Compounds Identified	References
Sulphur compounds	bark	1,2,4-trithiolane [512], 2,3,5-trithiahexane [513], dimethyltetrasulfide [514], tris-methylthiomethane [515], 2,3,4,6-tetrathiaheptane [516], 2,3,5,7-tetrathiaoctane [517], 2,4,5,7-	¹⁸⁹

	tetrathiaoctane [518], 2,4,5,6,8-pentathianonane [519], 2,3,4,6,8-pentathianonane [520], 2,4,5,7,9-pentathiadecane [521], 2,4,5,7,8,10-hexathiaundecane [522], 2,3,5,6,8,10-hexathiaundecane [523]	
seeds	2,4-dithiapentane [524], 2,3,4-trithiapentane [525], [512], 1,3,5-trithiacyclohexane [526], [513], 3-methyl-2,4,5-trithiahexane [527], [514], [515], [516], [517], [518], [519] 3,6-dimethyl-2,4,5,7-tetrathiaoctane [528], [522]	187
seeds	6-(methylsulfonyl)-1,2,3-dithiazinan-4-one [529]	188
bark	2,3,5-trithiahexane 5-oxide [530], 2,4,5,7-tetrathiaoctane 2-oxide [531], s-(methylthiomethyl)methanesulfonylthioate [532], methylthio(methylthio-methyl)sulfone [533], 2,3,5,7-tetrathiaoctane 3,3-dioxide [534], methylsulphonylmethylthiomethane [535], methylmethanethiosulfonate [536], bis-methyl-sulphonylmethane [537], bis-(methylthiomethyl)sulfone [538]	190
hydrocarbons	seeds	187
	tetradecane [201], tetradec-1-ene [539]	



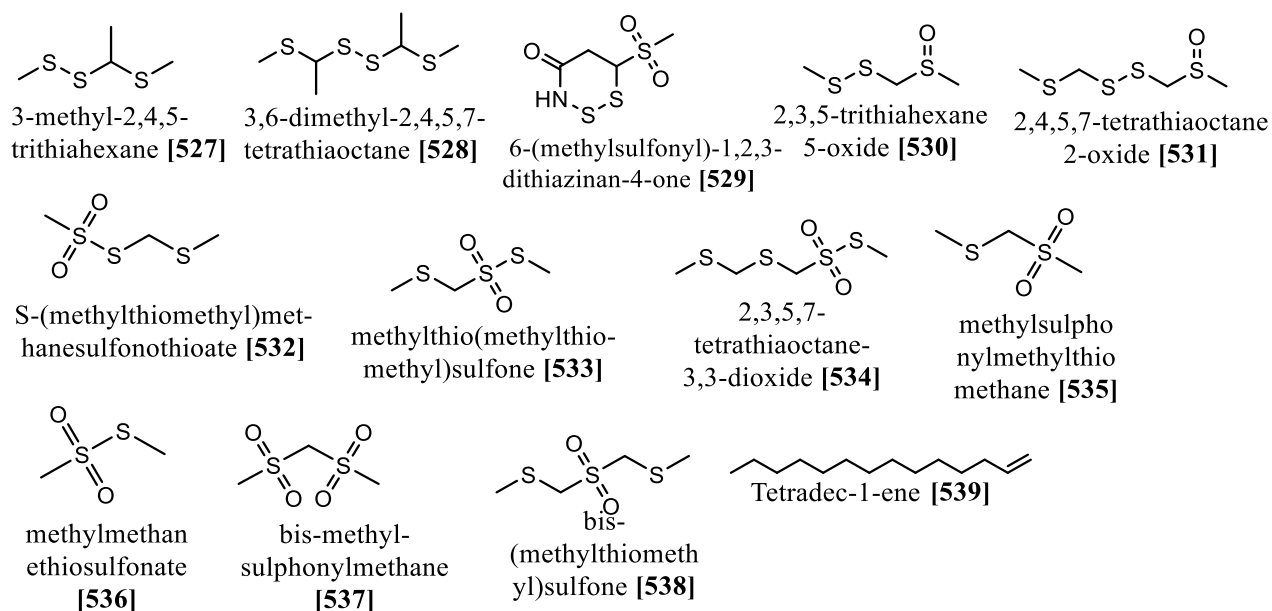


Figure 2-41 Structures of phytochemicals Identified in *S. Zenkeri*

2.8 Conclusion on Phytochemicals the phytochemicals of the Amherstieae tribe

The reviewed literature reveals that members of the Amherstieae tribe, to which *T. gentii* belongs, are chemically rich in triterpenoids, sterols, flavonoids, and phenolic derivatives that frequently exhibit antimicrobial and antiparasitic properties. However, research attention across the tribe remains uneven: while genera such as *Berlinia* and *Tamarindus* are well characterized, *Talbotiella* species have received little or no phytochemical or pharmacological investigation. This imbalance highlights a clear knowledge gap in the chemotaxonomic mapping of the tribe. Establishing the phytochemical composition of *T. gentii*, therefore, provides not only baseline data for an unstudied genus but also a rational foundation for exploring its bioactive potential. By isolating and characterizing its secondary metabolites, this study contributes to bridging that gap and expanding the chemical diversity documented within the Amherstieae tribe. Building on these observations, the next section examines the phytochemical trends and bioactivity patterns across the Amherstieae tribe to contextualize the rationale for studying *Talbotiella gentii* in greater detail.

2.9 Phytochemical Trends and Bioactivity within the Amherstieae Tribe

In the literature, several compounds have been isolated from species within the Amherstieae tribe, to which *Talbotiella gentii* belongs. Some of these compounds are common to multiple species within the tribe, indicating a shared phytochemical profile among members of this taxonomic group, while others have been reported in only a single species, suggesting that such compounds may be unique to specific taxa. This variability in compound distribution among Amherstieae species highlights the potential for novel phytochemical diversity within *T. gentii* and supports the rationale for conducting column chromatographic separation to isolate and identify compounds from this understudied species. The Amherstieae tribe, with its diverse range of bioactive compounds, therefore presents a promising avenue for drug discovery, particularly in the search for new natural products with therapeutic potential. Given the structural diversity observed in compounds from related species, further research on *T. gentii* may uncover new antitrypanosomal agents.

A comparative review of reported metabolites across the Amherstieae tribe reveals that terpenoids and flavonoids dominate the chemical profiles, frequently exhibiting bioactivity against microbial and parasitic pathogens¹⁹¹. While numerous compounds have been identified, systematic attempts to correlate chemical structure with biological function remain limited¹⁹². The paucity of structure–activity relationship (SAR) studies and bioassay-guided investigations represent a significant gap in current phytochemical research, especially given the recurring structural features shared among many taxa. Collectively, the literature suggests that while Amherstieae species share recurring classes of bioactive compounds, the absence of systematic structure–activity evaluations represent a major research gap. This gap forms the conceptual basis of the present study, which seeks to

isolate, characterize, and evaluate the biological relevance of triterpenoid and sterol constituents from *T. gentii* through both experimental and computational approaches.

2.10 Molecular Docking Insights into *Trypanosoma brucei* Enzyme Inhibition

Molecular docking is a computational method used to predict interactions between two structures, such as a protein target (receptor) and a ligand, by estimating their bonding potential through docking score¹⁹³. Molecular docking, crucial in structure-based drug design, identifies the optimal orientation of a ligand in the receptor's active site to form a stable complex¹⁹⁴. Docking is also a cost-effective strategy for predicting drug candidates before animal testing by identifying the most favourable ligand conformations within a receptor's active site¹⁹⁵.

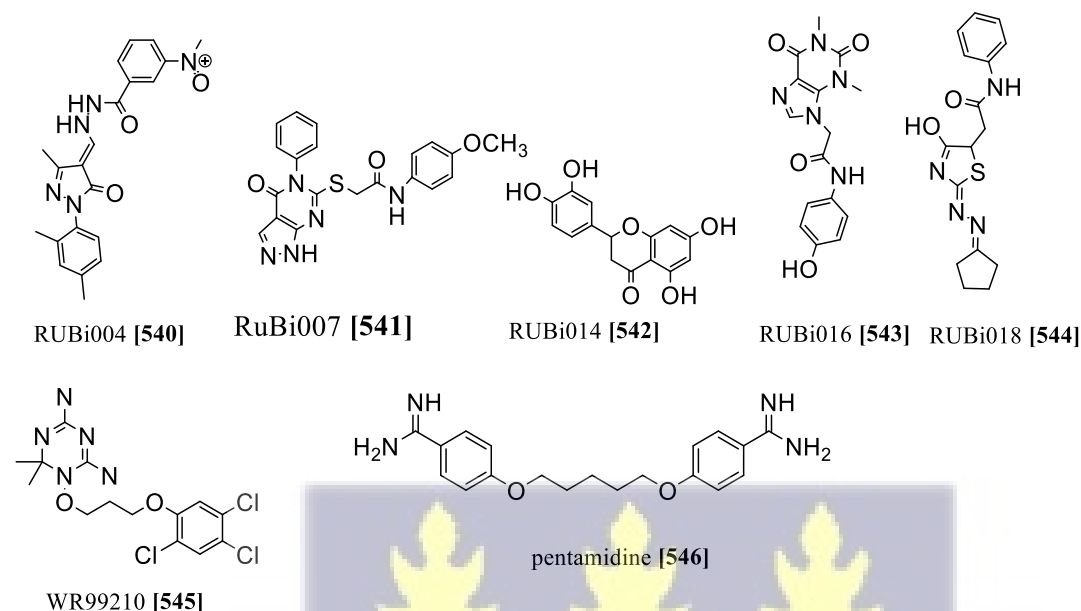
Since this thesis incorporates molecular docking studies, to understand the mechanisms of action of the antitrypanosomal studies carried out, subsequent sections will focus on the *in silico* screening for Trypanosomal parasites in drug discovery.

2.10.1 Studies on Molecular Docking Involving *Trypanosoma brucei*

In silico methods are advancing drug design, aiding the development of single and multitarget agents for diseases like Trypanosomiasis. Despite promising leads, current treatments are inadequate, highlighting the need for further research into natural compounds and their synthetic analogues to develop effective antitrypanosomal drugs¹⁹⁶. A study by Magambo et al, identified five compounds (RUBi004 [540], RUBi007 [541], RUBi014 [542], RUBi016, and RUBi018 [544]) as promising inhibitors of *Trypanosoma brucei* enzymes, particularly *TbDHFR* and *TbPTR1* (key targets in antifolate drug development for HAT) after screening a ligand library of 5742 compounds⁶⁹. The screening revealed high binding affinities to *TbDHFR*, with RUBi007 [541] (-9.5 kcal/mol) and RUBi004 [540] (-9.4 kcal/mol) showing the strongest interactions, followed by RUBi014 [542] (-8.7 kcal/mol), RUBi018 [544] (-7.9 kcal/mol), and RUBi016 (-7.6

kcal/mol). Similarly, docking against *TbPTR1* demonstrated strong affinities, particularly for RUBi004 [540] (-10.3 kcal/mol), RUBi014 [542] (-9.7 kcal/mol), RUBi007 [541] (-9.6 kcal/mol), and RUBi016 (-8.9 kcal/mol)⁶⁹. *In vitro* assays confirmed the trypanocidal activity of these compounds, with IC₅₀ values of 9.6 ± 3.2 µM (RUBi004 [540]), 34.9 ± 17.1 µM (RUBi007 [541]), 14.6 ± 9.9 µM (RUBi014 [542]), 25.4 ± 4.7 µM (RUBi016), and 12.7 ± 3.7 µM (RUBi018 [544]), indicating low micromolar inhibition of *T. brucei* growth. Compared to standard drugs, WR99210 [545] and pentamidine exhibited significantly lower IC₅₀ values of 0.5 ± 0.4 µM and 0.014 µM, respectively⁶⁹. However, when combined with the *TbDHFR* inhibitor WR99210 [545], RUBi004 [540], RUBi007[541], RUBi014 [542], and RUBi018 [544] displayed antagonism, suggesting potential competitive inhibition at the active site. In contrast, RUBi016 [543] exhibited an additive effect, indicating a distinct mode of interaction¹⁶³. These findings provide valuable insights into the dual inhibition of *TbPTR1* and *TbDHFR*, offering a foundation for further optimization of antifolate scaffolds in the development of novel therapeutics for HAT. Setzer et al⁷⁰ used *in-silico* molecular docking to explore potential targets of phytochemicals from 19 Nigerian medicinal plants with antitrypanosomal activity. The study examined 386 chemicals and discovered that triterpenoids and steroids targets sterol 14 α -demethylase; anthraquinones and berberine alkaloids bind to pteridine reductase 1; and chromenes and alkaloids interact with triose phosphate isomerase. Polyphenolic compounds, such as flavonoid gallates, showed versatility by binding to multiple proteins¹⁹⁷. These results highlight the potential of these phytochemicals in developing new antitrypanosomal agents. Studies done by Luscher et al showed that the inhibition of cytochrome P450 sterol 14 α -demethylase (CYP51) is critical for sterol biosynthesis and serves as a key target in the development of antiparasitic and antifungal drugs¹⁹⁸. Promising antiparasitic compounds, such as 4,15-isoatropicolide tiglate, have been identified for their potent activity

against *T. brucei rhodesiense*. However, the precise mechanisms through which these sesquiterpene lactones exert their effects remain to be fully elucidated^{199,200}.



2.10.2 Protein Targets for *Trypanosoma brucei*

For a drug to cure a disease, it must bind to a specific protein target to disrupt the life processes of the disease-causing parasite. The documented viable protein targets for *Trypanosoma brucei* are; Aldolase (*Tb*ALD), Alternative oxidase (*Tb*AOX), Cathepsin B (*Tb*CatB), Dihydrofolate reductase (*Tb*DHFR), Dihydroorotate dehydrogenase (*Tb*DHODH), Glyceraldehyde-3-phosphate dehydrogenase (*Tb*GAPDH), Glycogen synthase kinase 3 (*Tb*GSK3), Hexokinase 1 (*Tb*HK1), N-Myristoyltransferase (*Tb*NMT), Nucleoside 2-deoxyribosyltransferase (*Tb*NDRT), Nucleoside hydrolase (*Tb*NH), Oligopeptidase B (*Tb*OP), Ornithine decarboxylase (*Tb*ODC), Pteridine reductase 1 (*Tb*PTR1) [S78], Pyruvate kinase (*Tb*PyK), Rhodesain, Sphingolipid synthase (*Tb*SLS), Sterol 14 α -demethylase (*Tb*CYP51), Triosephosphate isomerase (*Tb*TIM), Trypanothione reductase (*Tb*TryR), Trypanothione synthetase (*Tb*TryS), and UDP-galactose 4'-epimerase (*Tb*UDPGE)²⁰¹. Parasitic trypanosomatids salvage pterins from their host organisms

using pteridine reductase (PTR1), and this enzyme is a potential target for antiparasitic drug development¹⁶⁸.

2.10.2.1 The *Trypanosoma brucei* Pteridine Reductase 1 (*TbPTR 1*) and the *Trypanosoma brucei* Dihydrofolate Reductase (*TbDHFR*) Enzymes

Trypanosomes lack the ability to synthesize folates and pterins, essential for critical cellular metabolic processes such as nucleic acid and protein synthesis²⁰². To survive, they obtain pterin precursors from their hosts^{203,204}, which are then reduced in two steps to active tetrahydro-derivatives²⁰³. The key enzymes involved in this reduction are dihydrofolate reductase (DHFR, EC 1.5.1.33) and pteridine reductase 1 (PTR1, EC 1.5.1.33)^{205,206}. DHFR, an NADPH-dependent enzyme, converts folate to 7,8-dihydrofolate (DHF) and then to 5,6,7,8-tetrahydrofolate (THF)^{206,207}. However, conventional antifolates like methotrexate (MTX) are less effective against trypanosomes due to the alternative pathway provided by PTR1, an NADPH-dependent enzyme in the short-chain dehydrogenase/reductase (SDR) family^{203,208}. PTR1 also reduces biopterin to 7,8-dihydrobiopterin (DHB) and then to 5,6,7,8-tetrahydrobiopterin (THB)^{206,207}. When DHFR is inhibited, PTR1 gene expression increases, ensuring the supply of reduced folates necessary for parasite survival^{203,209,210}. Studies on PTR1 gene knockdown and knockout in *T. brucei* have shown significant growth inhibition in both lab and animal models, suggesting that dual inhibition of DHFR and PTR1 could be a promising strategy for treating HAT^{208,211}. While PTR1 can reduce dihydrofolate, it cannot fully replace DHFR activity, highlighting DHFR as an important target for anti-trypanosomal drugs^{206,208}. The structure of *T. brucei* DHFR (*TbDHFR*) offers a foundation for developing effective antifolate drugs targeting trypanosomal DHFR, similar to other DHFRs. Comparing *TbDHFR* with *Plasmodium falciparum* DHFR (*PfDHFR*), for which both wild-type and PYR-resistant mutant structures are known²¹², could provide insights into designing effective

treatments. By integrating molecular docking with experimental validation, researchers can efficiently identify and optimize inhibitors for PTR1 and DHFR in trypanosomes, potentially leading to new therapeutic agents against diseases like African sleeping sickness and Chagas disease. Also, integrating these molecular targets with the phytochemical investigation of *T. gentii* provides a rational framework for identifying potential enzyme inhibitors from natural sources. This linkage between plant-derived secondary metabolites and trypanosomal protein targets strengthens the scientific rationale of the present study.

2.11 Conclusion on Trypanosomiasis and Related Molecular Studies

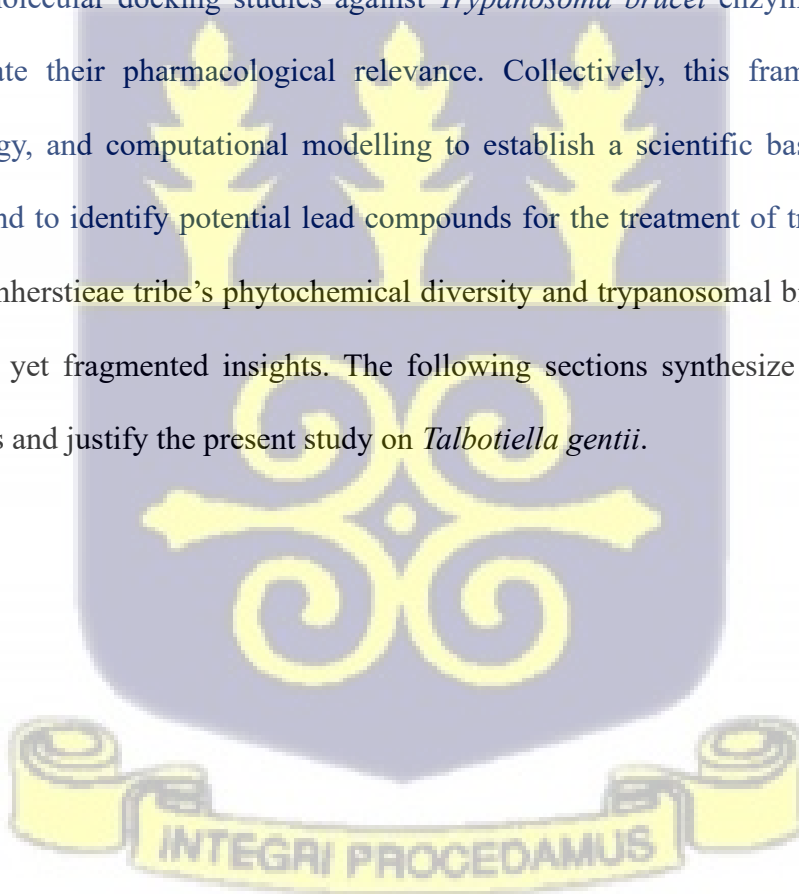
Despite advances in chemotherapy, Human African Trypanosomiasis (HAT) remains a significant public-health concern due to limited drug accessibility, toxicity, and resistance. The literature shows increasing interest in natural products and computational screening as complementary strategies for identifying new inhibitors of validated trypanosomal enzyme targets such as dihydrofolate reductase (*TbDHFR*) and pteridine reductase 1 (*TbPTR1*). However, few studies have combined the isolation of authentic plant metabolites with both *in vitro* and *in silico* evaluation against these enzymes. Addressing this gap, the present research investigates the *in vitro* antitrypanosomal activity and molecular docking profiles of compounds isolated from the dichloromethane extract of *T. gentii*. The outcome is expected to enhance understanding of structure–activity trends among triterpenoids and sterols and to identify promising natural scaffolds for further optimization.

2.12 Conceptual Framework

This study is grounded in the natural product drug discovery framework, which postulates that plants, particularly those within the Fabaceae family, are prolific sources of secondary metabolites with diverse biological activities. The research is guided by the principle that traditional medicinal

use can serve as a rational starting point for identifying pharmacologically active compounds. *Talbotiella gentii*, traditionally employed for managing stomach-related ailments often linked to microbial infections, was hypothesized to contain metabolites with antiparasitic potential.

The study's conceptual model, therefore, connects ethnomedicinal knowledge, phytochemical exploration, and biological validation. It assumes that the extraction and chromatographic fractionation of *T. gentii* will yield terpenoid and sterol scaffolds characteristic of Fabaceae plants. Structural elucidation through spectroscopic methods (^1H -NMR, ^{13}C -NMR, and 2D-NMR) provides molecular-level understanding of these isolates, while *in vitro* antitrypanosomal screening and molecular docking studies against *Trypanosoma brucei* enzymes (*TbDHFR* and *TbPTR1*) evaluate their pharmacological relevance. Collectively, this framework integrates chemistry, biology, and computational modelling to establish a scientific basis for the plant's traditional use and to identify potential lead compounds for the treatment of trypanosomiasis. A review of the Amherstieae tribe's phytochemical diversity and trypanosomal biochemical targets reveals valuable yet fragmented insights. The following sections synthesize these findings to identify key gaps and justify the present study on *Talbotiella gentii*.



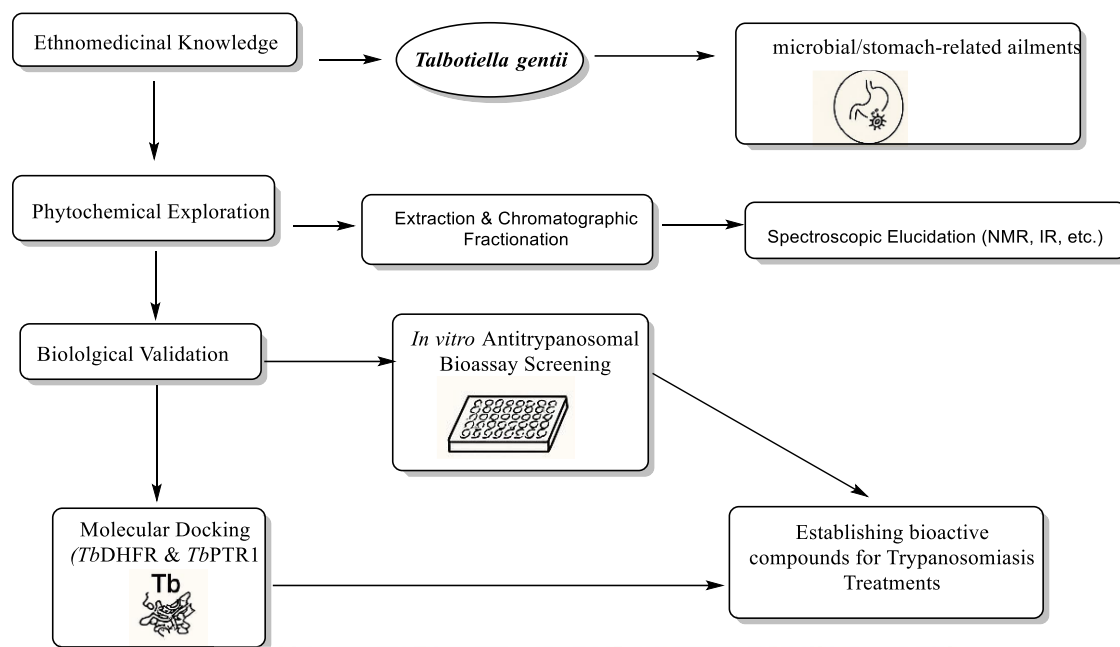


Figure 2-42: Linking Traditional Knowledge to Molecular Innovation: A Conceptual Framework for *T. gentii* Antitrypanosomal Potential

2.13 Chapter Conclusion

This chapter reviewed the current understanding of Human African Trypanosomiasis (HAT), focusing on its transmission, pathophysiology, treatment challenges, and the emerging role of natural products in drug discovery. Although several chemotherapeutic options exist, treatment remains hindered by toxicity, drug resistance, high costs, and limited accessibility, particularly in rural endemic areas. These limitations emphasize the urgent need for safer and more affordable therapies from plant-derived sources.

Within the Fabaceae family, the Amherstieae tribe has shown significant promise as a rich source of bioactive phytochemicals, including triterpenoids, flavonoids, sterols, and phenolic compounds with antimicrobial and antiparasitic activities. However, there is a gap in the chemotaxonomic exploration of this tribe. While genera such as *Tamarindus* and *Berlinia* are well characterized,

Talbotiella species have remained almost entirely unexplored in both phytochemical and pharmacological research.

Talbotiella gentii, the focus of this study, is traditionally used to treat stomach ulcers (which are found to be of microbial origin), yet no phytochemical or antitrypanosomal investigation has been reported on the species. This absence represents a clear gap in both chemotaxonomic and ethnopharmacological knowledge. Given its close relationship to chemically prolific genera, *T. gentii* may contain novel or therapeutically significant compounds worthy of exploration.

Although some Amherstieae species have yielded promising bioactive constituents, few studies have examined their structure–activity relationships. This lack of correlation between chemical structure and biological activity limits understanding of their pharmacological potential and slows the identification of lead compounds. Furthermore, most studies on plant-based antitrypanosomal activity have not combined compound isolation, in vitro evaluation, and molecular docking within a unified approach. The separation of these methods reduces the predictive strength of biological assessments. In addition, even though *Trypanosoma brucei* pteridine reductase 1 (*TbPTR1*) and dihydrofolate reductase (*TbDHFR*) are established molecular targets in antifolate drug development, few natural product studies investigate compounds against these enzymes.

Collectively, the review highlights five major research gaps that justify the present study:

1. The absence of phytochemical and pharmacological data on *Talbotiella gentii*.
2. An uneven distribution of chemotaxonomic research within the Amherstieae tribe.
3. A lack of SAR-focused studies for bioactive phytoconstituents in the tribe.
4. Limited integration of isolation, biological screening, and docking in previous studies.

5. Under exploration of natural products targeting validated trypanosomal enzymes (*TbPTR1* and *TbDHFR*).

By addressing these gaps, this study aims to provide foundational data on the chemical constituents of *T. gentii*, assess their antitrypanosomal potential through *in vitro* and *in silico* approaches, and contribute to the broader chemotaxonomic mapping of the Amherstieae tribe. These insights collectively define the scientific rationale and methodological direction for the experimental chapters that follow.



3.1 General Experimental Methods

The experimental workflow was designed to assess the antitrypanosomal constituents of the stem bark of *T. gentii*. This was achieved by sequentially extracting the plant material with solvents of increasing polarity as established by Ramluckan et al. (2014) and Taufik et al. (2024)^{213,214}. Soxhlet extraction was employed to exhaustively extract the air-dried and pulverized plant material using dichloromethane, ethyl acetate, and methanol in increasing order of polarity. This method was chosen because it minimizes solvent use and allows exhaustive extraction within a shorter time, as well as, enable the extraction and separation of different phytochemicals based on their polarities as reported by Ramluckan et al. (2014) and Eney et al. (2021)^{214,215}. Non-polar solvents first extract lipophilic compounds like fats, oils, and terpenoids, while subsequent polar solvents target more hydrophilic compounds such as flavonoids, alkaloids, and phenolics. This gradient approach improves extraction efficiency and compound selectivity as evidenced by Ramluckan et al. (2014) and Rodrigues et al. (2018)^{214,216}. Antitrypanosomal activities were then carried out on the three different extracts, and based on the activities, the most active extract, dichloromethane (DCM) was selected for phytochemical purification to isolate antitrypanosomal agents as performed by Feyera et al. (2014)²¹⁷. Extracts from medicinal plants exhibit varying antitrypanosomal activities due to differences in phytochemical solubility, with dichloromethane (DCM) extracts often demonstrating superior activity by extracting lipophilic bioactive compounds as supported by Feyera et al. (2014) and Bero et al. (2013)^{217,218}. This activity-guided approach of selecting the most potent extract, like DCM, for phytochemical purification is well established for isolating

novel antitrypanosomal agents, facilitating targeted drug discovery against trypanosomiasis as validated by Feyera et al. (2014) and Bero et al. (2013)^{217–219}.

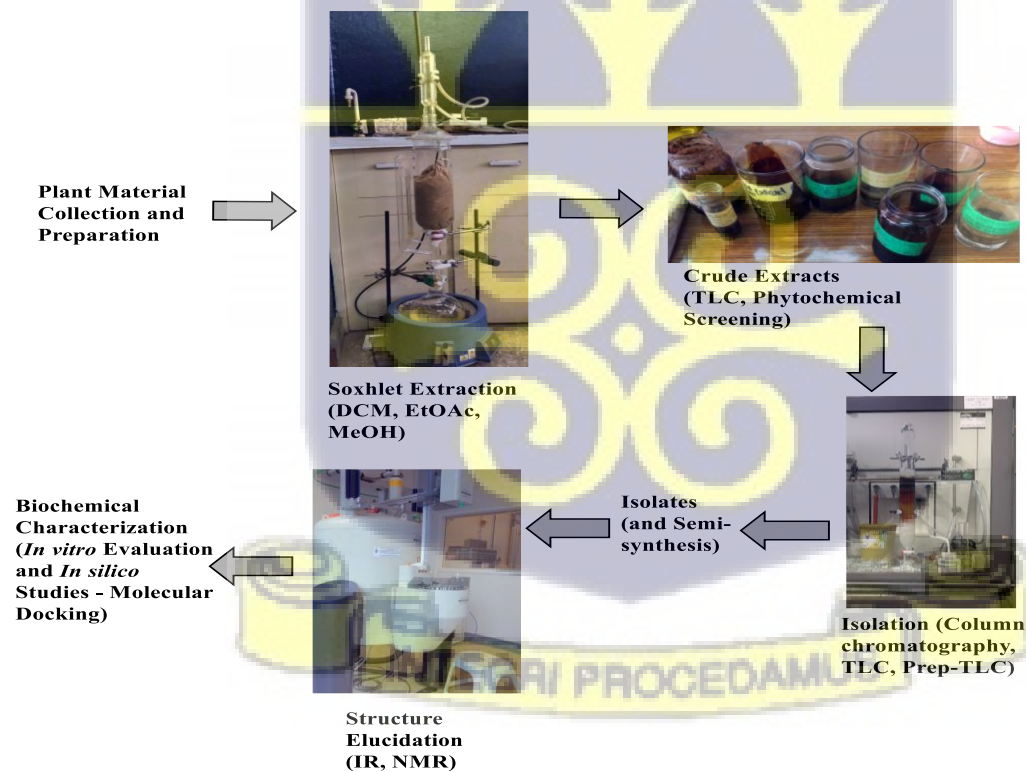
Gravity column chromatography was performed using DAVISIL® silica gel (pore size 60 Å, particle size 40–63 µm; equivalent to mesh size 230–400) as the stationary phase. Although this mesh size is more commonly associated with flash chromatography, it has been successfully used in gravity-based separation to isolate small molecules, such as β-sitosterol, friedelin, and coumarins like seselin and ulopterol, as demonstrated by Nour et al. (2023)²²⁰. The solvent flow was carefully regulated to minimise back pressure and channelling, enabling effective gravity-driven separation. Gravity column chromatography was selected to achieve effective fractionation under non-pressurized conditions suitable for thermolabile natural products. The separations were carried out in a fume hood, ensuring safe handling of volatile solvents. Gradient elution with hexane, dichloromethane, and ethyl acetate mixtures enabled stepwise fractionation according to polarity. Analytical-grade solvents were used and prepared volume-by-volume (v/v); for instance, Hex:DCM (80:20) represents 80 parts hexane to 20 parts dichloromethane. Fractions were monitored by TLC and verified by ¹H-NMR to confirm composition and detect overlapping components beyond single-spot TLC appearances. Fractions showing similar TLC profiles were pooled and further purified as needed.

TLC was used for rapid visualization and monitoring of fractions. Silica gel plates (Kiesegel 60 F254, 0.2 mm thickness) were examined under UV light at 254 nm and 365 nm, then derivatized with anisaldehyde spray reagent (prepared from 96% methanol, 2.5% H₂SO₄, and 1.5% p-anisaldehyde) to reveal terpenoids and sterols. Distinctive band colours were noted as indicative of different phytochemical classes. Where gravity column chromatography did not achieve adequate resolution, smaller columns or preparative TLC plates (silica gel 60G F₂₅₄, 0.5 mm, glass-

backed) were employed as complementary purification methods. Uncorrected melting points of isolated solids were determined using a Stuart Scientific Melting Point Apparatus.

Spectroscopic characterization involved NMR and, where available, IR analyses. Full sets of ^1H -NMR, ^{13}C -NMR, DEPT-135, HSQC, COSY, and HMBC spectra were recorded in deuteriochloroform (CDCl_3) using a Bruker AVANCE 400 MHz spectrometer (University of Surrey, UK), with TMS as reference. Spectroscopic analyses were interpreted systematically. IR absorptions were assigned to characteristic functional groups, while ^1H -NMR and ^{13}C -NMR spectra provided detailed structural information. Correlations through 2D-NMR experiments (COSY, HSQC, and HMBC) confirmed connectivities and resolved structural ambiguities. Minor unassigned signals were attributed to trace natural co-components inherent to plant extracts as explained in our response above. IR analyses were applied selectively where additional functional-group confirmation was required, as NMR offered the principal structural evidence. The combined use of IR and multidimensional NMR ensured accurate dereplication and structural validation. Although 1D-NMR dereplication is often sufficient for known compounds, selected known isolates were also subjected to 2D-NMR for enhanced confirmation, for student training purposes as well as keeping a library of known spectra, given that this was the first phytochemical investigation of *T. gentii*. IR spectra were obtained on a Perkin Elmer 2000 FTIR spectrometer (ATR mode) at the University of Ghana. Spectroscopic analyses were interpreted systematically. IR absorptions were assigned to characteristic functional groups, while ^1H - and ^{13}C -NMR spectra provided detailed structural information. Correlations through 2D-NMR experiments (COSY, HSQC, and HMBC) confirmed connectivity and resolved structural ambiguities. Minor unassigned signals were attributed to trace natural co-components inherent to plant extracts. IR spectroscopy is an important tool for confirming functional groups and assessing sample integrity.

In vitro antitrypanosomal screening of the plant extracts and isolated compounds was conducted at the Department of Biochemistry, Cell and Molecular Biology, University of Ghana, using bloodstream forms of *Trypanosoma brucei brucei* obtained from the Parasitology Department of the Noguchi Memorial Institute for Medical Research, under the guidance of Prof. Theresa Gwira. This assay was selected to evaluate the direct inhibitory potential of the isolates against the parasite and to establish structure–activity relationships among the compounds. Complementary molecular docking studies were performed in collaboration with Prof. Samuel Kwofie at the Department of Biomedical Engineering, University of Ghana, to predict the binding affinities and interaction modes of the bioactive compounds with key *T. brucei* enzymes (*TbDHFR* and *TbPTR1*). This integrative approach was designed to correlate *in vitro* activity with potential molecular targets and to rationalize the mechanisms underlying the observed antitrypanosomal effects.



Scheme 3-1 Graphical representation of experimental procedures

3.2 Materials

3.2.1 Collection and Preparation of Plant Materials

The stems, roots, and leaves of *Talbotiella gentii* were collected on the 2nd of July 2019 at the Sapawusu Forest Reserve Extension in the Eastern Region of Ghana. A Voucher specimen with the code MAC19-2 have been deposited at the Ghana Herbarium at the Department of Plant Science and Environmental Biology, University of Ghana. The plant was identified by Mr. Patrick Ekpe of the same department. Each plant part was air-dried for five weeks and pulverized with a miller.

3.2.2 Solvents and Chemicals

All solvents and reagents were of analytical grade. The solvents used were dichloromethane, ethyl acetate, methanol, hexane, and toluene (BDH®, ≥99% purity). Reagents for phytochemical screening included ferric chloride, bismuth nitrate, potassium iodide, picric acid, mercuric chloride, iodine, acetic anhydride, sulfuric acid, and p-anisaldehyde (Sigma-Aldrich®, USA). Derivatization employed pyridine (≥99%, Sigma-Aldrich®) and acetic anhydride (≥98%, Merck®). All aqueous solutions were prepared with distilled water.

3.2.2.1 Preparation of Anisaldehyde Spray Reagent

To prepare anisaldehyde spray reagent, 96% of cold methanol, 2.5% of H₂SO₄, and 1.5% of p-anisaldehyde were used according to the procedure of Langat (2014)²²¹. To visualize the compounds, the developed TLC plates were sprayed with the reagent and a distinctive colour of the TLC bands was then observed. The reagent was prepared fresh and used. This solution was used to detect most organic compounds, especially terpenoids and steroids. After developing a TLC plate, it is sprayed onto the dried plate, and the plate is then baked at 110 °C in an oven for about 10 minutes. Most organic compounds will stain as blue, violet, green, pink, yellow, red, cream and grey on a light pink background as documented by Spangenberg et al. (2010)²²²

3.2.3 Equipment and Instruments

Major instruments used in this study included a Soxhlet extractor (Quickfit®, UK) for exhaustive extraction, a rotary evaporator (Büchi Rotavapor R-210, Switzerland) for solvent removal, and a column chromatography setup (DAVISIL® silica gel 60 Å, 40–63 µm) for compound separation. Thin-layer chromatography (TLC) and preparative TLC were carried out using Kieselgel 60 F254, 0.2 mm thickness and EMD Millipore® silica gel 60 F₂₅₄ plates (20 × 20 cm, 2 mm layer) respectively. Spectroscopic analyses were performed using a Perkin Elmer 2000 FTIR spectrometer (ATR mode) and a Bruker AVANCE 400 MHz NMR spectrometer (University of Surrey, UK). Antitrypanosomal activity was assessed with a Varioskan Lux Elisa microplate reader (Thermo Fisher Scientific, USA).

3.2.4 Parasite and Cell Culture Materials

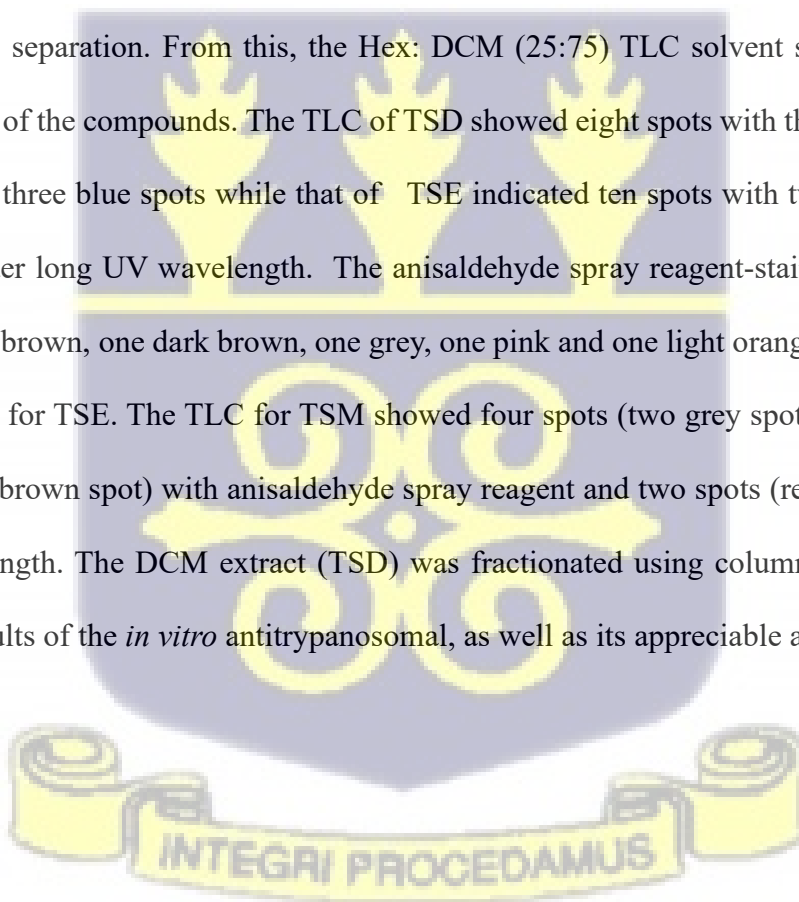
Trypanosoma brucei brucei (GUTat 3.1 strain) was obtained from the Noguchi Memorial Institute for Medical Research, University of Ghana. HM1-9 medium (Thermo Fisher Scientific, USA), fetal bovine serum (Gibco, UK), β-mercaptoethanol, penicillin G sodium, and streptomycin sulfate were used as specified.

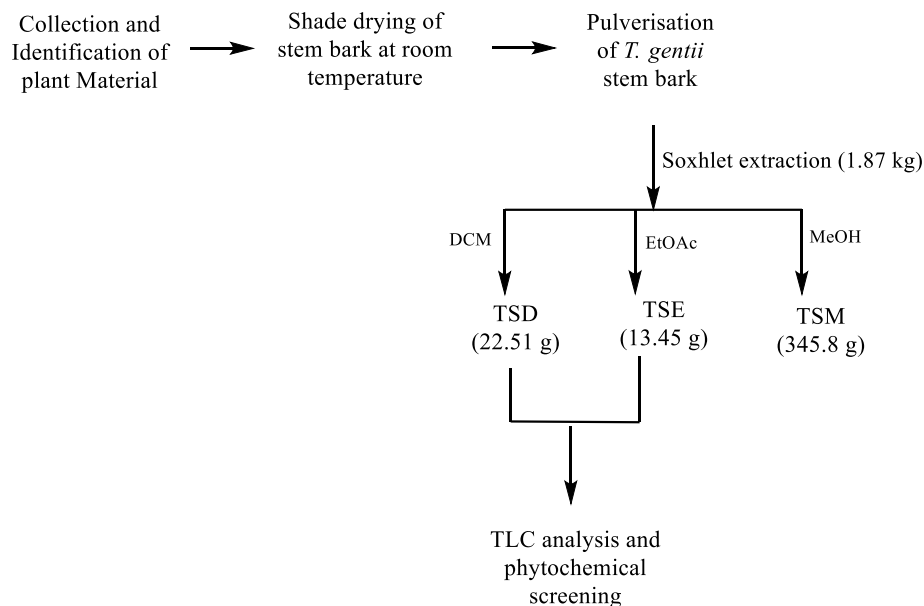
3.3 Methods

3.3.1 Solvent extraction of the stem bark of *Talbotiella gentii*

A 1.87 kg sample of pulverized stem material was extracted sequentially using the Soxhlet method with 4 L each of dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) using the approach of Taufik et al. (2024)²¹³. Each extraction was conducted for 24 hours based on similar methods carried out by Mustafa et al. (2022) and Autor et al. (2022)^{223,224}. The extraction temperature was however, maintained below the boiling point of each solvent to prevent thermal degradation and to optimize the recovery of both free and bound phytochemicals as reported by

Zhang et al. (2022)²²⁵. In place of standard cellulose thimbles, a woven cotton pouch was used to contain the powdered plant material during extraction²²⁶. The extract was subsequently cooled, filtered through cotton and Whatman No. 1 filter paper to remove particulates. The filtrates were then concentrated under reduced pressure using a Cole-Parmer rotary evaporator to afford the crude extracts. The DCM extract (coded TSD) yielded 22.51 g of a dark-brown residue, while the EtOAc (TSE) and MeOH (TSM) extracts afforded 13.45 g and 34.58 g, respectively. TLC was then performed for TSD, TSE, and TSM extracts using Hex: DCM (50:50) and Hex: DCM (25:75) as the solvent system and visualized with long UV wavelength and finally stained with anisaldehyde spray reagent. The purpose was to determine the right solvent system for the chromatographic separation. From this, the Hex: DCM (25:75) TLC solvent system resulted in better separation of the compounds. The TLC of TSD showed eight spots with three red spots, two green spots, and three blue spots while that of TSE indicated ten spots with two red, two green and six blue under long UV wavelength. The anisaldehyde spray reagent-stained TLC for TSD showed six (two brown, one dark brown, one grey, one pink and one light orange) as compared to five brown spots for TSE. The TLC for TSM showed four spots (two grey spots, one purple spot and one reddish-brown spot) with anisaldehyde spray reagent and two spots (red and blue) under long UV wavelength. The DCM extract (TSD) was fractionated using column chromatography based on the results of the *in vitro* antitrypanosomal, as well as its appreciable amount.





Scheme 3-2 Schematic representation of preparation of *T. gentii* stem extracts.

3.3.2 Phytochemical Screening

Since *T. gentii* had not been previously investigated, preliminary phytochemical screening was therefore conducted to guide solvent selection, prioritize bioactive fractions for isolation, and establish baseline chemotaxonomic data. Although the main aim of the study was to isolate pure compounds, this screening step was necessary to qualitatively identify the major classes of secondary metabolites present in each solvent fraction. The dichloromethane (TSD) and ethyl acetate (TSE) crude extracts of *T. gentii* stem bark were therefore screened separately for alkaloids, anthraquinones and anthracene derivatives, flavonoids, cardiac glycosides, tannins, terpenoids, steroids, and saponins using established methods^{227–231}.

Standard qualitative reagents and tests were employed, including Dragendorff's, Mayer's, Wagner's, and Hager's reagents for alkaloids; the Shinoda test for flavonoids; the Keller–Kiliani test for cardiac glycosides; the Borntrager's test for anthraquinones; the frothing test for saponins; the ferric chloride test for tannins; and the Liebermann–Burchard and anisaldehyde spray reagents

for terpenoids and steroids. All reagents and procedures followed established phytochemical protocols described by Harborne (1998)²²⁹, Sofowora²³², Trease & Evans (2002)²³⁰, and Wagner & Bladt (1996)^{221,233}.

3.3.2.1 Detection of Major Phytochemical Groups

Approximately 0.1 g of each crude extract was used for all qualitative tests. Each extract was analysed for the following groups of secondary metabolites:

Alkaloids: About 0.1 g of each extract was dissolved in 5 mL of 2 M HCl, filtered, and tested with Dragendorff's, Mayer's, Wagner's, and Hager's reagents following Cromwell²³⁴ and Harbone²²⁹, and Trease & Evans²³⁰. Formation of characteristic precipitates indicated alkaloids.

Anthraquinones and Anthracene Derivatives: Each of the crude extracts (0.1 g) was dissolved with distilled water (30 mL) in a test tube, and the resulting mixture was filtered. The filtrate was then tested for free and combined anthraquinones using the Borntrager's reaction as described in reference²²⁷.

Cardiac Glycosides: A 0.1 g portion of each extract was subjected to the Keller–Kiliani test; the appearance of a brown-red ring at the interface confirmed cardiac glycosides²²⁸.

Flavonoids: About 0.1 g of extract was dissolved in 10–15 mL of 80 % ethanol and analysed by the Shinoda test following Sofowora²³⁰. A pink or orange coloration indicated flavonoids.

Saponins: For the frothing test, 0.1 g of each extract was shaken vigorously with 10 mL of distilled water; persistent foam after standing indicated the presence of saponins²³¹.

Tannins: Approximately 0.5 g of extract was mixed with a few drops of FeCl₃ solution according to Harborne^{235,236}. Blue-black or green coloration indicated tannins or phenolics.

Terpenoids and Steroids: TLC was performed on 0.1 g of extract dissolved in suitable solvent using petroleum ether–ethyl acetate systems (7:3 and 8:2). Plates were visualized with Liebermann–Burchard and anisaldehyde spray reagents, prepared according to Wagner & Bladt (1996)^{221,233,237}. Red-violet coloration indicated terpenoids, while reddish-brown coloration signified steroids.

3.3.3 Column chromatographic separation of the dichloromethane fraction, TSD

A 12 g of TSD extract was dissolved in a 10 mL DCM to form a solution. A 6 g of silica gel was added to the solution, swirled, and placed in the fume hood overnight for it to dry. The resulting dried mixture was then stirred to give a consistent powdery mixture. A slurry of silica gel (310 g) and hexane (1 L) was then prepared and packed into a chromatographic column. The extract was loaded by the dry loading method explained earlier. A layer of cotton wool was placed on top of the column to ensure the integrity of the stationary phase during elution.

Based on the preliminary TLC profile of the TSD, elution was initially done with 500 mL of hexane, and 40 mL of the eluate fractions were collected into a beaker. Gradient elution was achieved by gradually increasing the polarity of the eluent from 100% hexane to Hex: DCM (25:75), 100% dichloromethane, and finally with 100% ethyl acetate. The eluates were collected and monitored with TLC and ¹H-NMR using deuterated chloroform. Fractions were refrigerated to facilitate precipitation. The resulting solids were either purified by trituration with acetone or recrystallisation. Other purification methods like PrepTLC and column (including Pasteur-scale) chromatography were attempted. Purity of isolates were determined by TLC and ¹H-NMR analyses and Subsequent characterization using FT-IR and NMR spectroscopy was done for the isolates. The physical parameters (R_f, nature of isolates, and corresponding melting points) of the characterized compounds were also noted. Based on the TLC profiles, similar eluates were

combined into four subfractions coded TSD-A (2.6 g), TSD-B (4.6 g), TSD-C (2.2 g), and TSD-D (1.7 g). The subfractions were worked on as below.

3.3.3.1 Work done on Subfraction TSD-A

Subfraction TSD-A ($\approx$ 2.6 g), obtained from the initial non-polar eluates (Hexane $\rightarrow$ Hex:DCM 90:10), showed faint or unresolved bands on TLC with very high R_f values (0.85–0.95) and weak response under UV light. $^1\text{H-NMR}$ (CDCl_3) spectra exhibited mainly aliphatic signals (δ 0.8–1.6 ppm) typical of long-chain hydrocarbons, waxes, and fatty esters, but no diagnostic triterpenoid resonances. Attempts at recrystallisation and small-scale prep-TLC did not yield distinct compounds; thus, TSD-A was archived for possible future profiling while isolation efforts focused on TSD-B–D, which contained well-resolved triterpenoid constituents.

3.3.3.2 Work done on Subfraction TSD-BA

Subfraction TSD-BA (1.5), from TSD-B was subjected to thin-layer chromatography (TLC) and proton nuclear magnetic resonance ($^1\text{H-NMR}$) analyses. Initial TLC using hexane:dichloromethane (60:40) revealed two spots under UV and after anisaldehyde staining. A subsequent TLC with hexane:dichloromethane (25:75) yielded better resolution of the two components, prompting the use of preparative thin-layer chromatography (prepTLC) with the latter solvent system.

PrepTLC (EMD Millipore® silica gel 60 F₂₅₄ glass plates, 20 $\times$ 20 cm, 2 mm layer, 10–12 μm particles, 60 Å pores).was employed to isolate and purify individual components based on prior TLC and $^1\text{H-NMR}$ data indicating the presence of multiple constituents. The prepTLC plate was marked 3 cm from the base with a 0.5 cm-wide application band. The sample was applied using a glass Pasteur pipette and allowed to dry thoroughly. The developing solvent

(hexane:dichloromethane, 75:25) was pre-equilibrated in a TLC chamber for 10 minutes prior to development.

The plate was developed to the top, dried in a fume hood, and subjected to a second development under the same conditions to enhance resolution. Visualization under short- and long-wavelength UV light revealed seven distinct bands, designated PT1 through PT7. These were scraped off with a metal spatula and extracted with 10 mL portions of dichloromethane, followed by filtration. The extraction process was repeated to ensure complete recovery.

The resulting eluates were analyzed via ^1H -NMR, and based on spectral overlay, the components were pooled into three major fractions: TSD-BA₁ (0 g), TSD-BA₂ (0.68 g), and TSD-BA₃ (0.82 g). The collected eluates were subjected to ^1H -NMR analysis, and based on spectral similarities observed through overlay, they were combined into three main fractions: TSD-BA₁, TSD-BA₂, and TSD-BA₃. These fractions were then stored in the fridge for further analysis.

3.3.3.2.1 Subfraction TSD-BA₁

TSD-BA₁ was found to contain no significant material, as confirmed by ^1H -NMR analysis (Appendix I-27), which showed only solvent peaks and no characteristic compound signals. This was likely due to the band consisting of solvent artifacts or non-detectable material under UV and staining conditions.

3.3.3.2.2 Subfraction TSD-BA₂

PT3 and PT4 exhibited similar ^1H -NMR spectra upon overlay and were therefore combined and coded as TSD-BA₂. The ^1H -NMR spectrum of TSD-BA₂ (Appendix I-28) showed a clean profile, including a sextet at 3.29 ppm, indicative of a proton adjacent to five others, likely within a methylene or methine group near an electronegative atom such as oxygen. A singlet at 2.31 ppm suggested protons adjacent to a carbonyl or similar electron-withdrawing group, while a singlet

around 2.1 ppm was consistent with a methyl group in a deshielded environment. Multiple peaks between 0.77 and 1.28 ppm indicated the presence of aliphatic methyl groups.

Despite the clean ^1H -NMR spectrum, the ^{13}C -NMR spectrum appeared unresolved. Attempts to further purify TSD-BA₂ using Pasteur pipette-scale column chromatography did not achieve any separation.

3.3.3.2.3 Subfraction TSD-BA₃

PT5, PT6, and PT7 displayed similar ^1H -NMR spectra upon overlay and were therefore combined and designated as TSD-BA₃. The ^1H -NMR spectrum of TSD-BA₃ (Appendix I-29) showed a vinylic proton signal at 5.12 ppm and an allylic proton signal at 2.04 ppm, indicative of a compound containing a double bond and adjacent aliphatic protons. A cluster of peaks around 0.88 ppm was consistent with terminal methyl groups at the end of a long-saturated hydrocarbon chain. Additionally, singlets observed at 1.68 ppm and 1.59 ppm suggested the presence of methyl or methylene groups in non-deshielded, aliphatic environments, likely not adjacent to electronegative atoms or other proton-containing groups.

3.3.3.3 Work done on Subfraction TSD-BB

White solids precipitated from subfraction TSD-BB (1.4 g) when stored in the refrigerator. The TSD-BB solution was decanted, and the solids were washed with acetone, dried, and labelled TSD-BB₁ (10.1 mg). These solids were soluble in dichloromethane and chloroform. Thin-layer chromatography (TLC) of the solid, run with Hex/DCM (70:30) and $\text{CHCl}_3/\text{EtOAc}$ (90:10), revealed single spots with R_f values of 0.72 and 0.86, respectively. The spots fluoresced blue under UV light and appeared pale brown when stained with anisaldehyde spray reagent. Proton NMR analysis of the solids showed characteristic peaks consistent with triterpenoids. A full set of NMR spectra was then obtained to confirm the identity of the compound.

A second crop of creamy, oily solids also precipitated from the solution, which was found to be soluble in DCM. However, due to the small amount obtained, further analysis could not be carried out on it.

3.3.3.4 Work done on Subfraction TSD-BC

Subfraction TSD-BC (0.9) precipitated white solids, which were washed with acetone. The solids were triturated with acetone and coded TSD-BC₁ (5.1 mg). The white solids were found to be soluble in dichloromethane and ethyl acetate with a melting point of 213 – 215 °C. TLC analysis on the solids using hex: dcm (25:75) and hex: EtOAc (50:50) indicated one blue spot under UV, which turned reddish brown when stained with anisaldehyde reagent spray with R_f of 0.43 and 0.5 respectively. H-NMR analysis on the solids revealed resolved peaks characteristic of triterpenoids. This information led to the full set of NMR analysis on the compound for characterisation.

3.3.3.5 Work done on Subfraction TSD-BD

Fraction TSD-BD (0.8 g) precipitated solids, which were then washed with acetone to afford white solids coded TSD-BD₁ (20.1 mg). TLC analysis on the solids using Hex: DCM (25:75) indicated four spots under UV and four spots (one reddish brown and three light brown) when stained with anisaldehyde reagent spray. A comparison of the TLC profile of TSD-BD₁ with that of TSD-BC₁ suggested that TSD-BD₁ contained traces of TSD-BC₁. Based on their TLC profiles and overlaid ¹H NMR spectra, TSD-BC₁ was identified as a relatively pure compound, while TSD-BD₁ appeared to be impure, likely due to the presence of co-eluting, structurally related constituents. Given the potential biological relevance of TSD-BD₁ and the challenges associated with achieving complete purification, the compound was selected for chemical derivatization.

3.3.3.5.1 Acetylation of TSD-BD₁

Acetylation was selected as the derivatization strategy to achieve two main objectives: first, to enhance the analytical purity of the isolated compound by modifying its hydroxyl group and improving separation from co-eluting impurities; and second, to generate a structurally modified analogue for *in vitro* antitrypanosomal screening, enabling preliminary assessment of structure–activity relationships. This decision was further supported by the biological results of the parent isolate, which showed promising antitrypanosomal activity, indicating that modification of its hydroxyl group could potentially enhance its bioactivity. The semi-synthesis was therefore performed to obtain an acetylated analogue for comparative evaluation, guided by experimental findings.

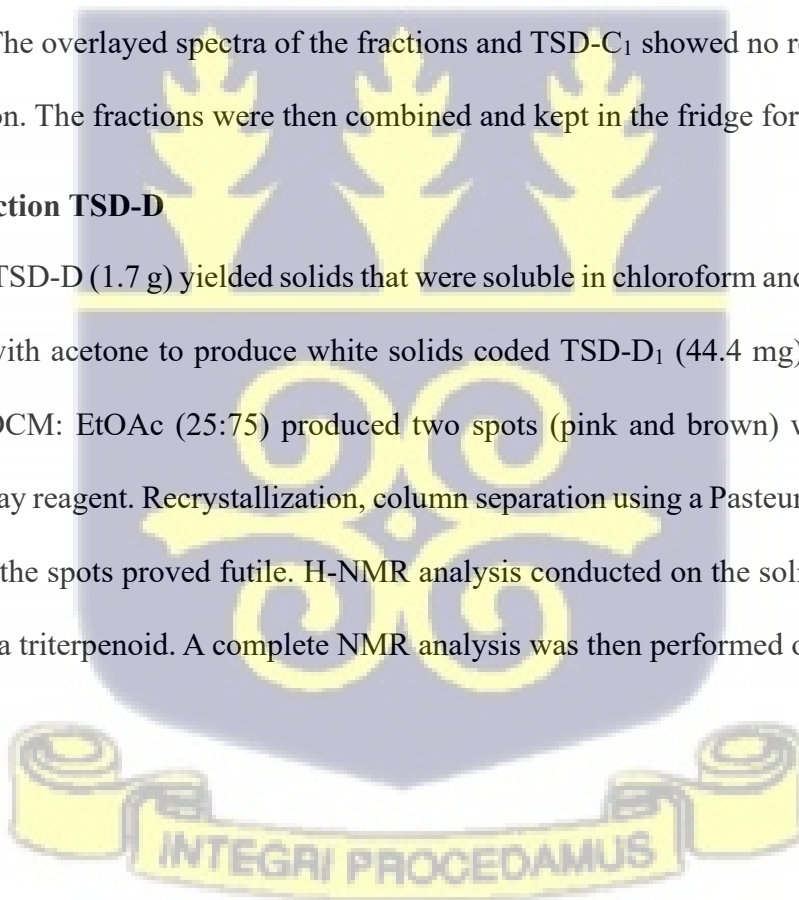
For the acetylation, pyridine (1 mL) and acetic anhydride (1 mL) were introduced to the TSD-BD₁ in a round-bottom flask. The reaction mixture was stirred at room temperature and allowed to proceed for 24 hours. Afterwards, methanol (5 mL) was added to quench the reaction, and the mixture was evaporated under reduced pressure to eliminate unreacted acetic anhydride. To remove residual pyridine, four portions of 5 mL of toluene were added sequentially and evaporated under reduced pressure. This was followed by five portions of methanol (5 mL each), also evaporated under reduced pressure, to eliminate any remaining toluene²²¹. The completion of the reaction and suitability of the resulting derivative TSD-BD_{1A} for chromatographic purification were assessed using TLC and ¹H NMR spectroscopy. Both the pure isolate TSD-BC₁ and the acetylated derivative TSD-BD_{1A} were subsequently subjected to *in vitro* antitrypanosomal testing against *Trypanosoma brucei*.

3.3.3.6 Work Done on Subfraction TSD-C

Subfraction TSD-C (2.2 g) yielded impure solids TSD-C₁. TLC analysis on the solids gave five spots. This impure solid was further subjected to column separation using Hex: DCM (25: 75) and monitored H-NMR. The fraction TSD-C₁ was dissolved in 10 mL of DCM. The mixture was then placed in the fume chamber for 2 hours to dry. A small column was packed with hexane and silica gel slurry. The dried mixture (TSD-C₁ and silica gel) was thoroughly stirred and loaded onto the column. The column was first eluted with 100 mL of Hexane, and 5 mL of fractions were collected into vials and monitored with H-NMR. The eluting solvent was then changed to Hex: DCM (25:75). 40 fractions were collected, and their spectra were compared by overlaying with TSD-C₁ and each other. The overlayed spectra of the fractions and TSD-C₁ showed no resolution from the column separation. The fractions were then combined and kept in the fridge for future analysis.

3.3.3.7 Subfraction TSD-D

The subfraction TSD-D (1.7 g) yielded solids that were soluble in chloroform and DCM. The solids were triturated with acetone to produce white solids coded TSD-D₁ (44.4 mg). TLC analysis of TSD-D₁ using DCM: EtOAc (25:75) produced two spots (pink and brown) when stained with anisaldehyde spray reagent. Recrystallization, column separation using a Pasteur pipette, and prep-TLC to separate the spots proved futile. H-NMR analysis conducted on the solids revealed peaks characteristic of a triterpenoid. A complete NMR analysis was then performed on the solids.



3.3.4 *In vitro* Antitrypanosomal Activity Evaluation

In vitro antitrypanosomal analysis using blood-stream forms of *Trypanosoma brucei brucei* were done on the extracts, isolated compounds and the derivatized compound using the Alamar blue method²³⁸.

3.3.4.1 Parasite Culture and Preparation

Bloodstream forms of *Trypanosoma brucei brucei* (GUTat 3.1 strain) were cultured in HM1-9 medium supplemented with 10% heat-inactivated fetal bovine serum, β -mercaptoethanol, penicillin G sodium (100 μ g/mL), and streptomycin sulfate (100 μ g/mL). The cultures were maintained at 37°C and subcultured every 72 hours to ensure optimal growth. For the assay, the parasite suspension was adjusted to a final density of 4×10^3 cells/mL.

3.3.4.2 Stock Solution Preparation and Assay Set-up

Stock solutions were prepared for the crude extract, fractions, and isolated compounds. Each sample, including the dichloromethane extract (TSD), ethyl acetate extract (TSE), methanol extract (TSM) fractions, and selected isolates (TSD-BB₁, TSD-BC₁, TSD-BD_{1A}, and TSD-D₁), was dissolved in 1 mL of dimethyl sulfoxide (DMSO) to achieve a concentration of 10 mg/mL. For isolates where less than 10 mg was available, 5 mg of the isolate was used. The solutions were filtered through a 0.45 μ m Millipore filter under sterile conditions and stored at -20°C until further use.

In the 96-well plate assay, a total volume of 100 μ L per well was maintained. Initially, 50 μ L of medium was used to dilute the test compounds, which were tested in triplicate across a concentration range from 100 to 0.196 μ g/mL. Following compound dilution, 50 μ L of a trypanosome suspension (at twice the desired final concentration) was added to each well, ensuring a final cell density of 4×10^3 cells/mL. Diminazene aceturate was included as a positive control.

3.3.4.3 Incubation, Viability Assessment, and Data Analysis

The inoculated plates were incubated at 37°C under a 5% CO₂ atmosphere for 72 hours. After the incubation, 10 µL of a 44 µM sodium resazurin solution (Alamar Blue) was added to each well to assess parasite viability. The plates were incubated for an additional 4 hours, and fluorescence was measured using a Varioskan Lux Elisa microplate reader (Thermo Fisher Scientific, USA) with an excitation wavelength of 536 nm and an emission wavelength of 588 nm.

The fluorescence readings were used to determine the percentage of viable cells relative to control wells. The data were plotted against the concentration of the test samples, and IC₅₀ values (the concentration required to inhibit 50% of the parasite population) were calculated using GraphPad Prism version 9.1.2.226.

3.3.5 Molecular docking studies on isolated and characterized compounds

Molecular docking studies were done to predict how strongly the isolates bind to the protein target of *Trypanosoma brucei brucei*, specifically, *Tb*PTR1 and *Tb*-DHFR by means of their binding affinities.

3.3.5.1 Targets and small molecules retrieval and preparation

The 3D structures of *Trypanosoma brucei*-Pteridine Reductase 1 (*Tb*-PTR1) and *Trypanosoma brucei*-dihydrofolate reductase (*Tb*-DHFR) were retrieved from the Protein Data Bank (PDB)²³⁹ with PDB identifications of 6RX6 and 3QFX solved at a resolution of 1.11 Å and 2.20 Å respectively. The *Tb*PTR1 structure (PDB ID: 6RX6) was originally co-crystallized with inhibitor 4 (NMT-C0026)²⁴⁰. The *Tb*-DHFR structure (PDB ID: 3QFX) was originally cocrystallized with pyrimethamine²⁴¹. All co-crystallized ligands and water molecules were removed to expose the binding site prior to docking.

Leveraging Biovia Discovery Studio visualizer V21.1.0.20298188, the bounded ligands and water molecules were removed before subjecting the structures to a 5,000-step energy minimisation using the steepest descent algorithm, applying the Optimised Potential Liquid Simulation (OPLS) all-atom force field. This is to ensure thermodynamically favourable docking process²⁴². The energy-minimized structures were then saved as AutoDock files before docking.

The structure Data File (SDF) 3D conformers of the isolates TSD-BB₁, TSD-BC₁, and TSD-D₁ as well as the semi synthesized derivative TSD-BD_{1A}, were retrieved from PubChem²⁴³. The energies of the compounds were minimised using the Universal Force Field (UFF)²⁴⁴ then saved as AutoDock files.

3.3.5.2 Molecular docking Procedure

To determine the binding affinity between the targets and the compounds and also their binding pose within the binding pockets of the proteins, AutoDock Vina²⁴⁵ was used to virtually screen the compounds against the targets.

The docking grid for *Tb*PTR1 was centered at (x = 37.04, y = 33.05 and z = 49.73 Å³) coordinates with a grid dimension of 26.20 × 26.70 × 22.18 Å³. For *Tb*DHFR, the grid box centered at (x = 40.29, y = 40.52 and z = 42.02 Å³) with dimensions 30.93 × 34.16 × 26.28 Å³.

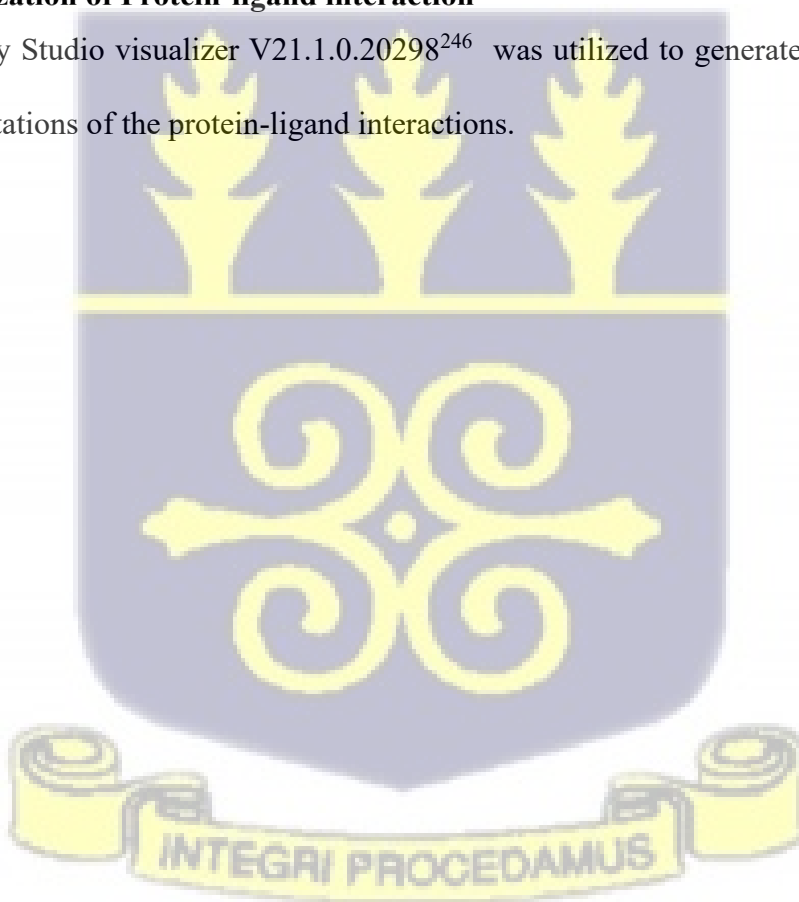
An exhaustiveness value of 8 was used for all runs. For validation and comparative analysis, pyrimethamine (CID 4993) and cycloguanil (CID 9049) were used as reference inhibitors for *Tb*DHFR, whereas CID 146675128, a known *Tb*PTR1 inhibitor, served as the reference ligand for *Tb*PTR1. The docking results were evaluated based on binding energies (kcal mol⁻¹) and interaction patterns within the active-site residues. The most negative binding-energy (lowest binding energy) pose for each ligand was selected for further interaction analysis.

Table 3-1 Docking Parameters of *Trypanosoma brucei* Target Proteins

Parameter	<i>Tb</i> PTR1 (Pteridine Reductase 1)	<i>Tb</i> DHFR (Dihydrofolate Reductase)
PDB ID	6RX6 ²⁴⁰	3QFX
Resolution (Å)	1.11 Å	2.20 Å
Experimental method	X-ray diffraction	X-ray diffraction
Co-crystallized ligand (bound ligand)	inhibitor 4 (NMT-C0026)	pyrimethamine
Grid box centre (x, y, z in Å)	37.04, 33.05, 49.73	40.29, 40.52, 42.02
Grid box dimensions (Å ³)	26.20 × 26.70 × 22.18	30.93 × 34.16 × 26.28
Reference inhibitor used for benchmarking	CID 146675128 (PTR1 inhibitor)	Pyrimethamine (CID 4993), Cycloguanil (CID 9049)

3.3.5.3 Visualization of Protein-ligand interaction

Biovia Discovery Studio visualizer V21.1.0.20298²⁴⁶ was utilized to generate and visualize 2D and 3D representations of the protein-ligand interactions.



CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Summary of Chapter

This chapter provides a comprehensive analysis of all the isolates TSD-BB₁, TSD-BC₁, and TSD-D₁ as well as the semi-synthesised derivative TSD-BD_{1A}, comparing the findings with existing literature, and offering insights into potential future research directions. In this current research work, gravity chromatographic separation was done on the dichloromethane extract of the stem of *T. gentiit*. Following chromatographic separation, the fractions were characterized and subjected to *in silico* molecular docking and *in vitro* investigation.

Column chromatography of the dichloromethane extracts (TSD) led to the isolation and characterization of three compounds, isolates TSD-BB₁, TSD-BC₁, and TSD-D₁ as well as the semi synthesized derivative TSD-BD_{1A}, two mixtures, and semi-synthesis (acetylation) of one of the isolated compounds. The compounds were mainly characterized using one and two-dimensional ¹H and ¹³C-NMR, and IR. In the case of mixtures, characteristic 1D and 2D NMR signals and comparison with literature values were used to identify compounds. The fraction TSD-BB afforded solids TSD-BB₁ which were characterized to be friedelan-3-one (friedelin [505]), fraction TSD-BC gave solids TSD-BC₁ which were characterized to be lup-20(29)-en-3-ol (lupeol, [38]) respectively. The second isolated solid TSD-D₁ which was a mixture from fraction TSD-D was found to contain stigmast-5-en-3 β -ol (β -sitosterol [44]) and 3 β -stigmast-5,22-dien-3 β -ol (stigmasterol [42]). *In vitro* antitrypanosomal activity investigation of six compounds and fractions derived from a plant, identified lupeol acetate [47] as the most potent compound with an IC₅₀ of 2.9 μ g/mL, followed by lupeol [38] with an IC₅₀ of 3.5 μ g/mL. Other compounds including friedelin, and stigmasterol/ β -sitosterol mixture showed moderate to less significant activity

compared to the reference drug diminazene aceturate. Further Molecular docking studies were then performed on the isolates to study their binding interactions, The *in silico* molecular docking study found that friedelin had the strongest binding affinity to *Trypanosoma brucei* enzymes *TbDHFR* and *TbPTR1*, surpassing known inhibitors, while other ligands like stigmasterol [42], β -sitosterol [44], and lupeol also showed promising affinities. These ligands could function as multi-target inhibitors, potentially reducing parasite resistance and enhancing antitrypanosomal drug effectiveness. While the findings align with previous studies, discrepancies in binding affinities, particularly for lupeol [38] and β -sitosterol [44], highlight the impact of different docking methodologies, with this study employing AutoDock Vina and energy-optimized models, whereas earlier research used Molegro Virtual Docker with rigid protein models and flexible ligands. These findings suggest that lupeol acetate [547] and lupeol [38] from the plant have potential for development as antitrypanosomal agents.

4.2 Phytochemical Screening of the Crude Extracts of *T. gentii* Stem Bark

A 1.87 kg sample of pulverized stems was extracted using 4 L of dichloromethane (DCM) via Soxhlet extraction for 24 hours. The filtered extract was evaporated, resulting in 22.51 g of a dark brown crude extract (coded TSD). Successive extractions with 4 L each of ethyl acetate (EtOAc) and methanol (MeOH) yielded 13.45 g (coded TSE) and 345.8 g (coded TSM) of their respective extracts.

Table 4-1: Phytochemical Screening on the crude extracts of the stem and Root of *Talbotiella gentii*

Phytochemicals	TSD	TSE
Alkaloids	-	-
Anthracene and Anthraquinone derivatives	-	+
Cardiac glycoside	+	+
Flavonoids	+	-
Saponins	+	+

Steroid	+	+
Tannins	+	+
Terpenoids	+	+

Legend: + means positive test; - means negative test

The phytochemical screening for the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, terpenoids, anthracene derivatives, and anthraquinone derivatives revealed varying results across the two different extracts of *T. gentii*. The dichloromethane (DCM) extract of the *T. gentii* stem (TSD) tested positive for all phytochemicals except alkaloids, anthracene, and anthraquinone derivatives. In reality, the DCM extract should not contain phytochemicals such as phenolics; however, some phenolics may occur as ester- or ether-linked derivatives of other phytochemicals, reducing their polarity and enabling partial extraction into the dichloromethane (DCM) fraction as reported by Mottaghipisheh et al. (2020)²⁴⁷. A study by Kılınç (2021) reported the isolation of several phenolic compounds, including luteolin-6-C-glucoside, luteolin-7-O-glucoside, caffeic acid, ursolic acid, 4-O-caffeoylquinic acid, and 3,5-O-dicaffeoylquinic acid, from the DCM extract of *Scabiosa sicula*²⁴⁸. Therefore, phytochemical screening for phenolic compounds was performed as an exploratory step to assess potential phenolics that might partition into less polar fractions. The ethyl acetate extract of the *T. gentii* stem (TSE), on the other hand, showed positive results for all phytochemicals except alkaloids and flavonoids. Table 4.1 contains the results of the preliminary phytochemical test done on the extracts.

4.3 Isolation and Semi- synthesis of compounds from TSD

A 12 g of the crude extract, TSD, was fractionated into four main fractions: TSD-A (2.60 g), TSD-B (4.60 g), TSD-C (2.20 g), and TSD-D (1.70 g). Fraction TSD-B, obtained from a hexane:

dichloromethane (25:75) solvent system, was further fractionated to yield four main subfractions TSD-BA (1.50 g), TSD-BB (1.40 g), TSD-BC (0.90 g), and TSD-BD (0.80 g), to separate closely eluting phytochemicals, improve purity, and facilitate the identification of individual constituents within the mixture. The scheme of the work done on TSD fraction is presented below in scheme 4-1.

For subfraction TSD-BA, TLC and ^1H -NMR analyses were conducted. TLC analysis using hexane: dichloromethane (60:40) revealed two spots under UV and anisaldehyde reagent spray. A second TLC analysis using hexane: dichloromethane (25:75) also gave two spots, but with better separation. This prompted the use of preparative TLC (prepTLC) with the latter solvent system. PrepTLC was employed to isolate and purify specific compounds from the fraction, especially as TLC and NMR suggested the presence of a mixture. It also aimed to separate co-eluting compounds and obtain purer materials for further analysis or biological testing. However, the separated bands remained impure and did not achieve sufficient resolution.

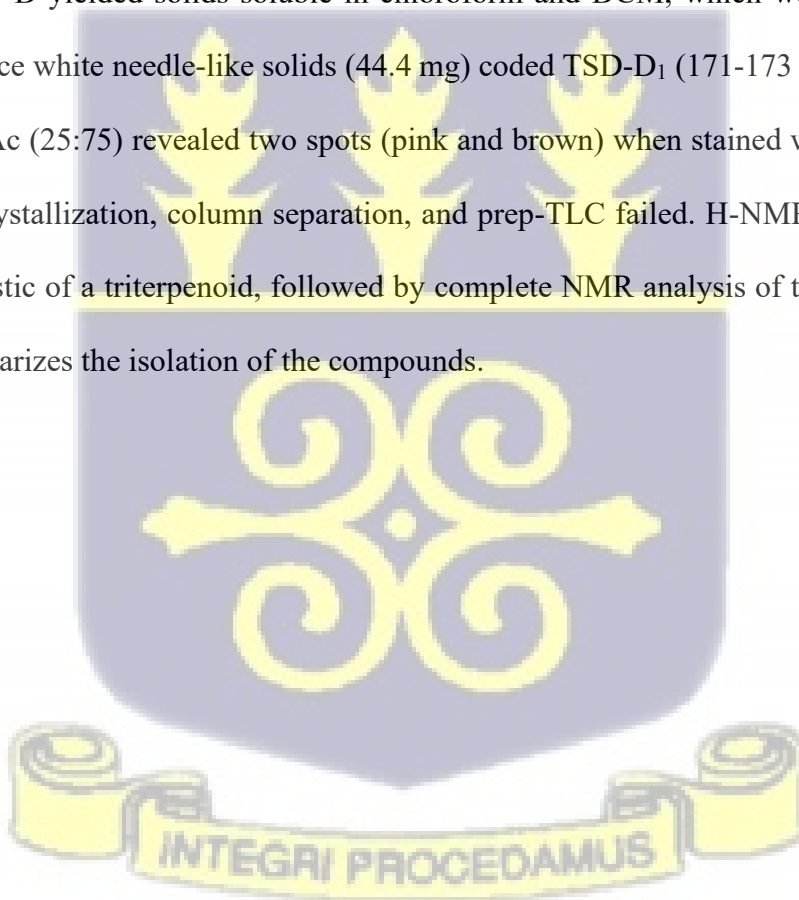
White solids precipitated from subfraction TSD-BB and were washed, dried, and labelled TSD-BB₁ (10.1 mg). These solids with a melting point of 261-263 °C were soluble in DCM and chloroform, and TLC and NMR analysis confirmed the presence of triterpenoids.

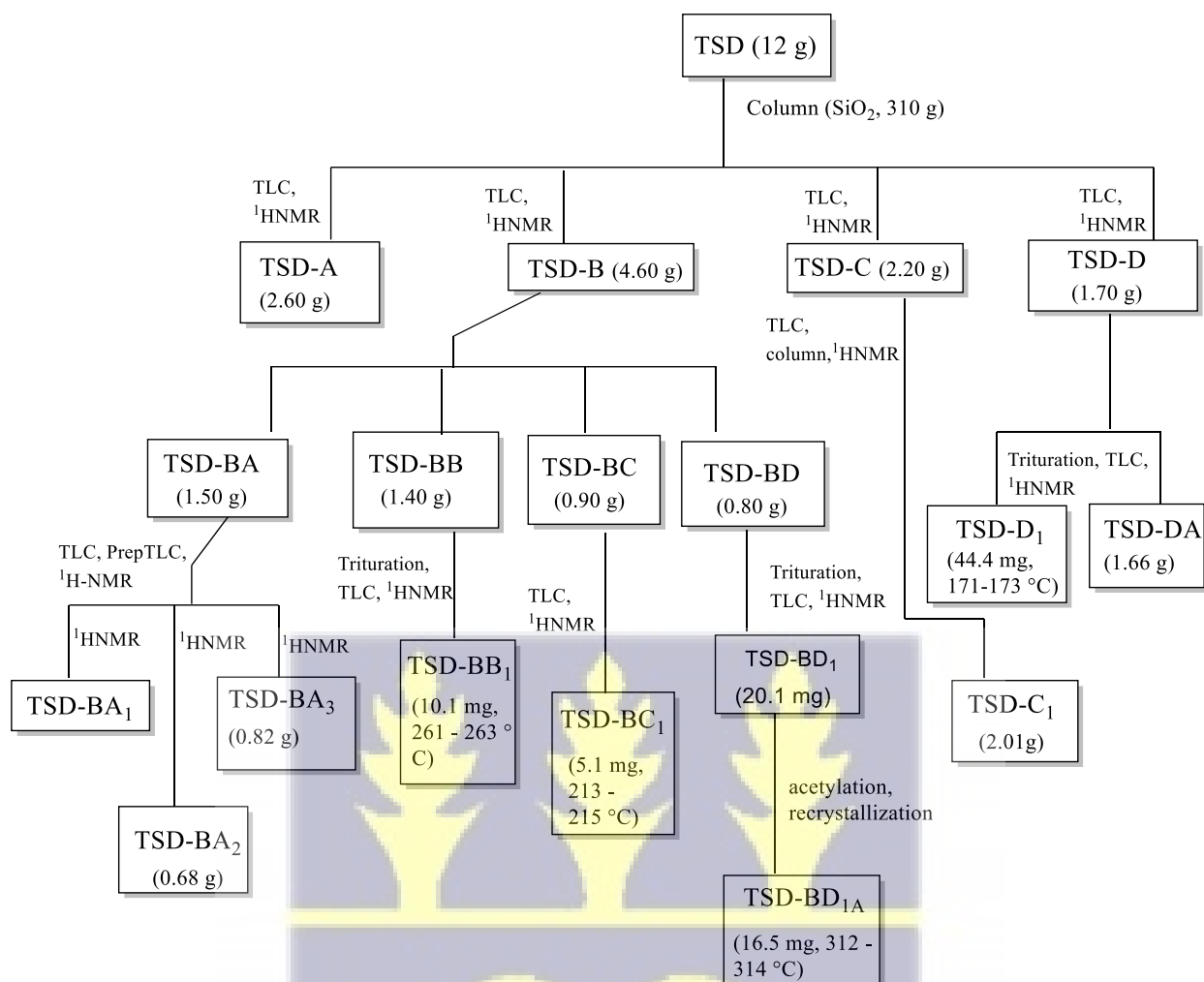
Subfraction TSD-BC and TSD-BD yielded solids TSD-BC₁ and TSD-BD₁ respectively. Subfraction TSD-BC₁ gave white solids (5.1 mg), soluble in DCM and ethyl acetate, with a melting point of 213–215 °C. TLC showed a single spot with R_f values of 0.43 and 0.5. ^1H -NMR analysis confirmed the presence of triterpenoids, leading to full NMR characterization.

Fraction TSD-BD precipitated white solids (20.1 mg), which were analyzed by TLC, showing four spots, including traces of TSD-BC₁. Further ^1H -NMR analysis confirmed that both subfractions

contained the same compound. Based on TLC and ^1H NMR comparison, TSD-BD₁ (20.1 mg) was impure, likely due to co-eluting compounds. It was then selected for acetylation to enhance purity and create a derivative for antitrypanosomal screening. TSD-BD₁ was then acetylated using an equimolar ratio of pyridine and acetic anhydride, resulting in the semi-synthesized product, TSD-BD_{1A} (16.5 mg). Derivatization was carried out on the fraction to alter the polarity of the target compound, improve its separation, and facilitate its efficient isolation from impurities. After acetylation, the derivative TSD-BD_{1A} (312-314 °C) was purified and assessed by TLC and NMR. Both TSD-BC₁ and TSD-BD_{1A} were tested in vitro against *Trypanosoma brucei*.

Subfraction TSD-D yielded solids soluble in chloroform and DCM, which were triturated with acetone to produce white needle-like solids (44.4 mg) coded TSD-D₁ (171-173 °C). TLC analysis with DCM:EtOAc (25:75) revealed two spots (pink and brown) when stained with anisaldehyde. Attempts at recrystallization, column separation, and prep-TLC failed. H-NMR analysis showed peaks characteristic of a triterpenoid, followed by complete NMR analysis of the solids. Scheme 4-1 below summarizes the isolation of the compounds.





Scheme 4-1: A Schematic representation of the processes for compound isolation and semi-synthesis.

4.4 Structure Elucidation of Isolated Compounds

4.4.1 Characterization of TSD-BB₁ from TSD-BB

The one-dimensional ¹³C-NMR spectrum of compound TSD-BB₁ exhibited 31 distinct and well-resolved carbon signals, characteristic of a triterpenoid framework. Complementary DEPT-135 analysis revealed eight methyl (CH₃), twelve methylene (CH₂), four methine (CH), and seven quaternary (C) carbons, a distribution consistent with a friedelane-type triterpenoid skeleton. Comprehensive interpretation of the 1D (¹H- and ¹³C-NMR) and 2D (HSQC, COSY, and HMBC)

spectra supported this conclusion. Comparison of the obtained spectral data with those reported for known friedelane-type triterpenoids in the literature confirmed the identity of TSD-BB₁ as friedelan-3-one [505]^{249–251}, figure 4-1 (Table 4-3).

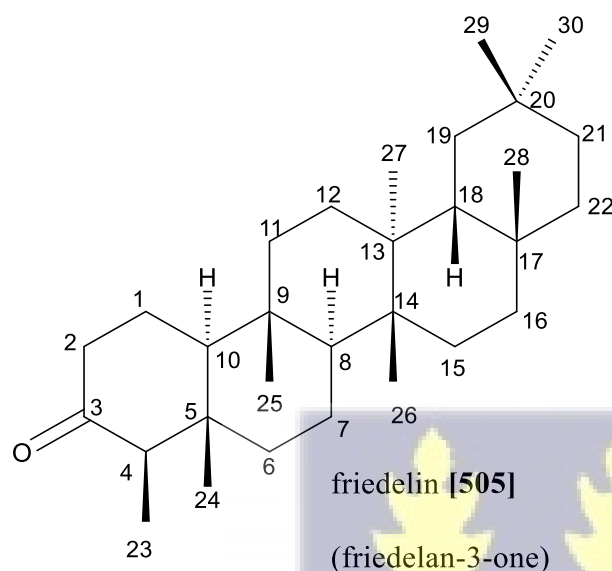


Figure 4-1: Structure of friedelan-3-one

The broadband ¹³C-NMR spectra corroborated the presence of the carbonyl carbon at 213.3 ppm for C3. Detailed ¹H and ¹³C-NMR data are provided in Table 4-2. The proton resonances for the eight methyl groups of friedelin were observed. Six methyl proton signals were recorded at δ_H 0.88 (3H, d, *J* = 6.7 Hz) for H23, δ_H 0.73 (s, 3H) for H24, δ_H 0.87 (s, 3H) for H25, and δ_H 1.18 (s, 3H) for H28. The chemical shifts at δ_H 1.01 (s, 3H) and δ_H 1.07 (s, 3H) confirmed the presence of two methyl groups in similar environments, assigned to H26 and H27, respectively. Additionally, resonances corresponding to the geminal dimethyl groups were observed at δ_H 1.00 (s, 3H) for H29 and δ_H 0.95 (s, 3H) for H30. The protons on C2 and C4 exhibited multiple signals between 2.4 and 2.2 ppm. Specifically, the methine proton at C4 appeared as a quartet at δ_H 2.24 (1H, q, *J* = 6.7 Hz) as a result of being split by the methyl protons H23, while the two diastereotopic protons of the methylene group at C2 were observed at δ_H H2a: 2.31 (dd, *J* = 7.0, 13.0 Hz) and H2b: 2.39

(dd, $J = 2.5, 5.0$ Hz). An additional resonance observed at δ_{H} 1.25 ppm, correlating to δ_{C} 29.7 ppm, was identified as trace grease residue rather than an additional molecular species, thereby confirming the structural integrity and purity of the isolate.

Prior NMR spectral analysis of friedelin²⁵² revealed the resonances of eight methyl protons, with signals at δ_{H} 1.18 (3H, singlet, H28); δ_{H} 0.95 (3H, singlet, H29) and δ_{H} 1.01 (3H, singlet, H30), indicating the presence of geminal dimethyl groups. Additional chemical shifts were observed at δ_{H} 0.87 (3H, singlet, H25); δ_{H} 1.05 (3H, singlet, H27); and δ_{H} 1.00 (3H, singlet, H26) on C-14, indicating two methyl groups in equivalent chemical environments. Further resonances were detected at δ_{H} 0.88 (3H, doublet, $J = 6.8$ Hz, H23) and δ_{H} 2.25 (1H, quartet, $J = 6.8$ Hz, H4), and at δ_{H} 0.73 (3H, singlet, H24). The methylene protons on C2 exhibited doublet of doublets signals at δ_{H} 2.39 (Ha, dd, $J = 2.5, 5.0$ Hz) and δ_{H} 2.31 (Hb, dd, $J = 7.0, 13.0$ Hz). The resonance signals for the eleven methylene groups were detected within the chemical shift range of 0.90 - 2.40 ppm. The chemical shifts of the methine protons are also detailed in Table 4-2.

In the HMBC spectrum (Appendix I-6), the resonance of H23 exhibited correlations with the carbon signals at δ_{C} 58.3 (C4), δ_{C} 42.2 (C5), and δ_{C} 14.7 (C24). The absence of a correlation between H23 and the carbonyl carbon C3 could be attributed to the possibility that some two- or three-bond correlations are either extremely weak or may not manifest. Therefore, the absence of an HMBC cross peak should not be interpreted as definitive evidence against a specific structural connection^{253,254}.

The COSY (Correlation Spectroscopy) (Appendix I-5) contour map revealed distinct cross-peaks, indicating important proton-proton interactions within the molecule. A notable cross-peak was observed between the resonances at 2.24 ppm (H4) and 0.88 ppm (H23), suggesting a direct connection. Another significant correlation appeared between 2.39 ppm (H2b) and 1.70 ppm

(H1a), highlighting their proximity in the molecular structure. The protons at 1.97 ppm (H1b) and 1.51 ppm (H10) also showed a clear interaction. Additionally, the resonance at 1.76 ppm (H6) was correlated with 1.38 ppm (H7), while 2.29 ppm (H2a) correlated with 1.96 ppm (H1b). A distinct interaction was also evident between 1.49 ppm (H21) and 0.94 ppm (H22), as well as between 1.44 ppm (H11) and 1.36 ppm (H12). Although the proton NMR spectrum provided limited information regarding the coupling constants, these COSY cross-peaks were essential for identifying the connectivity and spatial relationships between these protons within the molecular structure.

Overall, the spectra of TSD-BB₁ were considerably clean and matched reported data for this triterpenoid, confirming its identity. A minor signal at δ 1.25 ppm in the ¹H-NMR spectrum, correlating to δ 29.8 ppm in the ¹³C-NMR spectrum, was attributed to trace grease residue rather than an additional component, indicating that the isolate was structurally pure. TSD-BB₁ was therefore confirmed to be friedelan-3-one (commonly known as friedelin) after comparison with the literature^{252,255,256} (Table 4-3). TSD-BB₁ gave a measured mass of 10.1 mg. Its melting point was determined to be within the range of 261 to 262 °C and compared to literature value ranges of 259.7-261 °C²⁵² and 260-261 °C²⁵⁵.

Table 4-2 NMR assignments of friedelan-3-one (friedelin, TSD-BB₁) and comparison to literature

	Experimental Carbon, δ_c (ppm) (CDCl ₃ , 100 MHz)	HSQC/DEPT	Experimental Hydrogen, δ_H (ppm) (CDCl ₃ , 400 MHz)	HMBC	¹ H- ¹ H COSY	δ_c (ppm) (CDCl ₃ , 100 MHz) ²⁵²	δ_H (ppm) (CDCl ₃ , 400 MHz) ²⁵²
1	22.3	CH ₂	1.68 (dd, J= 5.1, 13.0), 1.96 (m, J= 2.5, 7.1, 13.0)		H10, H2	22.3	Ha-1: 1.97 (tdd, 2.5, 7.0, 13.0), Hb-1: 1.68 (dd, 5.0, 13.0)
2	41.6	CH ₂	2.29 (dd, J=7.0, 13 Hz), 2.38 (ddd, J=2.06, 5.25, 13.7 Hz)		H1	41.5	Ha-2: 2.31 (dd, 7.0, 13.0), Hb-2: 2.39 (dd, 2.5, 5.0)
3	213.3	C	-			213.2	-
4	58.3	CH	2.24 (q/m, J = 6.7 Hz)	C5, C24	H23	58.3	H-4: 2.25 (q, 6.8)
5	42.2	C	-			42.2	-
6	41.3	CH ₂	1.25, 1.76 (m)	C8	H7	41.5	H-6: 1.28

						Hb-6: 1.76 (dt, J = 2.5, 5.0)
7	18.3	CH ₂	1.37, 1.49 (m)	H6	18.3	Ha-7: 1.40 (m); Hb-7: 1.50 (m)
8	53.1	CH	1.39 (m)		53.1	H-8: 1.40 (m)
9	37.5	C	-		37.5	-
10	59.5	CH	1.53 (m)	H1	59.5	H-10: 1.53 (dd, J = 2.5, 12.5)
11	35.6	CH ₂	1.26, 1.44 (m)	H12	35.7	Ha-11: 1.26 (m); Hb-11: 1.48 (m)
12	30.5	CH ₂	1.35, 1.34 (m)	H11	30.5	Ha-12: 1.19(m); Hb-12: 1.35 (m)
13	39.7	C	-		39.7	-
14	38.3	C	-		38.3	-
15	32.4	CH ₂	1.33, 1.54 (m)	H16	32.4	Ha-15: 1.28 (m) Hb-15: 1.50 (m)
16	36.0	CH ₂	1.37, 1.58 (m)	H15	36.0	Ha-16: 1.36 (m) Hb-16: 1.58 (m)
17	30.0	C	-		30.0	-
18	42.8	CH	1.56 (m)	C27, C28	42.8	H-18: 1.57 (d, J = 5.0)
19	35.4	CH ₂	1.22, 1.37 (m)		35.4	Ha-19: 1.21 (m), Hb-19: 1.38 (m)
20	28.2	C	-		28.2	-
21	32.8	CH ₂	1.30, 1.49 (m)	H22	32.8	Ha-21: 1.53 (m), Hb-21: 1.30 (m)
22	39.3	CH ₂	0.93, 1.49 (m)	C20 H21	39.3	Ha-22: 0.94 (m), Hb-22: 1.51 (m)
23	6.8	CH ₃	0.88 (d, J = 6.7 Hz))	C4, C5, C24 H4	6.8	Me-23: 0.88 (d, J = 6.8)
24	14.7	CH ₃	0.73 (s)	C10	14.7	Me-24: 0.73 (s)
25	18.0	CH ₃	0.87 (s)		18.0	Me-25: 0.87 (s)
26	20.3	CH ₃	1.01 (s)	C8, C13, C14, C15, C16	20.3	Me-26: 1.00 (s)
27	18.7	CH ₃	1.05 (s)	C12, C13, C14, C17, C18	18.7	Me-27: 1.05 (s)
28	32.1	CH ₃	1.18 (s)	C16, C17, C18, C22	32.1	Me-28: 1.18 (s)
29	31.8	CH ₃	1.00 (s)	C19, C20, C30	31.8	Me-29: 0.95 (s)
30	35.0	CH ₃	0.95(s)	C19, C20, C21, C29	35.0	Me-30: 1.01(s)

Legend: J = coupling constant; s = singlet; d = doublet; dd = doublet of doublets; ddd = doublet of doublet of doublets; m = multiplets

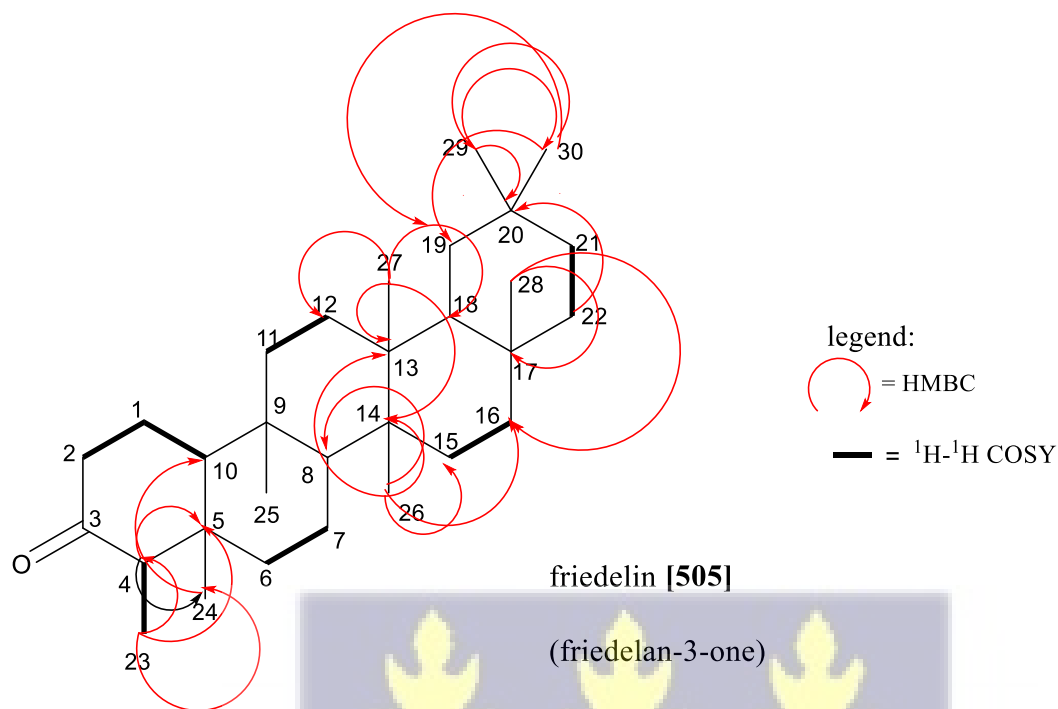


Figure 4-2: HMBC and ^1H - ^1H COSY correlations of friedelan-3-one

Friedelin [505] is a bioactive oleanane-type triterpenoid that was first isolated from bark of *Quercus suber* in the year 1807²⁵⁷, and has since been identified in various plant families; however, it has not been widely documented in species within the Amherstieae tribe. This study reports, for the first time, the isolation of friedelin [505] from the whole plant of *Talbotiella gentii*, and by extension, from the genus *Talbotiella*. Within the tribe Amherstieae, the presence of friedelin has been previously reported only in the branches of *Macrolobium acaciifolium*¹⁸⁵, a plant whose leaves are ethnomedicinally used in herbal baths and in the treatment of fever²⁵⁸. There is also mention of its presence in the leaves of *Macrolobium latifolium*¹⁸⁴, even though detailed reports on the medicinal uses of this plant remain limited. The pharmacological profile of friedelin [505] is well-documented and encompasses a broad spectrum of biological activities, including antipyretic, analgesic, anti-inflammatory²⁵⁹, antimicrobial²⁶⁰, and anticancer properties²⁶¹. In a study on the stem of *Tapinanthus bangwensis*, friedelin isolated from its ethyl acetate extract

demonstrated notable antioxidant potential, achieving 73.69% free radical scavenging activity, which was relatively close to the 93.96% observed for ascorbic acid²⁶².

4.4.2 Characterization of TSD-BC₁ from TSD-BC

TSD-BC₁ had a recorded mass of 5.1 mg, with its melting point observed between 215 and 216 °C. The ¹³C-NMR spectrum revealed the presence of thirty distinct carbon signals. These signals were further categorized using an HSQC-DEPT spectrum (Table 4-5), identifying six quaternary carbons, six methine carbons, seven methyl groups, and eleven methylene groups, all characteristic of a pentacyclic triterpenoid with a lupane skeleton.

Data from both one and two-dimensional ¹H- and ¹³C-NMR when compared with those of literature identified the TSD-BC₁ to be the known lupane-type triterpenoid lup-20(29)-en-3β-ol (Table 4-4, figure 4-3) also known as lupeol [38]^{249,250} with a literature melting point range of 214–216 °C²⁶³ and value of 213 °C²⁶⁴. The seven methyl groups were observed at δ_C: 28.00 (C23), 18.02 (C28), 16.14 (C25), 15.99 (C26), 15.39 (C24), 14.52 (C27), and 19.32 (C30). The six methine carbons exhibited chemical shifts at δ_C: 79.0 (C3), 55.3 (C5), 50.5 (C9), 38.1 (C13), 48.3 (C18), and 48.0 (C19). The eleven methylene groups resonated between δ_C 40.0 ppm (C22) and 18.3 ppm (C6). The six quaternary carbons produced signals at δ_C: 38.8 (C4), 40.8 (C8), 37.2 (C10), 42.8 (C14), 43.0 (C17), and 151.0 (C20). Notably, the signals at δ_C 151.0 ppm (C20) and δ_C 109.3 ppm (C29) were significantly deshielded, indicative of an olefinic bond between them. Additionally, the signal at δ_C 79.02 ppm assigned to C3 was deshielded due to the presence of a hydroxyl group attached to this carbon. A notable difference in chemical shifts between the geminal dimethyl groups C23 and C24, despite being on the same carbon atom, may be attributed to the proximity of C23 to an electronegative atom in close spatial arrangement.

The ^1H -NMR spectrum revealed seven singlets for the methyl protons at δ_{H} 0.76 (H24), 0.79 (H28), 0.83 (H25), 0.94 (H27), 0.97 (H23), 1.03 (H26), and 1.68 (H30). Two distinct multiplets were observed at δ_{H} 4.57 (1H, multiplet) and 4.69 (1H, doublet, $J=2.5$ Hz), corresponding to the olefinic protons H29a and H29b. These signals indicate the presence of a terminal isopropenyl group (exocyclic double bond), a characteristic feature of the lupane series of triterpenoids. Additionally, a multiplet at δ_{H} 2.38, attributed to H19 in the beta (β) configuration, is indicative of lupeol [38]²⁰³. The TSD-BC₁ spectra were significantly clean and matched with the reported literature, except for a very minor grease signal at 1.25 ppm correlating to 29.7 ppm in the ^{13}C -NMR spectrum that did not interfere with structural interpretation. Table 4-6 and Table 4-7 compare the ^{13}C -NMR and ^1H -NMR chemical shifts of lupeol [38] to literature values.

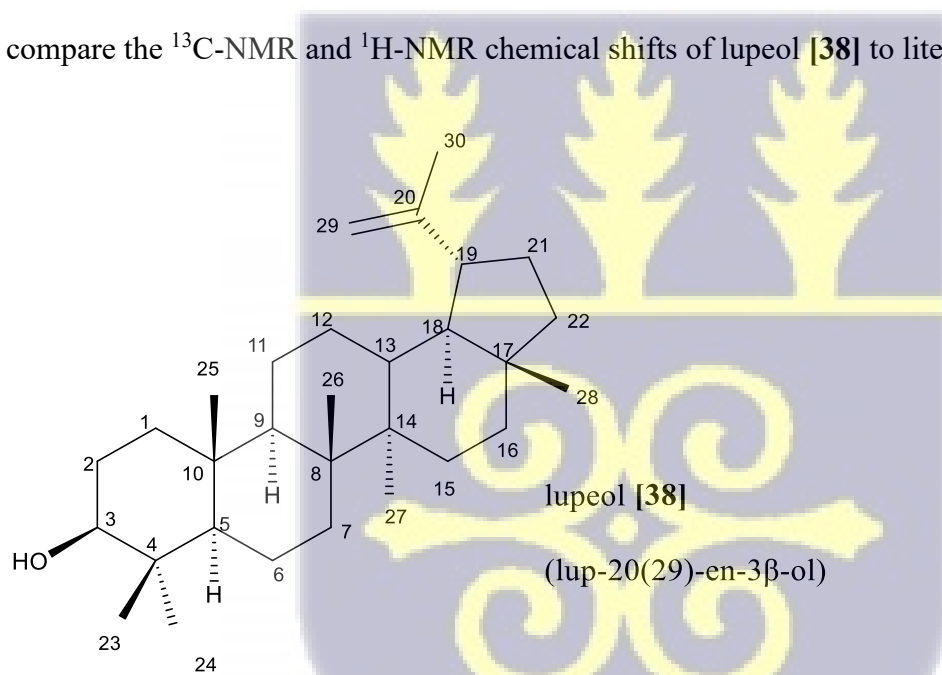


Figure 4-3: 3D structure of lup-20(29)-en-3 β -ol

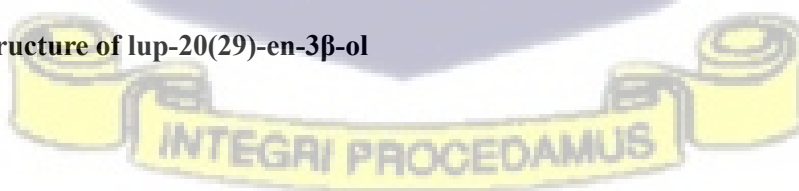


Table 4-3: NMR assignments of lup-20(29)-en-3 β -ol(lupeol [38], TSD-BC₁) and comparison to literature

	Experimental Carbon resonance in CDCl ₃ , δ_{ppm} (100 MHz)	DEPT	H-NMR Experimental CDCl ₃ , δ_{ppm} (400 MHz)	HMBC	COS Y	H-NMR 400 MHz, CDCl ₃ ²⁵⁰	C-NMR 400 MHz, CDCl ₃ ²⁵⁰
1	38.7	CH ₂	0.9 (m), 1.65 (m)			0.89m, 1.71m	38.71
2	27.4	CH ₂	1.59 (m), 1.68(m)		H3	1.59 m	27.43
3	79.0	CH	3.19 (dd)		H3	3.18 (dd)	79.0
4	38.8	C	-			-	38.9
5	55.3	CH	0.68 (dd)			0.66 (dd)	55.3
6	18.3	CH ₂	1.39 (m), 1.52 (m)		H7	1.38 (m)	18.3
7	34.3	CH ₂	1.39 (m)		H6	1.37(m)	34.3
8	40.8	C	-			-	40.8
9	50.5	CH	1.26 (m)			1.26 (m)	50.4
10	37.2	C	-			-	37.2
11	20.9	CH ₂	1.20, 1.41 (m)		H12	1.21 (m)	20.9
12	25.2	CH ₂	1.07 (m), 1.65 (m)		H11	1.07 (m), 1.65 (m)	25.1
13	38.1	CH	1.66 (m)	C19, C13		1.60 (m)	38.1
14	42.8	C	-			-	42.8
15	27.5	CH ₂	1.02, 1.60 (m)			1.59(m)	27.5
16	35.6	CH ₂	1.36, 1.48 (m)			1.32 (m)	35.6
17	43.0	C	-			-	43.0
18	48.3	CH	1.36, 1.39 (m)			1.37 (m)	48.3
19	48.0	CH	2.38 (td/m)			2.36 (m)	48.0
20	151.0	C	-			-	151.0
21	29.9	CH ₂	1.29 (o), 1.92 (m)			1.28 (m), 1.92 (m)	29.9
22	40.0	CH ₂	1.19, 1.38 (m)			1.17 (m), 1.35 (m)	40.0
23	28.0	CH ₃	0.97 (s)	C3, C4, C5, C24		0.94 (s)	28.0
24	15.4	CH ₃	0.76 (s)	C3, C4, C5, C23		0.75 (s)	15.4
25	16.1	CH ₃	0.83 (s)	C1, C5, C9, C10		0.82 (s)	16.1
26	16.0	CH ₃	1.03 (s)	C7, C8, C9, C14		1.02 (s)	16.0
27	14.6	CH ₃	0.94 (s)	C8, C13, C14, C15		0.96 (s)	14.6
28	18.0	CH ₃	0.79 (s)	C16, C17, C18, C22		0.78 (s)	18.0
29	109.3	CH ₂	4.57 (m), 4.69 (d)		H30	4.56(dd), 4.68 (d)	109.3
30	19.3	CH ₃	1.68 (s)		H29	1.67 (s)	19.3

Legend: J = coupling constant; s = singlet; d = doublet; dd = doublet of doublets; ddd = doublet of doublet of doublets; m = multiplets

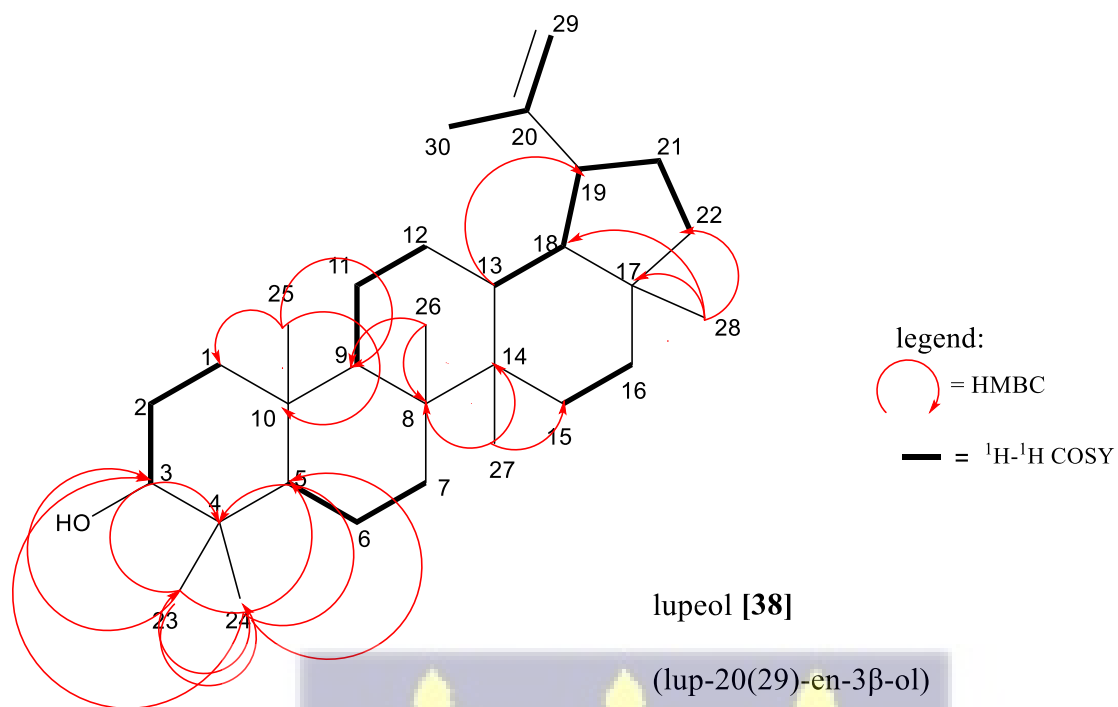


Figure 4-4: HMBC and ^1H - ^1H COSY correlations of lup-20(29)-en-3 β -ol

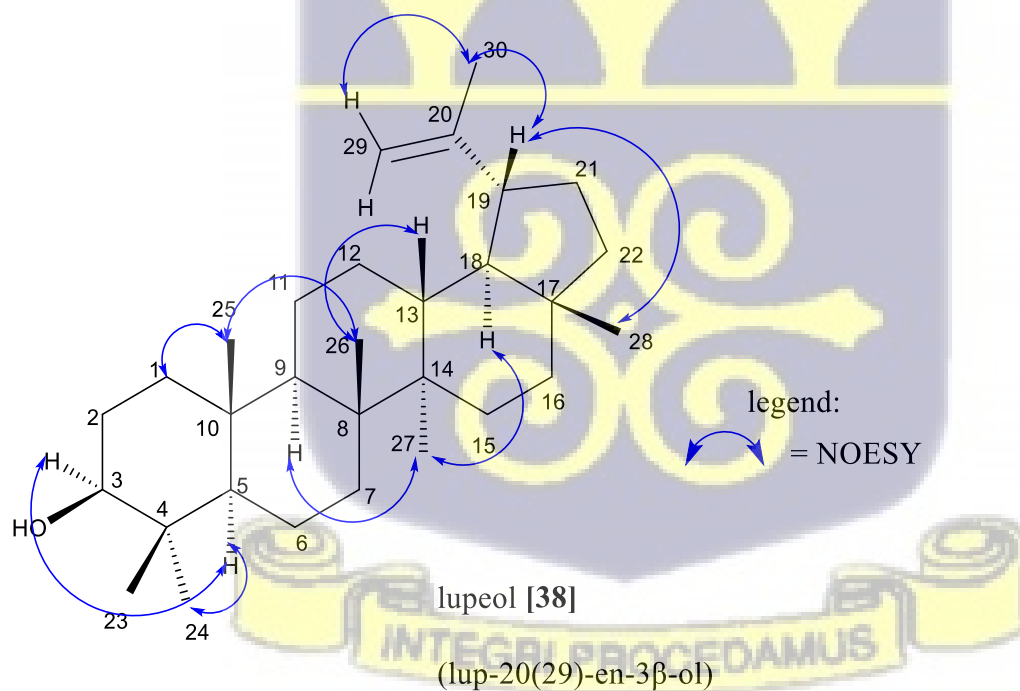


Figure 4-5: NOESY correlation of lup-20(29)-en-3 β -ol

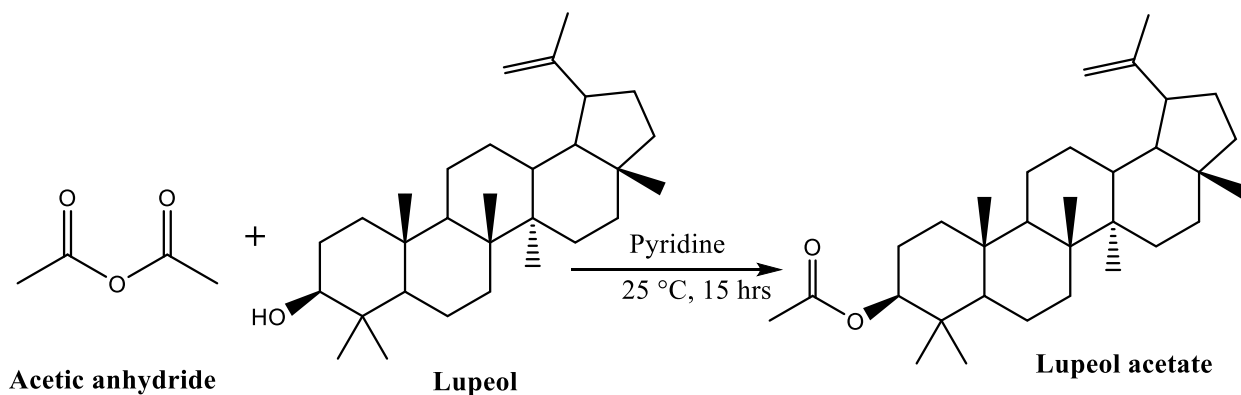
Lupeol [38] was first documented in 1908 following its isolation from the balata plant (*Manilkara bidentata*)²⁶⁵. Since then, it has been identified in numerous species across various plant families. This study reports, for the first time, the isolation of lupeol [38] from *Talbotiella gentii* and, more broadly, from the *Talbotiella* genus. Within the Amherstieae tribe, only a few species have been reported to contain lupeol. Notably, it has been isolated from the stems of *Anthonotha cladantha*¹²³ and *Humboldtia unijuga*¹⁷⁵, the leaves of *Macrolobium acaciifolium*¹⁸⁵, and the seeds of *Scorodophloeus zenkeri*¹⁸⁸. In addition, lupeol [38] has been detected in the leaves of *Julbernardia paniculata* through HPLC-DAD and GC-MS analyses¹⁸¹.

Lupeol [38] has garnered significant scientific interest due to its broad spectrum of pharmacological activities. It is considered a promising candidate for the prevention and treatment of several diseases, including cancer, diabetes, inflammatory and neurological disorders, and cardiovascular conditions²⁶⁶. Importantly, lupeol [38] has demonstrated selective cytotoxicity against cancerous cells. For instance, in studies involving human breast cell lines, it significantly reduced the viability of MCF-7 cancer cells, with an IC₅₀ value of 80 µM, while showing no toxic effects on normal human breast cells such as MCF-10A²⁶⁷. Additionally, lupeol has been reported to inhibit the invasion and intraerythrocytic growth of *Plasmodium falciparum*, with IC₅₀ values ranging from 7 to 28 mM, suggesting specific antiparasmodial activity and its potential as a novel target for antimalarial drug development²⁶⁸.

4.4.3 Acetylation of TSD-BD₁ to give the acetylated derivative, TSD-BD_{1A}

TSD-BD₁, containing lupeol [38], was subjected to acetylation to facilitate its structural elucidation. Given that lupeol [38] contains a hydroxyl functional group, its reaction with pyridine and acetic anhydride is expected to produce the corresponding ester, lupeol acetate [547], as depicted in Scheme 4-2. The derivatization was strategically employed to enhance the compound's

analytical profile. Notably, the acetylated product is anticipated to serve as a novel ligand with potential inhibitory activity against *Trypanosoma brucei* Pteridine reductase 1 (*TbPTR1*) and *Trypanosoma brucei* dihydrofolate reductase (*TbDHFR*) enzymes.



Scheme 4-2: Proposed reaction scheme for the acetylation of lup-20(29)-en-3 β -ol with acetic anhydride to form lupeol acetate [547]

4.4.3.1 Structure Elucidation of TSD-BD_{1A} (lupeol acetate)

TSD-BD_{1A} was obtained from the acetylation as white solids which had a measured mass of 16.5 mg. ¹H-NMR analysis of the acetylated product (Appendix I-14) was compared (figure 4-7) to the ¹H-NMR spectrum (Appendix I-7) of the precursor, TSD-BC₁. The overlay revealed distinct spectral differences consistent with the reported literature^{269–271}. Data from both one and two-dimensional ¹H- and ¹³C-NMR when compared with those of literature identified the TSD-BB₁ to be the known lupane-type triterpenoid lup-20(29)-en-3 β -yl acetate commonly known as lupeol acetate [547] (Figure 4.6, Table 4-6)^{269–271}. Its melting point was observed between 216 and 217 °C. This was in agreement with the literature values, which report ranges of 215 °C –217 °C²⁵¹ and 216 °C –219 °C²⁷². In the ¹H-NMR spectrum, the appearance of a singlet resonance at δ_H 2.04 ppm, attributable to the methyl of the acetyl moiety of the lupeol acetate [547], was observed. Additionally, there was a downfield chemical shift of the proton at position H3, shifting from 3.19

ppm (dd) in lupeol [38] (TSD-BC₁) to 4.47 ppm (dd) in lupeol acetate [547] (TSD-BD_{1A}). This downfield shift of H3 is due to the presence of the highly electronegative oxygen atoms within the ester functionality. There was also a signal detected at δ H 1.25 ppm, corresponding to δ C 29.7 ppm, was attributed to residual grease contamination rather than a component of the compound, thus supporting the isolate's structural integrity and purity.

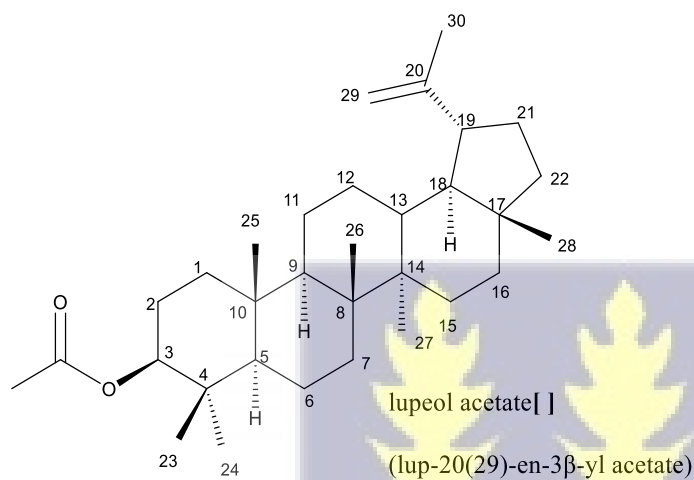


Figure 4-6 Structure of lupeol acetate [547]

The NMR analysis, with summarized data in Table 4-4, was conducted on the semi-synthesized compound to further confirm its structural identity.



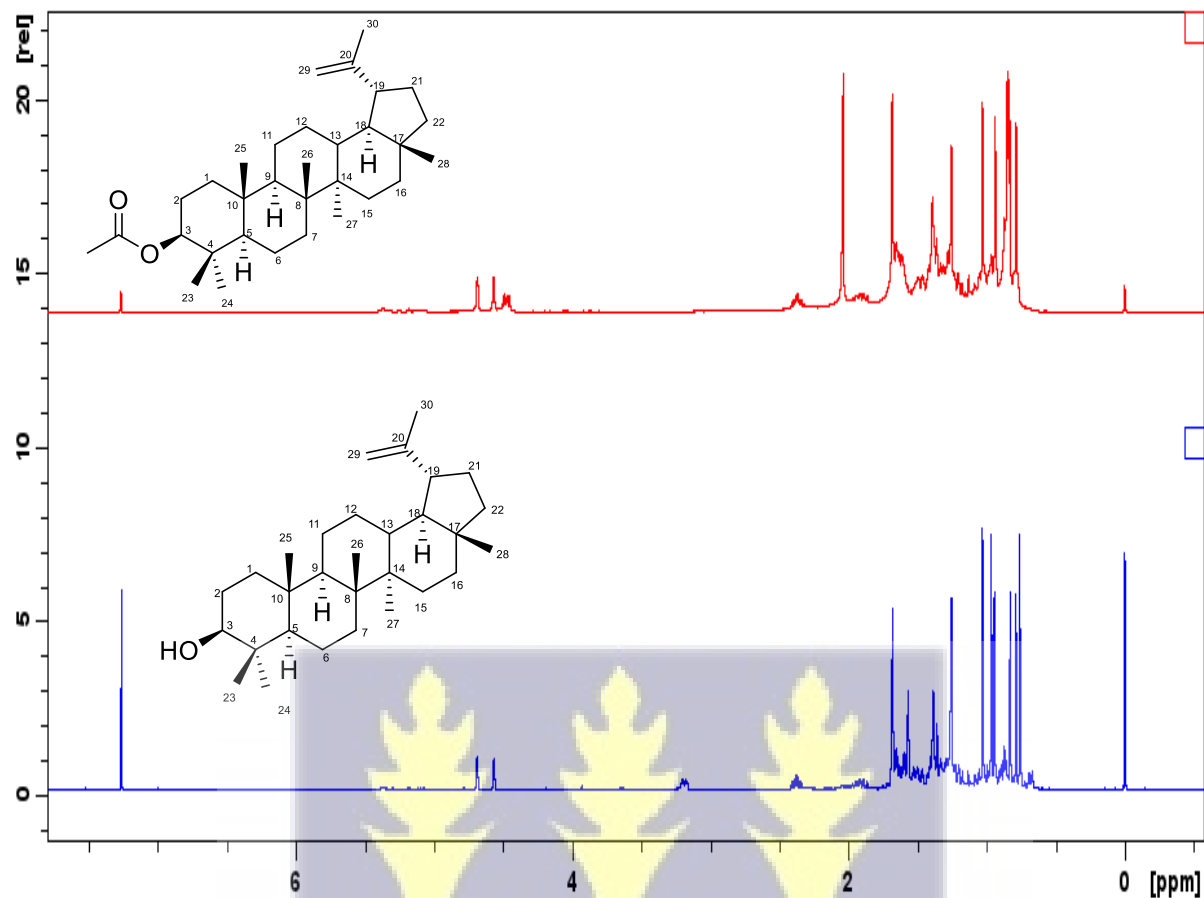


Figure 4-7: Overlaid ¹H-NMR spectra of TSD-BC₁ (lupeol) and TSD-BD_{1A}

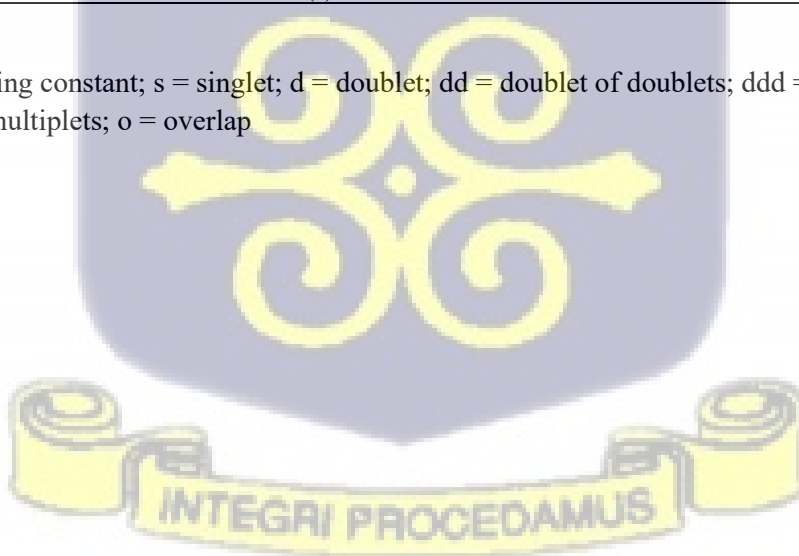
FTIR analysis done on TSD-BD_{1A} (Appendix II-1) showed a strong symmetric and asymmetric stretch of sp³ C–H bonds are observed at 2928 cm⁻¹ and 2853 cm⁻¹, respectively. A Fermi resonance peak around 3050 cm⁻¹ corresponds to the sp² C–H stretch. A prominent absorption at 1733 cm⁻¹ is attributed to the C=O stretching of the carbonyl group in the acetate. A weak C=C stretch appearing at 1639 cm⁻¹ in the IR spectrum corresponds to C=C stretching of the exocyclic double bond at C-20(29). Out-of-plane bending bands at 978 and 875 cm⁻¹ supported the presence of a terminal exocyclic double bond (=C–H₂) in the IR spectrum. Additionally, a medium-intensity peak at 1455 cm⁻¹ is assigned to sp³ C–H bending, while another medium peak at 1366 cm⁻¹ corresponds to the C–H bending of the gem-dimethyl groups at C23 and C24 on C4. The C–O stretching of the ester is evident from a strong absorption at 1244 cm⁻¹. The spectrum confirmed the successful acetylation of lupeol as in accordance with Muktar et al. (2018)²⁷³. The NMR spectra confirmed it was with no other correlation on the HSQCDEPT and HMBC. The TSD-BD_{1A} is therefore described as an enriched lupeol-acetate fraction. These findings (Figure 4.7) are consistent with previous reports in the literature^{209,210}, which also describe similar vibrational frequencies for these functional groups.

Table 4-4: Table showing the NMR data set for TSD-BD_{1A} (lupeol acetate [547]) and comparing the experimental ¹³C and ¹H-NMR chemical shift values with literature values under the same conditions.

	Experimental ¹³ C-NMR (CDCl ₃ , 100 MHz) δ_{ppm}	HSQC/ DEPT	Expt ¹ H- NMR (CDCl ₃ , 400 MHz) δ_{ppm}	HMBC	¹ H- ¹ H COSY	δ_c (ppm) (CDCl ₃ , 100 MHz) ²⁷⁴	δ_H (ppm) (CDCl ₃ , 400 MHz) ²⁷⁴
1	38.41	CH ₂	0.98 (m/o)		H2	38.4	0.99 (m)
2	23.73	CH ₃	1.61 (m/o)	C3	H3, H1	23.7	1.62 (m)
3	80.98	CH	4.47 (dd)	C24, C1'	H2	81	4.47 (dd)
4	37.81	C	-			37.8	-
5	55.4	CH	0.79 (m)		H6	55.4	0.79 (m)
6	8.22	CH ₂	1.39, 1.50 (m/o)		H5, H7	18.2	1.50, 1.38 (m)
7	34.23	CH ₂	1.39 (m/o)		H6	34.2	1.49, 1.38 (m)
8	40.86	C	-			40.9	-
9	50.34	CH	1.28 (m/o)			50.3	1.28 (m)
10	37.07	C	-			37.1	-
11	20.95	CH ₂	1.39, 1.26 (m)		H12	20.9	1.39, 1.21 (m)

12	25.11	CH ₂	1.07, 1.66 (m/o)		H11, H13	25.1	1.66, 1.05 (m)
13	38.05	CH	1.66 (m)		H12	38	1.65 (m)
14	42.83	C	-			42.8	-
15	27.45	CH ₂	1.67, 1.00 (m)	C27	H16	27.4	1.67, 1.00 (m)
16	35.59	CH ₂	1.46, 1.36 (m)		H15	35.6	1.47, 1.36 (m)
17	43.01	C	-			43	-
18	48.3	CH	1.36 (m/o)		H19	48.3	1.35 (m)
19	48.02	CH	2.38 (m)	C18, C20, C21, C28, C29, C30	H18, H21	48	2.40 (m)
20	150.95	C	-			151	-
21	29.85	CH ₂	1.91, 1.33 (m)		H19, H22	29.8	1.91, 1.33 (m)
22	40.02	CH ₂	1.20, 1.38 (m/o)	C28	H21	40	
23	27.97	CH ₃	0.85 (o)	C3, C5, C24		28	0.84 (s)
24	16.51	CH ₃	0.84 (o)	C3		16.5	0.83 (s)
25	16.2	CH ₃	0.85 (o)	C1, C9, C, 10		16.2	0.85 (s)
26	15.99	CH ₃	1.03 (s)	C7, C8, C9, C14		16	1.03 (s)
27	14.52	CH ₃	0.94 (s)	C13, C15		14.5	0.94 (s)
28	18.03	CH ₃	0.79 (s)	C16, C17 C18, C19, C22		18	0.78 (s)
29	109.38	CH ₂	4.69 (d), 4.57 (dd)	C18, C19, C20, C30	H30	109.4	4.69, 4.57 (d)
30	19.31	CH ₃	1.68 (s)	C19, C20, C29	H29	19.3	1.68 (s)
1'	171.01	C=O	-			171.1	-
2'	21.34	CH ₃	2.04 (s)	C1'		21.3	2.05 (s)

Legend: J = coupling constant; s = singlet; d = doublet; dd = doublet of doublets; ddd = doublet of doublet of doublets; m = multiplets; o = overlap



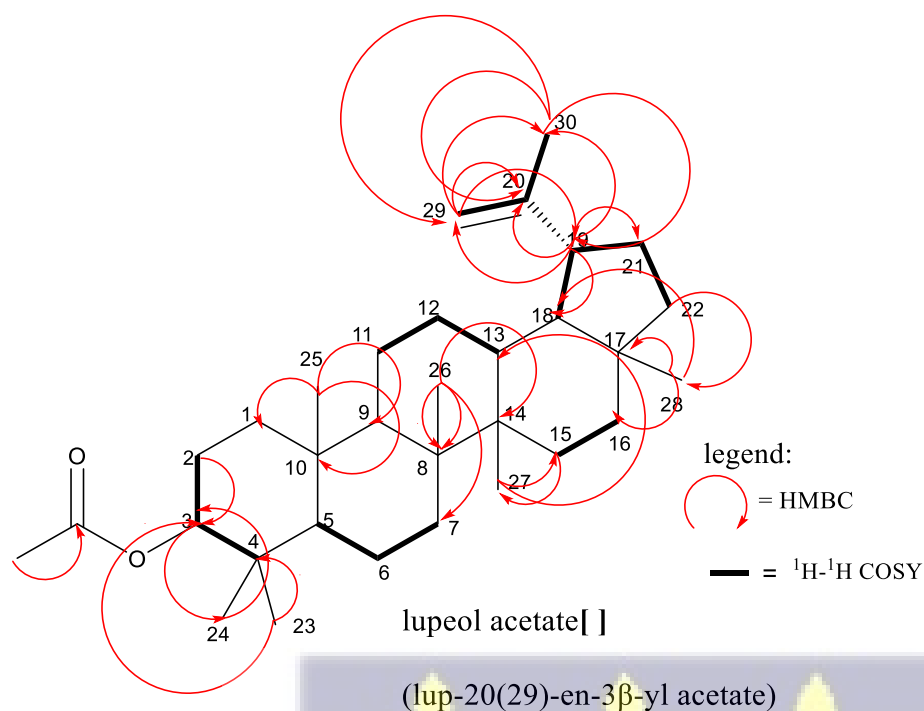


Figure 4-8 HMBC and ^1H - ^1H COSY correlations of Lup-20(29)-en-3 β -yl acetate

Another documented procedure involves stirring lupeol with acyl chloride and zinc dust for several hours, followed by purification steps to yield lupeol acetate [547]²⁷⁵. While the exact date or first report of this semi-synthesis is not specified in the provided sources, the method is well-established in the literature and has been widely used to produce lupeol acetate [547] from naturally isolated lupeol²⁷⁵.

To the best of my knowledge, there has been no report of isolation or detection of lupeol acetate [547] in the Amherstieae tribe. Semi-synthesis is therefore preferred because lupeol is more abundant than lupeol acetate [547] in plants, making it a more practical and efficient precursor for obtaining higher yields of the acetate derivative²⁷⁶. Moreover, semi-synthetic methods provide a more consistent and chemically pure product compared to direct extraction from complex plant matrices²⁷⁷. They also enable the derivatization of lupeol for structure–activity relationship (SAR) studies and the optimization of pharmacological properties²⁷⁸. Importantly, semi-synthesis ensures

an adequate and reproducible supply of lupeol acetate [547] for biological screening and potential therapeutic development²⁷⁶.

Lupeol acetate [547] has been extensively studied for its wide-ranging pharmacological activities, including anti-inflammatory, antioxidant, and anticancer effects²⁷⁹. It also exhibits antiprotozoal activity, which aligns with the direction of this study²⁷⁶. Additionally, the compound has demonstrated notable antimicrobial properties, with minimum inhibitory concentration (MIC) values of 12.5 µg/mL against *Staphylococcus aureus* and *Salmonella typhi*²⁷³. These findings support the biological relevance of semi-synthesizing lupeol acetate [547] and justify its continued investigation in antitrypanosomal research.

4.4.4 Characterization of TSD-D₁ from TSD-D

TSD-D₁ was identified as a mixture after taking the TLC and ¹H-NMR data. NMR data obtained on the different experiments on the solid (Table 4-5 and 4-6) and comparison with literature^{197,214,216} indicated the solid was a mixture. The complete NMR analysis, incorporating DEPT-135, HSQC-DEPT, ¹³C-NMR, and ¹H-NMR spectra (Appendix I-21 to Appendix I-26), revealed four characteristic olefinic carbons, six methyl peaks, and three quaternary carbon signals, which are indicative of the presence of stigmastane-type core. Data from both one and two dimensional ¹H-and ¹³C- NMR when compared with those of literature identified the solid to be made of β-sitosterol [44] and stigmasta-5,22-dien-3-ol (commonly known as stigmasterol [42])^{255,280–283}, Figure 4-9.

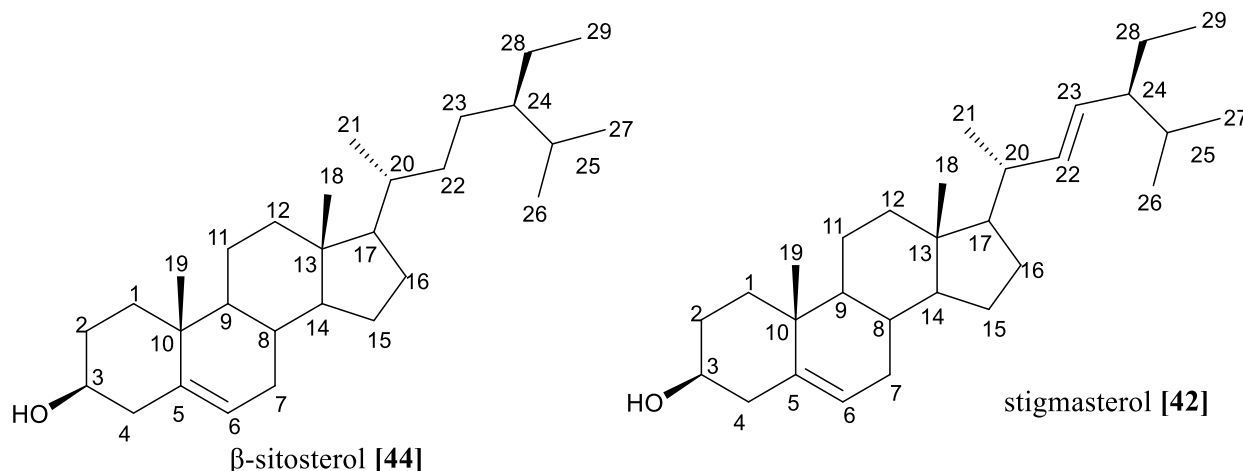


Figure 4-9 Structures of TSD-D₁

The ^1H -NMR spectrum exhibited chemical shifts ranging from 0.68 ppm (H18) to 5.34 ppm (H6). Specifically, six methyl signals consistent with those of β -sitosterol [44] were observed at δ_{H} 0.92 (3H, d, J = 6.5 Hz, H21), 0.69 (3H, s, H18), 1.01 (3H, s, H19), 0.84 (3H, m, H29), 0.83 (3H, d, J = 7.1 Hz, H26), and 0.81 (3H, d, J = 7.1 Hz, H27). Its proton signal corresponding to H3 appears as a septet at δ 3.52 (1H, J = 5.0, 6.1, 5.1 Hz). The olefinic proton signal for β -sitosterol [44] was observed at δ_{H} 5.38 (m, H6), alongside a residual solvent peak at δ 5.30 ppm, corresponding to δ 53.9 ppm in the ^{13}C -NMR spectrum, attributable to trace dichloromethane from sample preparation. This does not represent an impurity but a common solvent artifact. Also, an extra δ_{H} 1.25 ppm resonance correlating to δ_{C} 29.7 ppm was identified as trace grease rather than a constituent of the sample, confirming the structural fidelity and cleanliness of the isolate. In β -sitosterol [44], the angular methyl groups of C18 and C19 resonate at δ_{H} 0.69 (s) and 1.01 (s), respectively (Appendix I-21). The ^{13}C -NMR spectrum (Appendix I-22), further supported the presence of a double bond characteristic resonances at δ_{C} 140.7 ppm and 121.7 ppm for C5 and C6, respectively for β -sitosterol [44]. The three quaternary carbon atoms were detected at δ_{C} 140.7 (C5), 36.5 (C10), and 42.3 (C13). The C3, resonated at δ_{C} 71.8 ppm, while the methyl carbon

signals were observed at δ_C 11.9 (C18), 19.4 (C19), 18.8 (C21), 19.8 (C26), 19.1 (C27), and 12.0 (C29).

In contrast, stigmasterol [42] showed corresponding methyl peaks at δ_H 1.02 (3H, m, H21), 0.68 (3H, s, H18), 1.00 (3H, s, H19), 0.84 (3H, m, H29), 0.83 (3H, d, $J = 7.1$ Hz, H26), and 0.80 (3H, d, $J = 7.1$ Hz, H27). The angular methyl protons, appearing at δ_H 0.68 (s) and 1.00 (s), correspond to C18 and C19 of stigmasterol [42] respectively. The proton signal corresponding to H3 of the sterol moiety of both compounds appears as a septet at δ 3.52 (1H, $J = 5.0, 6.1, 5.1$ Hz). Additionally, the 1H -NMR spectrum (Appendix I-21) identified signals for the olefinic protons at δ_H 5.378 (m, H6), δ_H 5.14 (1H, dd, $J = 8.7, 15.1$ Hz, H22), and δ_H 5.03 (1H, dd, $J = 8.7, 15.1$ Hz, H23) for stigmasterol [42]. The ^{13}C -NMR spectrum (Appendix I-22), also confirmed the structure by showing characteristic resonances at δ_C 140.7 ppm and 121.8 ppm for the olefinic carbons C5 and C6, respectively, for stigmasterol [42]. The methyl signals appeared at δ_C 11.8 (C18), 19.4 (C19), 21.2 (C21), 19.8 (C26), 18.9 (C27), and 12.0 (C29). The chemical shifts for C22 and C23 were observed at δ_C 138.3 ppm and 129.3 ppm, respectively, for stigmasterol [42]. The carbon atom bearing the sterol moiety, C3, resonated at δ_C 71.8 ppm, whereas the three quaternary carbon atoms were detected at δ_C 140.7 (C5), 36.5 (C10), and 42.3 (C13).

In the IR spectrum (Appendix II-2), a broad peak at 3446 cm^{-1} is attributed to the hydrogen bonded O–H stretching frequency. The vibrational peaks at 2918 cm^{-1} and 2850 cm^{-1} correspond to the symmetric and asymmetric stretching of sp^3 C–H bonds. The absorptions at 1190 cm^{-1} and 1048 cm^{-1} are assigned to the C–O stretching of a secondary alcohol. A weak absorption at 1463 cm^{-1} corresponded to C=C vibrations, consistent with Δ^5 -sterol structures such as β -sitosterol and stigmasterol. An additional absorption at 1708 cm^{-1} , characteristic of a carbonyl group, was observed but is not diagnostic of sterol cores; it is attributed to a systematic error of the FTIR

instrument used in the analysis. Overall, the IR profile supports the sterol nature of the mixture and is consistent with previous literature^{255,284}, which reports similar vibrational frequencies for these functional groups. In complex mixtures or materials with overlapping spectral characteristics, sp² carbon signals may be present but can be difficult to differentiate from other signals in the IR spectrum²⁸⁵.

Stigmasterol [42] is often the predominant phytosterol present in natural mixtures containing β -sitosterol [44]. This co-occurrence is well-documented in the literature^{280–283}, which reports that isolating stigmasterol [42] in its pure form remains challenging due to its close structural similarity to β -sitosterol [44]. The only difference being the presence of a C22/C23 double bond in stigmasterol [42] which is absent in β -sitosterol [44].

This study reports the first isolation of β -sitosterol [44] from *Talbotiella gentii*, and consequently, the first within the entire *Talbotiella* genus. β -Sitosterol was initially discovered in 1897 in the germ of cereal grains²⁸⁶ and has since been widely identified across numerous plant species from various families. Within the Amherstieae tribe, its presence has been documented in eight species. For instance, β -sitosterol [44] was detected via GC-MS in the seeds of *Brachystegia eurycoma*, a plant traditionally used for wound healing and treatment of colorectal cancer¹³⁸, as well as in *Cynometra iripa*, known in Ayurvedic medicine for its anti-inflammatory properties^{157,171}. It has also been isolated from the seeds of *Scorodophloeus zenkeri*¹⁸⁸, the leaves and branches of *Macrolobium latifolium*^{184,185}, and the roots of *Anthonotha cladantha*¹²³. Within the *Humboldtia* genus, β -sitosterol [44] has been reported in three species: from the aerial parts of *H. vahliana*¹⁷⁸, the bark of *H. laurifolia*¹⁷⁶, and the roots and stems of *H. unijuga*¹⁷⁵. Pharmacologically, β -sitosterol [44] exhibits a wide range of bioactivities. Antibacterial testing against *Staphylococcus aureus* using the broth dilution method revealed a minimum inhibitory concentration (MIC) of

1.24 mg/mL and a minimum bactericidal concentration (MBC) of 2.208 mg/mL²⁸⁷. Beyond its antibacterial properties, β -sitosterol [44] has demonstrated significant anti-inflammatory, antinociceptive, anticancer, antifertility, angiogenic, antioxidant, immunomodulatory, and antidiabetic activities, all with minimal toxicity²⁸⁸.

Stigmasterol [42] is reported here for the first time in the *Talbotiella* genus. Historically, stigmasterol was first isolated in 1906 from the Calabar bean (*Physostigma venenosum*)²⁸⁹. Within the Amherstieae tribe, its occurrence has been documented in a few species across different genera. It has been isolated from the seeds of *Anthonotha cladantha*¹²³ and identified in the leaves and branches of *Macrolobium latifolium*^{184,185}. Additionally, GC/MS analysis confirmed the presence of stigmasterol in the seeds of *Anthonotha macrophylla*⁶⁷, while both HPLC-DAD and GC/MS techniques detected it in the leaves of *Julbernardia paniculata*¹⁸¹.

Stigmasterol [42] exhibits a broad spectrum of pharmacological activities. In particular, stigmasterol isolated from the leaves of *Annona muricata* demonstrated significant cytotoxic activity against Vero and HeLa cancer cell lines, with IC₅₀ values of 173.8 μ g/mL and 11.58 μ g/mL, respectively, and a high selectivity index²⁹⁰. Molecular docking analysis revealed strong binding affinity to the vaccinia virus H1-related phosphatase (PDB ID: 3F81), with a binding energy of -6.6 kcal/mol and an estimated inhibitory constant (K_i) of 14.35 μ M, suggesting potential for cervical cancer treatment²⁹⁰. Other reported bioactivities of stigmasterol include anticancer, anti-inflammatory, anti-osteoarthritis, antidiabetic, and immunomodulatory effects²⁹¹.

Table 4-5 Table comparing the ^{13}C -NMR chemical shifts of β -sitosterol and stigmasterol to literature values

β -sitosterol				Stigmasterol		
	DEPT-135	Experimental ^{13}C -NMR, δppm (100 MHz, CDCl_3)	δ_{C} (ppm) (CDCl_3 , 100 MHz) ²⁵⁵		Experimental ^{13}C -NMR, δppm (100 MHz, CDCl_3)	δ_{C} (ppm) (CDCl_3 , 100 MHz) ²⁵⁵
1	CH_2	37.27	37.3	CH_2	37.2	31.8
2	CH_2	31.6	30.9	CH_2	31.6	28.4
3	CH	71.8	71.8	CH	71.8	71.9
4	CH_2	42.2	42.3	CH_2	42.2	42.4
5	C	140.7	140.8	C	140.7	140.9
6	CH	121.7	121.7	CH	121.8	121.8
7	CH_2	31.9	31.7	CH_2	31.9	29.3
8	CH	31.9	31.9	CH	31.9	36.3
9	CH	50.2	50.1	CH	50.1	50.2
10	C	36.5	36.5	C	36.5	36.6
11	CH_2	21.1	21.1	CH_2	21.0	24.5
12	CH_2	39.7	39.8	CH_2	39.8	39.8
13	C	42.3	42.3	C	42.3	42.3
14	CH	56.8	56.8	CH	56.8	56.9
15	CH_2	24.3	24.3	CH_2	24.4	24.5
16	CH_2	28.3	28.3	CH_2	28.9	28.4
17	CH	56.0	56.1	CH	56.1	56.2
18	CH_3	11.9	11.9	CH_3	11.8	12.0
19	CH_3	19.4	19.4	CH_3	19.4	19.5
20	CH	36.2	36.2	CH	40.5	40.6
21	CH_3	18.8	18.8	CH_3	21.2	18.9
22	CH_2	34.0	33.9	CH	138.3	138.4
23	CH_2	26.1	26.1	CH	129.3	129.4
24	CH	45.9	45.9	CH	51.2	51.3
25	CH	29.2	29.2	CH	31.9	32.0
26	CH_3	19.8	19.8	CH_3	19.8	19.9
27	CH_3	19.1	19.0	CH_3	18.9	19.1
28	CH_2	23.1	23.1	CH_2	25.5	25.5
29	CH_3	12.0	12.0	CH_3	12.0	12.2



Table 4-6 Table comparing the ^1H -NMR chemical shifts of β -sitosterol and stigmasterol to literature values

β -sitosterol				stigmasterol		
	DEPT-135	Experimental ^1H -NMR, δppm (400 MHz, CDCl_3)	δ_{H} (ppm) (CDCl_3 , 400 MHz) ²⁰⁴		Experimental ^1H -NMR, δppm (400 MHz, CDCl_3)	δ_{H} (ppm) (CDCl_3 , 400 MHz) ²⁰⁴
1	CH_2	1.84, 1.12 (2H, <i>m</i>)	1.87, 1.10 (2H, <i>m</i>)	CH_2	1.50, 1.04	1.52 (1H, <i>m</i> , H1)
2	CH_2	1.49, 1.84 (2H, <i>m</i>)	1.55 (2H, <i>m</i>)	CH_2	1.83	1.85 (1H, <i>m</i> , H2)
3	CH	3.53 (1H, septet, $J=5.0$, 6.1, 5.1 Hz)	3.54 (1H, <i>m</i>)	CH	3.52 (1H, septet, $J=5.0$, 6.1, 5.1 Hz)	3.53 (1H, <i>m</i> , H3),
4	CH_2	2.27 (<i>m</i>)	2.29 (2H, <i>m</i>)	CH_2	2.26 (1H, <i>m</i>)	2.26 (1H, <i>m</i> , H4),
5	C	-	-	C	-	-
6	CH	5.35 (<i>brd</i>)	5.37 (1H, <i>t</i>)	CH	5.34 (1H, <i>m</i>)	5.35 (1H, <i>m</i> , H6),
7	CH_2	1.57 (1H, <i>m</i>), 1.99 (1H, <i>m</i>)	2.20 (2H, <i>m</i>)	CH_2	1.25 (1H, <i>m</i>), 1.43 (1H, <i>m</i>)	1.67 (1H, <i>m</i> , H7),
8	CH	1.44 (1H, <i>m</i>)	1.98 (1H, <i>m</i>)	CH	1.97 (1H, <i>m</i>)	1.37 (1H, <i>m</i> , H8),
9	CH	0.93 (<i>m</i>)	0.94 (1H, <i>m</i>)	CH	0.92 (1H, <i>m</i>), 1.47 (1H, <i>m</i>)	0.93 (1H, <i>m</i> , H9)
10	C	-	-	C	-	-
11	CH_2	1.50, 1.45 (<i>m</i>)	1.51 (2H, <i>m</i>)	CH_2	0.84 (1H, <i>m</i>), 1.49 (1H, <i>m</i>)	1.56 (1H, <i>m</i> , H11),
12	CH_2	2.00 (1H, <i>m</i>), 1.16 (1H, <i>m</i>)	1.50 (2H, <i>m</i>)	CH_2	1.15, 1.99 (<i>m</i>)	2.00 (1H, <i>m</i> , H12),
13	C	-	-	C	-	-
14	CH	0.99 (1H, <i>m</i>)	1.06 (1H, <i>m</i>)	CH	1.00 (<i>m</i>)	1.02 (1H, <i>m</i> , H14),
15	CH_2	1.05 (1H, <i>m</i>), 1.57 (1H, <i>m</i>)	1.60 (2H, <i>m</i>)	CH_2	1.56, 1.03 (<i>m</i>)	1.58 (1H, <i>m</i> , H15),
16	CH_2	1.83 (1H, <i>m</i>), 1.25 (1H, <i>m</i>)	1.85 (2H, <i>m</i>)	CH_2	1.30 (2H, <i>m</i>)	1.26 (1H, <i>m</i> , H16)
17	CH	1.12 (1H, <i>m</i>)	1.12 (1H, <i>m</i>)	CH	1.11 (1H, <i>m</i>)	1.13 (1H, <i>m</i> , H17),
18	CH_3	0.69 (<i>s</i>)	0.70 (3H, <i>s</i>)	CH_3	0.68 (3H, <i>s</i>)	0.69 (1H, <i>m</i> , H18).
19	CH_3	1.01 (<i>s</i>)	1.02 (3H, <i>s</i>)	CH_3	1.00 (3H, <i>s</i>)	1.02 (1H, <i>m</i> , H19),
20	CH	1.35 (<i>m</i>)	1.38 (1H, <i>m</i>)	CH	2.03 (1H, <i>m</i>)	2.05 (1H, <i>m</i> , H20),
21	CH_3	0.92 (3H, <i>d</i> , $J=6.5$)	0.94 (3H, <i>d</i>)	CH_3	1.02 (3H, <i>m</i>)	0.93 (1H, <i>m</i> , H21)
22	CH_2	1.02 (1H, <i>m</i>), 1.33 (1H, <i>m</i>)	1.04 (2H, <i>m</i>)	CH	5.14 (1H, <i>dd</i> , $J=8.7$, 15.1 Hz)	5.16 (1H, <i>dd</i> , 15.1, 8.6, H22)
23	CH_2	1.16 (1H, <i>m</i>), 1.18 (1H, <i>m</i>)	1.17 (<i>m</i> , 2H)	CH	5.03 (1H, <i>dd</i> , $J=8.7$, 15.1 Hz)	5.03 (1H, <i>dd</i> , 15.1, 8.6, H23)
24	CH	0.93 (1H, <i>m</i>)	0.95 (1H, <i>m</i>)	CH	1.52 (1H, <i>m</i>)	1.54 (1H, <i>m</i> , H24),
25	CH	1.66 (1H, <i>m</i>)	1.68 (<i>m</i> , 1H)	CH	1.43 (1H, <i>m</i>)	1.45 (1H, <i>m</i> , H25),
26	CH_3	0.83 (3H, <i>d</i> , $J=7.1$ Hz)	0.86 (3H, <i>d</i>)	CH_3	0.83 (3H, <i>d</i> , $J=7.1$ Hz)	0.85 (1H, <i>m</i> , H26),
27	CH_3	0.81 (3H, <i>d</i> , $J=7.1$ Hz)	0.84 (3H, <i>d</i>)	CH_3	0.80 (3H, <i>d</i> , $J=7.1$ Hz)	0.82 (1H, <i>m</i> , H27),
28	CH_2	1.23 (1H, <i>m</i>), 1.28 (1H, <i>m</i>)	1.26 (<i>m</i> , 2H)	CH_2	1.40 (2H, <i>m</i>)	1.43 (1H, <i>m</i> , H28),
29	CH_3	0.84 (3H, <i>m</i>)	0.86 (3H, <i>t</i>)	CH_3	0.84 (3H, <i>m</i>)	0.82 (1H, <i>m</i> , H29),

Legend: J = coupling constant; s = singlet; d = doublet; dd = doublet of doublets; m = multiplets

4.5 Conclusion of Phytochemical Studies

Four known triterpenoids TSD-BB₁, TSD-BC₁, and TSD-D₁, (a mixture of two compounds) (Figure 4–10) were successfully identified from the phytochemical investigation of the dichloromethane extract of *Talbotiella gentii* stem bark, representing the first report of these metabolites from this species. Two isolates were obtained: TSD-BB₁ was identified as the oleanane-type triterpenoid friedelin [505], and TSD-BC₁ as the lupane-type triterpenoid lupeol [38], based on detailed spectroscopic analysis and comparison with literature data. A semi-synthetic derivative, TSD-BD_{1A}, was prepared from lupeol and confirmed as lupeol acetate [547]. The remaining isolate, TSD-D₁, comprised a co-eluting sterol mixture of β -sitosterol [44] and stigmasterol [42], both well-documented plant sterols frequently co-occurring in natural matrices.

While the compounds isolated are known, and 1D-NMR dereplication may suffice for identification, multidimensional NMR ensured accurate dereplication and structural validation. In this study, selected known isolates were also subjected to 2D-NMR for enhanced confirmation, given that this was the first phytochemical investigation of *T. gentii* stem. The isolated compounds and the semi-synthesized compound were obtained in a purity range of 90-95% based on the integration ratio of the isolated compound and the minor impurity on the ¹H-NMR spectrum of each. These minor impurities (0-10%), including residual solvent, grease, and co-eluting natural products, are characteristic of natural product isolation^{225,292}. Studies have implied that natural products with purity ranges of at least 93% purity are acceptable for further characterization and bioactivity studies^{293–295}. For example, Bisson et al. (2015) reported that natural products with 90–95% purity are suitable for bioactivity testing, and Izzo et al. (2020) similarly noted that such purity levels are adequate for reliable pharmacological evaluation^{296,297}. Even though there were no mass spectra analyses because all the compounds isolated are known, the comprehensive ¹H-

NMR, ^{13}C -NMR, and 2D-NMR data, supported by literature comparison, provided reliable structural assignments.

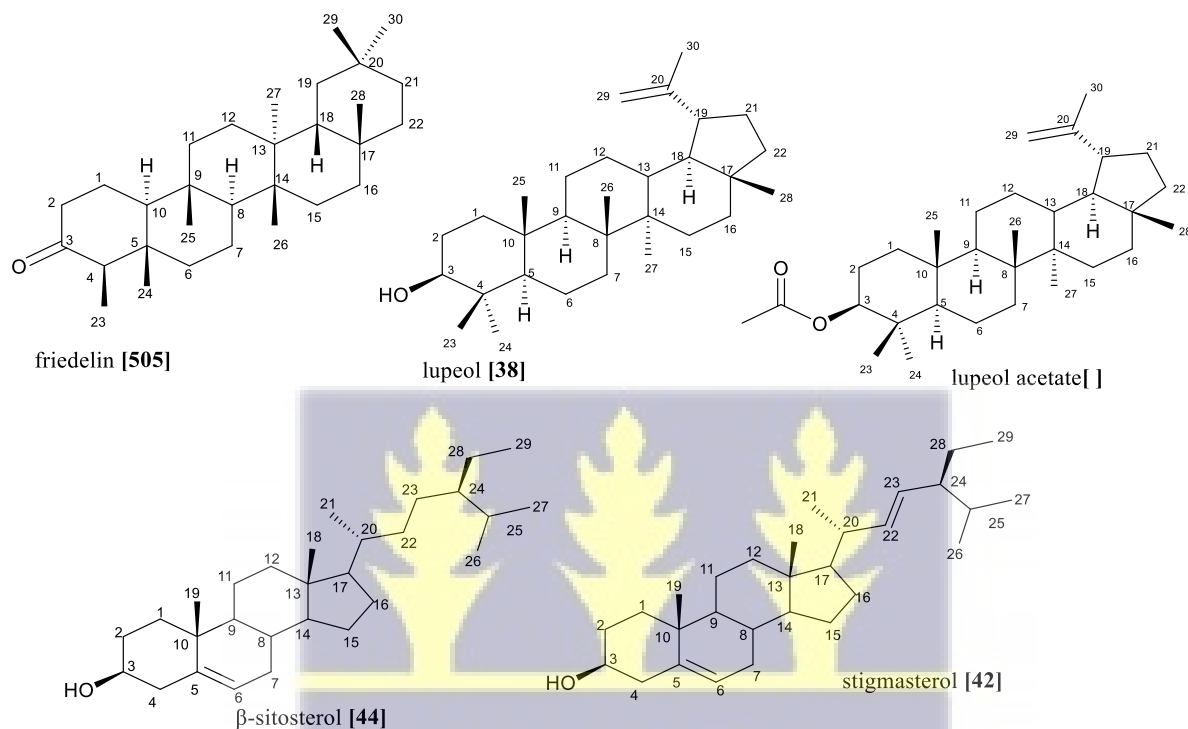


Figure 4-10 Structures of compounds from *T. gentii*

4.6 *In vitro* Antitrypanosomal activity Studies

4.6.1 *In vitro* Antitrypanosomal activities of *T. gentii* stem extracts against *T. b. brucei*

Among the *T. gentii* stem extracts tested, all showed activity against *T. b. brucei*, with the DCM extract exhibiting the most promising potency. The dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) extracts recorded IC_{50} values of $2.02 \pm 0.53 \mu\text{g/mL}$, $9.85 \pm 1.41 \mu\text{g/mL}$, and $24.67 \pm 4.69 \mu\text{g/mL}$, respectively (Appendix IV, Table 1). Although the extracts demonstrated varying degrees of potency, the DCM extract stood out with the strongest activity. The very good activity of the DCM extract suggests a higher concentration of bioactive constituents such as triterpenoids and sterols, consistent with solvent polarity principles and previous reports on similar fractions^{298,299}. This extract was therefore selected for further purification by column chromatography. These findings constitute the first report of the antitrypanosomal activity of *T. gentii* extracts against *T. b. brucei*.

4.6.2 *In vitro* Antitrypanosomal activities of compounds against *T. b. brucei*

In this study, two pure compounds, one mixture, and one derivatised compound from *T. gentii* stems were evaluated for their *in vitro* antitrypanosomal activity against *Trypanosoma brucei brucei* (Appendix IV, Table 2). The compounds included friedelin [505], lupeol [38], a mixture of β -sitosterol [44] and stigmasterol [42], as well as the derivatised compound lupeol acetate [547]. Among these, lupeol acetate [547] exhibited the highest potency ($IC_{50} = 2.90 \pm 0.80 \mu\text{g/mL}$), followed by lupeol [38] ($IC_{50} = 3.50 \pm 0.70 \mu\text{g/mL}$). Friedelin [505] and the β -sitosterol [44]/stigmasterol [42] mixture displayed moderate activity ($IC_{50} = 45.50 \pm 6.20 \mu\text{g/mL}$ and $13.90 \pm 0.80 \mu\text{g/mL}$, respectively), compared with the standard diminazene aceturate ($IC_{50} = 0.05 \mu\text{g/mL}$).

Our search of the literature on the antitrypanosomal activity of friedelin (TSD-BB₁) against *T. b. brucei* indicated that only three activities were documented; available reports indicate varying degrees of inhibition. These reported IC₅₀ values range widely ($\approx$ 12 to >64 $\mu\text{g/mL}$), depending on the strain of *T. b. brucei* used in the *in vitro* analyses^{249,250,240,300}. For instance, friedelin isolated from *Celaenodendron mexicanum* exhibited an IC₅₀ greater than 30 μM (> 12.8 $\mu\text{g/mL}$) against bloodstream form trypomastigotes of *T. b. brucei* (strain S427)³⁰⁰. Friedelin isolated from *Severinia buxifolia* leaves showed no activity (IC₅₀ > 64.00 $\mu\text{g/mL}$) when tested against Trypomastigotes of *T. brucei* Squib-427 strain (suramin-sensitive)²²⁰. Friedelin isolated from *Dichapetalum albidum* stem bark also showed no activity (IC₅₀ of 53.47 $\mu\text{g/mL}$) when tested against GUTat 3.1 bloodstream form of *T. b. brucei* parasite³⁰¹. In the present study, friedelin, when tested against bloodstream forms of *T. b. brucei* (GUTat 3.1 strain), similarly showed no activity (IC₅₀ = 45.5 $\mu\text{g/mL}$). The slight differences in IC₅₀ values among studies may be attributed to the different strains of *T. b. brucei* parasites used. Squib-427 and GUT 3.1 are distinct strains of *T. b. brucei* with biological and experimental differences. Squib-427 (variant of the well-studied 427 strain) is a laboratory-adapted, monomorphic strain that grows rapidly, lacks the ability to differentiate into short stumpy forms, and is widely used for molecular biology studies due to its ease of culture and genetic manipulation³⁰². In contrast, GUT 3.1 is a fly-transmissible strain capable of completing the natural parasite lifecycle and differentiation stages, including short stumpy forms, making it more representative of natural infections³⁰³. These differences affect IC₅₀ outcomes in antitrypanosomal assays: Squib-427's monomorphic and faster growth characteristics typically yield more consistent but possibly lower IC₅₀ values because the strain may be more sensitive to drugs. GUT 3.1's ability to differentiate and more complex lifecycle stages can influence drug susceptibility, potentially resulting in higher or variable IC₅₀ values due to physiological states

that affect drug uptake and metabolism. Therefore, IC₅₀s can differ between these strains not only because of genetic variability but also due to differences in parasite biology and drug interaction dynamics^{304–306}. In summary, the antitrypanosomal activity of friedelin against *T. b. brucei* shows variable and generally weak inhibition across the compared literature, with differences in IC₅₀ values largely attributable to the distinct characteristics of strains like Squib-427 and GUTat 3.1, which affected friedelin's susceptibility and assay outcomes. The absence of polar functional groups capable of hydrogen-bond formation or ionic interactions may limit its affinity toward target enzymes in *T. b. brucei*^{307–309}. However, the low activity observed in this study may not entirely rule out that friedelin might have potential as a lead compound; it suggests that further structural optimization hence further optimization may enhance its efficacy.

Lupeol, on the other hand, demonstrated a much stronger inhibitory effect (IC₅₀ = 3.50 ± 0.70 µg/mL). A comparable study employing similar assay reported a higher IC₅₀ of 19.3 ± 1.6 µg/mL for lupeol when evaluated against *T. b. brucei* (strain 427) bloodstream forms trypomastigotes³¹⁰. Also, for a similar assay protocol by Tlhapi et al., the lupeol-rich fraction from *Rauvolfia caffra* stem methanol extract displayed an IC₅₀ of 18.5 µg/mL when tested against bloodstream form *T. b. brucei* (strain Lister 427) trypomastigotes³¹¹. The higher potency (IC₅₀ = 3.50 ± 0.70 µg/mL) obtained in this study, compared to IC₅₀ of 19.3 ± 1.6 µg/mL and IC₅₀ of 18.5 µg/mL from the literature, could be attributed to differences in the strain of *T. brucei* used. The compared literature both assessed the *in vitro* antitrypanosomal activity of lupeol against *T. b. brucei* (strain 427), whereas this study used *T. b. brucei* (GUTat 3.1 strain. This highlights the potential of lupeol as a potent antitrypanosomal agent. Even though lupeol has shown some activity against *T. b. brucei* across studies, its mechanism of action remains largely unexplored. Therefore, further investigation into its mode of action is strongly warranted.

The acetylated derivative of lupeol [38], lupeol acetate [547], showed the highest potency among the compounds tested, with an IC_{50} of $2.90 \pm 0.80 \mu\text{g/mL}$. There are limited *in vitro* studies on lupeol acetate [547] against *T. b. brucei*; however, a few studies have investigated its activity against other *Trypanosoma* species. This result is consistent with previous reports where lupeol acetate [547] demonstrated antitrypanosomal activity with *T. b. brucei* and *T. cruzi*. For instance, petroleum ether extract from *Kleinia odora*, which was found to contain lupeol acetate [547] was shown to eliminate *T. cruzi* Tulahuen CL2 trypomastigotes with an IC_{50} of $5.7 \pm 1.6 \mu\text{g/mL}$ and $0.5 \pm 0.1 \mu\text{g/mL}$ for *T. brucei* Squib-427 (suramin-sensitive) trypomastigotes³¹². Furthermore, lupeol acetate [547] induced death in *T. cruzi* Y-strain (TcII) — extracellular (trypomastigotes) & intracellular(amastigote) forms with an EC_{50} of $15.82 \pm 3.7 \mu\text{g/mL}$ and inhibited the amastigote stage with an IC_{50} of $32.55 \pm 1.2 \mu\text{g/mL}$ ³¹³. From this, it can be inferred that lupeol acetate exhibits significant antitrypanosomal activity, particularly against *T. b. brucei*, and this aligns with previous findings showing its potency against both *T. brucei* and *T. cruzi*, suggesting it is a promising lead compound for further antitrypanosomal drug development.

lupeol [38], a pentacyclic triterpenoid bearing a hydroxyl group at C-3, demonstrated strong activity ($IC_{50} = 3.5 \mu\text{g/mL}$) while, lupeol acetate [547], exhibited slightly improved activity ($IC_{50} = 2.9 \mu\text{g/mL}$), suggesting that modest modification at C-3 did not compromise biological potency. This enhancement may stem from the acetylation, allowing better intracellular access while retaining the pharmacophoric features of the lupane skeleton^{314,315} even though the exact mechanism through which lupeol acetate [547] exerts its trypanocidal effects remain to be elucidated.

Stigmasterol [42] and β -sitosterol [44], both phytosterols, were tested as a mixture against *T. b. brucei* (GUTat 3.1 strain) in this study and showed significantly weaker activity with an IC_{50} of

13.90± 0.80 µg/mL compared to the reference diminazene aceturate (0.05 µg/mL). The activity of these compounds against *T. b. brucei* has been inconsistent in the literature. Individually, these sterols often show weak or variable trypanocidal effects, with reported IC₅₀ values sometimes exceeding 100 µg/mL^{316–319}. The improved activity of the mixture may indicate additive or synergistic interactions. The mixture of β-sitosterol/stigmasterol isolated from *Morinda lucida* leaves showed a minimum inhibitory concentration (MIC) of 12.5 µg/mL against *T. b. brucei* S427 bloodstream forms³²⁰. Another study confirmed minimal activity of the β-sitosterol [44]/stigmasterol [42] mixture isolated from *Flacourtia rukam* leaves with an IC₅₀ of >10 µg/mL when tested against³¹⁶. β-sitosterol [44] and stigmasterol [42] isolated from *Avicennia lanata* stems exhibited poor inhibitory activity against *T. b. brucei* (strain S427), with MIC values of 142.30 µM (59.04 µg/mL) and 126.40 µM (52.18 µg/mL), respectively^{317,321}. Another study also reported that β-sitosterol [44] from *Buchholzia coriacea* seeds displayed modest activity against *T. b. brucei* (strain S427) with an MIC of 12.5 µg/mL³¹⁸. Stigmasterol [42], in particular, has shown IC₅₀ values exceeding 100 µg/mL when tested against *T. b. brucei* TC221 in another study³¹⁹, indicating poor activity. The β-sitosterol/stigmasterol mixture demonstrates weak and inconsistent antitrypanosomal activity, and this variability may be due to differences in parasite strains and assay parameters used across studies. Strains such as *T. b. brucei* S427 and TC221 differ in biological characteristics and drug susceptibility, which contributes to variations in reported inhibitory values³²². Additionally, IC₅₀ (the concentration required to inhibit 50% of parasite viability) and MIC (the lowest concentration that completely inhibits visible growth) are not equivalent– IC₅₀ is a quantitative measure of partial inhibition, while MIC is a threshold for total suppression– making direct comparison across studies difficult. Overall, although β-sitosterol and stigmasterol consistently show only weak to moderate activity and require high concentrations to

exert inhibition, their recurring bioactivity across different studies suggests they may serve as weak lead compounds or basic scaffolds, but they would require structural modification or derivatization and/or in combination with other active compounds to become viable antitrypanosomal drug candidates.

A mixture of friedelin, lupeol, β -amyrin, α -amyrin, β -friedelanol, campesterol, stigmasterol [42], and β -sitosterol [44], as identified in the hexane extract of *Tibouchina stenocarpa* seeds, was shown to be active against *T. cruzi* with an IC_{50} of 23.6 $\mu\text{g/mL}$ ³²³. Although this involved a different *Trypanosoma* species, the findings suggest that the combined effects of triterpenoids and sterols may enhance antiparasitic activity. In this study, a mixture of stigmasterol [42] and β -sitosterol [44] showed weaker activity against *T. b. brucei* than the standard, but potential synergistic effects merit further study.

To conclude, the isolated compounds exhibited varying antitrypanosomal activities, with lupeol acetate [547] and lupeol [38] showing the highest potency, the β -sitosterol [44]/stigmasterol [42] mixture displaying moderate effects, and friedelin [547] being the least active. The results are consistent with previous reports on triterpenoids and sterols, confirming measurable bioactivity across all isolates. These findings confirm the ethnomedicinal relevance of *T. gentii* and highlight its triterpenoids and sterols as promising leads for antitrypanosomal drug development, underscoring the potential of Amherstieae metabolites in natural product-based chemotherapy. Although limited by sample size³²⁴, these findings provide a preliminary rationale for subsequent molecular docking analysis, which explores the specific enzyme–ligand interactions that may underlie the observed trends^{325,326}.

4.7 Results of the Molecular Docking Studies of Isolated Ligands from *T. gentii* DCM Stem Fractions against *TbPTR1* and *TbDHFR*

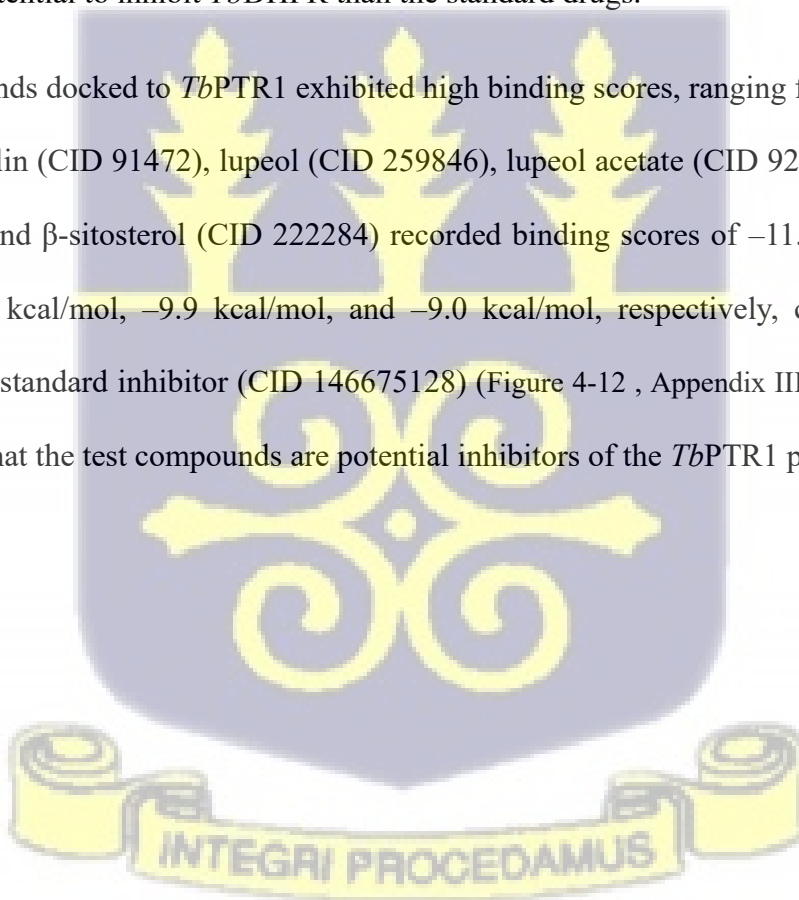
Molecular docking determines how two molecules, mostly a ligand and a protein or two proteins fit together and utilizes a scoring function to estimate the affinity between the two molecules^{327,328}. Molecular docking has been reported to provide significant prima facie evidence of inhibition³²⁹. Given its consistent activity across different studies, lupeol warrants further investigation, particularly regarding its mechanism of action, which remains largely unexplored. Given that inhibition of *TbDHFR* and *TbPTR1* gives rise to antitrypanosomal activity, the potential of the compounds to interact with and inhibit these enzymes was investigated. Inhibitors of *TbDHFR* and *TbPTR1*, pyrimethamine, cycloguanil and CID 146675128 respectively, were used as standards. *TbDHFR* and *TbPTR1* were selected as molecular targets due to their essential roles in the folate and pterin metabolic pathways of *Trypanosoma brucei*. These enzymes are critical for parasite survival and proliferation, particularly in the synthesis of nucleotides and maintenance of redox balance. Inhibition of either enzyme disrupts these key metabolic functions, and since *T. brucei* can compensate for DHFR inhibition via PTR1, dual inhibition of both enzymes is considered a promising therapeutic strategy. Therefore, assessing the binding potential of the test compounds against both *TbDHFR* and *TbPTR1* provides a rational approach for evaluating their antitrypanosomal potential.

Studies have shown that a binding energy of -7.0 kcal/mol is considered a strong indicator of binding affinity, effectively differentiating between active and inactive compounds³³⁰. Since nearly 97.7% of inhibitors exhibit binding energies below -7.0 kcal/mol³³¹, using -7.0 kcal/mol as a threshold effectively excludes about 95% of non-inhibitors³³². This value can be used as a threshold

in the absence of known inhibitors^{241,333}. Generally, the more negative the binding energy, the stronger the binding affinity.

All the five tested compounds against *Tb*DHFR demonstrated significant binding scores. Friedelin (CID 91472) [505], lupeol (CID 259846) [38], lupeol acetate (CID 92157) [547], β -sitosterol (CID 222284) [44], and stigmasterol (CID 5280974) [42] showed stronger binding affinities to *Tb*DHFR, with docking scores ranging from -10.8 to -8.7 kcal/mol. These scores are more favourable than those of the reference inhibitors cycloguanil (CID 9049, -8.0 kcal/mol) and pyrimethamine (CID 4993, -7.2 kcal/mol) (Figure 4-11 , Appendix III-A Table 1), suggesting that the test compounds may have a higher potential to inhibit *Tb*DHFR than the standard drugs.

All five compounds docked to *Tb*PTR1 exhibited high binding scores, ranging from -11.2 to -9.0 kcal/mol. Friedelin (CID 91472), lupeol (CID 259846), lupeol acetate (CID 92157), stigmasterol (CID 528079), and β -sitosterol (CID 222284) recorded binding scores of -11.2 kcal/mol, -10.9 kcal/mol, -10.5 kcal/mol, -9.9 kcal/mol, and -9.0 kcal/mol, respectively, compared to -9.5 kcal/mol for the standard inhibitor (CID 146675128) (Figure 4-12 , Appendix III-B Table 1). These results suggest that the test compounds are potential inhibitors of the *Tb*PTR1 protein target.



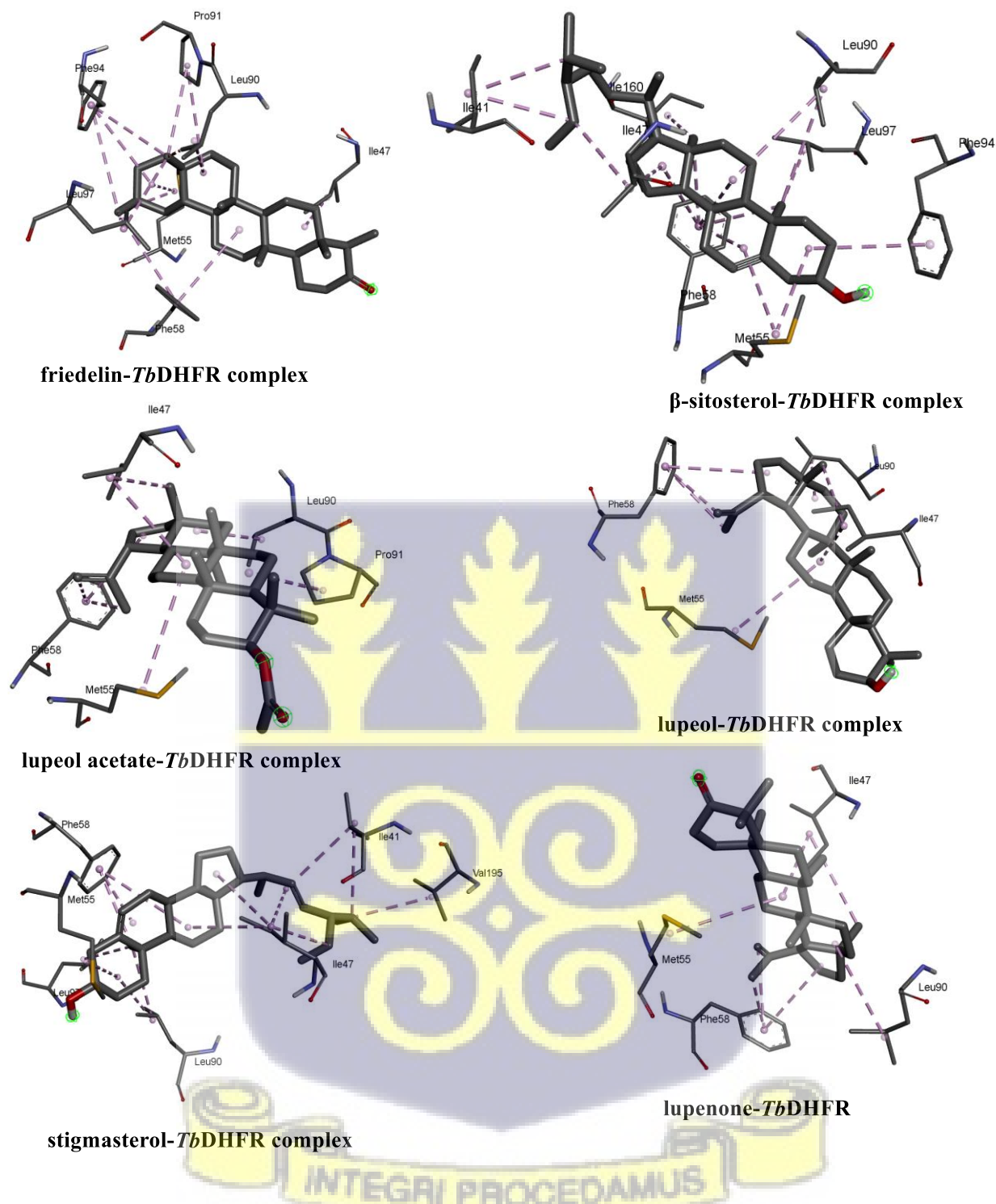
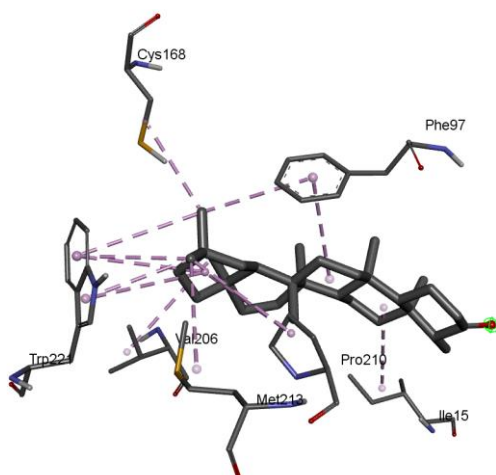
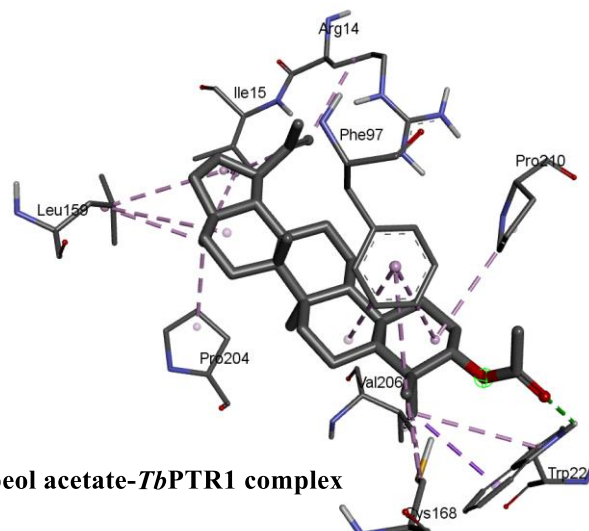


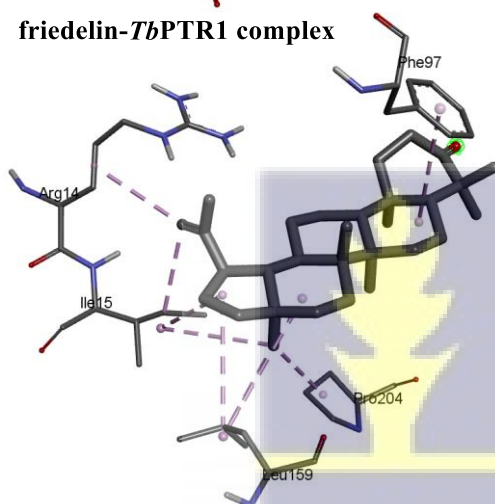
Figure 4-11 Binding modes of screened ligands against *Tb*DHFR



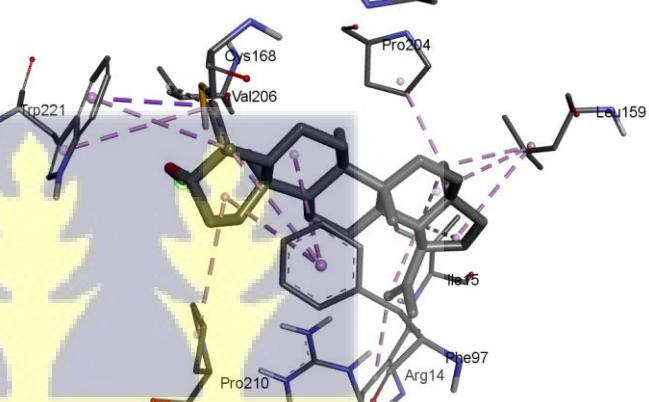
friedelin-*Tb*PTR1 complex



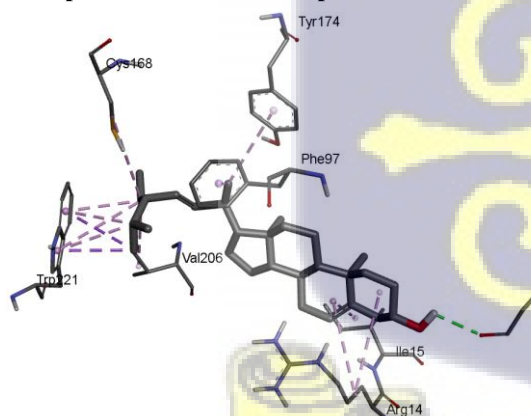
lupeol acetate-*Tb*PTR1 complex



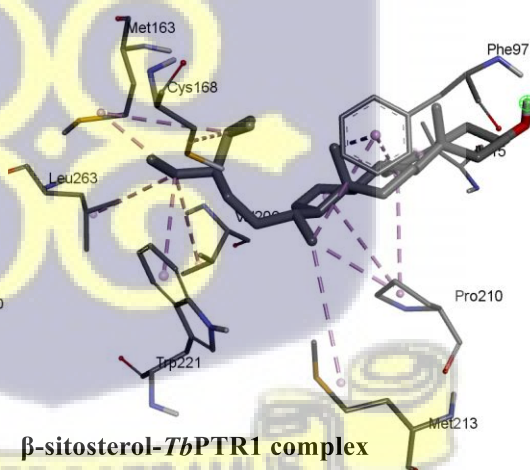
lupenone-*Tb*PTR1 complex



lupeol-*Tb*PTR1 complex



stigmasterol-TbPTR1 complex



β -sitosterol-*TbPTR1* complex

Figure 4-12 Binding modes of screened ligands against *TbPTR1*

4.7.1 Protein-ligand interaction

For a compound to inhibit a target, it must interact with the active site residue of the target³³³. In the case of *Tb*DHFR, effective interaction requires binding to specific active site residues, Met55, Asp54, Phe58, Thr86, Leu90, Phe94, Ile160, Tyr166 and Ser89²⁴¹. Similarly, interaction with *Tb*PTR1 involves key residues, Phe113, Trp221, His267, Met213, Val206, Leu209, Pro210, Leu208, Arg14, Ser95, Asp161, Phe97 and Tyr174²⁴⁰. All the compounds interacted with at least one of the active site residues of both *Tb*DHFR and *Tb*PTR1 as summarized in Table 4-13 and 4-15 below and shown in appendix III-A and appendix III-B, hence might impede the binding of their substrate, hence inhibiting them^{69,334}.

4.7.1.1 Interaction Profile of *Tb*DHFR Complexes

There were different kinds of interaction observed in the protein-ligand complexes. For the friedelin-*Tb*DHFR complex, molecular docking analysis revealed key interactions between friedelin and the *Tb*DHFR protein. Friedelin formed two π -alkyl interactions with PHE58 and PHE94, along with four alkyl-alkyl interactions involving Leu94, Pro91, Ile47, and Met55. Additionally, it engaged in Van der Waals interactions with eleven residues: Ser89, Ile160, Thr86, Gly161, Gly162, Ile41, Tyr166, Val33, Ala34, Arg59, and Arg100 (appendix III-A 1). Alkyl-alkyl interactions were observed between ring B and Ile47, as well as between ring D and both Leu90 and Pro91. Ring E also formed an alkyl-alkyl interaction with Met55, while Met55 further interacted with C30 through an alkyl-alkyl interaction. π -alkyl interactions were identified between ring C and Phe58, and between ring E and Phe94. Additionally, Phe97 exhibited π -alkyl interactions with C28 and C29 while Leu97 engaged in alkyl-alkyl interactions with the same carbon atoms. Pro91 also formed an alkyl-alkyl interaction with C28, whereas Leu90 displayed an unfavourable donor-donor interaction with C28.

For the lupeol-*Tb*DHFR complex, lupeol formed a π -alkyl interaction with PHE58 and three alkyl-alkyl interactions with MET55, ILE47, and LEU90. Additionally, the ligand engaged in Van der Waals interactions with eight residues: GLU50, TRP49, ASP54, ILE160, THR86, SER89, PRO91, and THR46. Alkyl-alkyl interactions were observed between ring C and both MET55 and ILE47, as well as between ring D and LEU90. Furthermore, ILE47 formed an alkyl-alkyl interaction with C28. A π -alkyl interaction was identified between C29, C30, and ring E with PHE58. These interactions are illustrated in Appendix III-A-2.

For the lupeol acetate-*Tb*DHFR complex, lupeol acetate formed one π -alkyl interaction with PHE58 and four alkyl-alkyl interactions with MET55, PRO91, ILE47, and LEU90. Additionally, the ligand engaged in Van der Waals interactions with seven amino acid residues: GLU50; TRP49; ASP54; ILE160; THR86; SER89; and THR46. The observed alkyl-alkyl interactions occurred between ring B and PRO91; ring C and both MET55 and ILE47; ring D and LEU90; and between ILE47 and C28. Additionally, π -alkyl interactions were identified between PHE58 and ring E, as well as between C29 and C30. These interactions are illustrated in Appendix III-A-2.

For the stigmasterol-*Tb*DHFR complex, two π -alkyl interactions were observed between the ligand and the amino acid residues PHE58 and ILE47. Additionally, six alkyl-alkyl interactions occurred with MET55, LEU90, VAL195, ILE41, ILE47, and LEU97. Fourteen van der Waals interactions were observed with PRO91, ILE160, THR86, GLY161, GLY162, GLY163, GLY44, GLY42, THR46, SER89, TYR166, VAL33, ALA34, and PHE94. The alkyl-alkyl interactions involved ring A and the protein residues MET55 and LEU90; ring C, ring D, and C28 with ILE47; C19 with LEU90 and LEU97; and C25 with ILE41 and VAL195. The observed π -alkyl interactions occurred between C22 and the residues ILE41 and ILE47; between PHE58 and C19; and between ring B and ring C. These interactions are illustrated in Appendix III-A-3.

For the β -sitosterol-*Tb*DHFR complex (Appendix III-A-3), Three pi-alkyl interactions were observed between the ligand and the amino acid residues PHE58, PHE94, and MET55. Six alkyl-alkyl interactions were detected between MET55, LEU90, LEU97, ILE41, ILE47, and ILE160. The ligand also formed twelve van der Waals interactions with the residues ARG59, THR86, SER89, GLY161, GLY162, THR46, GLY42, VAL195, GLY163, TYR166, VAL33, and ALA34. The alkyl-alkyl interactions involved MET55 with rings A and B; ILE41 with C25 and C28; ILE47 with ring D and C28; LEU90 with ring C; and ILE160 with C18. For pi-alkyl interactions, PHE58 interacted with ring B, ring C, ring D, C19, and C18. Additionally, another pi-alkyl interaction was observed between ring A and PHE94.

Table 4-7 Interactions Existing in the *Tb*DHFR-ligand Complexes

Active site Amino acid residue	Interactions Observed Between Active Site Residue and Ligand				
	friedelin	lupeol	lupeol acetate	stigmasterol	sitosterol
Met55	alkyl-alkyl	alkyl-alkyl	alkyl-alkyl	alkyl-alkyl	pi-alkyl
Asp54	-	Van der Waal	Van der Waal	-	-
Phe58	pi-alkyl	pi-alkyl	pi-alkyl	pi-alkyl	pi-alkyl
Thr86	Van der Waal	Van der Waal	Van der Waal	Van der Waal	Van der Waal
Leu90	unfavourable donor-donor	alkyl-alkyl	alkyl-alkyl	alkyl-alkyl	alkyl-alkyl
Phe94	pi-alkyl	-	-	Van der Waal	pi-alkyl
Ile160	Van der Waal	Van der Waal	Van der Waal	Van der Waal	alkyl-alkyl
Tyr166	Van der Waal	-	-	Van der Waal	Van der Waal
Ser89	Van der Waal	Van der Waal	Van der Waal	Van der Waal	Van der Waal

4.7.1.2 Interaction Profile of *Tb*PTR1 Complexes

For the ligand (friedelin)-*Tb*PTR1 complex (Appendix III-B-1), the alkyl-alkyl interactions were as follows: Ile15 interacted with ring B; Val206 with C28; ring E with Pro210; and C29 with Pro210, Met213, and Cys168. The π -alkyl interactions included Phe97 with ring C; Trp221 with ring E and C30; and Phe97 with C30. Additionally, ten van der Waals interactions were identified with Gly16, Asn93, Tyr174, Ser95, Pro204, Ser207, Arg14, Leu208, Leu209, and Gly205.

For the lupeol-*TbPTR1* complex (Appendix III-B-2), a π - σ interaction was observed between Trp221 and C23, while Trp221 also engaged in a π -alkyl interaction with C24 of lupeol. Additional π -alkyl interactions were identified between Phe97 and ring A, ring B, and C24, as well as between Arg14 and Ile15 with C29. The alkyl-alkyl interactions included Pro210 with ring A; Val206 with C23; Cys168 with both C23 and C24; Pro204 with C28; Leu159 with ring D, ring E, and C28; and Ile15 with C28 and ring E. Furthermore, van der Waals interactions were observed between lupeol and the following residues: Met213, Leu209, Ser207, Leu208, Asn93, Gly205, Asp161, Tyr174, Lys178, Ser95, and Asn93.

For the lupeol acetate-*TbPTR1* complex (Appendix III-B-2), a π - σ interaction was observed between Trp221 and C23. Additionally, the carbonyl oxygen of the acetate group formed a conventional hydrogen bond with Trp221. The π -alkyl interactions involved Phe97 with ring A, ring B, and C24, as well as Arg14 and Ile15 with C29. Alkyl-alkyl interactions were identified between Pro210 and ring A; Val206 and C23; Cys168 with both C23 and C24; Pro204 and C28; Leu159 with ring D, ring E, and C28; and Ile15 with C28 and ring E. Furthermore, van der Waals interactions were observed between lupeol acetate and the following residues: Met213, Leu209, Ser207, Leu208, Asn93, Glu217, Gly205, Asp161, Tyr174, Lys178, and Ser95.

For the stigmaterol-*TbPTR1* complex (Appendix III-B-3), a conventional hydrogen bond was formed between the hydroxyl (-OH) group of stigmaterol and Gly10. A π - σ interaction was observed between Trp221 and C27, as well as between Phe97 and C20. The π -alkyl interactions involved Trp221 with C28 and C25, Tyr174 with C20, and Ile15 and Arg14 with ring B. Alkyl-alkyl interactions were observed between C28 and the amino acids Val206 and Cys168. Van der Waals interactions occurred between stigmaterol and the following residues: Leu263, Gly205,

Met163, Ser207, Leu208, Gly16, Lys13, Asn93, Ala94, Ser95, Leu159, Pro204, Met213, Leu209, and Pro210.

For the β -sitosterol–TbPTR1 complex (Appendix III-B-3), a π – σ interaction was observed between Phe97 and C18. Additionally, π –alkyl interactions were identified between Trp221 and C28, Ile15 and ring B, and Phe97 with both ring C and C20. Several alkyl–alkyl interactions were also noted: Pro210 with ring C, ring D, and C20; Met213 with C20; Cys168 with C26; Met163 with C28 and C26; and C28 with Leu263 and Val206. Finally, van der Waals forces were observed with Tyr174, Asp161, Gly205, Leu209, Ser207, Leu208, Arg14, Ala94, Ser95, and Asn93.

Table 4-8 Interactions Existing in the *Tb*PTR1-ligand Complexes

Active site	Interactions Observed Between Active Site Residue and Ligand				
Amino acid residue	friedelin	lupeol	lupeol acetate	stigmaterol	sitosterol
Tyr174	-	Van der Waal	Van der Waal	pi-alkyl	Van der Waal
Phe113	-	-	-	-	-
Trp221	pi-alkyl	Pi-sigma bond	Pi-sigma bond; H-bond	Pi-sigma	pi-alkyl
His267	-	-	-	-	-
Met213	alkyl-alkyl	Van der Waal	Van der Waal	Van der Waal	alkyl-alkyl
Val206	alkyl-alkyl	alkyl-alkyl	alkyl-alkyl	alkyl-alkyl	alkyl-alkyl
Leu209	Van der Waal	Van der Waal	Van der Waal	Van der Waal	Van der Waal
Pro210	alkyl-alkyl	alkyl-alkyl	alkyl-alkyl	Van der Waal	alkyl-alkyl
Leu208	Van der Waal	Van der Waal	Van der Waal	Van der Waal	Van der Waal
Arg14	Van der Waal	pi-alkyl	pi-alkyl	pi-alkyl; alkyl-alkyl	Van der Waal
Ser95	Van der Waal	Van der Waal	Van der Waal	Van der Waal	Van der Waal
Asp161	-	Van der Waal	Van der Waal	-	Van der Waal
Phe97	pi-alkyl	pi-alkyl	pi-alkyl	Pi-sigma	Pi-sigma

The *in silico* molecular docking studies suggest that friedelin, lupeol, lupeol acetate, stigmaterol, and β -sitosterol may inhibit *Tb*DHFR, while friedelin, lupeol, lupeol acetate, and stigmaterol are potential inhibitors of *Tb*PTR1. Therefore, friedelin, lupeol, lupeol acetate, and stigmaterol could act as dual inhibitors of both *Tb*DHFR²⁴¹ and *Tb*PTR1²⁴⁰, contributing to their observed antitrypanosomal activity. Dual inhibition of these critical enzymes may enhance antitrypanosomal efficacy; however, inhibition of a single target could equally contribute to the overall activity.

These findings highlight the therapeutic potential of these natural compounds against trypanosomal infections.

4.7.2 Comparison and Synergy Analysis of Experimental Binding Affinities

The binding affinities of various ligands against *Trypanosoma brucei* Dihydrofolate Reductase (*TbDHFR*) and Pteridine Reductase 1 (*TbPTR1*) were determined experimentally. For *TbDHFR*, the binding affinities of the tested ligands were as follows: friedelin (-10.8 kcal/mol), stigmasterol (-9.7 kcal/mol), β -sitosterol (-9.5 kcal/mol), lupeol (-9 kcal/mol), and lupeol acetate (-8.7 kcal/mol). The known inhibitors, cycloguanil and pyrimethamine, showed binding affinities of -8.0 kcal/mol and -7.2 kcal/mol, respectively. In the case of *TbPTR1*, friedelin exhibited the strongest binding affinity at -11.2 kcal/mol, followed by lupeol (-10.9 kcal/mol), and lupeol acetate (-10.5 kcal/mol). Stigmasterol and β -sitosterol displayed weaker affinities of -9.9 kcal/mol and -9.0 kcal/mol, respectively, while the reference inhibitor, CID 146675128, showed a binding affinity of -9.5 kcal/mol.

The experimental binding affinities of the tested ligands against *Trypanosoma brucei* dihydrofolate reductase (*TbDHFR*) and pteridine reductase 1 (*TbPTR1*) reveals promising results. Friedelin exhibited the strongest binding affinity to both enzymes, with -10.8 kcal/mol against *TbDHFR* and -11.2 kcal/mol against *TbPTR1*, outperforming the reference inhibitors, cycloguanil and pyrimethamine for *TbDHFR*, and CID 146675128 for *TbPTR1*. Other ligands such as stigmasterol, sitosterol, and lupeol also demonstrated significant binding affinities to both targets. Lupeol, for instance, showed affinities of -9.0 kcal/mol and -10.9 kcal/mol against *TbDHFR* and *TbPTR1*, respectively. These results suggest that these natural products could be more potent inhibitors of these enzymes than the currently known inhibitors.

Molecular docking simulations showed significant binding affinities of the ligands to both *Tb*DHFR and *Tb*PTR1 (see Tables 4-11 and 4-12, respectively). Given the key role of these enzymes in *T. brucei* metabolism, their simultaneous inhibition suggests a synergistic, multi-target effect that could more effectively disrupt the pathway and limit resistance. Moreover, the superior performance of these natural products over known inhibitors highlights their potential as lead compounds for new anti-trypanosomal therapies, with possible enhanced efficacy at lower doses.

4.7.3 Comparison of Experimental Binding Affinities with Literature

The results obtained in this study for the binding affinities of friedelin, lupeol, and stigmasterol against *Trypanosoma brucei* proteins are generally consistent with the limited literature available. An extensive review of the literature indicates that the only documented *in silico* molecular docking studies involving these compounds (ligands) were conducted by Setzer and Ogungbe in 2012⁷⁰. For instance, friedelin's binding to *Tb*PTR1 reported in the literature was observed to have a higher affinity (-16.3 kcal/mol)¹⁹⁷ compared to the experimental value of -11.2 kcal/mol in this study. They also recorded stronger binding of friedelin to other *T. brucei* proteins, and *Tb*DHFR (-18.0 kcal/mol), indicating a potential for higher selectivity towards these targets. However, no docking to *Tb*Rhodesain was observed, which aligns with the current findings where friedelin was not docked to *Tb*Rhodesain in this study either¹⁹⁷.

Lupeol's docking results show some variability when compared to literature. The binding affinity (Table 4-12) of lupeol against *Tb*PTR1 in this study (-10.9 kcal/mol) is lower than the literature value of -18.5 kcal/mol¹⁹⁷. Similarly, for *Tb*DHFR, the literature reports a significantly stronger binding affinity of -17.4 kcal/mol¹⁹⁷ compared to the experimentally (Table 4-11) obtained value of -9.0 kcal/mol in this study.

β -sitosterol showed binding affinities of -9.5 kcal/mol (Table 4-12) and -9.0 kcal/mol (Table 4-13) against *Tb*DHFR and *Tb*PTR1, respectively, in this study. The literature, however, reports significantly stronger affinities, with values as high as -23.7 kcal/mol for *Tb*DHFR and -24.4 kcal/mol for *Tb*PTR1¹⁹⁷. In a similar study by Setzer et al., stigmasterol showed binding affinity of -24.1 kcal/mol and -23.5 kcal/mol against *Tb*DHFR and *Tb*PTR1 respectively, compared to this study (-9.7 kcal/mol for *Tb*DHFR and -9.9 kcal/mol for *Tb*PTR1). While the experimental results presented here generally align with literature values, several discrepancies were noted, particularly in the binding affinities of lupeol and β -sitosterol. This discrepancy may be attributed to differences in experimental conditions or computational approaches used for the docking studies, highlighting the importance of considering these elements in computational studies. These factors are confirmed in the subsequent paragraph.

The results in this study were compared to a similar study by Setzer et al. According to the compared literature, Setzer et al. performed docking using Molegro Virtual Docker with *Tb*PTR1 (PDB ID: 3JQ7) and *Tb*DHFR (PDB IDs: 3RG9 and 3QFX)¹⁹⁷. In contrast, this study utilized AutoDock Vina with *Tb*PTR1 (PDB ID: 6RX6) and *Tb*DHFR (PDB ID: 3QFX). Furthermore, the method used by Setzer et al. employs a rigid protein model with flexible ligands and does not include protein energy minimization. Instead, it focuses on repetitive docking with different ligand orientations¹⁹⁷. Conversely, the method used in this study optimizes the energy of both the protein and ligands for greater thermodynamic stability, employs predefined grid boxes to identify binding sites, and evaluates ligand poses based on binding energy. Another point to consider is that the literature emphasizes flexibility in ligand docking¹⁹⁷, whereas the method in this study offers a more refined and energetically stable docking process. In summary, the two docking methods differ in their approaches to protein preparation, ligand flexibility, and docking algorithms.

The stronger affinities reported in the literature might suggest that certain ligands have the potential for higher binding efficacy than observed in the current study. However, the lack of comprehensive literature on lupeol acetate binding to *TbPTR1* and *TbDHFR* limits the ability to compare these results directly.



5

CHAPTER FIVE

5.1 CONCLUSION

This study marks the first comprehensive phytochemical and biological investigation of *Talbotiella gentii*, a critically endangered plant species with notable ethnomedicinal relevance. The isolation of triterpenoids and sterols from the dichloromethane extract, together with the semi-synthesis of lupeol acetate, provides valuable insight into the chemical profile of this underexplored species. *In vitro* antitrypanosomal assays confirmed measurable bioactivity of the isolates, with lupeol acetate showing the highest potency, followed by lupeol and the sterol mixture. Molecular docking studies further revealed strong binding affinities of these compounds toward key *Trypanosoma brucei* enzymes—dihydrofolate reductase (*TbDHFR*) and pteridine reductase 1 (*TbPTR1*)—suggesting potential inhibitory effects on essential parasitic pathways.

These findings lend preliminary scientific support to the traditional use of *T. gentii* in managing stomach ulcer, which may involve microbial infections. Although trypanosomiasis is caused by a different pathogen, the observed antitrypanosomal activity indicates a broader antimicrobial potential that could contribute to the plant's traditional applications. While the isolated compounds are known triterpenoids and sterols commonly distributed within the Fabaceae family, this study provides the first baseline phytochemical and biological data for *T. gentii*, offering an understanding of its chemotaxonomy, biosynthesis, and pharmacological studies as well as setting the foundation for future studies. The results underscore the importance of continued research on Ghana's endemic flora, as even species yielding known compounds can reveal valuable insights into structure–activity relationships, ecological adaptations, and indigenous medicinal knowledge. Conservation of *T. gentii* therefore remains significant—not for chemical novelty alone, but for its

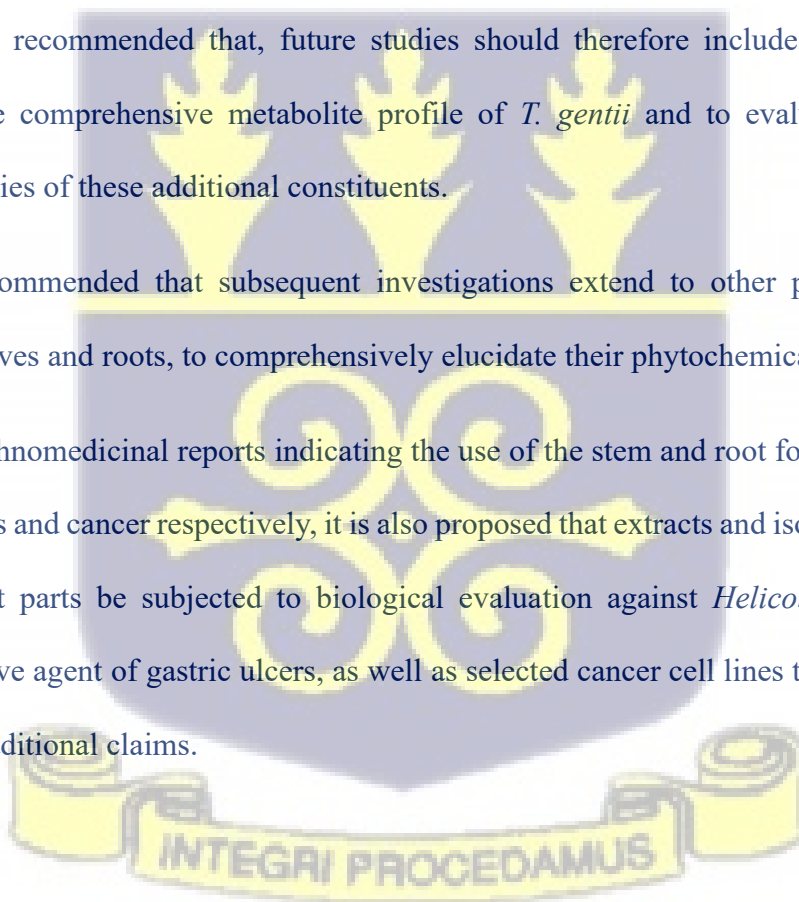
contribution to biodiversity, potential genetic variation in secondary metabolism, and the preservation of Ghana's unique ethnobotanical heritage.

5.2 RECOMMENDATIONS

While the DCM fraction yielded known triterpenoid and sterol constituents, phytochemical screening of the ethyl acetate (TSE) and methanol extracts (TSM) indicated the presence of other secondary metabolite classes in the stem that were not characterized due to scope and resource limitations. It is recommended that, future studies should therefore include these extracts to establish a more comprehensive metabolite profile of *T. gentii* and to evaluate the potential biological activities of these additional constituents.

It is further recommended that subsequent investigations extend to other parts of *T. gentii*, including the leaves and roots, to comprehensively elucidate their phytochemical profiles.

In view of the ethnomedicinal reports indicating the use of the stem and root for the management of stomach ulcers and cancer respectively, it is also proposed that extracts and isolated constituents from these plant parts be subjected to biological evaluation against *Helicobacter pylori*, the principal causative agent of gastric ulcers, as well as selected cancer cell lines to substantiate and validate these traditional claims.



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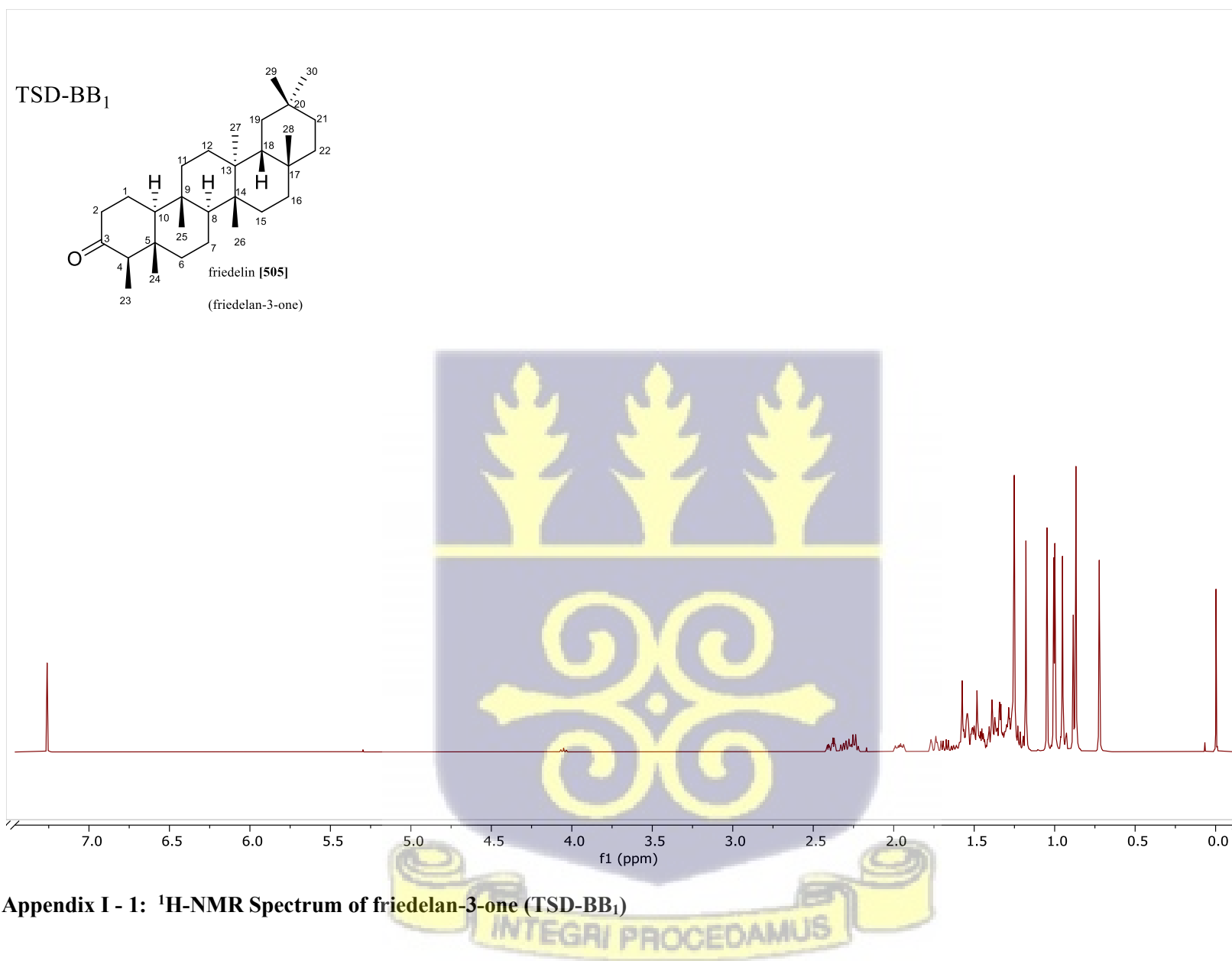


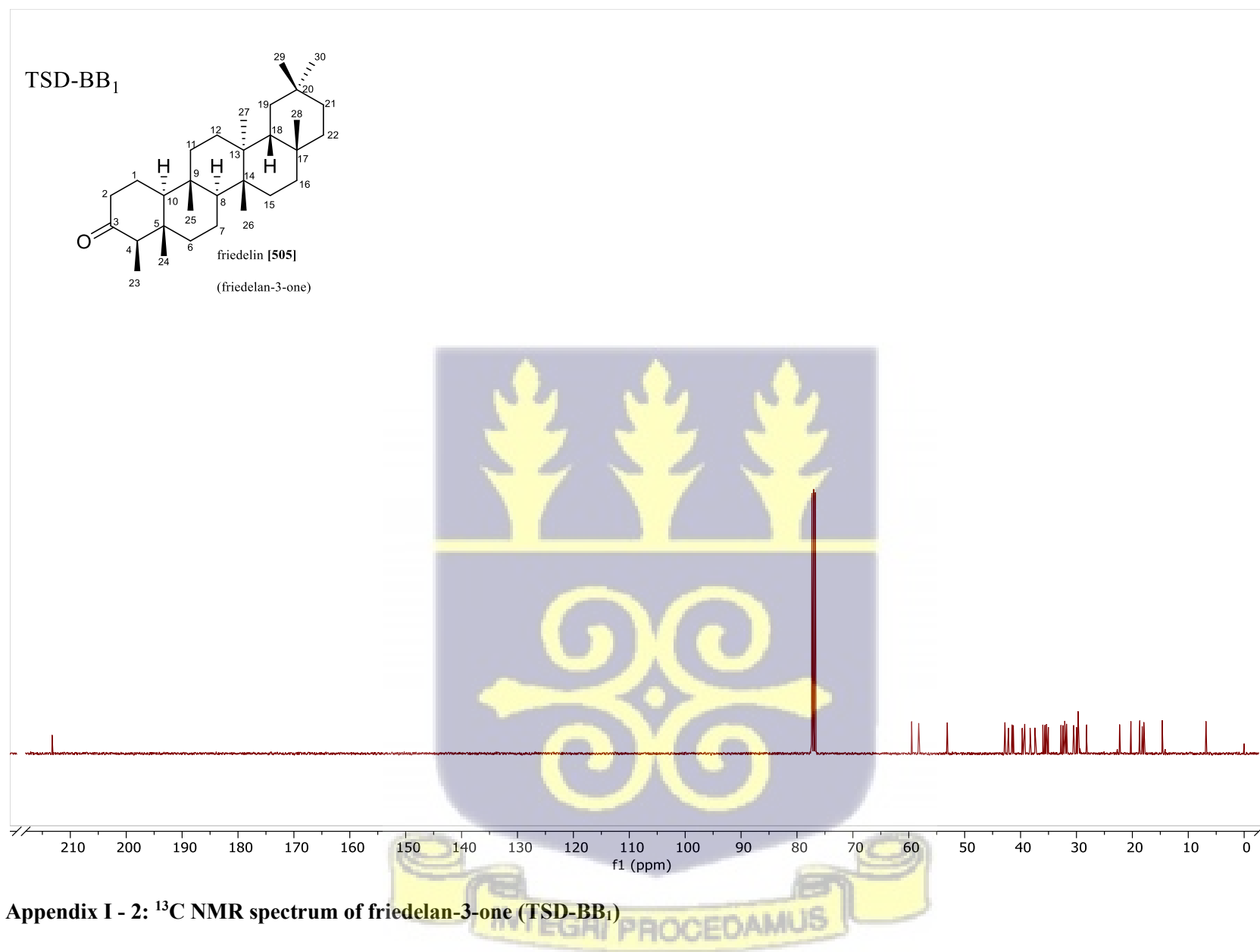
APPENDIX I: NMR SPECTRA

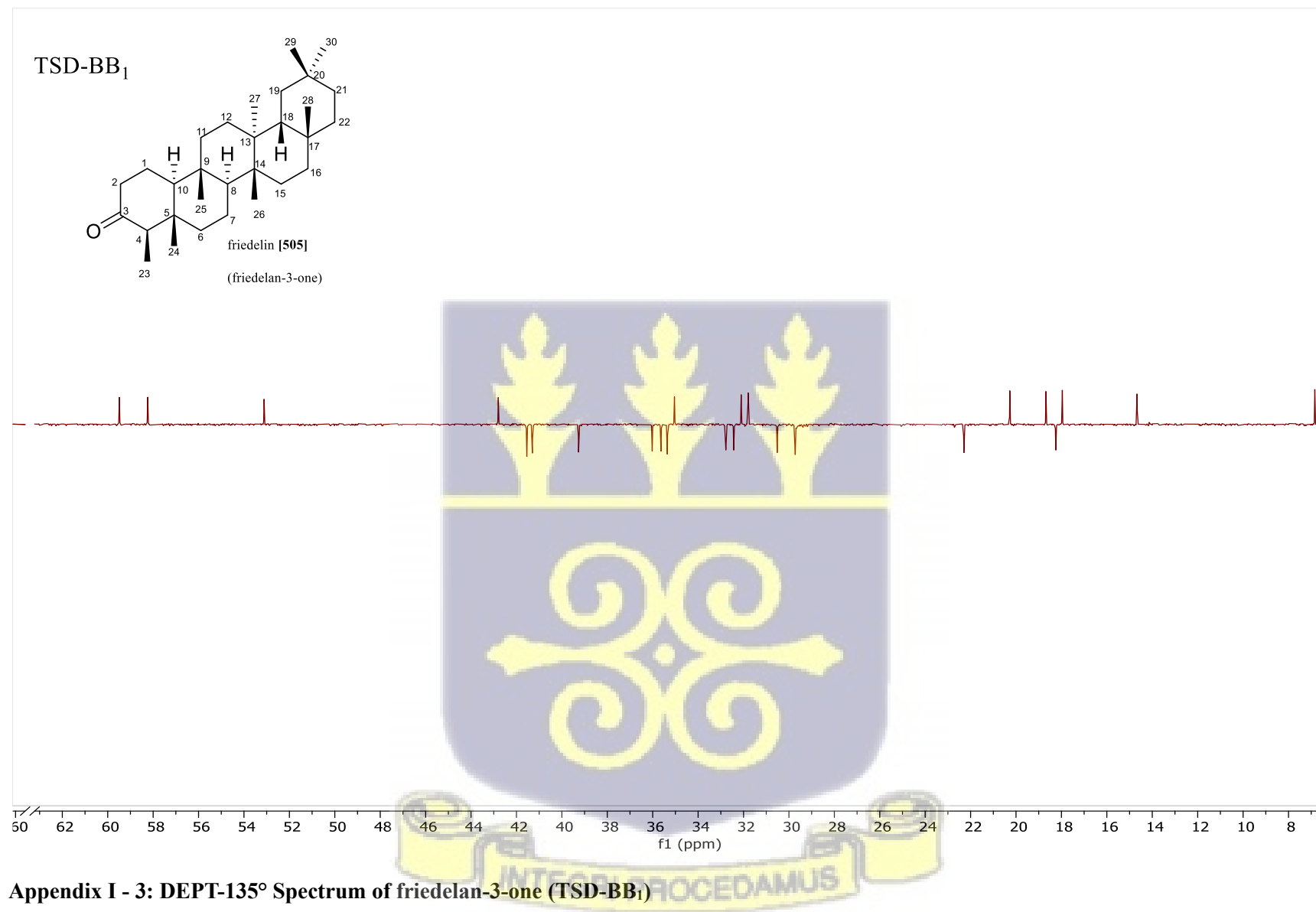
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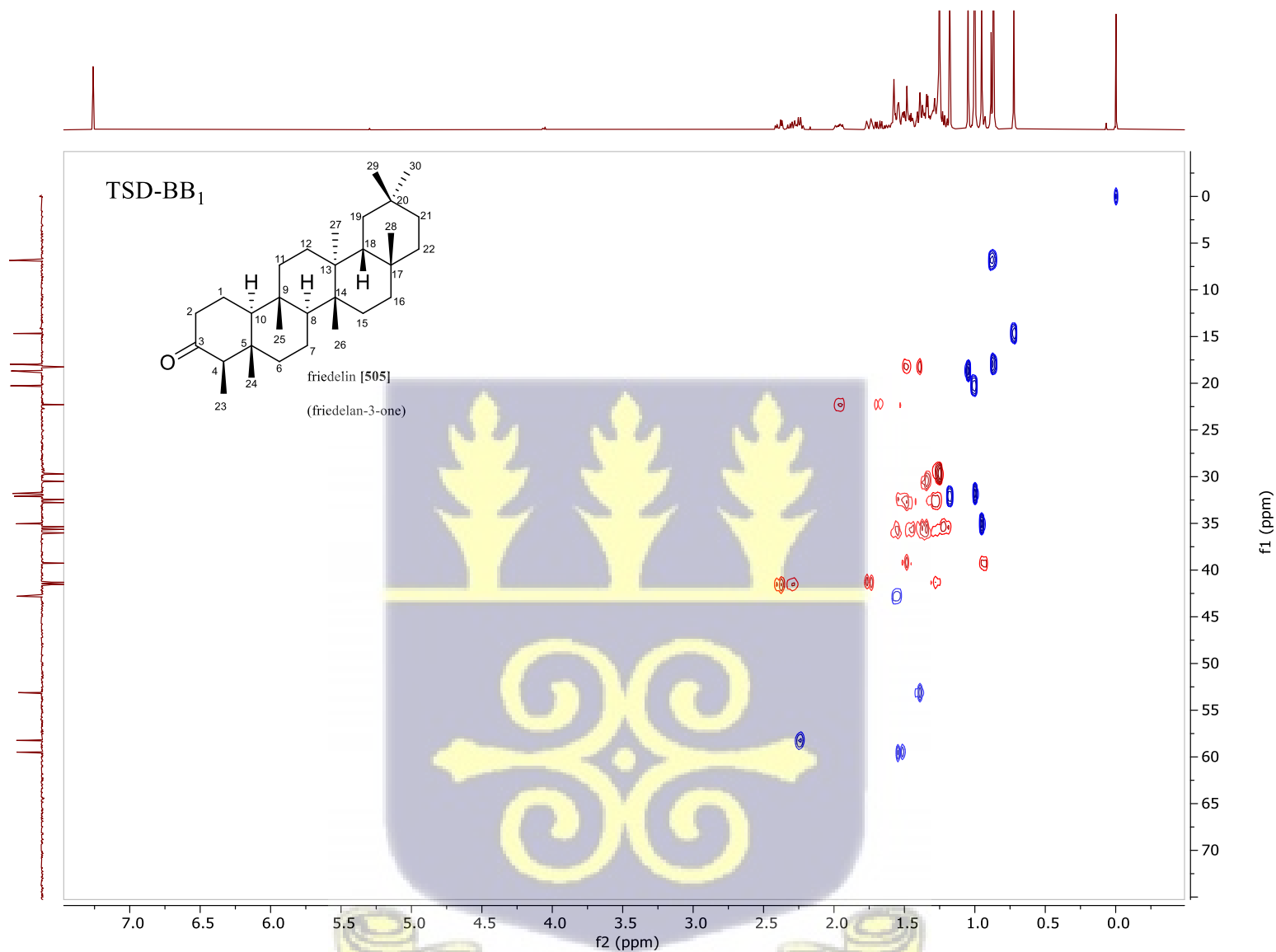
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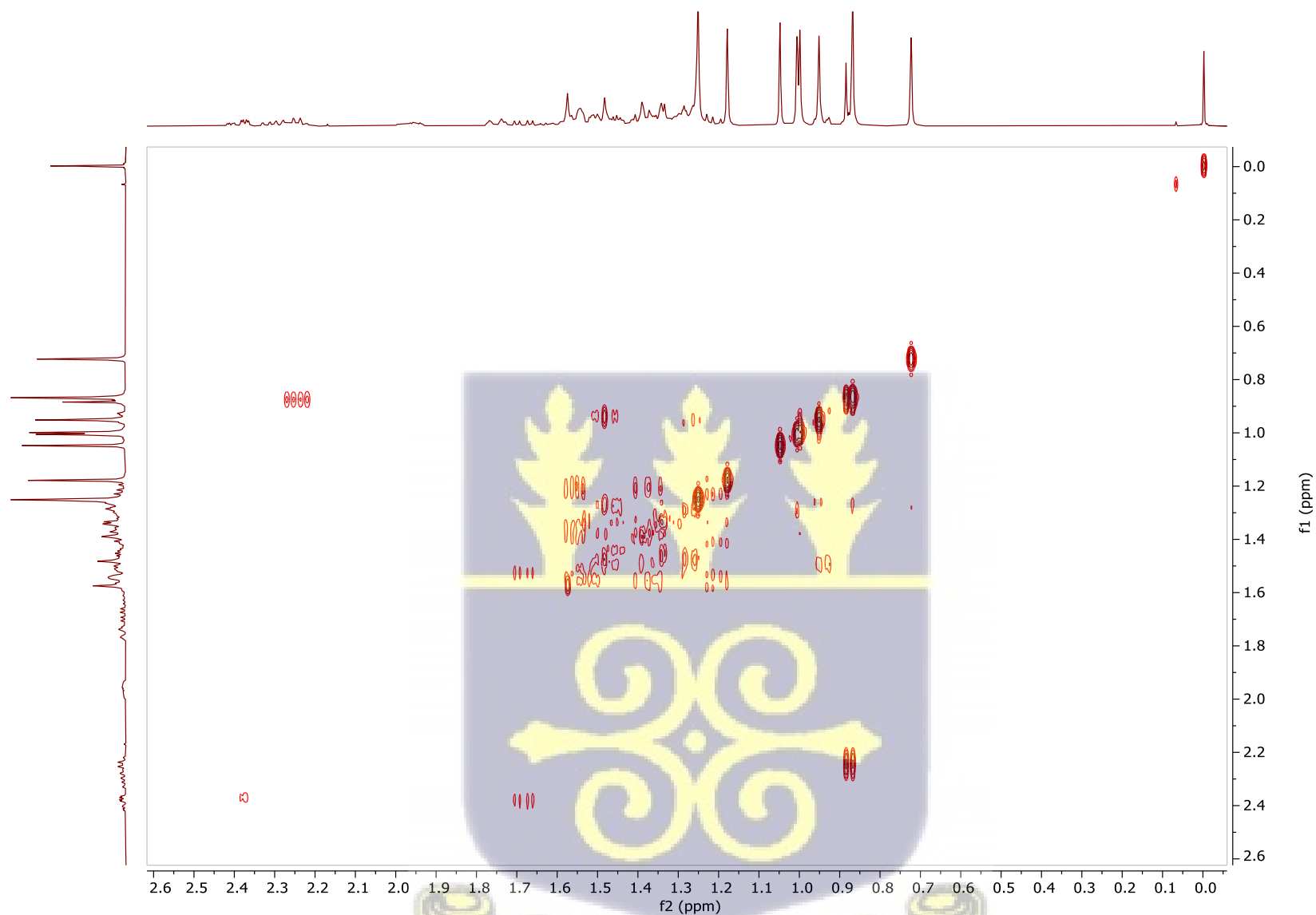




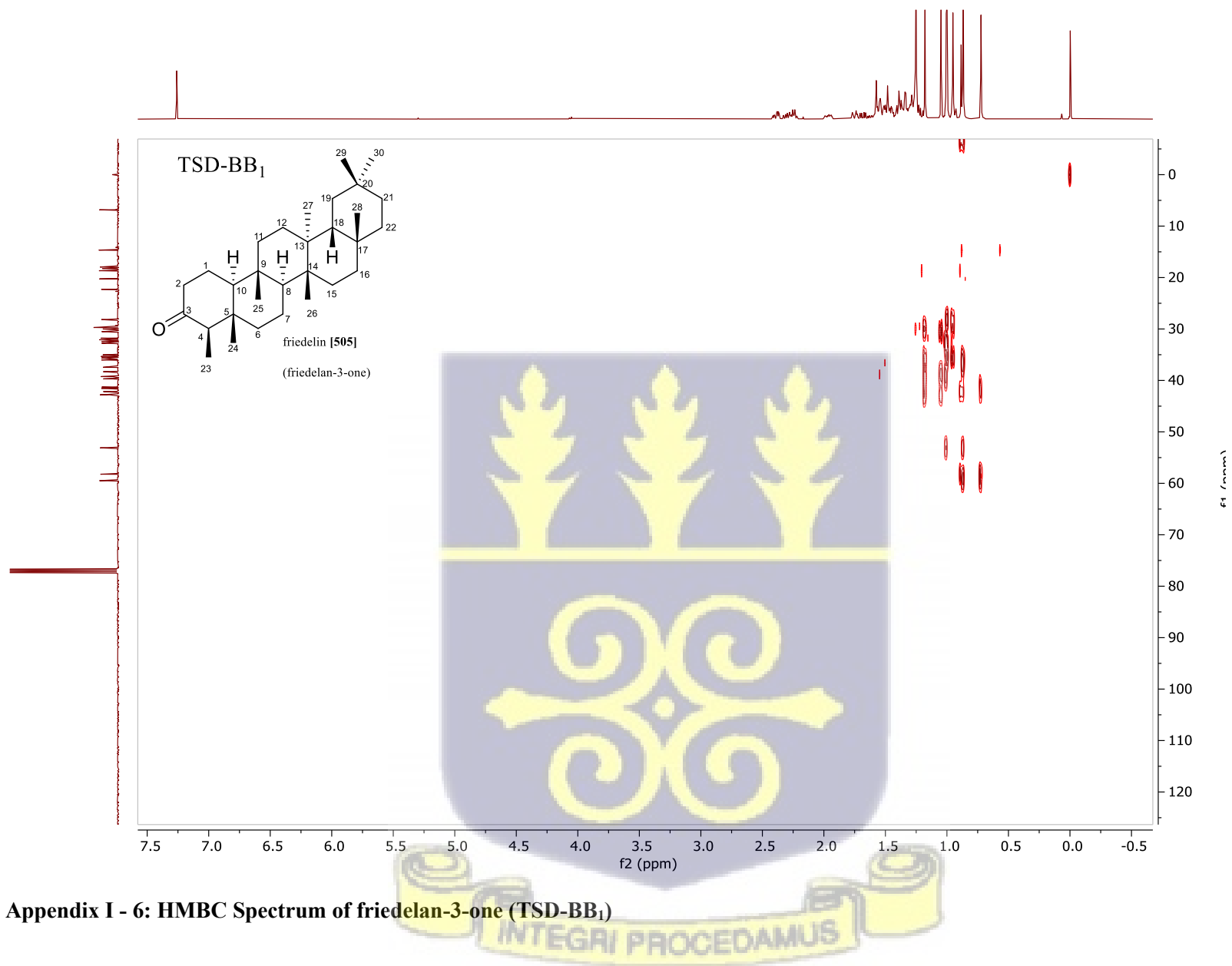




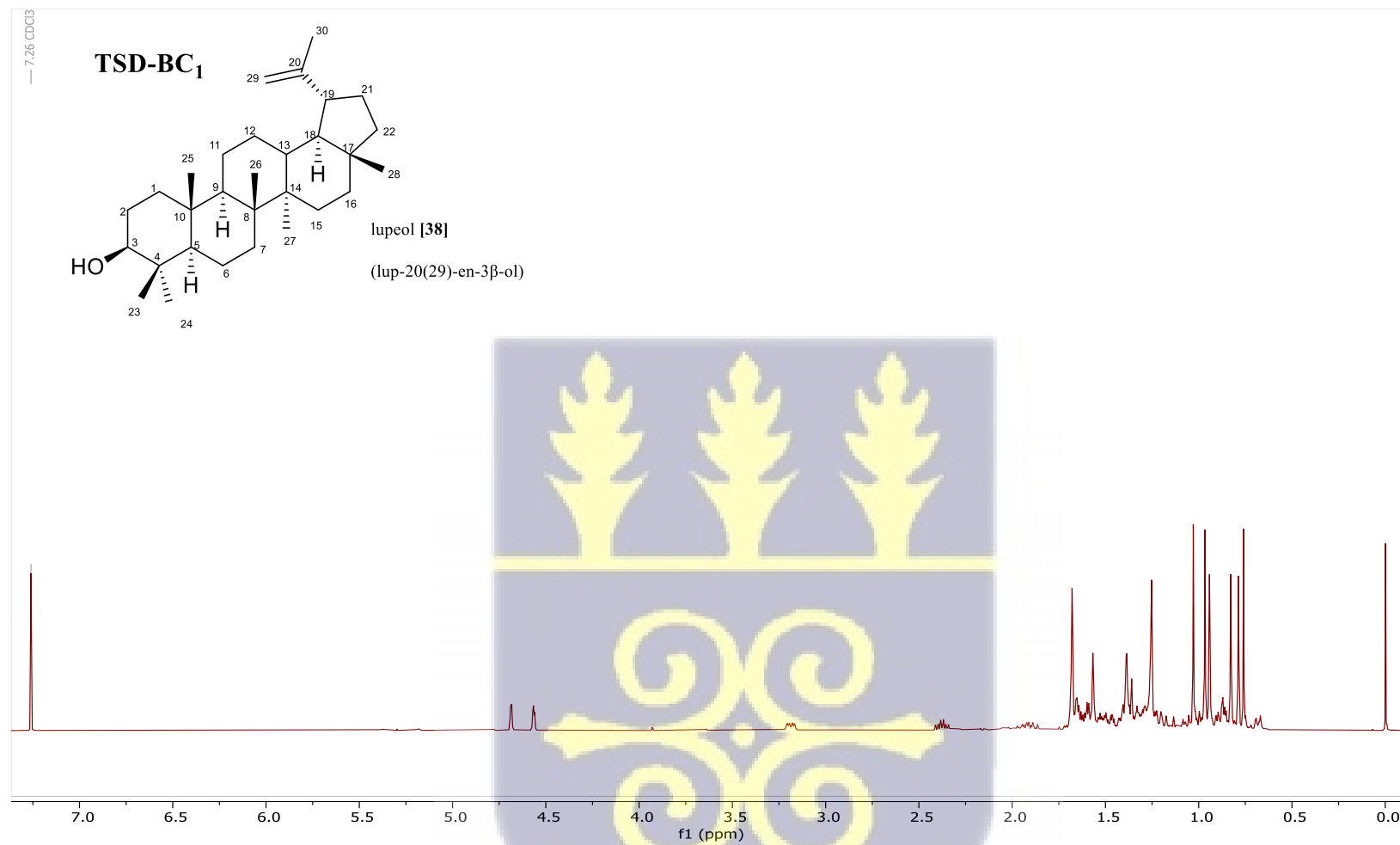
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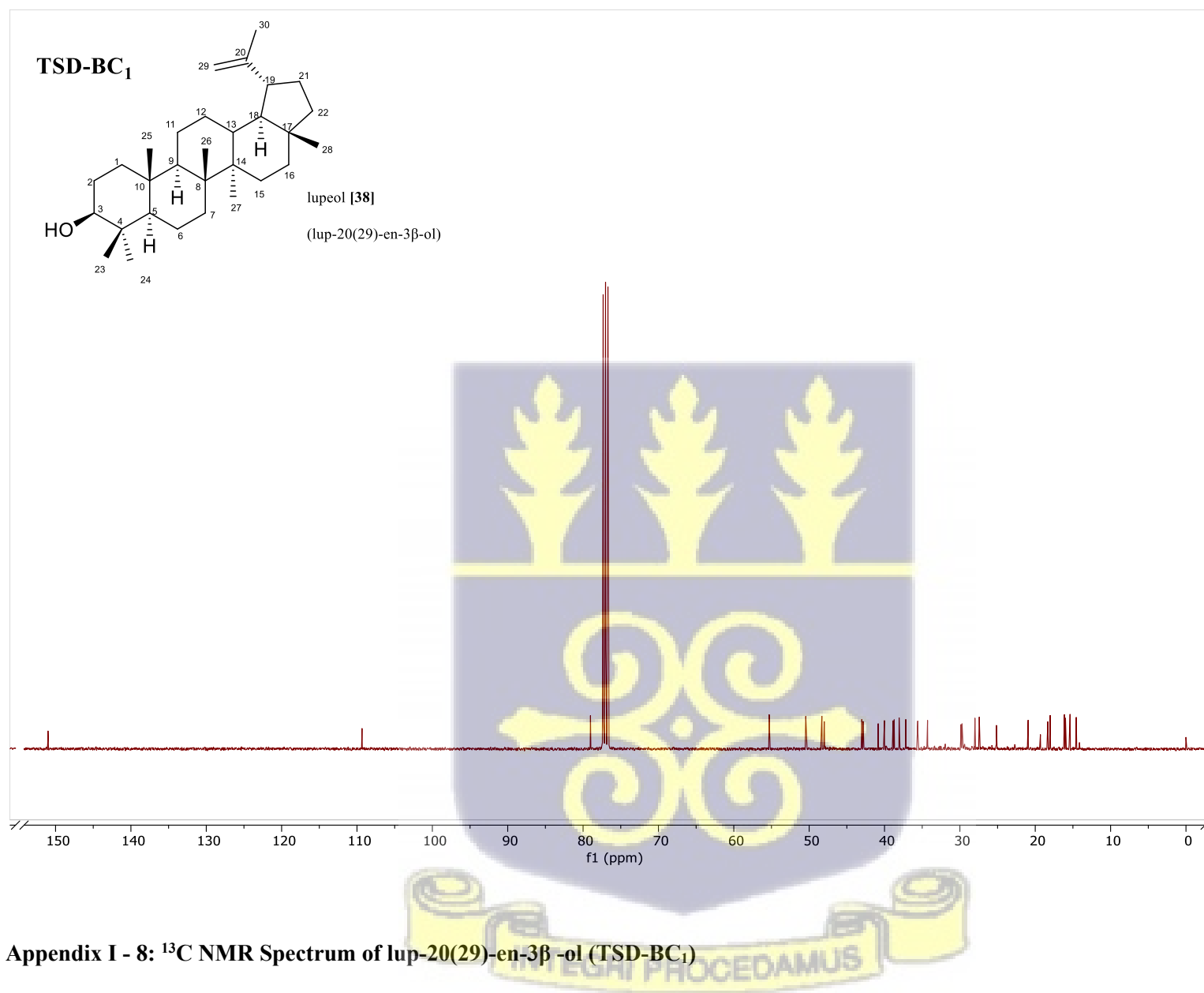
Appendix I - 5: ^1H - ^1H COSY Spectrum of friedelan-3-one (TSD-BB₁)

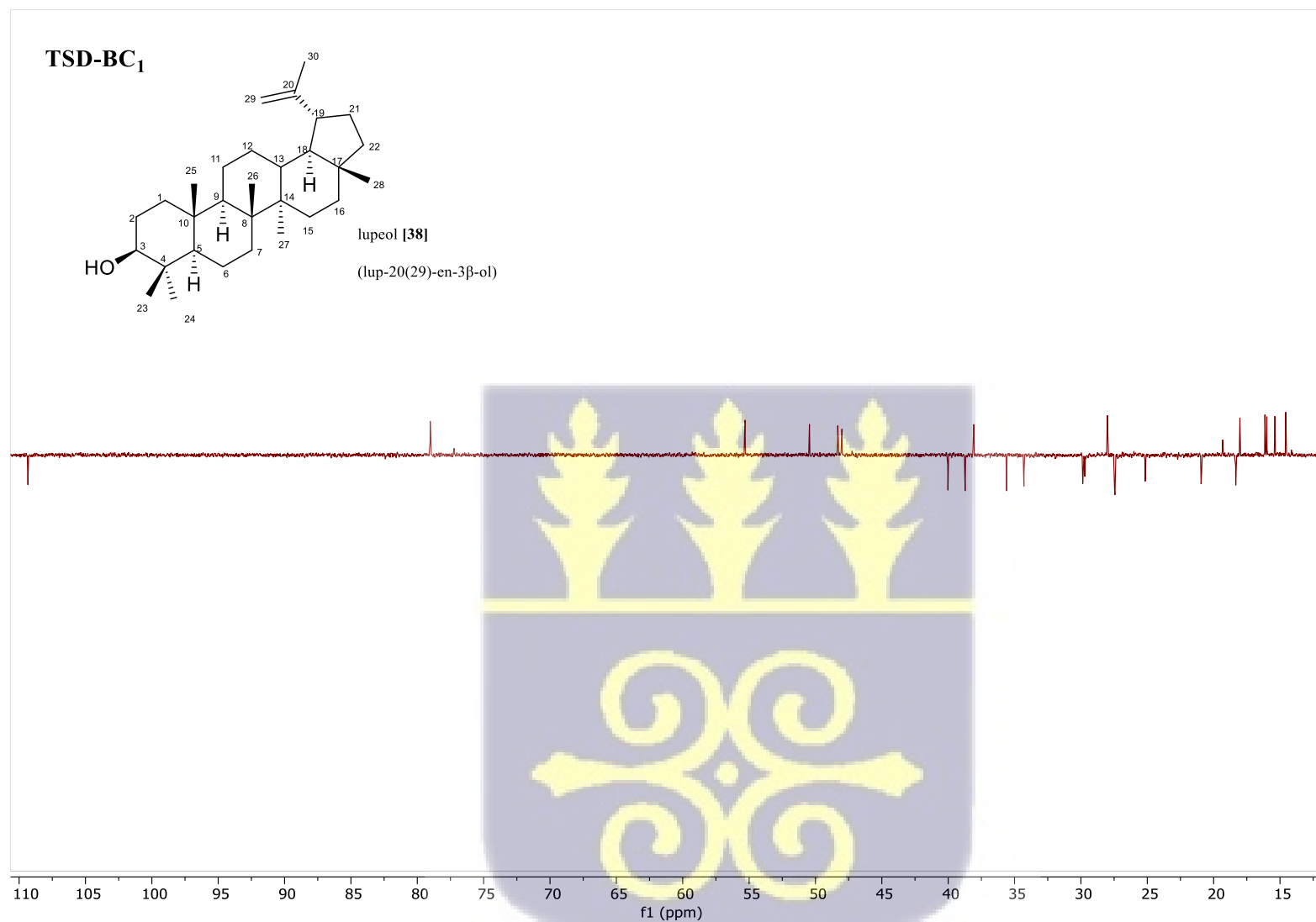


Appendix I - 6: HMBC Spectrum of friedelan-3-one (TSD-BB₁)

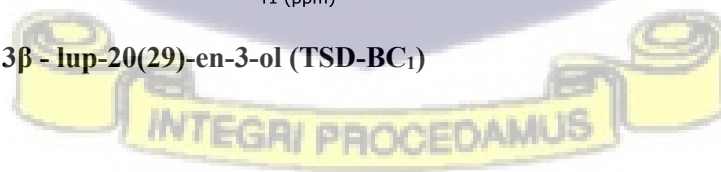


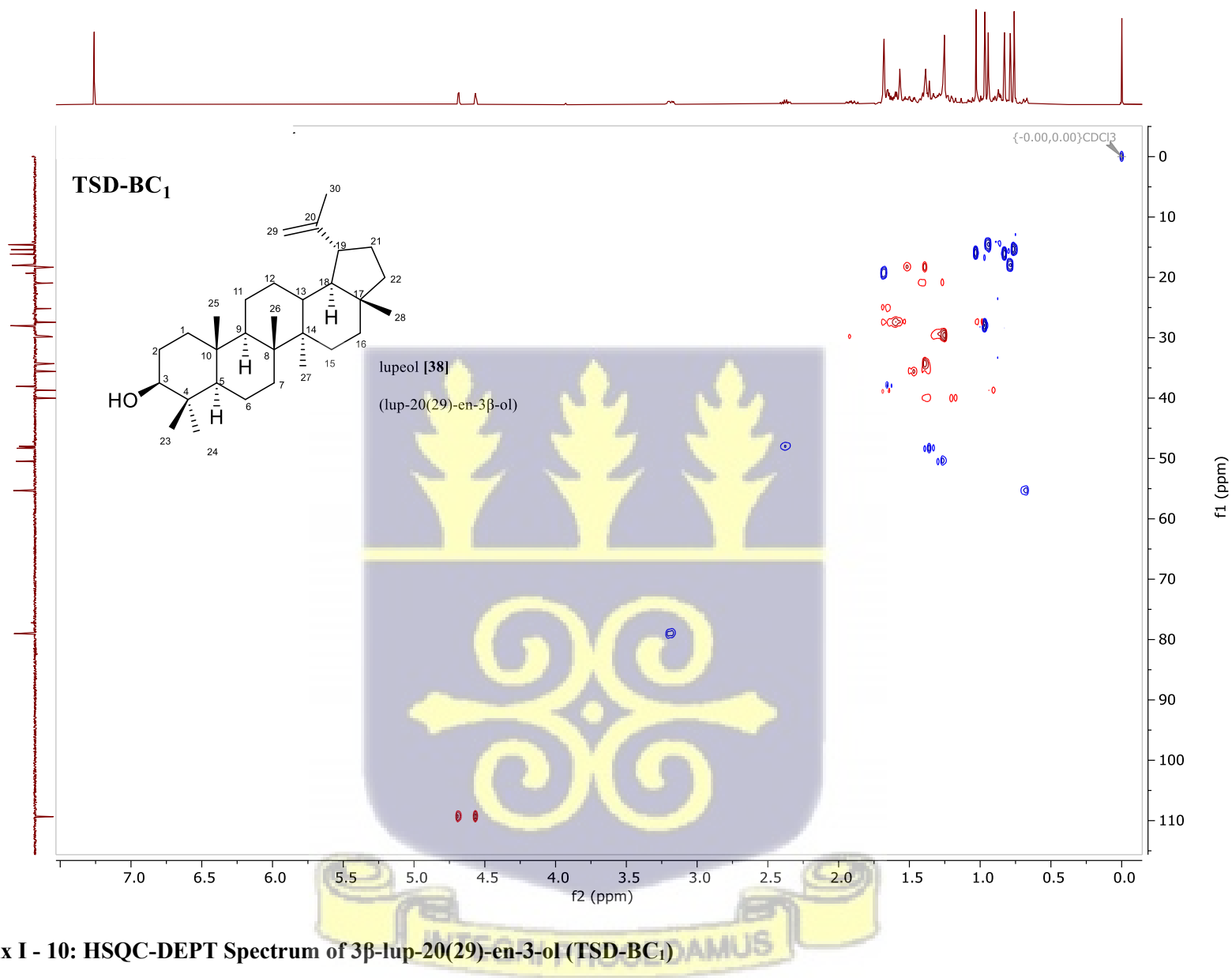
Appendix I - 7: ¹H NMR Spectrum of lup-20(29)-en-3 β -ol (TSD-BC₁)



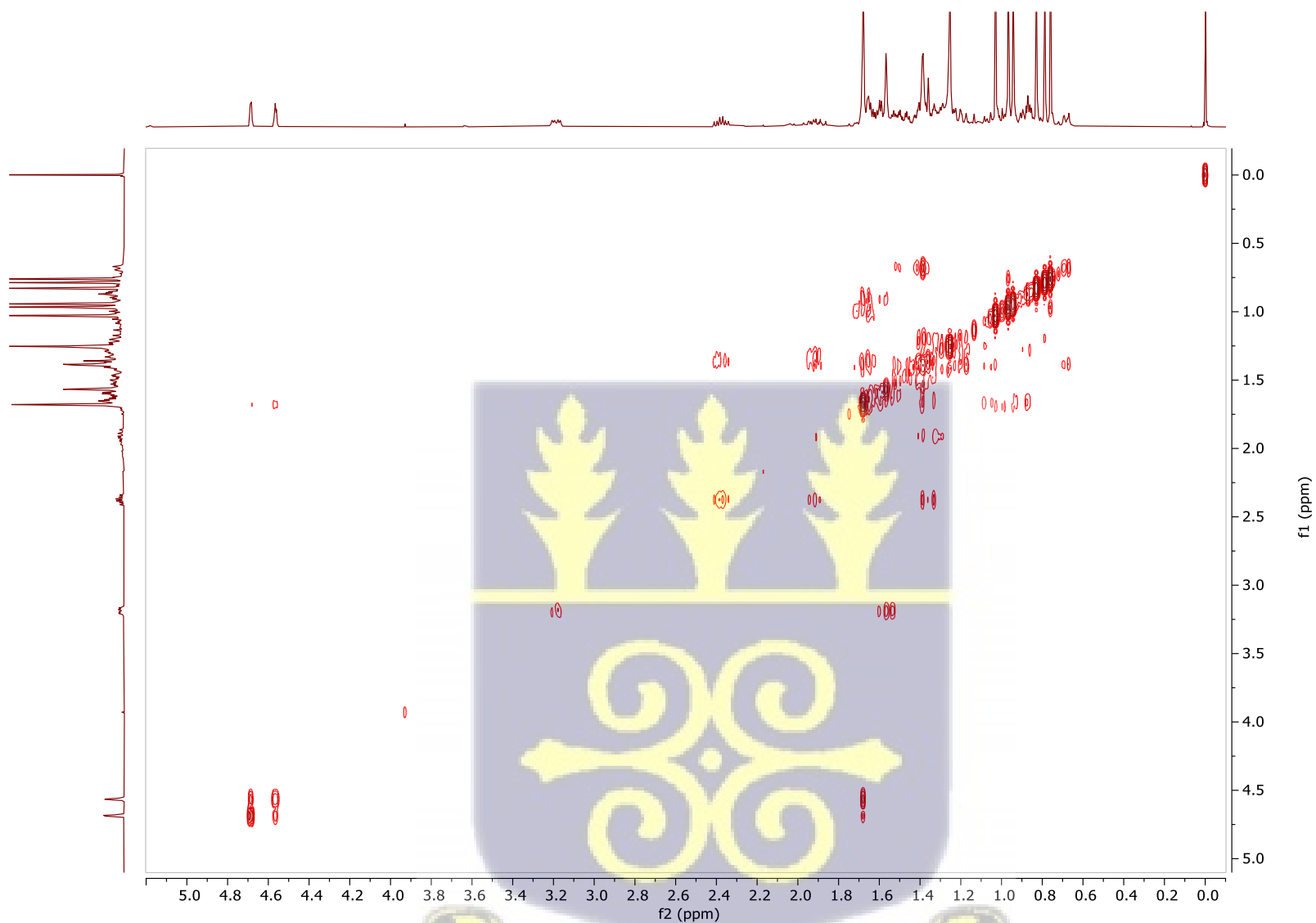


Appendix I - 9: DEPT-135 Spectrum of 3 β - lup-20(29)-en-3-ol (TSD-BC₁)

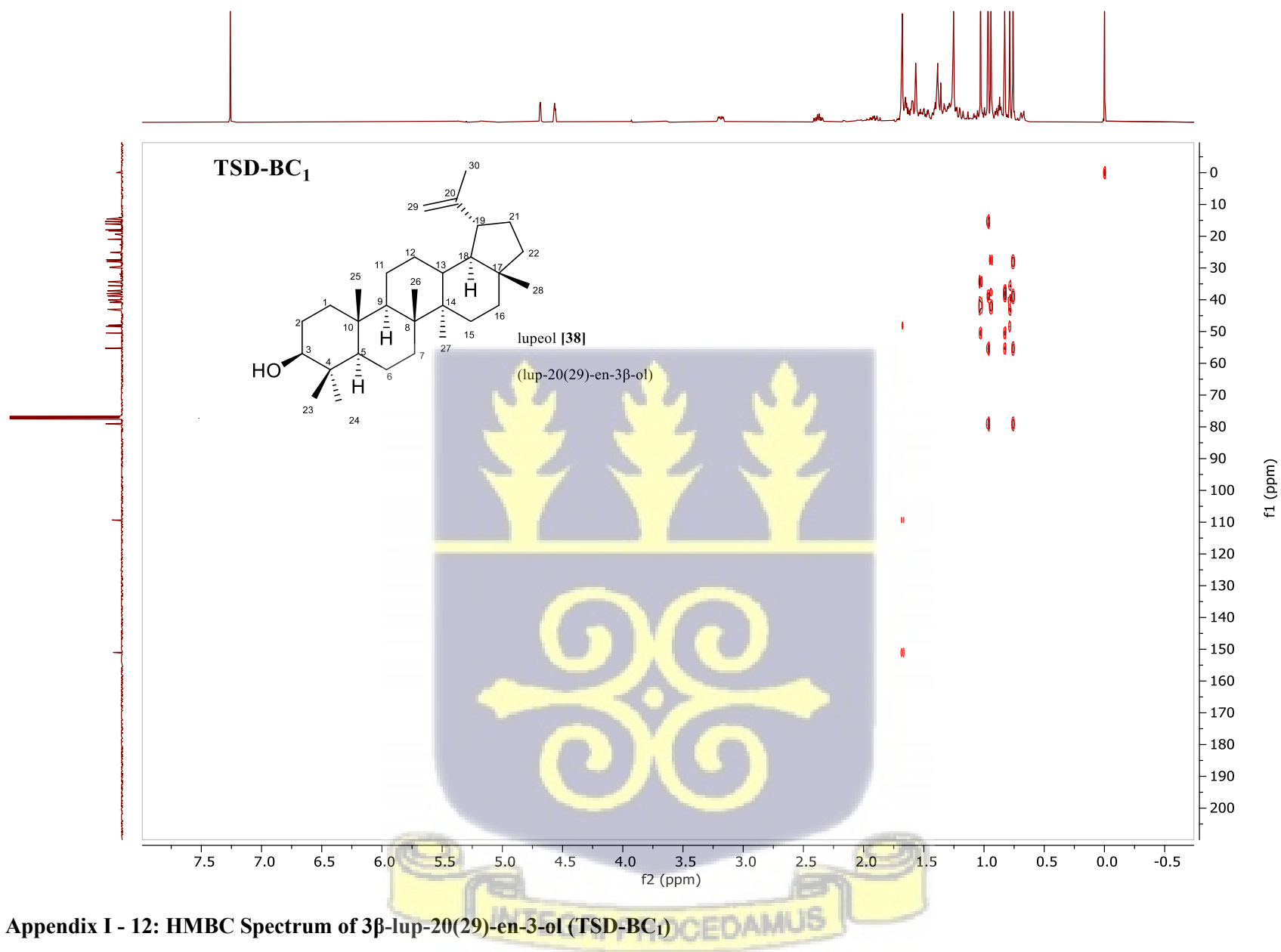




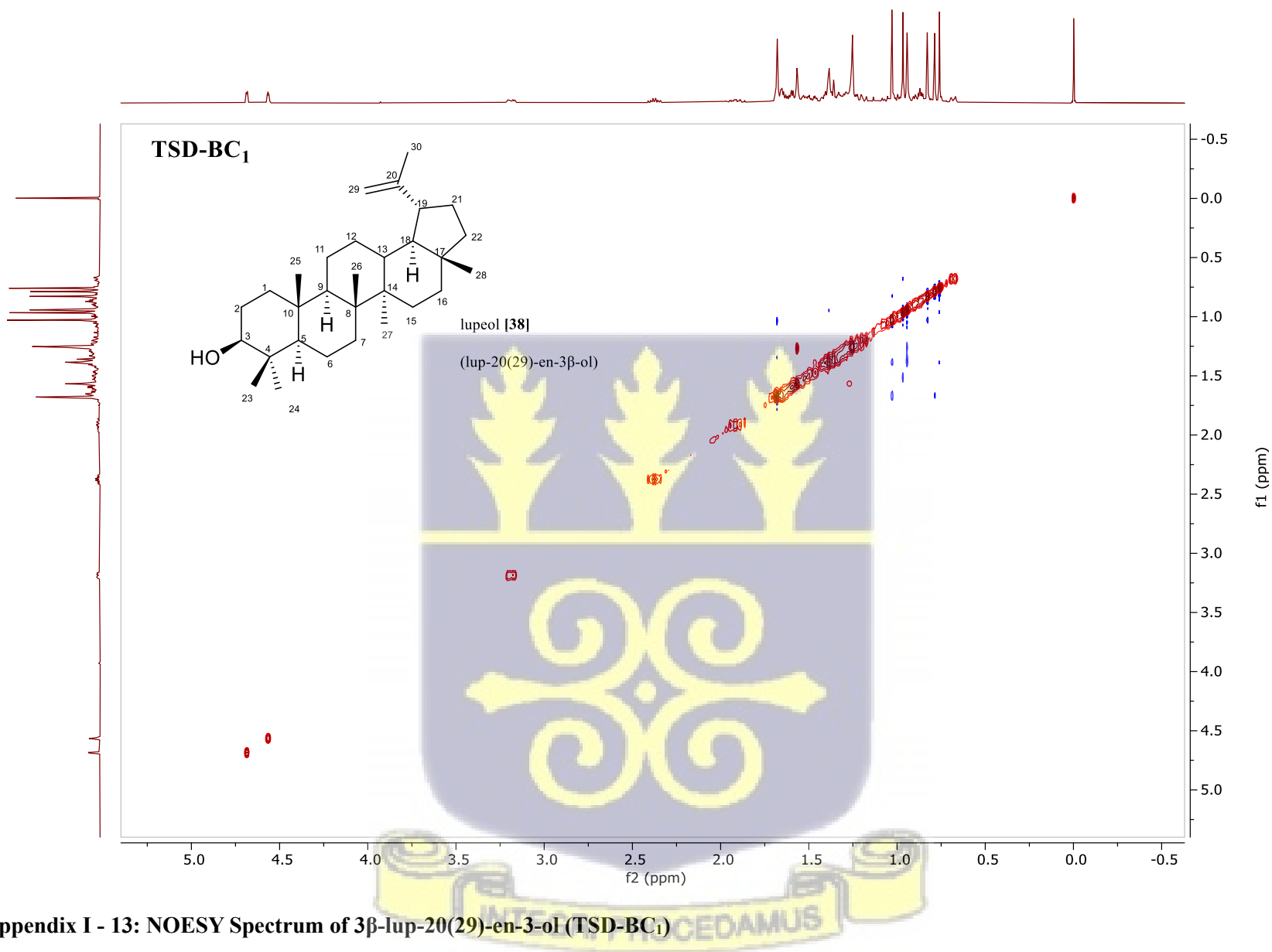
Appendix I - 10: HSQC-DEPT Spectrum of 3 β -lup-20(29)-en-3-ol (TSD-BC₁)

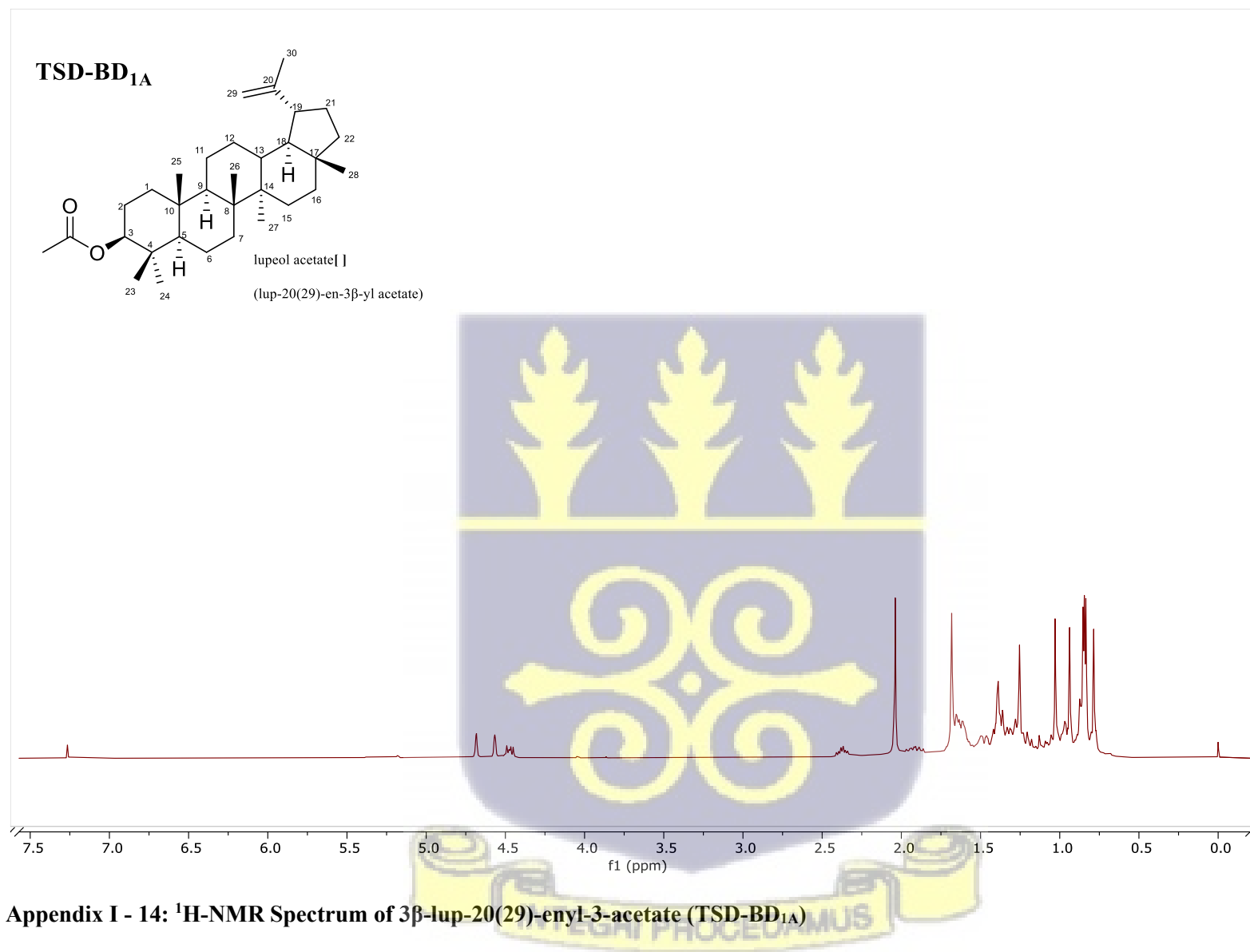


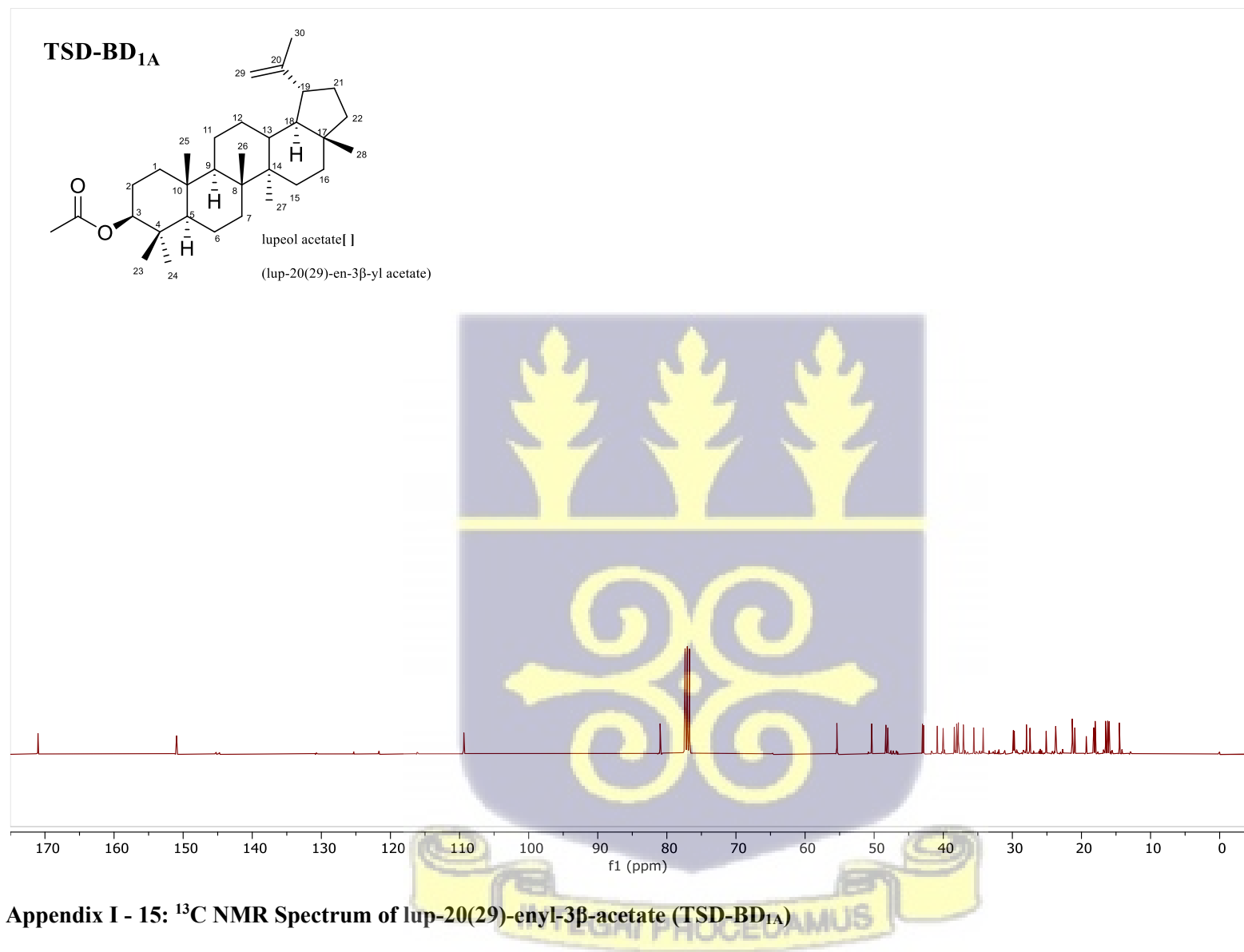
Appendix I - 11: ^1H - ^1H COSY Spectrum of 3β -lup-20(29)-en-3-ol (TSD-BC₁)

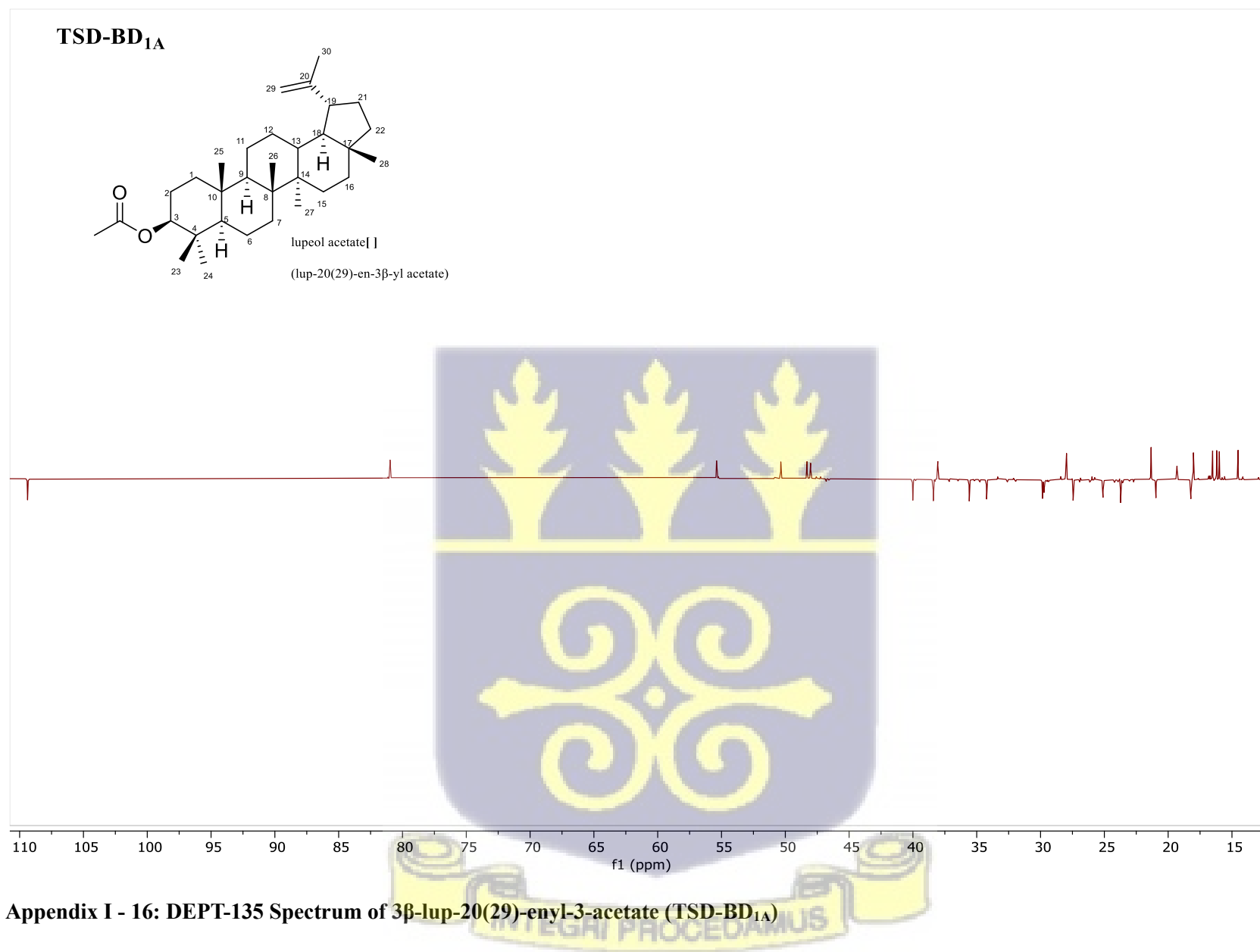


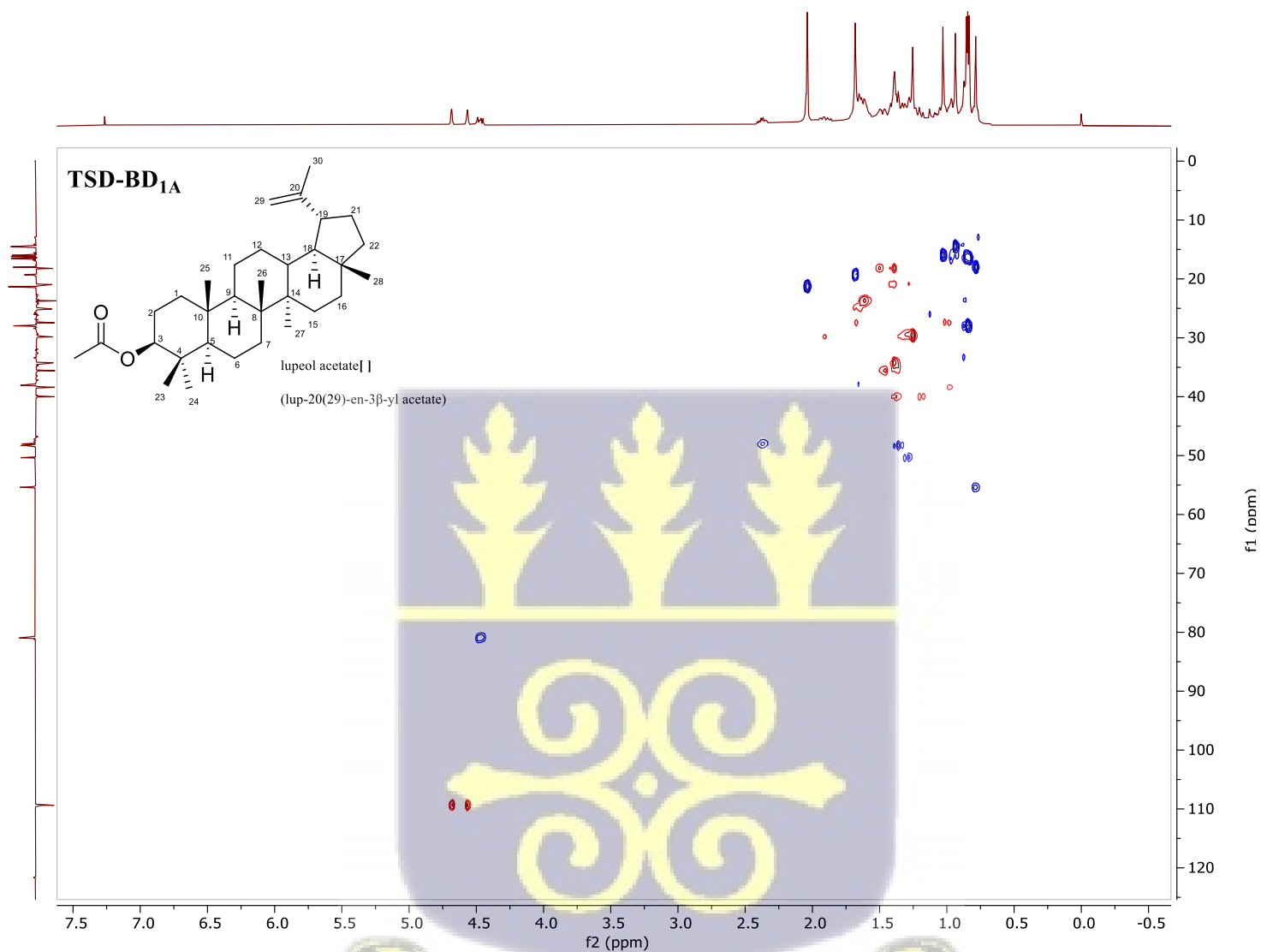
Appendix I - 12: HMBC Spectrum of 3 β -lup-20(29)-en-3 β -ol (TSD-BC₁)



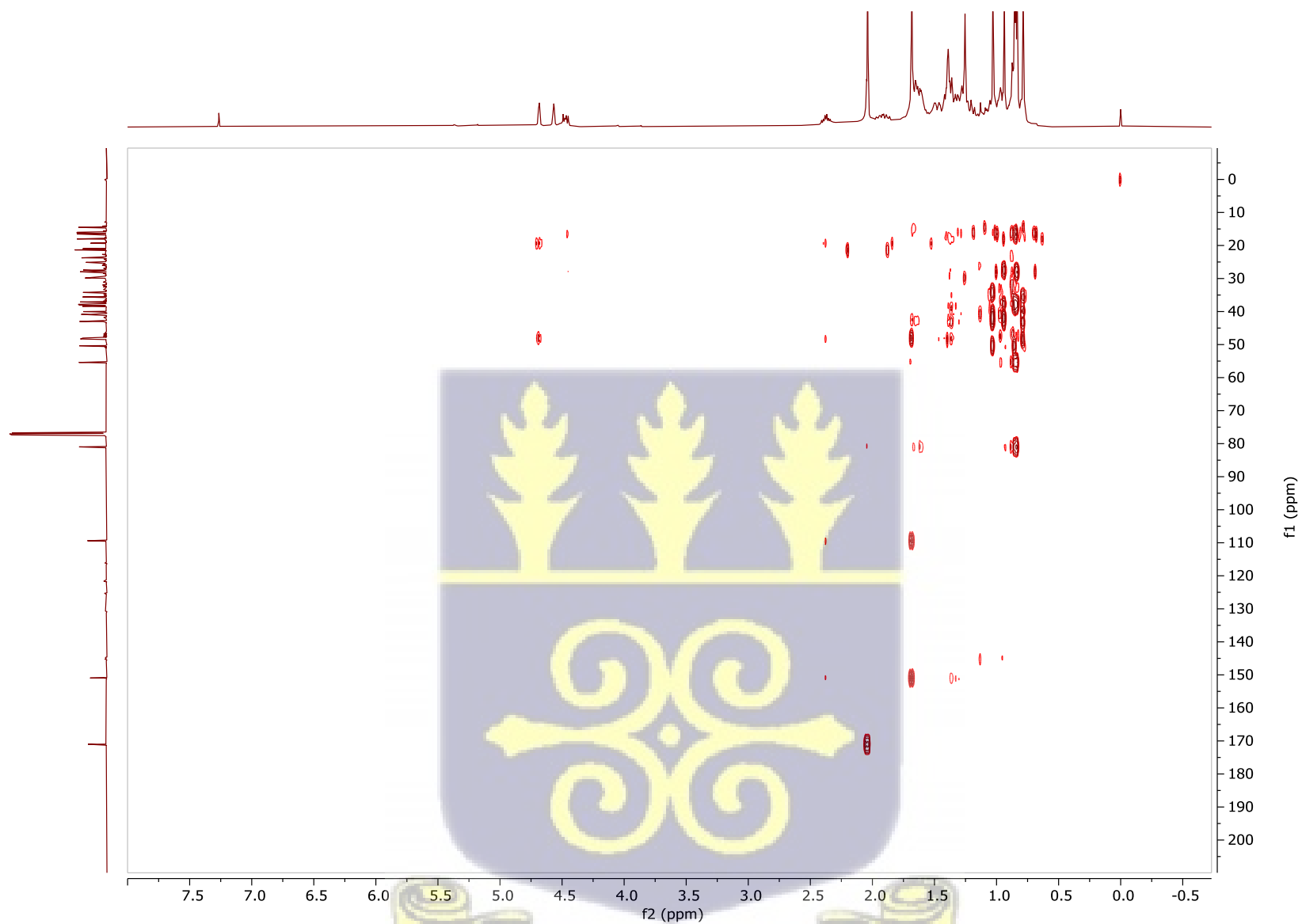




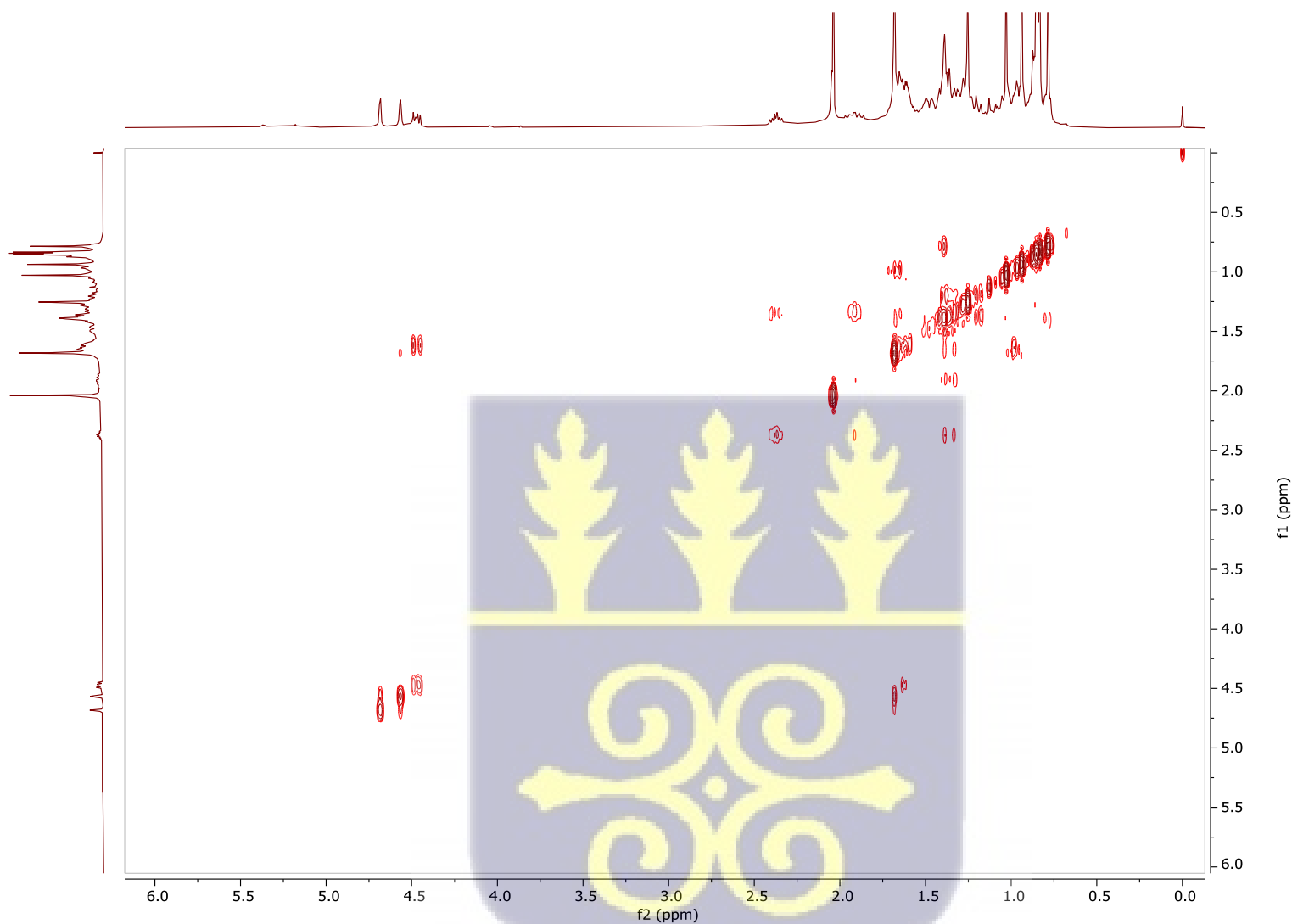




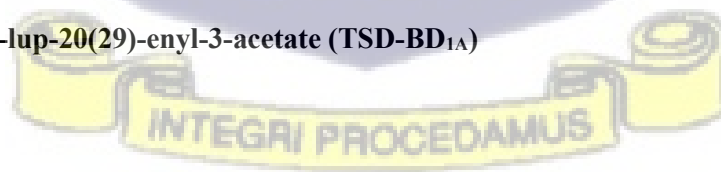
Appendix I - 17: HSQC-DEPT Spectrum of 3β-lup-20(29)-enyl-3-acetate (TSD-BD_{1A})



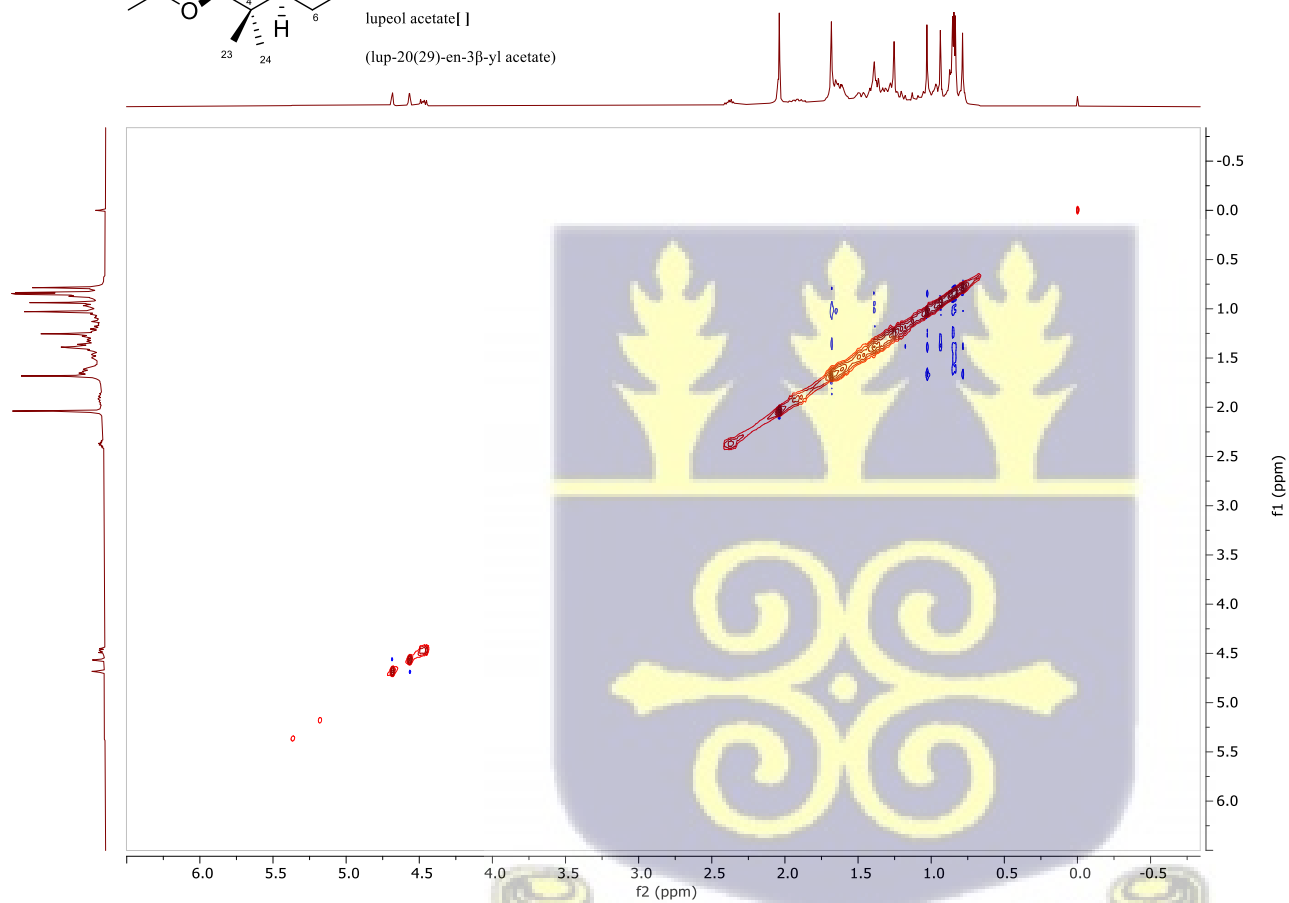
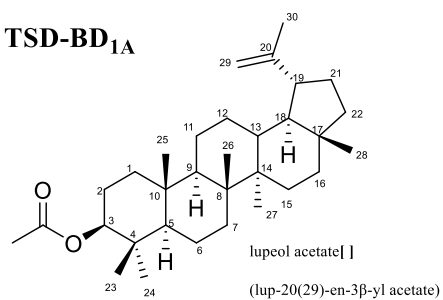
Appendix I - 18: HMBC Spectrum of 3 β -lup-20(29)-enyl-3-acetate (TSD-BD_{1A})



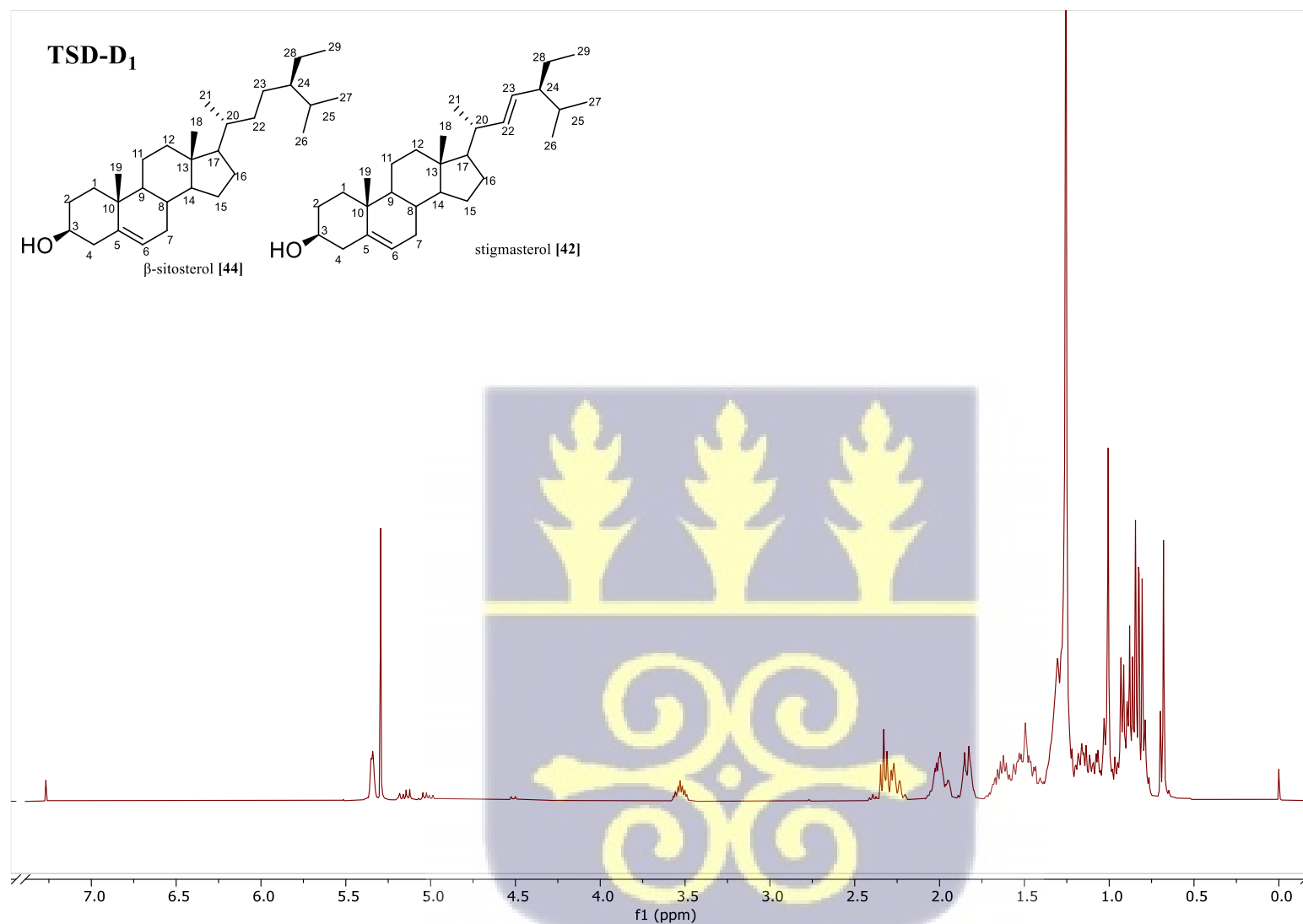
Appendix I - 19: COSY Spectrum of 3 β -lup-20(29)-enyl-3-acetate (TSD-BD_{1A})



TSD-BD_{1A}

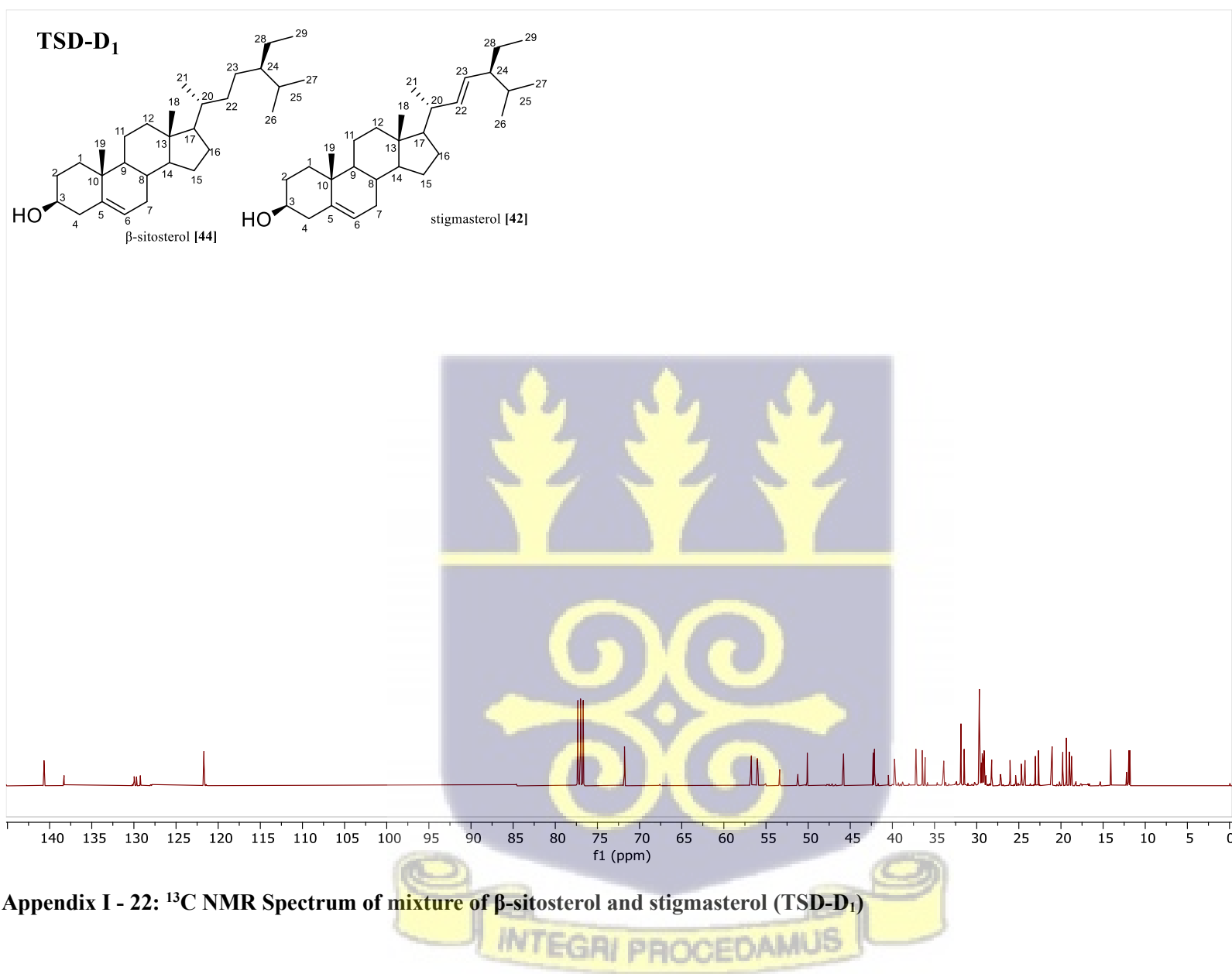


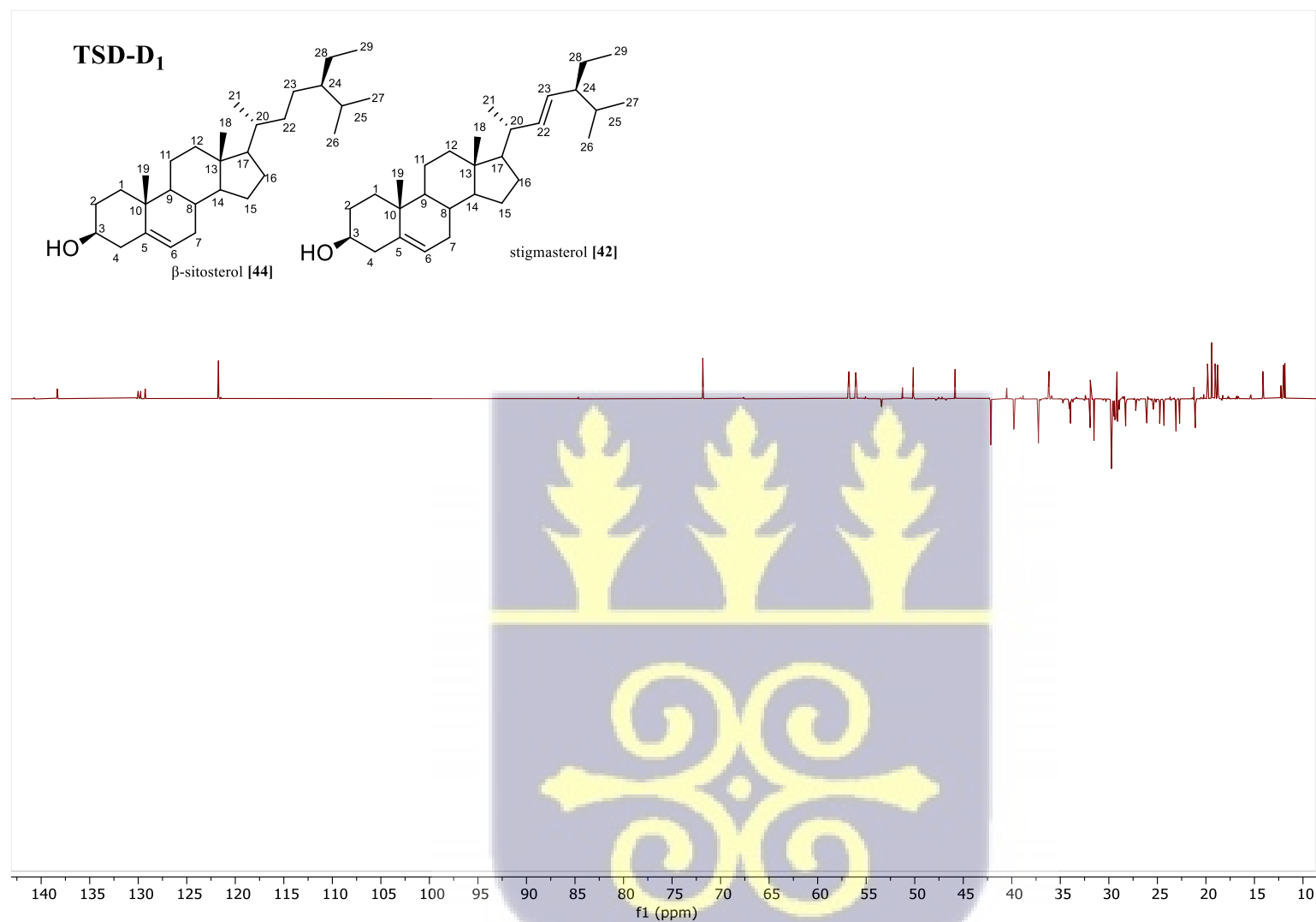
Appendix I - 20: NOESY Spectrum of 3β-lup-20(29)-enyl-3-acetate (TSD-BD_{1A})



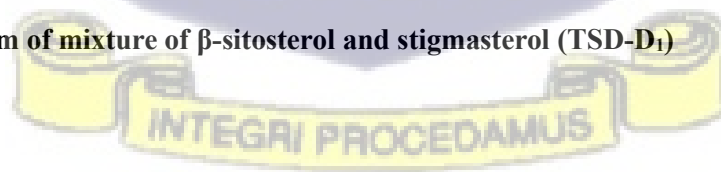
Appendix I - 21: ^1H -NMR Spectrum of mixture of β -sitosterol and stigmasterol (TSD-D₁)



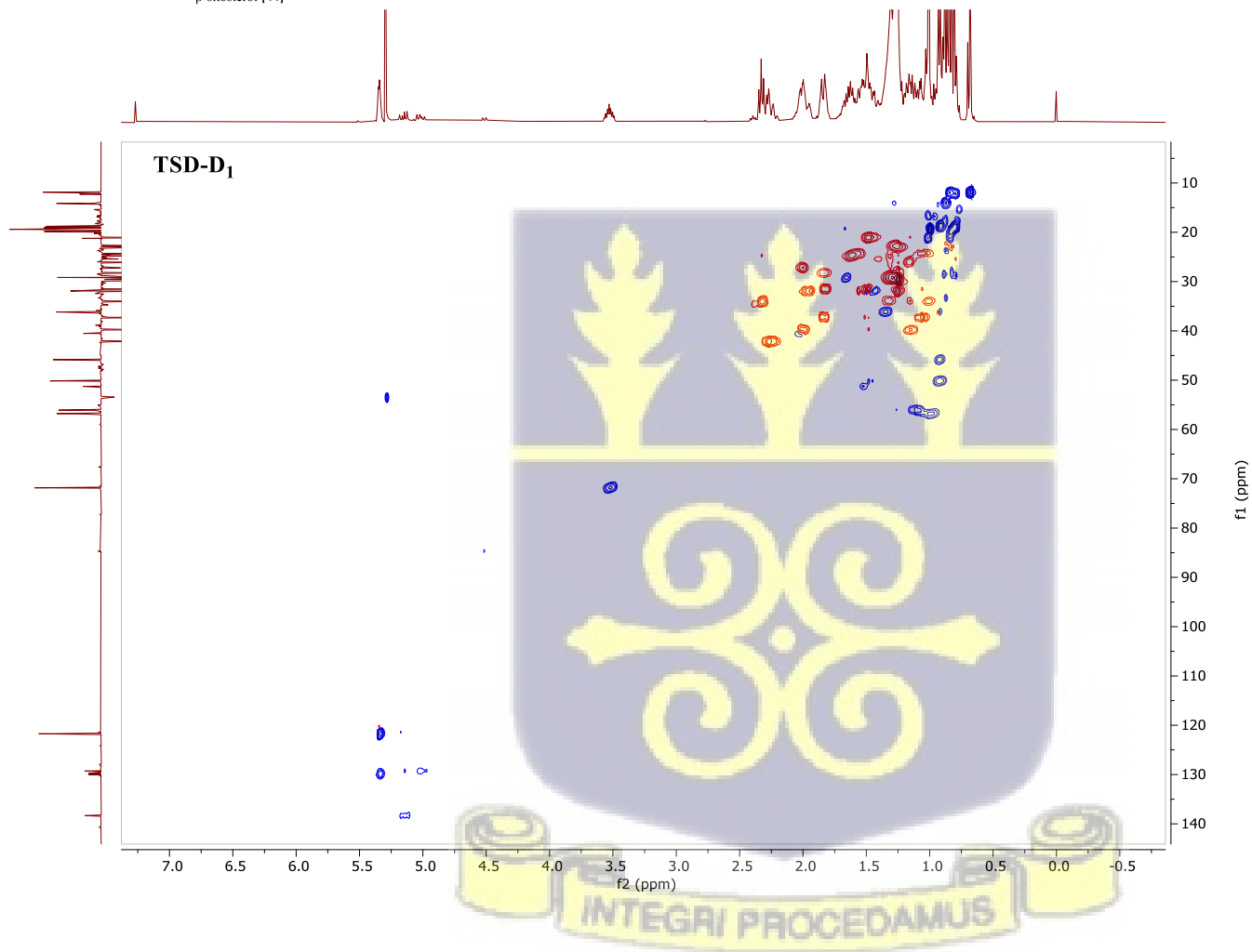
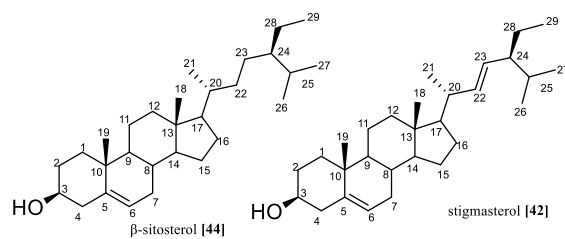




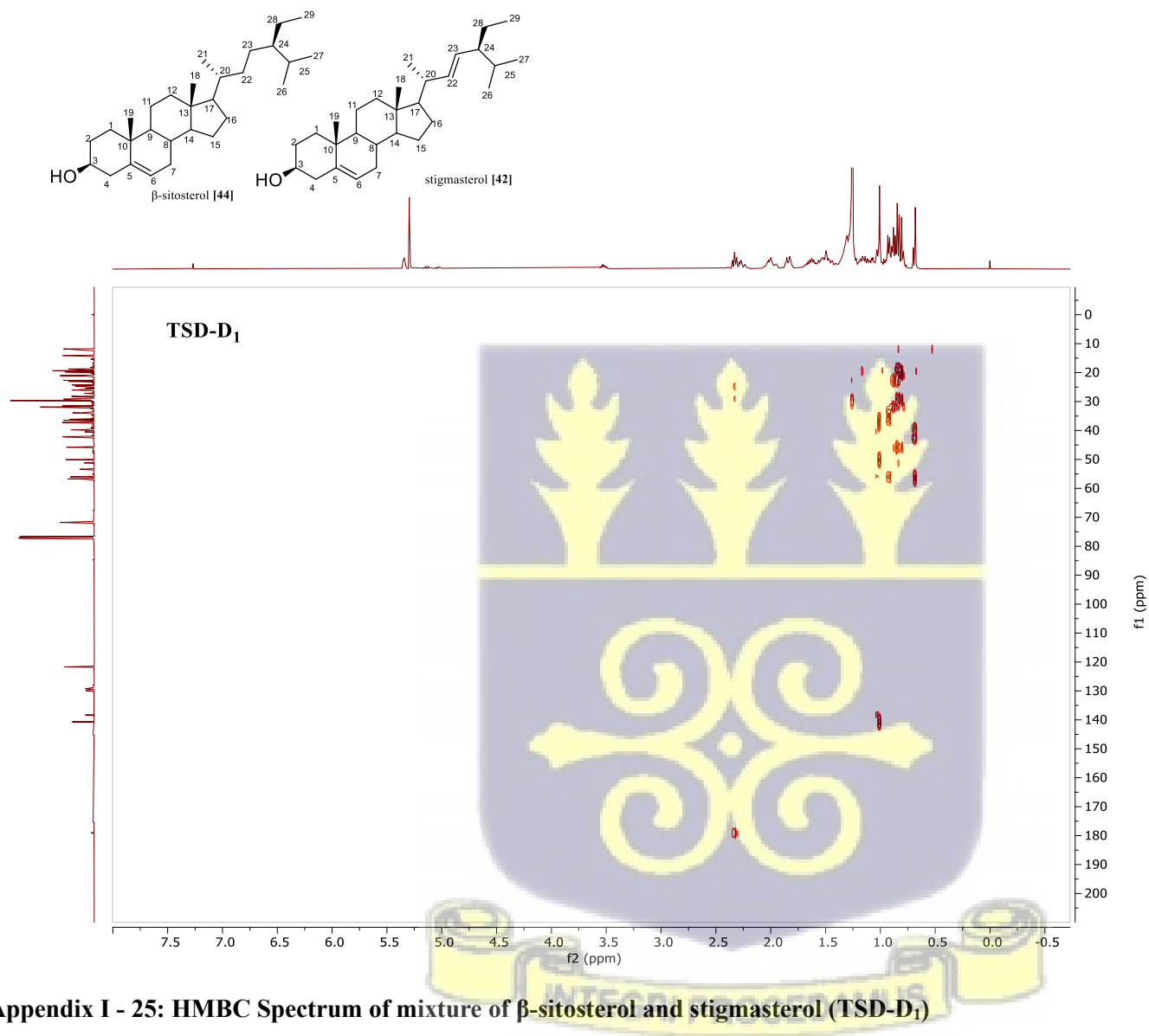
Appendix I - 23: HSQC-DEPT Spectrum of mixture of β -sitosterol and stigmasterol (TSD-D₁)





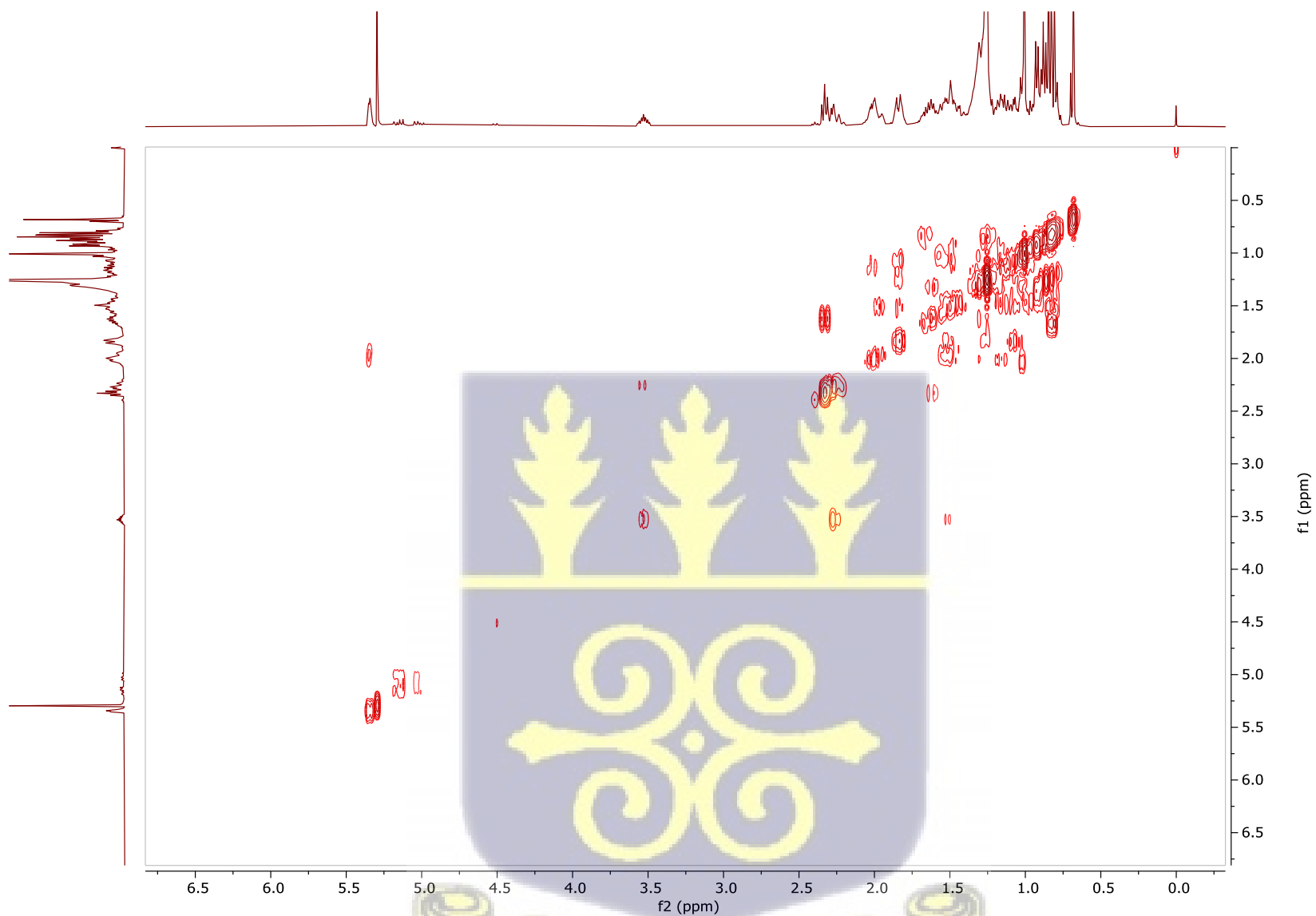


Appendix I - 24: HSQC-DEPT Spectrum of mixture of β -sitosterol and stigmasterol (TSD-D₁)

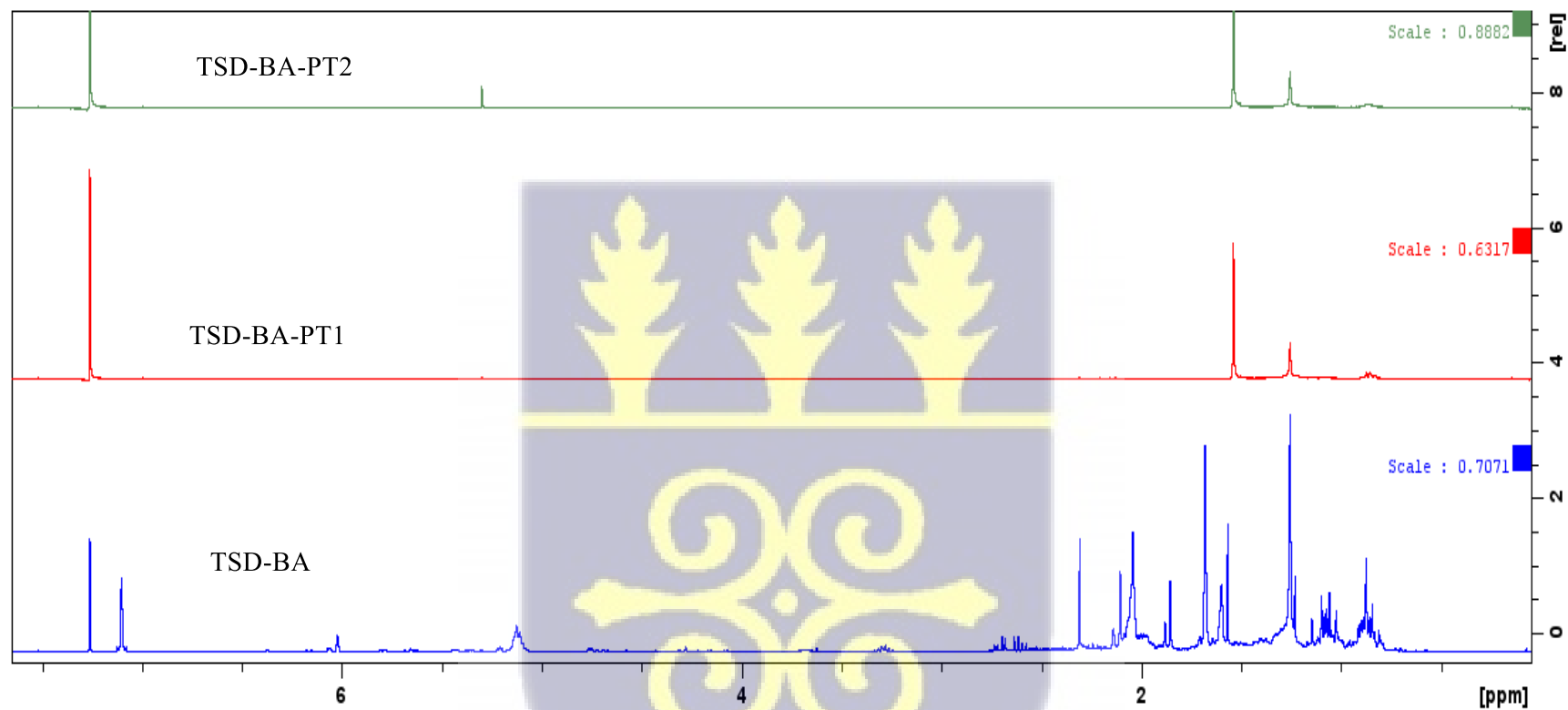


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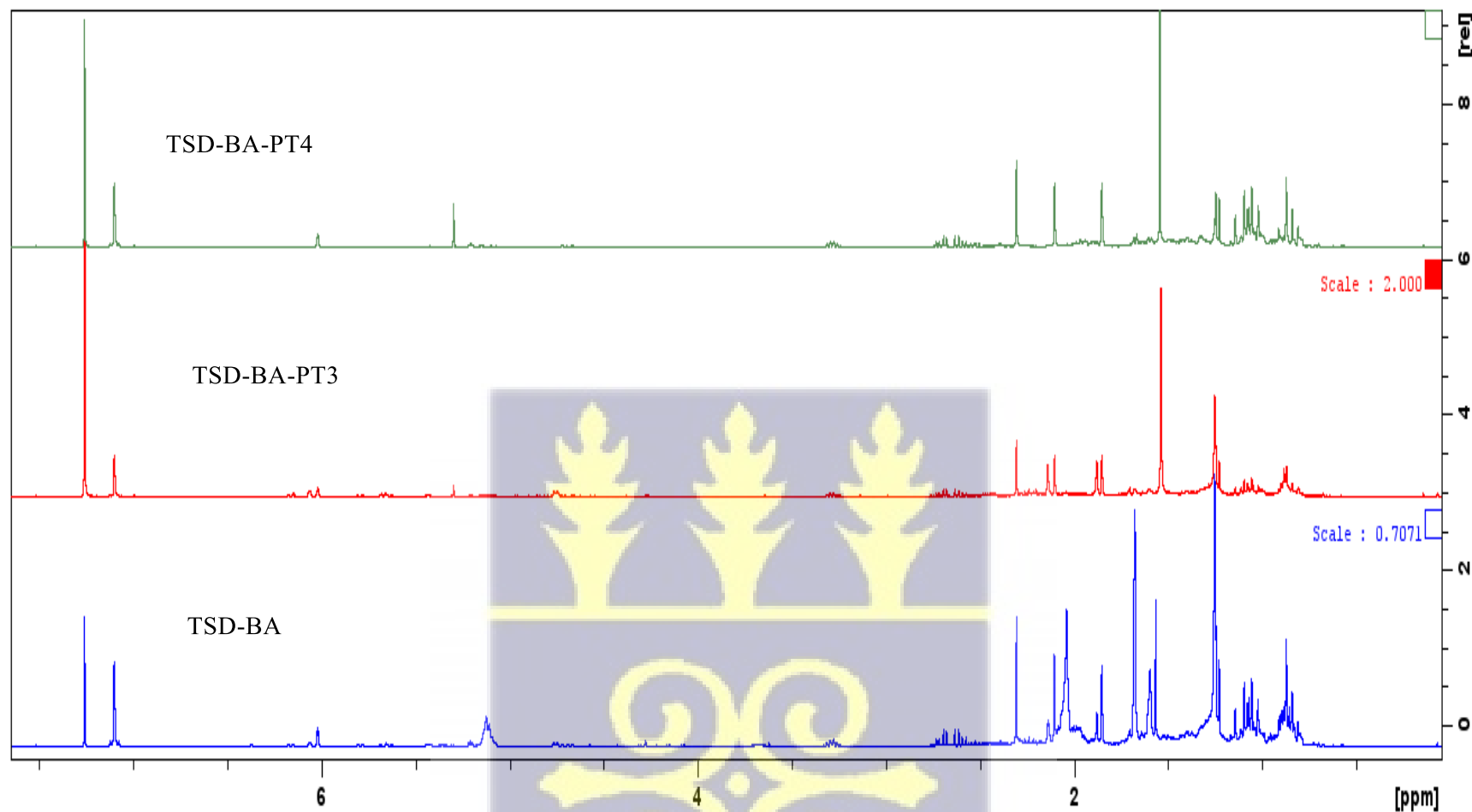




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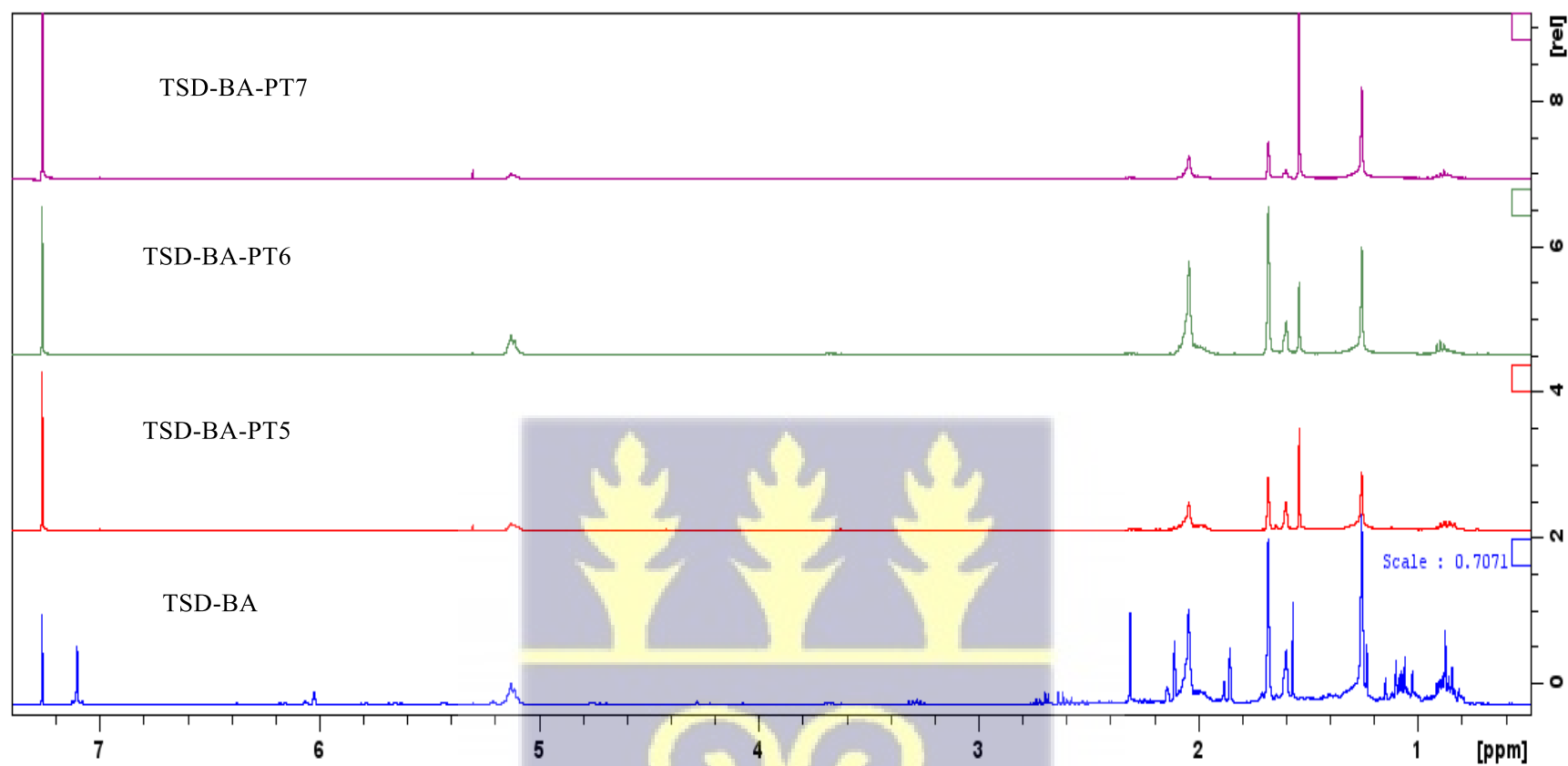


Appendix I - 27: Overlaid spectra of TSD-BA₁



Appendix I - 28: Overlaid spectra of TSD-BA₂





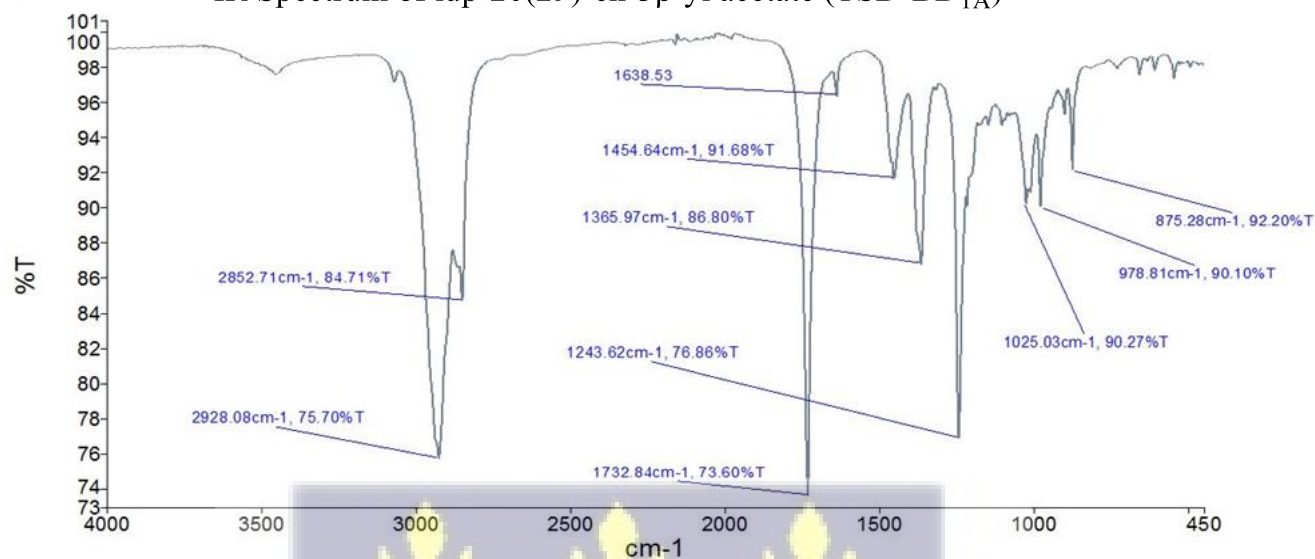
Appendix I - 29: Overlaid spectra of TSD-BA₃

APPENDIX II: IR SPECTRA

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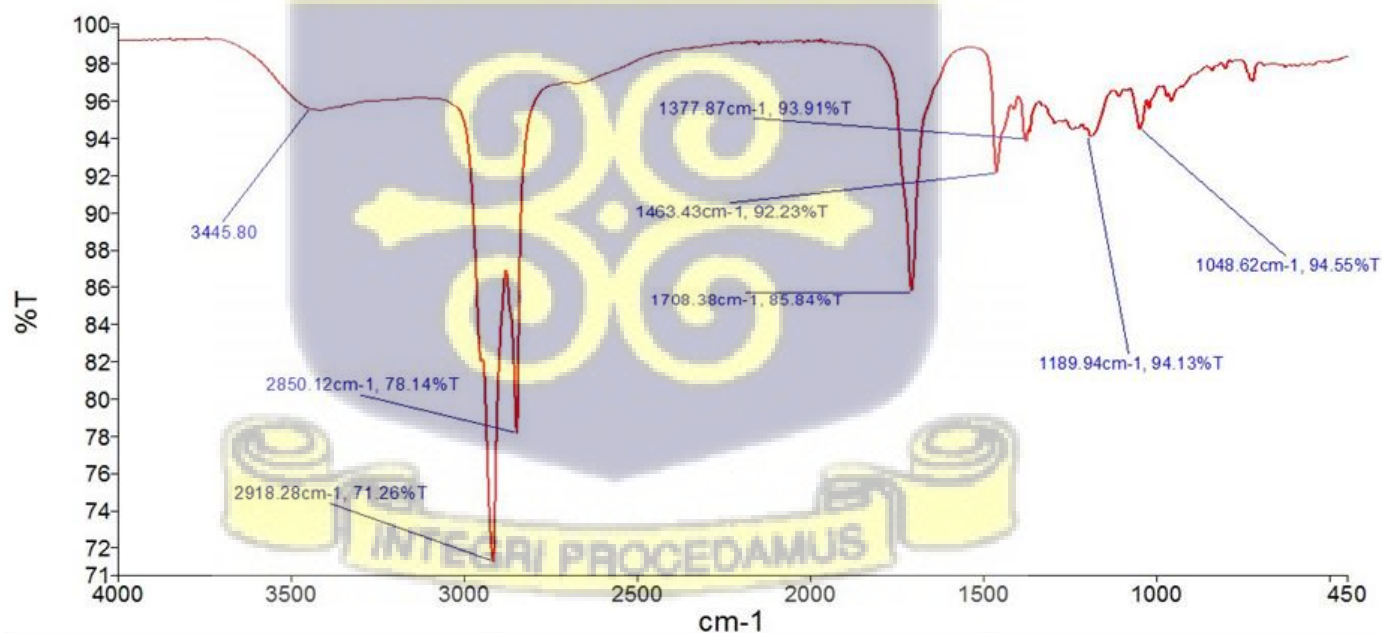


IR Spectrum of lup-20(29)-en-3 β -yl acetate (TSD-BD_{1A})



Appendix II- 1: IR Spectrum of lup-20(29)-en-3 β -yl acetate (TSD-BD_{1A})

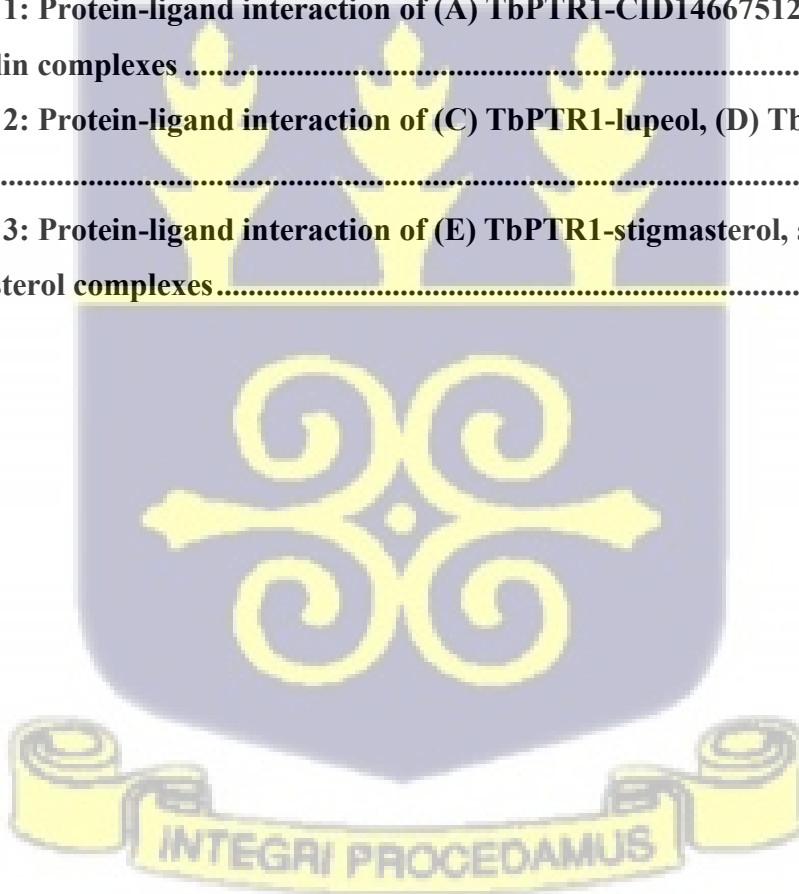
IR Spectrum of of mixture of β -sitosterol [44] and stigmasterol [42] (TSD-D₁)



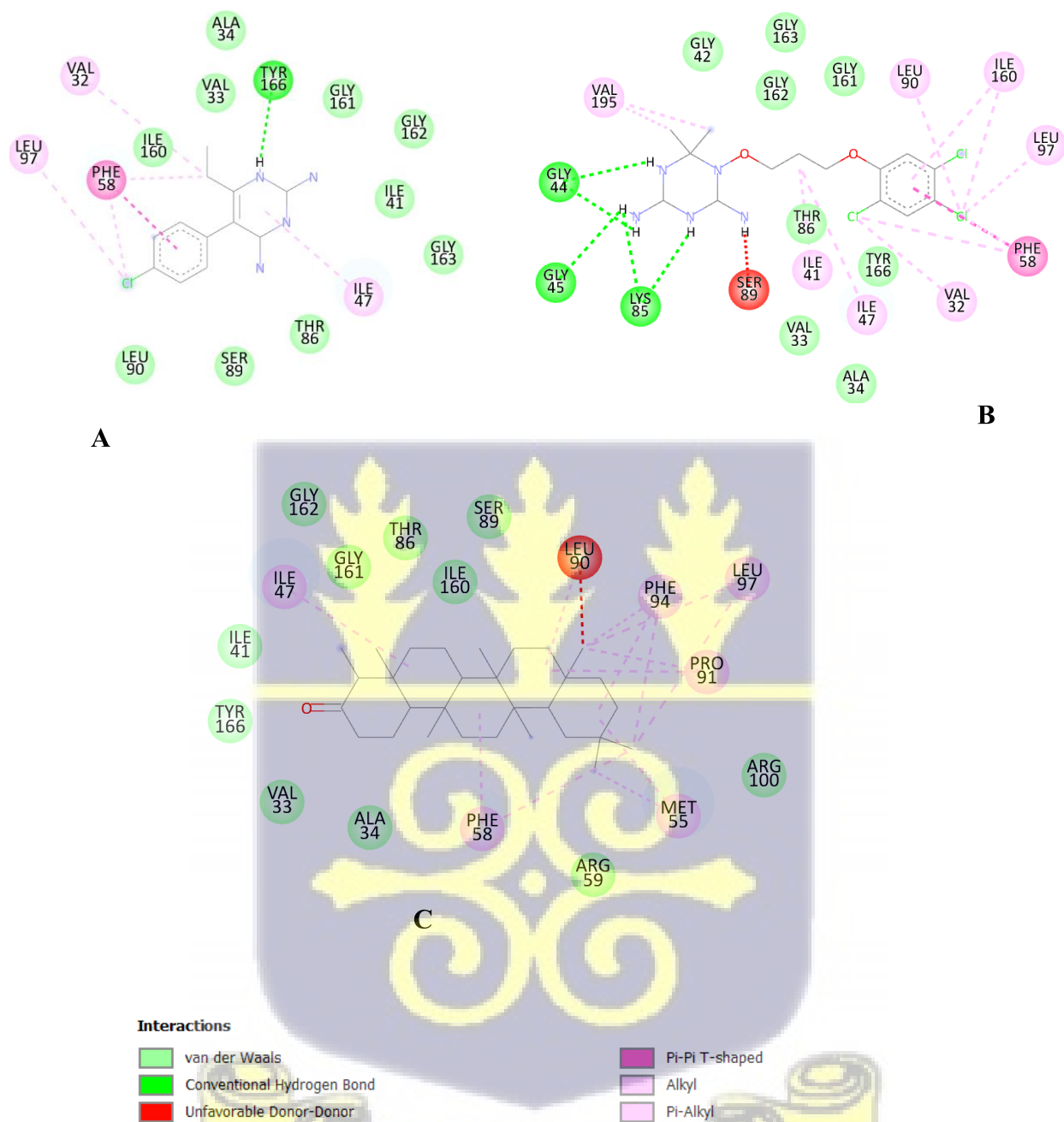
Appendix II- 2: IR Spectrum of of mixture of β -sitosterol [44] and stigmasterol [42] (TSD-D₁)

APPENDIX III: *Trypanosoma brucei* Enzyme-Ligand Interactions

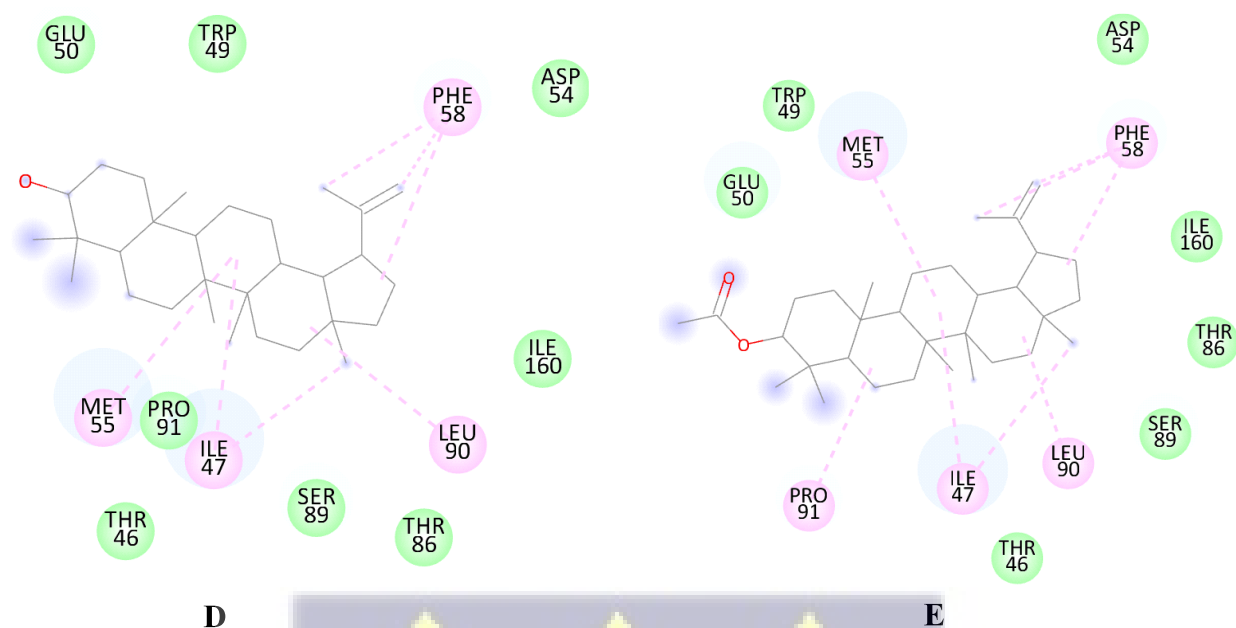
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APPENDIX III-A: *Trypanosoma brucei* dihydrofolate reductase (*Tb*DHFR) - Ligand Interactions



Appendix III-A 1 : Protein-ligand interaction of (A) *Tb*DHFR-pyrimethamine, (B) *Tb*DHFR-WR99210 [545], (C) *Tb*DHFR-friedelin complexes

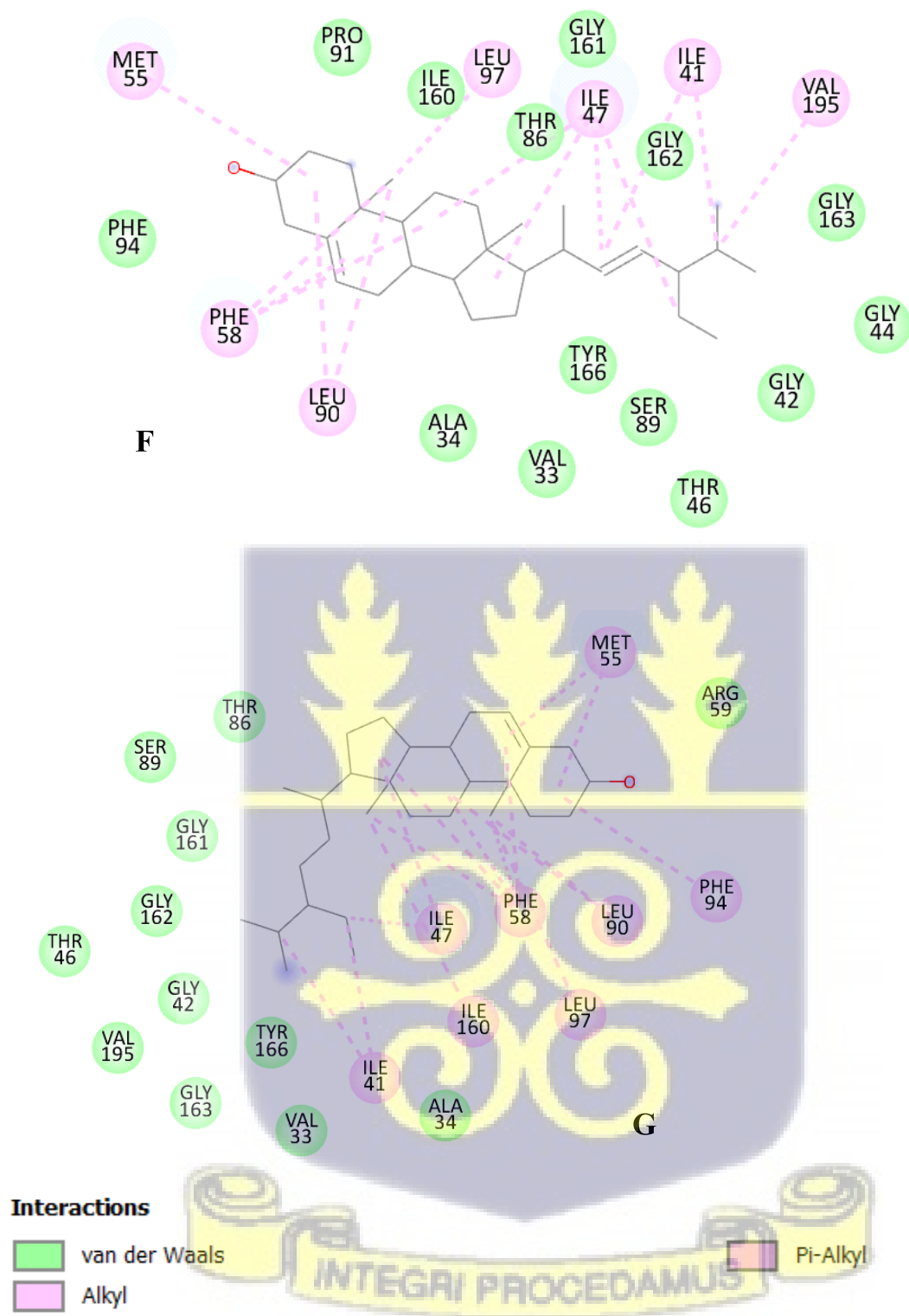


Interactions

	van der Waals		Pi-Pi T-shaped
	Conventional Hydrogen Bond		Alkyl
	Unfavorable Donor-Donor		Pi-Alkyl

Appendix III-A 2: Protein-ligand interaction of (D) *Tb*DHFR-lupeol, (E) *Tb*DHFR-lupeol acetate,





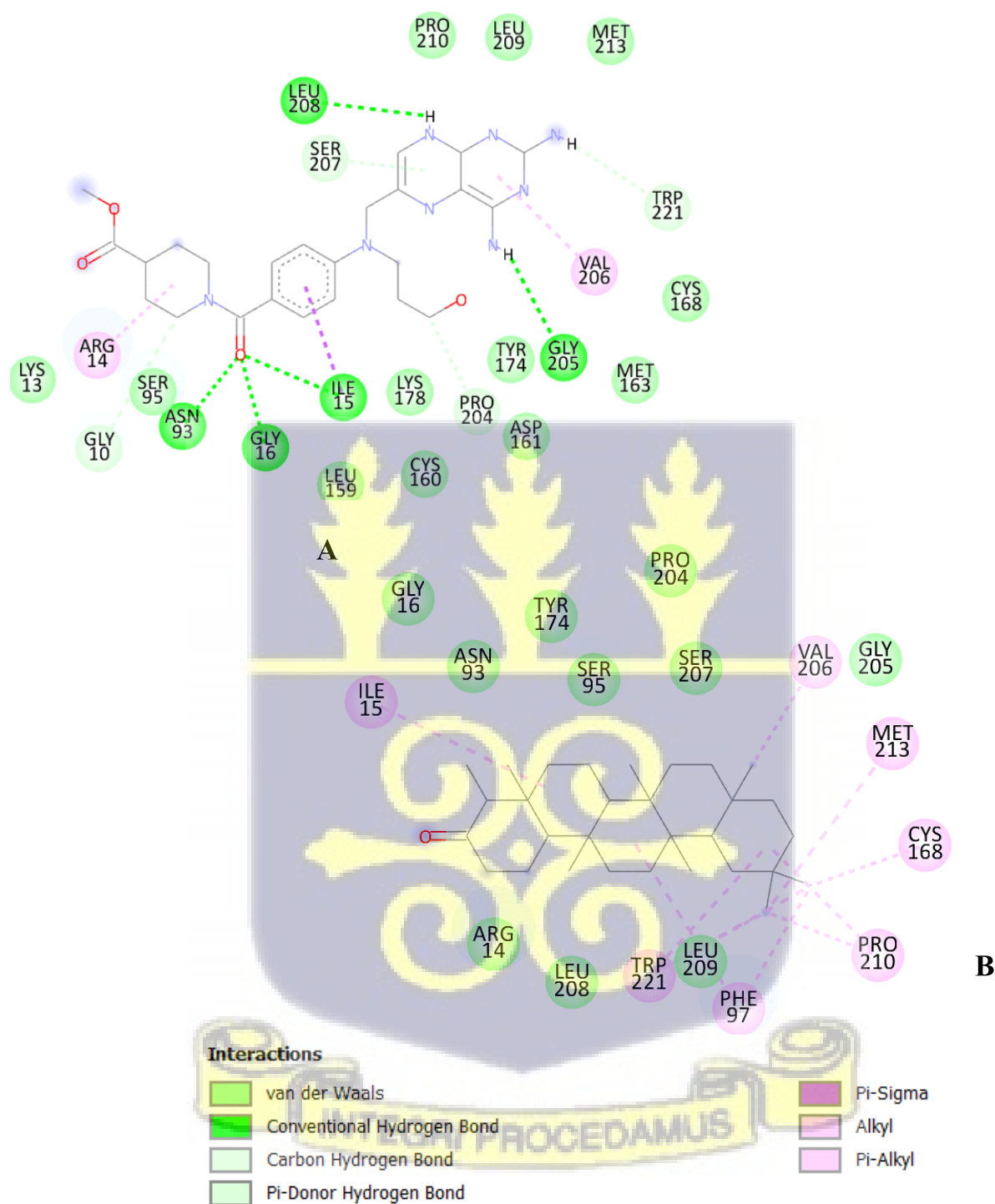
Appendix III-A 3: Protein-ligand interaction of (F) *Tb*DHFR-stigmasterol, and (G) *Tb*DHFR- β -sitosterol complexes

Appendix III-A Table 2: Docking results of compounds against *Tb*DHFR

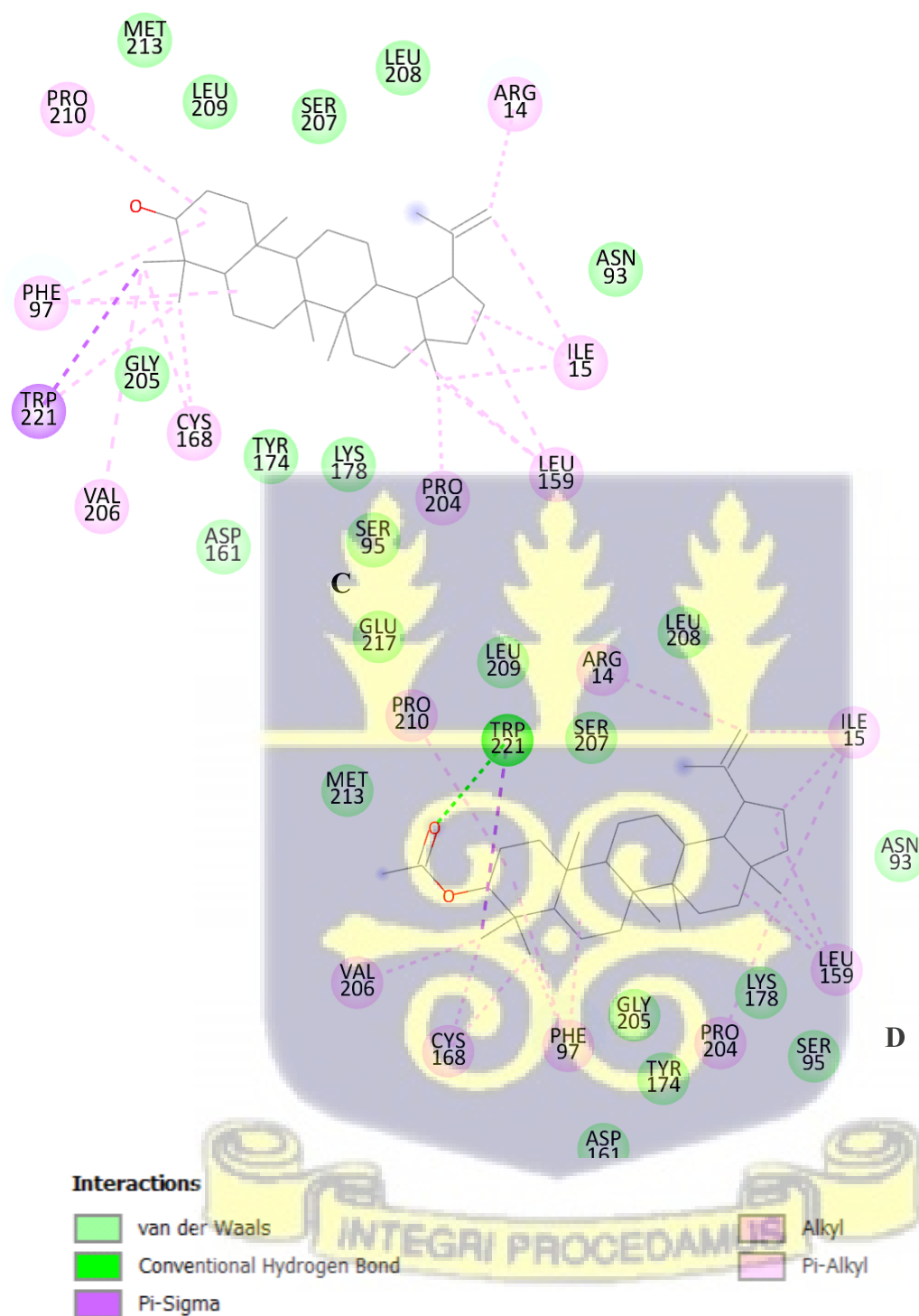
Ligand	PubChem compound identifier (CID)	Binding Affinity (kcal/mol)	Protein Residue Interacting with Compound
friedelin	91472	-10.8	ILE 47, LEU 97, PHE 58, PHE 94, PRO 91, MET 55, LEU 90, GLY 161, GLY 162, ILE 41, ILE 160, THR 86, SER 89, TYR 166, VAL 33, ALA 34, ARG 59, ARG 100
stigmasterol	5280974	-9.7	PHE 58, LEU 90, MET 55, LEU 97, ILE 47, ILE 41, VAL 195, PHE 94, PRO 91, ILE 160, THR 86, GLY 161, GLY 162, GLY 163, GLY 44, GLY 42, TYR 166, SER 89, THR 46, VAL 33, ALA 34
β -sitosterol	222284	-9.5	MET 55, PHE 94, PHE 58, LEU 90, LEU 97, ILE 41, ILE 47, ILE 160, ARG 59, THR 86, SER 89, GLY 161, GLY 162, THR 46, GLY 42, GLY 163, VAL 195, VAL 33, TYR 166, ALA 34
lupeol	259846	-9	PHE 58, MET 55, ILE 47, LEU 99, ASP 54, GLU 50, TRP 49, THR 46, PRO 91, SER 89, THR 86, ILE 160
lupeol acetate	92157	-8.7	PHE 58, LEU 90, ILE 47, MET 55, PRO 91, ASP 54, TRP 49, GLU 50, ILE 160, THR 86, SER 89, THR 46
cycloguanil	9049	-8	GLY 45, GLY 44, LYS 85, ILE 41 ILE 47, VAL 195, VAL 33, TYR 166, GLY 161, ALA 34, THR 86, THR 46, SER 89, GLY 42, GLY 162, GLY 163
pyrimethamine	4993	-7.2	PHE 58, TYR 166, ILE 47, LEU 97, VAL 32, ILE 160, VAL 33, ALA 34, GLY 161, GLY 162, GLY 163, ILE 41, THR 86, SER 89, LEU 90



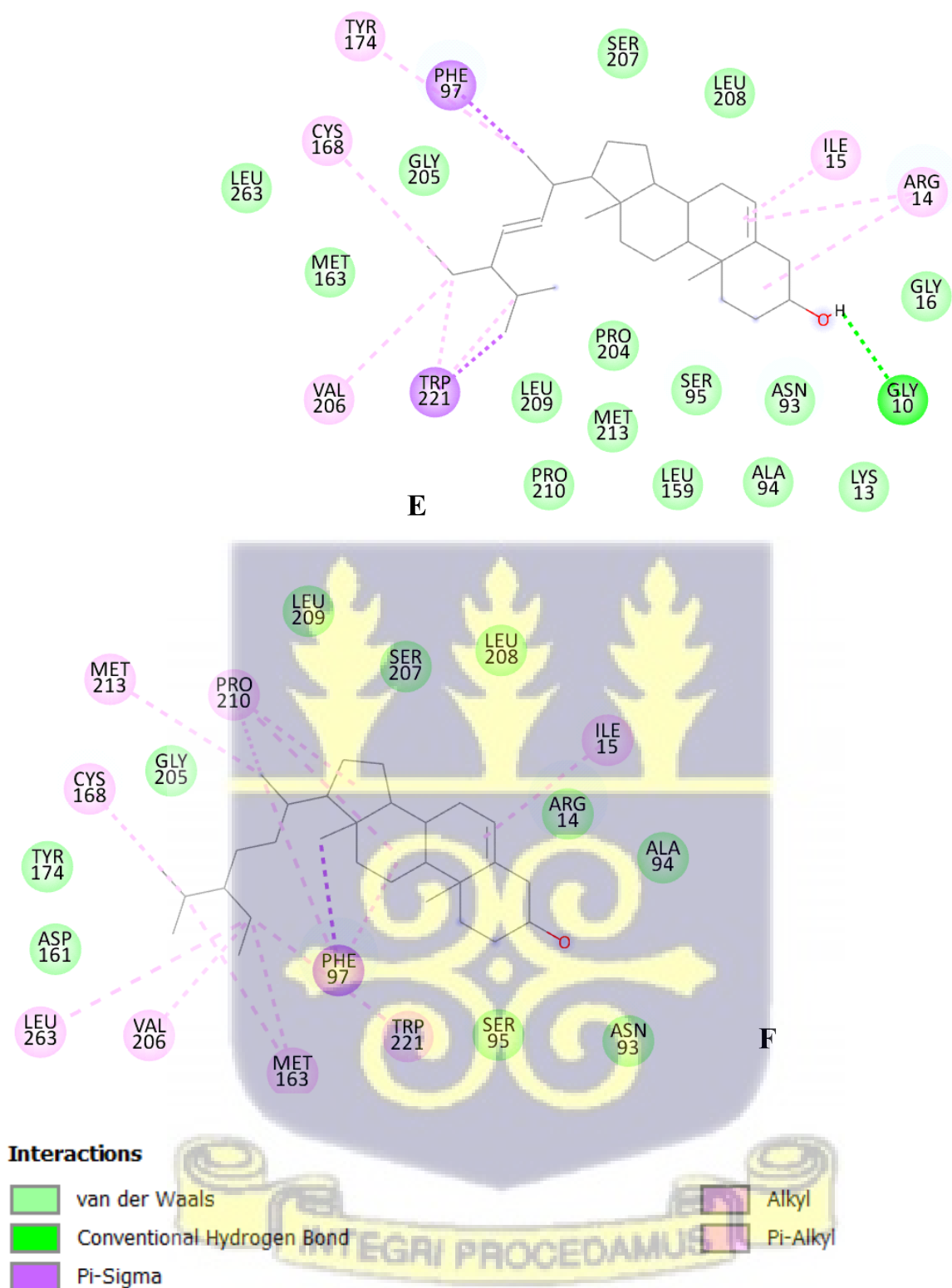
APPENDIX III-B: *Trypanosoma brucei* pteridine reductase 1 (*Tb*PTR1)-Ligand Interactions



Appendix III-B 1: Protein-ligand interaction of (A) *Tb*PTR1-CID14667512, and (B) *Tb*PTR1-friedelin complexes



Appendix III-B 2: Protein-ligand interaction of (C) *TbPTR1*-lupeol, (D) *TbPTR1*-lupeol acetate



Appendix III-B 3: Protein-ligand interaction of (E) *TbPTR1*-stigmasterol, and (F) *TbPTR1*- β -sitosterol complexes

Appendix III-B Table 2: Docking results of *Tb*PTR1

Ligand	PubChem compound identifier (CID)	Binding Affinity	Proteins Interacting with Compound
friedelin	91472	-11.2	LE 15, VAL 206, MET 213, CYS 168, PRO 210, PHE 97, TRP 221, GLY 16, ASN 93, TYR 174, SER 95, SER 207, GLY 205, ARG 14, LEU 208, LEU 209, PRO 204
lupeol	259846	-10.9	PHE 97, TRP 221, CYS 168, VAL 206, PRO 204, LEU 159, ILE 15, ARG 14, PRO 210, GLY 205, ASP 161, TYR 174, LYS 178, SER 95, ASN 93, LEU 208, SER 207, LEU 209, MET 213
lupeol acetate	92157	-10.5	TRP 221, PRO 210, ARG 14, ILE 15, LEU 159, PRO 204, PHE 97, CYS 168, VAL 206, MET 213, GLU 217, LEU 209, SER 207, LEU 208, ASN 93, LYS 178, SER 95, GLY 205, TYR 174, ASP 161
stigmasterol	528079	-9.9	GLY 10, PHE 97, TRP 221, TYR 174, CYS 168, VAL 206, ARG 14, ILE 15, GLY 16, LEU 208, SER 207, GLY 205, LEU 263, MET 213, SER 95, LEU 159, ALA 94, ASN 93, LYS 13
methyl 1-[4-[[2,4-bis(azanyl)pteridin-6-yl]methyl-(3-oxidanylpropyl)amino]phenyl]carbonylpiperidine-4-carboxylate	146675128 (standard Inhibitor)	-9.5	Leu208, Pro210, Leu209, Met213, Trp221, Val206, Cys168, Gly205, Met163, Tyr174, Pro204, Asp161, Lys178, Ile15, Cys160, Leu159, Gly16, Asn93, Ser95, Arg14, Lys13, Gly10, Ser207
β -sitosterol	222284	-9	PHE 97, ILE 15, PRO 210, MET 213, CYS 168, LEU 263, VAL 206, MET 163, TRP 221, SER 95, ASN 93, ALA 94, ARG 14, LEU 208, SER 207, LEU 209, GLY 205, TYR 174, ASP 161



APPENDIX IV: *In vitro* antitrypanosomal results of extracts and isolates against *T. b. brucei*

Appendix IV-Table 1: Antitrypanosomal Activity of *T. gentii* Stem Extracts Against *T. brucei brucei* (IC₅₀ Values)

<i>T. gentii</i> extracts	IC ₅₀ (µg/mL) mean ± SD
<i>T. gentii</i> stem DCM	2.02 ± 0.53
<i>T. gentii</i> stem EtOAc	9.85 ± 1.41
<i>T. gentii</i> stem MeOH	24.67 ± 4.69
Diminazene Aceturate	0.05 ± 0.02

Appendix IV-Table 2: *In vitro* antitrypanosomal screening of *T. gentii* isolates against *T. b. brucei*

<i>T. gentii</i> extracts	IC ₅₀ (µg/mL) mean ± SD
<i>T. gentii</i> stem DCM	2.02 ± 0.53
<i>T. gentii</i> stem EtOAc	9.85 ± 1.41
<i>T. gentii</i> stem MeOH	24.67 ± 4.69
Diminazene Aceturate	0.05 ± 0.02

