

PHARMACOGNOSTIC AND ANTICONVULSANT STUDIES ON *GLOSSONEMA BOVEANUM* DECNE (APOCYNACEAE)

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(M.Sc/PHARM-SCI/16604/11 - 12)**

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE IN PHARMACOGNOSY

**DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,
FACULTY OF PHARMACEUTICAL SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA - NIGERIA**

AUGUST, 2015

DECLARATION

I declare that the work in this dissertation entitled **Pharmacognostic and Anticonvulsant Studies on *Glossonema boveanum* Decne (Apocynaceae)**, was carried out by me in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria under the supervision of **Prof. M.S. Abubakar and Dr. A.M. Musa**. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation has been previously presented for another higher degree or diploma at any University.

Salisu Shehu

Date

CERTIFICATION

This Dissertation entitled ‘**Pharmacognostic and Anticonvulsant Studies on *Glossonema boveanum* Decne (Apocynaceae)**, by Salisu Shehu meets the Regulations governing the award of the degree of Masters of Science in Pharmacognosy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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To Almighty Allah, for his countless blessings and favours upon me and for inspiring in me the zeal and wisdom to the successful conduct of my work.

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I acknowledge the sufficient grace of God that has sustained me through the difficult moments encountered during the course of my work.

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ABSTRACT

Glossonema boveanum Decne is a hairy canescent perennial herb belonging to the Apocynaceae. Traditionally, the powdered whole plant is use for the treatment of many illnesses including epilepsy. This study is aimed at establishing some of the pharmacognostic features of the powdered whole plant; investigating the phytochemical compounds of the plant and evaluating the anticonvulsant property of its aqueous ethanol extract. The powdered whole plant material was examined by microscopy and its physicochemical parameters such as: Moisture content, extractive values and ash values were also determined. Thin layer chromatographic technique was used for the phytochemical screening of the various fractions of the aqueous ethanol extract of the plant. Using silica gel column chromatography and preparative thin layer chromatography, a more detailed phytochemical screening was carried out on the n-Hexane fraction of the extract. Maximal electroshock-induced seizure model in chicks and Pentylenetetrazole (PTZ)-induced seizure model in mice were used for the anticonvulsant studies. The intra-peritoneal median lethal dose (LD₅₀) of the extract on mice and chicks was also determined. Microscopical examination of the powdered whole plant of *G. boveanum* identified the presence of polygonal epidermal cells, anisocytic stomatal cells, covering trichomes with cystolith incrustated in them, Starch grains, suberins, lignins and aleurone grains were also found to be present. Thin layer chromatographic analysis on the various fractions of aqueous ethanol extract revealed the presence of phenolic compounds, triterpenoids, flavonoids, and anthracene derivatives. Column chromatography and Preparative thin layer chromatography carried out on the n-Hexane fraction of the extract led to the isolation of two pentacyclic triterpenoids; Compound SAL₁ and compound SAL₂ which were later identified using NMR spectroscopic analysis as α -amyrin acetate and lupeol respectively. At the highest dose used (140 mg/kg), the extract conferred 40% protection against maximal electroshock induce seizure in chicks with no significant decrease in

recovery period against it. Phenytoin (20 mg/kg), the standard anticonvulsant employed conferred 90% protection against maximal electroshock-induced seizure. The extract did not exhibit any significant effect against pentelenetetrazole induced seizure in mice. The intraperitoneal median lethal dose of the extract on mice and chicks was determined to be 1246 mg/kg and 471 mg/kg respectively. All these findings on *G. boveanum* are being reported for the first time. The result suggests that the aqueous ethanol extract of the plant contains pharmacologically active compound(s) that may be valuable in management of generalized tonic-clonic and or partial seizures.

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LIST OF ABBREVIATIONS

CC	Column chromatography
°C	Degree Celsius
Fig.	Figure
g/gm	Gram
Kg	Kilogram
Hz	Hertz
<i>J</i>	Coupling constant
m	Multiplet
Mg	Milligram
NMR	Nuclear Magnetic Resonance
ppm	Parts Per Million
R_f	Retention factor
s	Singlet
TLC	Thin Layer Chromatography
UV	Ultra Violet
v/v	Volume by Volume
w/w	Weight by Weight
α	Alpha
β	Beta
δ	Delta
C	Carbon
^{13}C NMR	Carbon Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
1D	One Dimensional Spectroscopy

2D	Two Dimensional Spectroscopy
d	doublet
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation
¹ H - NMR	Proton Nuclear Magnetic Resonance
LD ₅₀	Median Lethal Dose
PTZ	Pentylentetrazole
MES	Maximal Electroshock
CAM	Complementary or Alternative Medicine
HCl	Hydrochloric acid
TLC	Thin layer Chromatography
MS	Mass Spectroscopy
HPLC	High Performance Liquid Chromatography
Mag.	Magnification
s	Singlet peak of proton signal
m	Multiplet peaks of proton signals
t	Triplet peaks of proton signals

CHAPTER ONE

1.0 INTRODUCTION

Plants are important sources of medicines, especially in developing countries that still use plant-based traditional medicine for their health care. Even though a more recent figure is not available, the WHO has estimated that up to 80% of the population in Africa and the majority of the populations in Asia and Latin America still use traditional medicine for their primary health care needs (WHO, 2003). In developed countries, plant-based traditional medicines or phytotherapeutics are often termed complementary or alternative medicine (CAM), and their use has increased steadily over time (Blumenthal *et al.*, 2006). However, such “botanical dietary supplements” are regulated as foods rather than drugs by the United States Food and Drug Administration. Phytomedicines have always formed the basis of traditional medicaments in China, India, and Africa and in many other cultures over the centuries (Iqbal *et al.*, 2006).

The use of medicinal plants in the form of crude extracts presents several difficulties. The amount of the bioactive compound(s) from plant may vary with both locality and the season in which they are collected. Also, bioactive molecules of many plants are powerful poisons when taken in excess, and if the plant extract contains a lower content of bioactive compound(s) than usual, suboptimal dosage may not be effective. Medicinal properties of many plants are also rapidly lost on storage, for example foxglove leaf's bioactive molecules decompose on long term storage, unless dried quickly after collection (Loiset *et al.*, 2001). Furthermore, crude extracts from many medicinal plants may contain, in addition to the bioactive molecules, other constituents which have harmful effects. For example aristolochic acids present in a Chinese plant, *Aristolochia fangch* (*Aristolochiaceae*) used for the treatment of infection, are nephrotoxic and carcinogenic compounds closely associated with renal failure (Loiset *et al.*, 2001).

It is therefore, important to isolate and identify the bioactive molecules from plant extracts. The advantage of using pure compound drugs instead of crude extracts include, among others, accurately prescribed dosage, structural modification of isolated compound may allow an improvement in the efficacy and moderation of side effects. Pure bioactive molecules can frequently be synthesized economically, thus preventing dependence on plants as sources of drugs (Williams, 1947).

1.1 Definition and Scope of Pharmacognosy

The word Pharmacognosy is derived from the Greek word "Pharmakon", meaning a drug or poison and "gnosis" meaning to acquire knowledge of and literally meaning "the entire knowledge of drug". It is defined as the scientific study of the structural, physical, chemical and sensory characters of crude drugs of vegetable, animal and mineral origin. It includes the study of various naturally occurring drugs, its history, sources, distribution, methods of cultivation, collection, preparation, active constituents, medicinal uses, identification tests, preservation methods, substituents and adulterants (Ghani, 1990).

It is the science of biogenic or naturally derived pharmaceuticals and poisons. It deals with medicinal plants as crude herbs or extracts pure natural compounds and foods having health benefits (Heinrich *et al.*, 2004).

Pharmacognostic studies helps in identification and characterization of the medicinal plant material. And the process of its standardization can be achieved by stepwise pharmacognostic studies (Ozarkar, 2005). The standardization of a crude drug is an integral part of establishing its correct identity. Thus, before any crude drug is included in herbal pharmacopoeia, pharmacognostic as well as other standard parameters must be established (Abere *et al.*, 2007).

A large proportion of the world population has already adopted 'back to nature' strategy to maintain a healthier life and still there is an increasing trend of using natural medicines based on complementary and alternative therapies in developed and as well developing countries (Namraj 2013). As a result of this, there is a need for more researches in the field of Pharmacognosy. With aim at evaluating the quality (identity, purity, consistency), efficacy (therapeutic indications, clinical studies, pharmacological investigations) and safety (adverse reactions, drug interactions, contraindications, precautions) and standardization of these medicinal substances.

The scope of Pharmacognosy includes the study of physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources (American society of Pharmacognosy, 2005). It forms an essential domain of modern pharmaceutical science. It is a multidisciplinary high-tech science of natural medicines that deal with the systematic study of natural medicines in terms of purity, potency consistency and safety. Thus, clinical Pharmacognosy, analytical Pharmacognosy and industrial Pharmacognosy have been established as the specialized and professional offshoots of Pharmacognosy to meet the contemporary advancements in the field of Pharmacognosy. Furthermore, molecular Pharmacognosy, genomic Pharmacognosy and metabolomic Pharmacognosy have been deemed as the promising approaches of Pharmacognosy research to accommodate future demands in molecular biology, biotechnology and analytical chemistry of natural medicinal plants (Namraj, 2013). Other fields of Pharmacognosy researches includes, animals, bacteria, fungi , marine organisms and mineral, as these serves as some important promising sources of traditional and modern medicines.

1.2 Importance of Medicinal Plants in Pharmacognosy

Medicinal plants have been useful in the development of new drugs and continue to play an important role in the drug discovery process (Farnsworth, *et al.*, 1994; Cragg *et al.*, 1997). The correlations between the ethno-medicinal uses of medicinal plants and modern medicines discovered from those plants have been studied by Fabricant (Fabricant *et al.*, 2001). Base on their analysis, 88 single chemical entities isolated from 72 medicinal plants have been introduced into modern therapy, many of which have the same or similar therapeutic purpose as their original ethno-medicinal use (Fabricant and Farnsworth, 2001). Many synthetic drugs owe their origin to plant-based complementary medicine (Howes *et al.*, 2003). A number of plants in the world have been used in traditional medicine remedies

(Barbosa-Filho *et al.*, 2006, Agra *et al.*, 2007). Many plants were known for their anticonvulsant activity and Various phytochemical and pharmacological studies have been carried out on some of these anticonvulsant plants (Chauhan *et al.*, 1988, Nsour *et al.*, 2000).

Plants produce a variety of natural products with highly diverse structures. These products are commonly termed “secondary metabolites”. Primary metabolites are essential for plant growth and development while Secondary metabolites are known to exhibit some other important physiological functions to the plant. In addition to their physiological function in plants, secondary metabolites or commonly known as ‘natural products’ have a strong impact on human culture and have been used throughout human history as condiments, pigments, and pharmaceuticals. Categories of secondary metabolites commonly encountered include waxes and fatty acids, polyacetylenes, terpenoids (e.g., monoterpenoids, iridoids, sesquiterpenoids, diterpenoids, triterpenoids), steroids, essential oils (lower terpenoids and phenylpropanoids), phenolics (simple phenolics, phenylpropanoids, flavonoids, tannins, anthocyanins, quinones, coumarins, lignans), alkaloids, and glycosidic derivatives (e.g., saponins, cardiac glycosides, flavonoid glycosides) (Satyajit, *et al.*, 2006).

1.3 Medicinal Plant Screening

The knowledge associated with traditional medicine (complementary or alternative herbal products) has promoted further investigations of medicinal plants as potential medicines and has led to the isolation of many natural products that have become well known pharmaceuticals. Spectroscopic methods coupled with good extraction techniques like chromatography, have contributed phenomenal success of natural product chemistry. A sound isolation strategy has helped in the isolation and characterization of many bioactive molecules. Nowadays, bioassay guided fractionation of medicinal plants is a feature of routine in the attempt to isolate bioactive components from natural sources (Satyajit, *et al.*, 2006).

In practice, as soon as the plant material is collected, it needs to be identified by a taxonomist so as to ascertain the correct identity of the material. The plant part(s) collected are dried quickly to avoid degradation of the components by moisture or by microbes. Once the material has been dried to constant weight, it is ground to smaller particles and extracted using a suitable solvent and extraction method.

Once the extract has been obtained, the activity within can be demonstrated by bioassay methods using both the crude extract or by using the fractionated extract. One of the simplest separation methods is partitioning which is widely used method as an initial extract purification step. The isolation of the bioactive compound can be achieved by Chromatographic techniques such as column chromatography, preparative thin layer chromatography and High performance liquid chromatography (HPLC). Finally, the structure of the isolated compound(s) is elucidated using classical spectroscopic technique such as nuclear magnetic resonance (NMR), 1-D and 2-D proton NMR as well as C-13 NMR, Infrared (IR) and mass spectroscopy (MS) (Leland *et al.*, 2006).

1.4 Epilepsy

Epilepsy is the second most common neurological disorder after stroke, effecting at least 50 million persons worldwide. It is a chronic and often progressive disorder characterized by the periodic and unpredictable occurrence of epileptic seizures, i.e., involuntary contraction of striated muscle repeatedly. Convulsion arises due to sudden excessive and rapid discharge of cerebral neurons in the grey matter of the brain (Rang *et al.*,2007).

All antiepileptic drugs are tested extensively in animal models for efficacy and safety before their use in humans, as animal seizure models provide some knowledge of the possible clinical use of new compounds. For example, it is predicted that the compounds which show efficacy in the maximal electroshock seizure test may be used for the treatment of generalized tonic clonic seizures in humans and compounds which show efficacy in the pentylenetetrazole model may be used for the treatment of myoclonic seizures in human patients (White,1997, Holmes *et al.*, 2008,). Animal seizure models are developed by using chemical or electrical stimulus-evoked paradigms to generate seizure activity in vivo or in vitro.

It has been argued that the use of these models will detect the antiepileptic drug similar to those which are already in market and therefore potentially useful compounds may be missed using these models. For example, the maximal electroshock seizure and pentylenetetrazole tests failed to identify the anticonvulsant activity of levetiracetam. However, levetiracetam was subsequently found to be active in other seizure models (Gower *et al.*, 1995, Klitgaard *et al.*, 1998). Therefore, it is suggested that along with these models, other seizure models should also be used for development of new antiepileptic drug since no single test can satisfactorily demonstrate the anticonvulsive properties of new compounds.

1.5 Statement of Research Problem

Epilepsy is an important global health problem; Second most common neurological disorder after stroke, affecting at least 50 million persons worldwide (Suresh *et. al.*,2012). In developing countries 75% of people with epilepsy do not receive effective treatment (Suresh *et. al.*, 2012). Despite its traditional use for the treatment of epilepsy, *G. boveanum* has not been scientifically validated for this claim and a number of its medicinal constituents have not been identified.

1.6 Justification of the research

Many therapeutically active drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity such as antiepileptic property. Thus, a lot of drug discovery researches were largely focused on validating some of these ethno-medicinal claims (Ratnasooriya *et al.*, 2005).

Glossonema boveanum has been reported to have some important ethno-medicinal uses such as its use in lactation and in the treatment of epilepsy. Previously, none of these medicinal claims have been validated scientifically. Also, there was little information on the phytochemical constituents of the

plant. It is therefore, very paramount to scientifically validate some of these ethno-medicinal claims and as well investigate some of its phytochemical constituents that may be responsible for some of its biological activities.

1.7 Research hypothesis

The extract of *G. boveanum* (Decne) has constituent(s) with anticonvulsant activity.

1.8 Overall Aim

The aim of the research is to carryout pharmacognostic and anticonvulsant studies on the aerial part of *G. boveanum* in order to provide the scientific basis for the use of the plant in traditional medicine for the treatment of epilepsy.

1.8.1 Specific Aims

- i. To evaluate some pharmacognostic properties of the powdered *G. boveanum*, which could serve useful in its identification
- ii. To carryout phytochemical studies on the extract and fractions of *G. boveanum*
- iii. To evaluate the anticonvulsant activity of the extract of *G. boveanum*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction to Apocynaceae Family

Family Apocynaceae, commonly known as the Dogbane family, consists of approximately 424 genera distributed among three sub-families known as Rauvolfioideae, Apocynoideae and Asclepiadeae (Liede, *et. al.*, 2005). The Asclepiadaceae comprises of three sub-families; Asclepiadoideae, periplocoideae and Secamonoideae. Many of these plants are large trees with buttress roots found in rainforests, while some are smaller, evergreen or deciduous trees, shrubs or climbers found in warm and temperate regions of the world (Bensuzan, 2009). The sap of most of the plants is milky latex, which has economic importance for medicinal uses and for the production of rubber (Liede, *et.al.*,2005, Bensuzan, 2009).

2.2 Introduction to Asclepiadoideae

Asclepiadaceae was formally separated from Apocynaceae. The separation was based on the morphological characters of androecium pollen transfer system, gynoecium and the more or less presence of extra whorl of corona attached to the petal which is present in Asclepladaceae and lacking in Apocynaceae (Al Nawaihi *et al.*, 2006). On the other hand, new evidence from more detailed and extensive morphological and palynological evidences as well as the rapidly growing body of molecular information, some taxonomist suggests that Brown delimitation does not reflect natural relationship and support the recognition of a single entity (Sherif, 2013). Endress and Bruyns (2000) concluded that the Asclepiadaceae is an apomorphic derivative of the Apocynaceae; thus making the later family monophyletic and are better considered *Asclepiadaceae* as a sub-family of the latter, a view which is also held by Angiosperm phylogeny group (Sherif, 2013). *Asclepiadaceae*, is further sub-classified into three tribes, *Asclepiadoideae*, the largest and cosmopolitan, *Periplocoideae* and *Secamonoideae*, both are smaller sub-families and restricted to limited areas in Asia and Africa (Verhoeven and Johan, 2001, Meve and Heneiek, 2005). The *Asclepiadoideae* sub-family consists of approximately 3000 species distributed among 177 genera, across Asia, Africa and Europe (Shaza, 2011).

2.3. Taxonomy of Asclepiadoideae

In the sub-family *Asclepiadoideae*, Albers and Meve (2004) recognized five tribes: *Fockeeae*, *marsdenieae*, *stapeliaceae*, *Golonobeae*, and *Asclepiadeae*. Six years later, Endress and Bruyns (2000) reduced the tribes to three by abandoning *Fockeeae* and placing it into the tribe *Marsdenieae* and abandoning *Golonobeae* and placing within the tribe *Asclepiadeae*. Further, the name *Ceropegieae* was adopted for the tribe formally known as *stapeliaceae*. The current three tribes of *Asclepiadoideae* sub-family in their treatment are thus: *Marsdenieae*, *Ceropegieae*, and *Asclepiadeae* (Sherif, 2013).

The tribal sub-division of *Asclepiadoideae* is based largely on the organization of the androecium (Endress and Bruyns 2000); The pollinaria are directly attached to the corpusculum as in *Marsdenieae* or attached to the corpusculum via caudicles, additional arm-like appendages of the translator that are synopomorphic for the other two tribes of the sub-family (*Asclepiadeae* and *Ceropegieae*). The pollinia in the pollen sacs are oriented upwardly in tribes *Ceropegieae* and *Marsdenieae* or horizontally to the pundulus in tribe *Asclepiadeae* in relation to the translator. In addition to the orientation of the pollen sacs, the morphology of the anther (whether or not embedded in the tissue of the anther wings) and the position of anther wings with respect to the anther sacs were suggested as supplementary characters for tribal classification of the sub-family (Endress and Bruyns, 2000). The characters of gynoecium, particularly the presence or absence of true styles and the sharp constriction between stigma-head and ovaries have also been suggested as useful in differentiating *Asclepiadeae* and *Ceropegieae* (Swarupanandan *et al.*, 1996).

2.4 Chemical Constituents and Pharmacological Actions of Asclepiadoideae

Members of family *Asclepiadaceae* are reputed to contain a number of biologically-active compounds such as indolequinoline alkaloids, flavonoids, triterpenes, cardenolides, pregnane and C/D-cis-polyoxypregnane esters and glycosides (Braca *et al.*, 2002). A study conducted on some 11 Egyptian species (*Glossonema boveanum* included) of *Asclepiadoideae* sub family were found to produce flavonol glycosides, which include quercetin 3-O-galactoside (i), quercetin-3-O-glucoside (ii), quercetin-3-O-rutinoside (iii), Kaempferol-3-O-neohesperidoside and Kaempferol-3-O-rutinoside (iv) (Heneidak *et al.*, 2006) (Fig.1). Phenolic compounds were also reported in some medicinal plants from *Asclepiadoideae* and *Periplocoideae* (Surveswaran *et al.*, 2010).

Studies on some species of the genus *Cynanchum* of *Asclepiadoideae* which have close affiliations with *C. acutum* have been done and the following products have been distinguished: steroidal glycosides (Liu *et al.*, 2007), carbohydrates (Yi-Bin *et al.*, 2004), phenolic compounds (Lou *et al.*, 1993) and triterpenes (Konda *et al.*, 1990).

Phytochemical investigations on *Cynanchum acutum* L. (*Asclepiadaceae*) have revealed the presence of several natural compounds including β - sitosterol(v), lupeol(vi), lupyl acetate and α -amyryn (vii) (Halim

et al.,1990), sarcostine (viii), quercetin (ix) and quercetin 3-O-β- D-galactoside (El-Sayed *et al.*, 2012) (Fig.1) four flavonoid glycosides: quercetin di-O-hexoside, quercetin 3-O- rhamnosyl (1→2) glycoside, quercetin 3-O-galactoside and quercetin 3-O-xyloside(x) (Heneidak *et al.*, 2006) and 2 simple coumarins: scopoletin (xi) and scoparone (xii) (El-Demerdash *et al.*, 2009) as well as of seven other flavonoids (Ghada *et al.*, 2008). A tribe member of *Glossonema boveanum*; *Solenostemma argel* (Del.) was found to Contain Acylated phenolic glycosides, namely argelin and argeloside, choline, flavonoids, monoterpene and pregane glucoside, sitosterol and a triterpenoid saponin (Shafek *et al.*, 2012).

Asclepiadaceae has an abundance of esterified polyoxypregnane glycosides with significant antitumor activity (Braca *et al.*, 2002). Many members of this sub-family serve as an important source of cytotoxic and cardiac glycosides and contain highly valuable potential products for curing many diseases. For example, resinoid (galitoxin), a toxic principle found in the milky latex of poisonous species of *Asclepias curassavica* (L). Several glycosides (cardiac glycosides) and an alkaloid have been isolated from the plant. Root extracts of this plant are widely used in South America as an emetic and laxative. A decoction of *A. curassavica* (L) is used as an abortifacient. Roots are known as 'Pleurisy root" and used as an expectorant for pneumonia, lung problems, employed to treat warts, fever, etc. It also used as anti-ovulatory, astringent, cardiogenic and for abdominal tumor, haemorrhages, and headache. The plant contains highly potential esterified polyhydroxy pregnane glycoside that shows antitumour and anticancer property (Chandrashekara *et al.*, 2012). There was a report on antioxidant activity of some members of the family *Asclepiadoideae* and *Periplocoideae* (Surveswaran *et al.*, 2010).

The *Cynanchum* genus from *Asclepiadoideae* reported for their use in folk medicine as antifebrile, antitumour, antitussive, diuretic, expectorant, anticonvulsant, anodyne, and tonic agent, and are effective against chronic hepatic (Tawfiq, 1991). *Solenostemma argel* (Del.) was reported to exhibit these pharmacological actions; Anti-inflammatory activity, antimicrobial activity and larvicidal activity (Shafek *et al.*, 2012).

A thorough study of literature showed that a large number of taxa in the family *Asclepiadoideae* are medicinally important and contain different kinds of secondary metabolites.

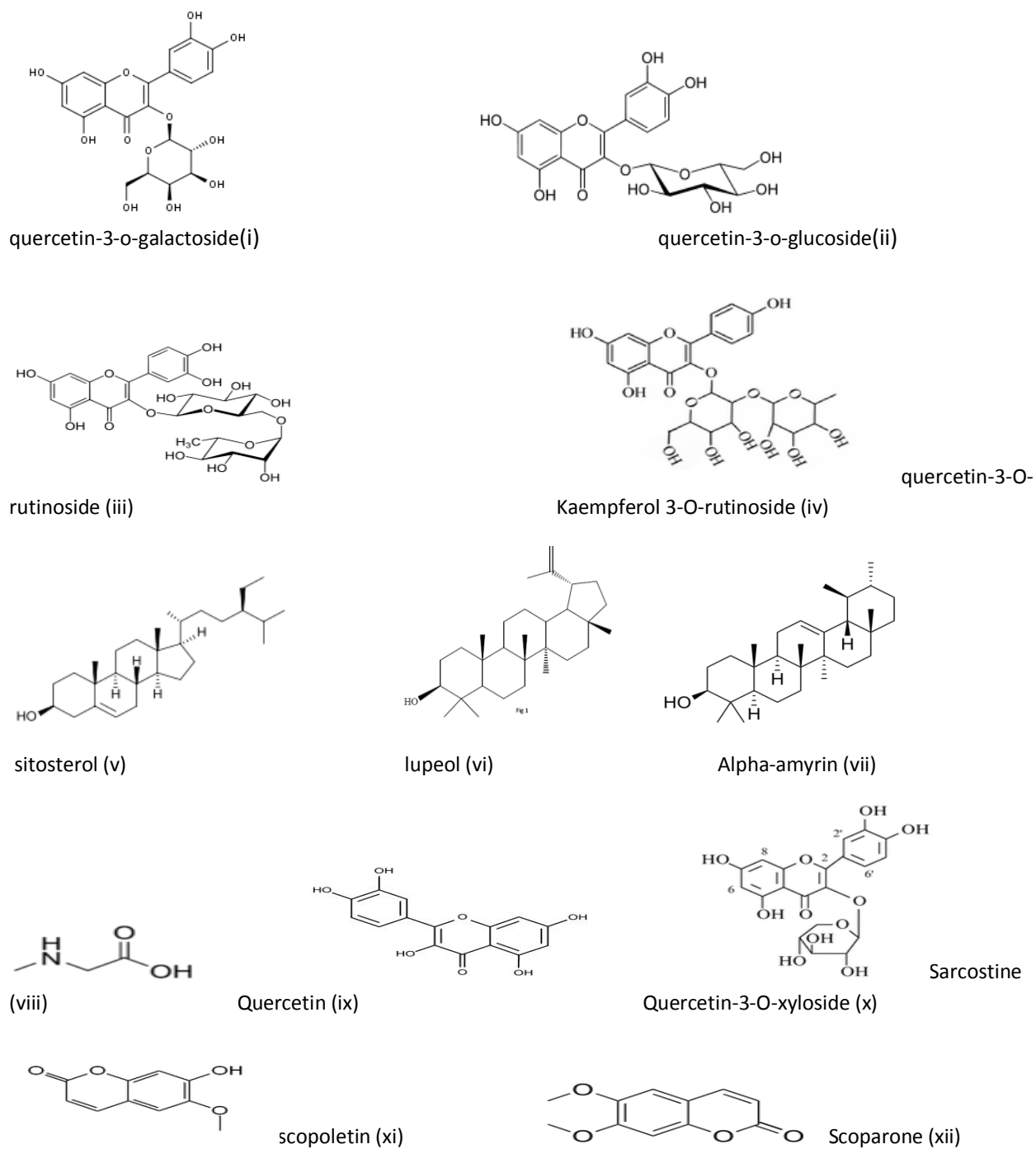


Fig 1. Chemical Structures of Some Isolated Compounds from *Asclepiadoideae*

2.5 *Glossonema* Genus

The genus belongs to the sub-tribe Glossonematinae of the tribe Asclepiadeae. It was previously thought to be monophyletic with *Odonthanthera* and *Solenostemma* genera, but were now shown to be non monophyletic and are closely allied to *pentarrhinum*, an African genus of five species belonging to subtribe *cynanchinae*, based on molecular, karyological and morphological evidences. (Liede *et al.*, 2002). This is further supported by the relationships as expressed in the 'tree', based on RAPD analysis (Sherif, 2013).

The present results indicate that the small Afro-Arabian genera *Pentarrhinum*, *Glossonema* and *Odonthanthera* are monophyletic, and together form a subclade within *Cynanchum* (Liede *et al.*, 2002). This clade is characterized by thick-walled, ornamented fruits. However, as no further morphological or chemical evidence could be found, name changes are left to a species-level revision of the group (Liede *et al.*, 2002). The genus comprises of five species, which include [Glossonema boveanum](#), [G. revoilii](#), [G. thruppii](#), [G. varians](#) and *G. macrocephalum* (Bullock, 1955).

2.6 Description of *Glossonema boveanum*

2.6.1 Taxonomic Classification of *G. boveanum*

Kingdom: Plantae

Phylum: Magnoliophyta

Class : Magnoliopsida

Order : Gentianales

Family : Apocynaceae

Sub family: Asclepiadoideae

Genus : *Glossonema*

Specie : *Glossonema boveanum* (Decne)



Plate I. *Glossonema boveanum* in its Natural habitat

2.6.2 Botanical Description of *Glossonema boveanum*

Glossonema boveanum (Decne), called "Taaringida" in Hausa, is a hairy canescent perennial herb that reaches up to 10-30 cm long. Stems are much-branched from the base. Leaves are 1-2 cm long and 0.5-1.2 cm wide, ovate, or ovate-lanceolate, with cuneate or truncate base, acute or mucronate apex, and usually undulate margins. Flowers are with whitish-yellow corolla and deeply lobed calyx and arranged in sessile cymes. The fruit is cylindrical or ovoid, acuminate, pubescent, echinate, and greenish follicle with flattened, ovate, brown seeds with a narrow white margin. (Hepper and Friis., 1994).

2.7 Geographical Distribution of *G. boveanum*

The plant is a weed of cultivation and is also found in waste places of the Sahel zone, from Mauritania to Northern Nigeria and Western Cameroon, and extending throughout North and North Eastern Africa to Saudi Arabia and North Western India (Burkill 1985, Neuwinger, 2000).

2.8 Ethno-medicinal Uses of *G. boveanum*

The plant is edible raw, especially the young flowering top and fruits. It provides a good fodder for all stock, and serves as a famine-food for man. It contains a copious amount of milky sap and is taken by women in Northern Nigeria to increase lactation, hence the Hausa epithet for the plant (Burkill 1985, Neuwinger, 2000). The powdered whole plant suspended in local diary milk has been used for the treatment of epilepsy in some parts of Northern Nigeria.

2.9 Plants with Anticonvulsant Activity

A thorough survey of literature reveals that a number of plants such as *Acorus calamus*, *Crocus sativus*, *Embllica officinalis*, *Ginkgo biloba*, *Hypericum perforatum*, *Matricaria recutita*, *Panax ginseng*, *Passiflora incarnata*, (Suresh *et. al.*, 2012), *Carissa edulis* (*Apocynaceae*) (Jamilu, *et.al.*, 2007) have been reported to exhibit anticonvulsant activity. A number of plant based formulations from various systems of medicines like Brahmi Rasayana (Ayurvedic medicine), Chaihu-longu-muli tang (Chinese medicine), Habb-e-Shifa (Unani medicine), TJ-960 (Japanese kampo medicine) and Nigerian herbal recipe have shown anticonvulsant potential in battery of animal models (Suresh *et. al.*, 2012).

Various classes of phytoconstituents obtained from plants, such as alkaloids, lipids, terpenes, triterpenoids, flavonoids and coumarins have also been reported to possess anticonvulsant activity (Suresh *et. al.*, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 List of Materials/Equipment

3.1.1 Materials used for Extraction

These include; 500ml separating funnels, glass funnels, conical flasks, measuring cylinder, beakers, evaporating dish and water bath.

3.1.2 Materials Used for Chromatographic Materials

Glass column 75cm long and 3.2cm diameter, Silica gel (60-120 mesh) for column chromatography (Merck Germany), Pre-coated thin layer chromatography (TLC) aluminium plates (Silica gel 60 F₂₅₄) for TLC analysis (Merck Germany) and Pre-coated Thin layer chromatography(TLC) glass plates for preparative TLC. Other materials used include; glass rod, 50ml-beakers crucibles, Bunsen burner, Oven and microscope.

3.1.3 Equipment/Materials used for Anticonvulsant Studies

Ugo Basile electro-convulsive machine (model 7800), Pestle and mortar and 1ml- syringes, 20ml- beakers and glass vials.

3.2 List of chemicals/Reagents

All reagents and chemicals used were of analytical grade.

3.2.1 List of Chemicals

Methanol (Sigma Aldrich St. Louis, MO, USA), Ethanol (Qualikems fine chem., Nandesari, India), Ethyl acetate (Qualikems fine chem., Nandesari, India), Chloroform (Qualikems fine chem., Nandesari, India), N-butanol (Qualikems fine chem., Nandesari, India), Acetone (Qualikems fine chem., Nandesari, India), N-hexane (Qualikems fine chem., Nandesari, India), Pentylene-tetrazole (Sigma chemical CO USA) and Tween-80

3.2.2 Drugs used

Valproic acid (Sanofi-Synthelabo Ltd. UK) and Phenytoin (Hovid pharm Ltd)

3.2.3 List of reagents

Universal detecting reagent

p-anisaldehyde-sulphuric acid and Iodine vapour

Specific Detecting Reagent

Liebermann-Burchard reagent for steroids and triterpenoids, Dragendorff reagent for alkaloids and other nitrogen-containing compounds, Ferric chloride solution for Phenolic Compounds Aluminium chloride solution for Flavonoids and Borntragger's Reagent for anthracenes

All the above mentioned detecting reagents were freshly prepared at the point of use. The method of their preparation is as shown in appendix J.

Reagents Used for Microscopy

Chloral hydrate solution, dil. Glycerol, Phloroglucinol, Sudan red, N/50 iodine solution, Hydrochloric acid, Iodinated zinc chloride and sulphuric acid.

3.2.4 Animals used

- i) Forty three (43) Swiss albino mice (16-25g) obtained from animal house at the Department of Pharmacology and Therapeutics, ABU, Zaria.
- ii) Sixty three (63) one day-old cockerel chicks (26-41g) obtained from NAPRI/ABU Shika, Zaria.

3.3 Collection, Identification and Preparation of the Plant Material

The whole plant of *G. boveanum* was collected from Kauran Namoda, Zamfara State in January 2014. The herbarium specimen of the freshly collected plant material was prepared and taken to the herbarium section of the Department of Biological Sciences, where it was identified by the Taxonomist (Mal. Namadi Sunusi) and a voucher number 617 deposited for future reference. The collection was then shade dried to constant weight and ground to a fine powder using pestle and mortar. Its weight was determined and then stored in a moist proof, light proof plastic container.

3.4 Pharmacognostic Examination of Powdered *G. boveanum*

Pharmacognostic study on the powdered whole plant involved macroscopic and microscopic evaluation of the plant powder.

3.4.1 Macroscopic Examination of Powdered *G. boveanum*

The plant powder was examined for organoleptic characters namely odour, taste, colour. Examinations were done using sense organs like the nose, tongue, and the naked eyes as described in Evans (2009).

3.4.2 Microscopic Examination of Powdered *G. boveanum*

Small amounts of ground powdered *G. boveanum* were used for the microscopy. The sample was cleared in chloral hydrate solution in test tubes by boiling on water bath. It was mounted using dilute glycerol and viewed under the microscope (Evans, 2009).

3.4.2.1 Chemo microscopical Studies of Powdered *G. boveanum*

Small amount of ground powdered *G. boveanum* was cleared in chloral hydrate solution in test tube by boiling on water bath. The mountant used was dilute glycerol and the reactions were observed under a light microscope following World Health Organisation guidelines (2011).

i) Cell wall Materials

- a) **Cellulose cell walls:** Iodinated zinc chloride (2 drops) was added to the cleared sample on a slide, and this was allowed to stand for few minutes. Sulphuric acid (1 drop) was added. The sample was covered with a slip and observed.
- b) **Lignified cell walls:** Few drops of phloroglucinol was added to the cleared sample and allowed to stand until almost dry. Sulphuric acid (1 drop) was added, then a drop of glycerol and covered with a slip.
- c) **Suberised cell walls:** Sudan red (2 drops) was added to the cleared sample on a slide, cover slip was applied and then gently heated over hot water bath for 2 minutes.

ii) **Cell Content**

- a) **Calcium carbonate and Calcium oxalate:** To the cleared sample, 2 drops of hydrochloric acid were added, cover slip applied.
- b) **Starch:** Few drops of iodine solution were added to the cleared sample and cover slip applied.
- c) **Aleurone grains:** Few drops of ethanolic iodine solution were added to the cleared sample and cover slip applied.

3.4.3 Determination of Physicochemical Parameters of the powdered *G. boveanum*

3.4.3.1 Total ash of *G. boveanum*

About 2g of the ground air-dried plant material was weighed into a previously ignited and tared crucible. The plant material was then spread evenly and ignited gradually until it became white, indicating the absence of carbon. It was then cooled in a dessicator and weighed. The weight of ash was then determined by subtracting the weight of the tared crucible from the total weight. The percentage weight of the ash with respect to the weight of original plant material is calculated as the total ash value. The same procedure was repeated for two more times in each case determining the total ash value (WHO 2011).

3.4.3.2 Acid- Insoluble Ash of *G. boveanum*

To the crucible containing the total ash, 25ml of dil. HCl was added, covered with watch-glass and boiled gently for 5 min. The watch-glass was then rinsed with 5ml of hot water and the rinse added to the crucible. The content of the crucible was then filtered through an ash-less filter paper and the residue washed with hot water until the filtrate became neutral to litmus paper. The ash-less filter papers containing the insoluble matter were then transferred to the original crucible, dried on hot plate, ignited to constant weight and cooled in a dessicator for 30min. The weight of the insoluble matter was then determined and its percentage with respect to the original weight of the plant material was regarded as the acid insoluble ash. The same procedure was repeated for two more times in each case determining the acid-Insoluble ash value (WHO 2011).

3.4.3.3 Determination of Water Soluble-Ash of *G. boveanum*

To the crucible containing the total ash, 25ml of water was added and boiled for 5 min. The content of the crucible was then filtered through an ashless filter paper, the residue was then washed with hot water and ignited for 15 min. The weight of the residue was then subtracted from the weight of the total ash to obtain the weight of water soluble ash and its percentage with respect to the original weight of the plant material calculated as the water soluble ash value. The same procedure was repeated for two more times in each case determining the water soluble ash value (WHO 2011).

3.4.3.4 Determination of Moisture Content of Powdered *G. boveanum* (loss on drying Method)

About 3g of powdered *G. boveanum* was weighed into previously heated and tarred crucible and heated to a constant weight in an oven at 105^oC. The loss in weight was determined by subtracting the weight of the crucible and the sample after heating from the weight of crucible and content before heating. The procedure was repeated for two more times in each case determining the percentage loss in weight (WHO 2011).

3.4.3.5 Determination of Extractive values of *G. boveanum*

Water extractive (Cold Maceration Method) of *G. boveanum*

About 4g of air-dried, coarsely powdered *G. boveanum* whole plant was macerated in 100ml of water in 500ml closed round bottom flask and then shaken frequently for the first 6 hours using shaker. It was then allowed to stand for 18 hours and filtered immediately. 25ml of the filtrate was evaporated to dryness in a tarred, flat bottomed, shallow dish on water bath and dry at 105^oC to constant weight. The procedure was repeated two more times in each case determining the weight of the dried extract. The percentage weight of the dried extract with respect to the original weight of the plant material was calculated as the water soluble extractive (WHO, 2011).

Ethanol extractives (Cold Maceration Method) of *G. boveanum*

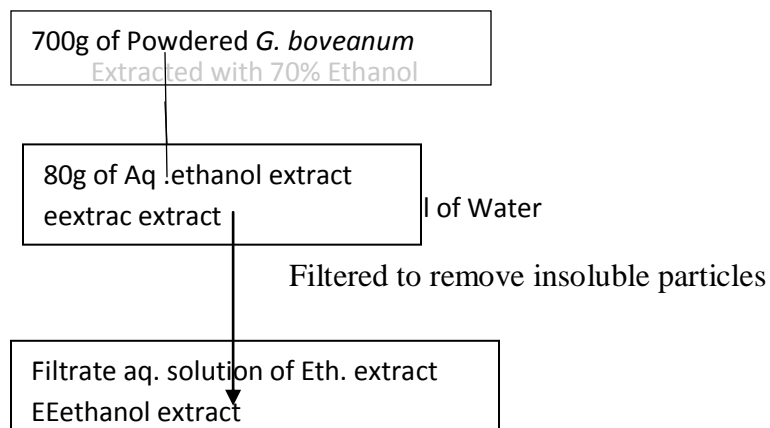
About 5g of the air-dried drug sample, coarsely powdered was macerated in 100ml of 90% ethanol in a closed 500ml round bottom flask and then shook frequently for the first 6 hours using shaker. It was then allowed to stand for 18 hours and filtered immediately. 25ml of the filtrate was evaporated to dryness in a tarred, flat bottomed, shallow dish on water bath and dried at 105°C to constant weight. The procedure was repeated two more times in each case determining the weight of the dried extract. The percentage weight of the dried extract with respect to that of the original weight of the plant material was calculated as the alcohol soluble extractive (WHO, 2011).

3.5 Extraction of Powdered *G. boveanum*

About 700g of the dried, powdered whole plant of *G. boveanum* was macerated in a glass jar with 2L of aqueous ethanol (70%^{v/v}) at room temperature for 3 days (72hrs). The content of the jar was then filtered through a cotton plug and finally through a filter paper (Whatman no.1). The filtrate was concentrated to dryness on water bath set at 50°C. The aqueous ethanol extract obtained was weighed and kept in a desiccator.

3.6 Solvent-solvent Partitioning of the Aqueous Ethanol Extract of *G. boveanum*

A portion of the aqueous ethanol extract (80g) was suspended in water (400ml) and then partitioned with n- Hexane (2×300ml), Chloroform (2×300ml), Ethyl acetate (3×200ml), and n-butanol (2×200ml) successively. The Ethylacetate and the n-butanol partitioned fractions were collected and concentrated and coded EA and NB fractions respectively. The N- hexane fraction (HF) and chloroform fraction (CF) were concentrated at room temperature likewise.



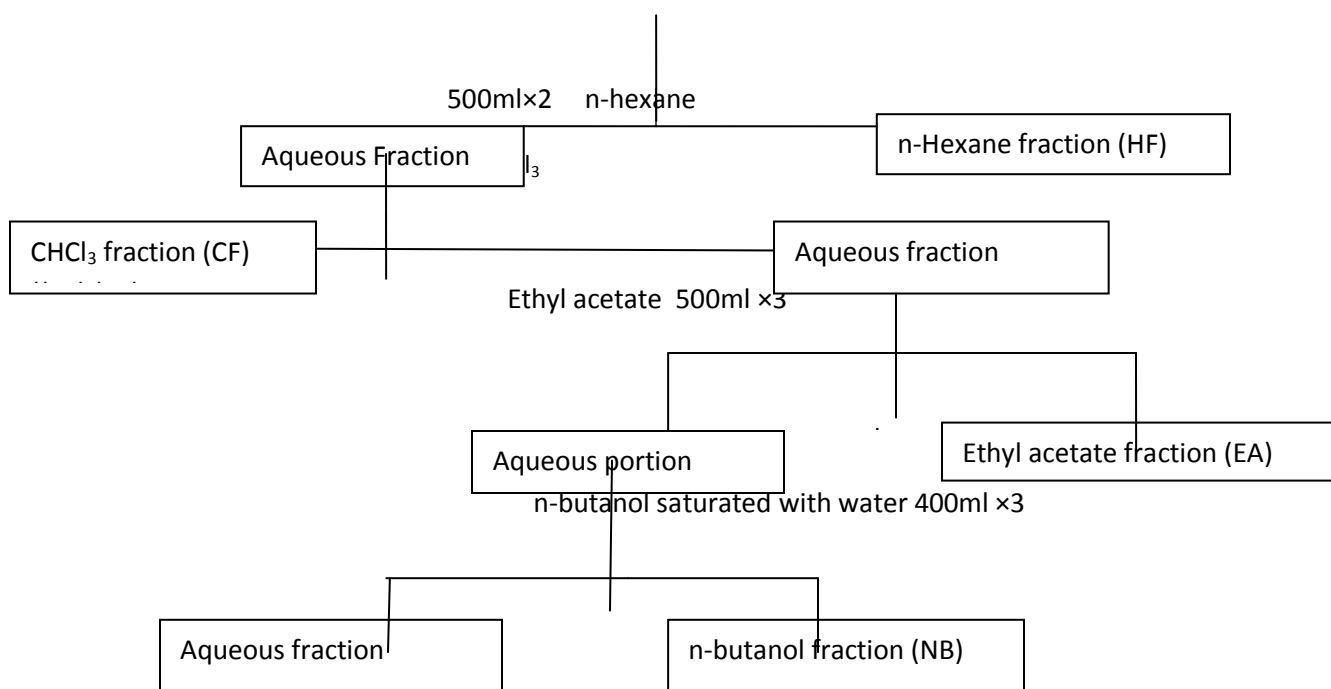


Fig. 2. Fractionation Chart of 70% Ethanol extract of *G. boveanum* (Woo *et al.*, 1980)

3.7 Thin layer chromatographic studies of the extract of *G. boveanum*

In this analysis, the crude extract and its various fractions were dissolved in their appropriate solvent and applied on pre-coated silica gel TLC plates as spots with the aid of capillary tubes at one end of the plate in a straight line, about 0.5 cm above the bottom edge and 0.5 cm away from the sides. The spots were dried and the plates placed in a chromatographic tank containing the mobile phase (the solvent system) that has been prepared in the tank at least 30 minutes earlier. The mobile phase ran along the TLC plate in an ascending manner due to capillary action, carrying with it the components of the extract or the mixture. When the mobile phase reached the desired distance, the plate was removed, the solvent front marked immediately with the aid of a pencil and the plate dried. The separated components were located by observing the chromatogram under ultra-violet light for fluorescence. This was followed by spraying with their respective detecting reagents and heating at 110°C. This method was used for all TLC analysis (Ghani, 1990). Both general and specific detecting reagents were used to reveal the various groups of Phytochemical compounds present in the plant. After development, the various R_f (retention factor) of the different chromatograms were determined.

3.7.1 Thin layer Chromatography of the Crude Extract

The crude aqueous ethanol extract was developed in the TLC tank using n-butanol/acetic acid/water (6:1:1) as the mobile phase. The plate was then sprayed with p-anisaldehyde-sulphuric acid and subsequently heated in oven at 110°C for 5 minutes to reveal the nature of the chromatograms on the plate (Ghani, 1990).

3.7.2 Thin layer Chromatography of the Various Fractions of the Extract

Thin layer Chromatography for the various fractions of the extract were developed using different solvent systems and the chromatograms detected by day light (using spraying reagents) and or with UV-light (short and long wave length) as in the table below:

Fraction	Solvent system	Detecting reagent
n-Hexane	Hexane:ethylacetate (7:3)	p-anisaldehyde,ferric chloride,LB,AlCl ₃ ,BT,DG
Chloroform	Hexane: ethylacetate (6:4)	p-anisaldehyde,ferric chloride,LB, AlCl ₃ ,BT,DG
Ethyl acetate	Hexane: ethylacetate (6:4)	p-anisaldehyde,ferric chloride, AlCl ₃ ,BT,DG
n- butanol	Butanol:acetic acid:water (6:1:1)	p-anisaldehyde,ferric chlororide,AlCl ₃ ,BT

LB; Libbermann buchard reagent, BT; Borntegers reagent, DG;Dragendoff's reagent

3.8 Column chromatography of n-Hexane fraction (HF) of Ethanol Extract of *G.*

Boveanum

3.8.1 Packing of the Column:

Wet slurry method as described by Brain and Turner (1975) was adopted. The lower end of the glass column (10cm long, and 1.5cm diameter) was plugged with glass wool. The eluting solvent was then passed to remove any air bubbles that may be trapped. A slurry containing 100g of the silica with twice

its volume of eluting solvent was prepared in a beaker and pour into the column gradually while the side of the column was tapped gently with a glass rod to even the compaction of the silica as it settles. The eluting solvent was then drained until when a small solvent head was left on top of the column. The column was allowed to stabilize for 6hrs and to ensure that the air bubbles were not trapped in the column.

3.8.2 Elution Procedure

About 2g of n-Hexane fraction of *G. boveanum* was pre-adsorbed with about 5g of silica gel, which was then allowed to dry. It was then mounted on the column and eluted with 100% n-hexane for 5 collections of the eluate (40ml in each aliquot). Thereafter, the polarity of eluting solvent was increased to 95% (hexane/chloroform) for 10 collections, and 90% for another 10 collections. Continuously, after several collections, based on the TLC profile, the solvent system polarity either maintained or increased using chloroform until 80 column fractions (1-80) coded CF₁ to CF₈₀ were collected. The polarity of the different solvent systems used at different points of collection is as shown on table 4.5. The various column fractions having similar TLC profile were pooled together. Column fraction CF₃₀ to CF₃₃ and CF₄₈ to CF₅₂ having the major spots were separately pooled together and coded as CLF1 and CLF2 respectively. Finally the column was washed with methanol.

3.8.3 Purification of CLF1 from n-Hexane fraction of the Ethanol Extract of *G. boveanum*

Fraction CLF1 (0.3g) was purified by column chromatography using a small column. The elution was made with 100% hexane, and solvent polarity gradually increased base on the TLC profile to 95%, 90%, and 80% (hexane/ethylacetate) successively, in each case collecting 20ml of the eluate up to a stage where 42 column fractions coded as cf1 to cf42 were collected. The various column fractions having similar TLC profiles were pooled together. Column fraction cf23 and cf24 with the major spot and less number of spots were pooled together and coded as CLF3. From this purified fraction (CLF3), the first compound coded as SAL₁ was isolated by preparative TLC.

3.8.4 Isolation of Compound SAL₁

The mobile phase (100ml) hexane/ethylacetate (40:1) was prepared and introduced into the chromatographic tank which was allowed to saturate for 30min. The sample was then streaked, allowed to dry. The plate was then introduced into the tank and developed. The single chromatogram identified was then scraped, dissolved in chloroform, centrifuged, filtered, concentrated and finally obtained and labelled as SAL₁.

3.8.5 Purification of CLF2

Fraction CLF2 (0.4g) was purified by column chromatography using a small column. The elution was made with 100% hexane, and solvent polarity gradually increased base on the TLC profile to 99%, 98%, and 95% (hexane/ethylacetate) successively, in each case collecting 20ml of the eluate up to a stage where 64 column fractions coded as cf1 to cf64 were collected. The various column fractions having similar TLC profiles were pooled together. Column fraction cf52 and cf57 with the major spot and less number of spots were pooled together and coded as CLF4. From this purified fraction (CLF4), the second compound coded as SAL₂ was isolated by preparative TLC.

3.8.6 Isolation of Compound SAL₂

SAL₂ was obtained from Preparative TLC in a similar way as SAL₁ but here, using 100ml of the mobile phase hexane/ethylacetate (8:2) as the mobile phase. This was prepared and introduced into the chromatographic tank which was allowed to saturate for 30min. The sample was then streaked, allowed to dry. The plate was then introduced into the tank and developed. The single chromatogram identified was then scraped, dissolved in chloroform, centrifuged, filtered, concentrated and finally obtained and labelled as SAL₂

3.8.7 Physicochemical Studies on the Isolated Compounds

In this section, some physicochemical studies were carried out on the isolated compounds; SAL₁ and SAL₂.

3.8.7.1 Thin layer Chromatography of Compound SAL₁ and SAL₂

Thin layer chromatography was carried out on compound SAL₁ and SAL₂ using hexane/ethylacetate mixture 40:1 and 9:1 solvent systems respectively. Their R_F values was then determined.

3.8.7.2 Melting Point Determination of Compound SAL₁ and SAL₂

Melting points of SAL₁ and SAL₂ were determined using Electrothermal Melting Point apparatus. Small amount of each of the sample was transferred into a capillary tube and inserted into the melting point apparatus. With the aid of a thermometer, the melting point was recorded.

3.8.8 Chemical Test on the Isolated Compounds

Liebermann buchard test was carried out on the isolated compounds (compound SAL₁ and SAL₂) to identify the nature or type of the compounds.

3.8.9 Spectroscopic Analysis of the Isolated Compound(s)

Nuclear magnetic resonance (NMR) spectroscopic analyses were carried out on the isolated compounds; SAL₁ and SAL₂ at School of Physics and Chemistry, University of Kwazulu Natal, South Africa and these include: 1D (¹H, ¹³C, DEPT-90 and DEPT-135) and 2D (¹H, COSY, HSQC, and HMBC). The analysis was conducted on Bruker AVANCE spectrometer, using the residual solvent peaks as internal standard. Chemical shift values were reported in parts per million (ppm). NMR solvent used was deuterated chloroform. The NMR spectra obtained were used for structure elucidation of compounds SAL₁ and SAL₂.

3.9 Biological Evaluation of the Extract of *G. boveanum*

In this section, evaluation of anticonvulsant activity of *G. boveanum* extract was carried out using two animal models. Acute toxicity studies were as well conducted on the two animal species used in the anticonvulsant studies.

3.9.1 Acute Toxicity Studies on the Aqueous Ethanol Extract of *G. boveanum*

The acute toxicity study employed was median lethal dose LD₅₀ determination as described by Lorke 1983.

3.9.1.1 LD₅₀ Determination in Mice

The method consists of two phases. In the first phase, three groups of three mice each, were administered with the crude ethanol extract of *G. boveanum* at doses of 10, 100 and 1000 mg/kg body weight (*i.p*) and observed for signs of toxicity and death within 24hr. In the second phase, four groups of

one mouse each received 600, 1000, 1600, and 2900 mg/kg doses of the extract intraperitoneally and were observed for signs of toxicity and death within 24hrs. The LD₅₀ was then determined by calculating the geometric means of the lowest dose that caused death and the highest dose for which the animals survived from the result of the second phase.

3.9.1.2 LD₅₀ determination in Chicks

The method consists of two phases. In the first phase, three groups of three chicks each, were administered with the crude ethanol extract of *G. boveanum* at doses of 10, 100 and 1000 mg/kg (*i.p*) and observed for signs of toxicity and death within 24hr. In the second phase, four groups of one chick each received 140, 225, 370, 600 mg/kg doses of the extract intraperitoneally and were observed for signs of toxicity and death within 24hrs. The LD₅₀ was then determined by calculating the geometric means of the lowest dose that caused death and the highest dose for which the animals survived from the result of the second phase.

3.9.2 Anticonvulsant Studies on the Aqueous Ethanol Extract of *G. boveanum*

3.9.2.1 Maximal Electroshock (MES)-induced Seizure in Chicks

The method employed in this study was described by Swinyard and Kupferberg (1985). A day old chicks (cockerel) were randomly divided into five groups, each of ten (10) chicks. The first group received normal saline (10ml/kg) *i.p*. the second, third and fourth groups were treated with 35, 70 and 140 mg/kg via the *i.p* route respectively. The fifth group was injected with 20mg/kg of phenytoin *i.p*. Thirty minutes later, maximal electroshock was administered to induce seizure in the chicks using Ugo Basile electroconvulsive machine (Model 7800). The shock duration, frequency, current and pulse width were set and maintained at 0.6s, 150 pulse/sec, 60mA and 0.6ms, respectively. The ability of the extract to prevent tonic hind limb extension (THLE) or reduce the recovery period from the tonic hind limb extension was considered as an indication of anticonvulsant activity of the extract.

3.9.2.2 Pentylentetrazole (PTZ)-induced seizure in Mice

The method described by Swinyard *et al.*, (1989) was employed. Thirty mice were divided into five groups, each of six mice. The first group received normal saline (10 ml/kg) via the *i.p* route. The second, third and fourth groups received 90, 180 and 360 mg/kg of the extract respectively via the *i.p* route. The fifth group was given Valproic acid at a dose of 200 mg/kg body weight via the *i.p* route. Thirty minutes post treatment, mice in all the groups received pentylentetrazole (85mg/kg) subcutaneously(*s.c*) and were observed over a period of 30min. Absence of an episode of clonic spasm of at least 5s duration indicates the extract ability to abolish the effect of PTZ on seizure threshold.

3.10 Statistical Analysis

All data were expressed as mean \pm SEM and percentage (%) where appropriate. One way analysis of variance (ANOVA) was used to determine the level of significance obtained. Results were regarded significant at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Results of Pharmacognostic Examination of Powdered *G. boveanum*

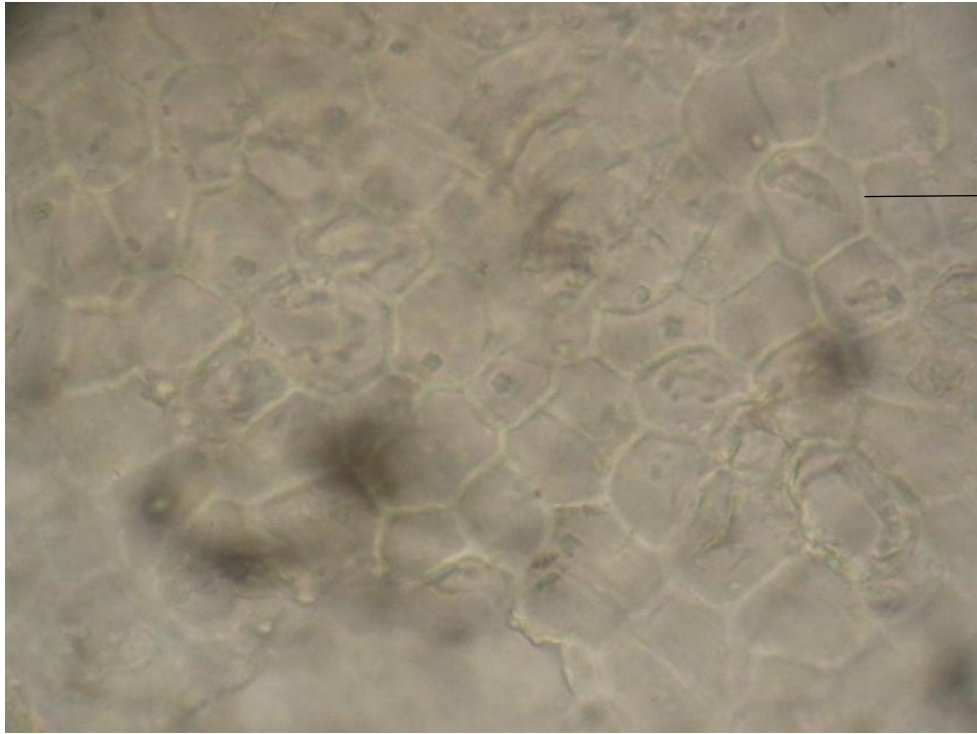
This section showed the results of pharmacognostic studies carried out on powdered whole plant of *G. boveanum*.

4.1.1. Organoleptic Characters

The organoleptic observations of the powdered *G. boveanum* showed that; the powder is green in colour, aromatic in odour, wool-like in appearance and have a characteristic taste.

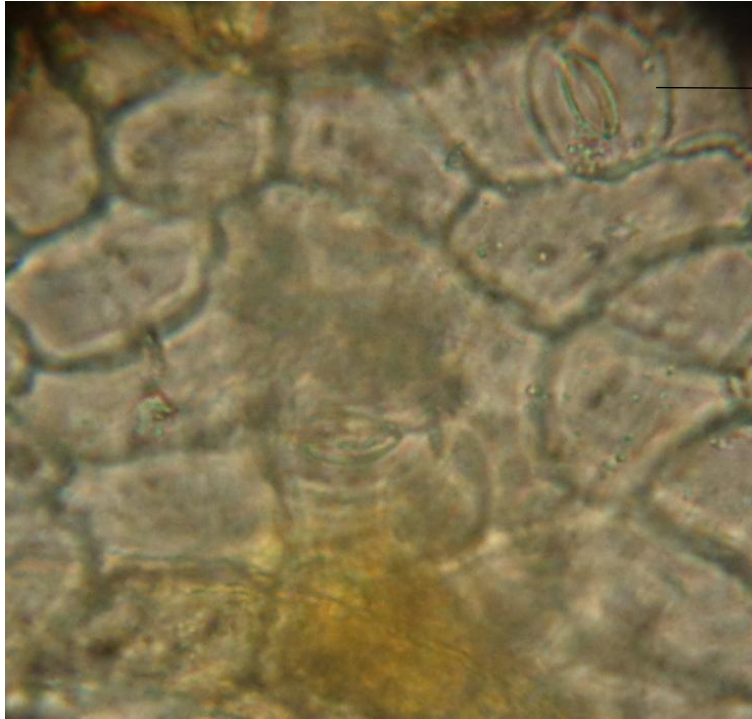
4.1.2 Microscopical Examination of Powdered Sample

The microscopy of the powdered *G. boveanum* revealed the presence of straight-walled polygonal epidermal cells, anisocytic stomatal cells, fragment of fibres, intact unicellular covering trichomes and parenchyma cells. Photomicrographs of some of these features are as presented in plates II–IV.



epidermal cells

Plate II: Photomicrograph of Cleared Powdered *G. boveanum*, Showing polygonal epidermal cells.
(Mag.x100)



Anisocytic stomata

Plate III: Photomicrograph of Cleared Powdered *G. boveanum* Showing anisocytic stomata. (Mag.x400)

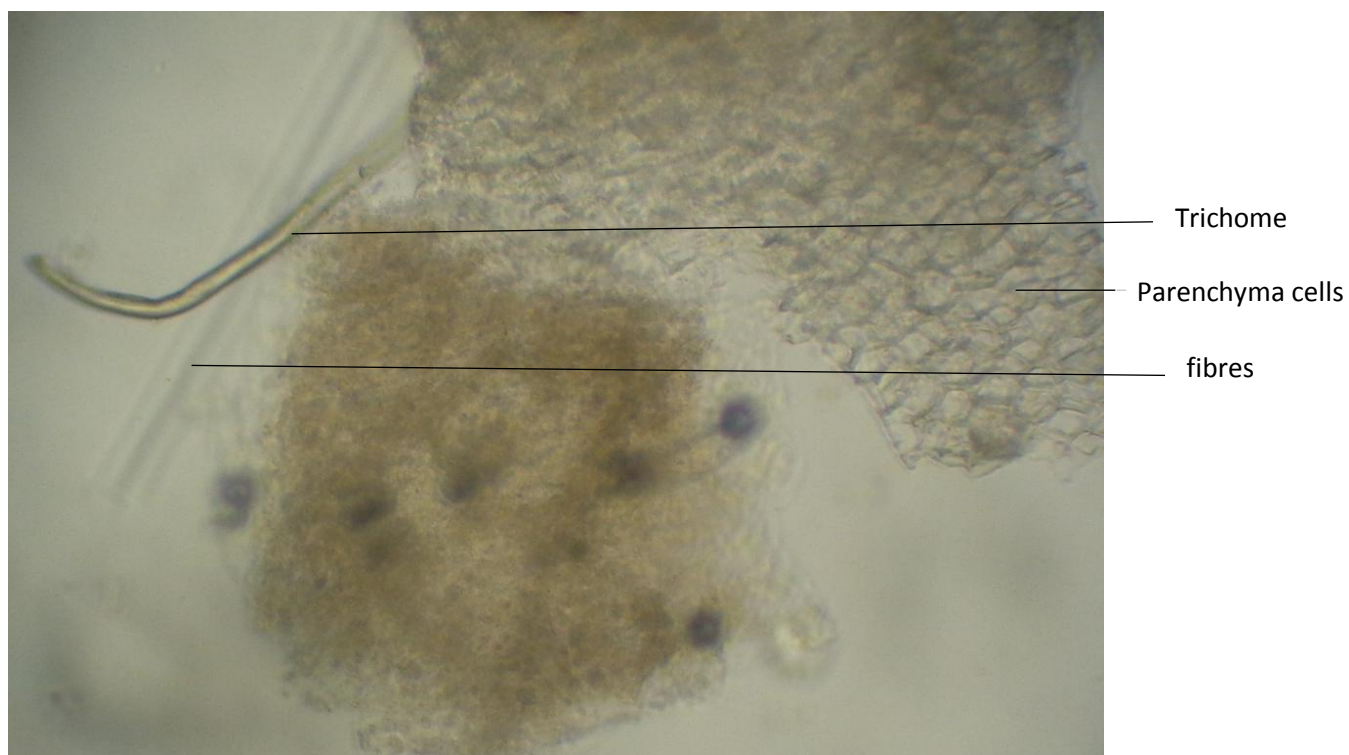


Plate IV: Photomicrograph of Cleared Powdered *G. boveanum* Showing covering trichomes, Parenchyma cells, and fibres. (Mag.x100)

4.1.2.1 Chemomicroscopical Studies of Powdered *G. boveanum*

The result of chemomicroscopic studies carried out on powdered whole plant of *G. boveanum* is as tabulated on table 4.1

i) Cell wall Materials

- a) **Cellulose cell walls:** Blue colour was observed around the epidermal cells indicating the presence of cellulose cell wall.
- b) **Lignified cell walls:** Cherry pink colour was observed indicating the presence of lignins around the xylem vessels.

c) **Suberised cell walls:** Orange-red colour was observed around the epidermal cells indicating the presence of suberised cell walls.

ii) Cell Content

a) **Calcium carbonates:** Crystals of calcium carbonates incrustated within the trichomes were observed to dissolve with effervescence.

b) **Aleurone grains:** Yellowish brown globules within the endospermic cells were observed.

Table 4.1: Chemo microscopic features of *G. boveanum* Powdered whole Plant

Constituent	Observation	Inference
Cellulose	blue colouration of cell walls of the epidermis	+
Lignin	cherry-pink colour around the xylem vessels	+
Suberins	orange-red colour around the epidermal cells	+
Calcium carbonate	Crystals dissolve with effervescence	+

Aleurone grains yellowish brown colour +
 in the endosperm cells

+ sign indicates the presence of constituent

4.1.3 Physicochemical parameters of the Powdered whole plant of *Glossonema boveanum*

The physicochemical constants of powdered *G. boveanum* whole plant determined are 18.33% as the total ash, 8.16% the acid insoluble ash, 2.53% the water soluble ash, 3.29% the alcohol soluble extractive, 9.33% the water soluble extractive and the percentage moisture content as 13.99%. All these values are as shown on table 4.2.

Table 4.2: Physicochemical Parameters of Powdered *G. boveanum* in Percentages

Physicochemical parameter	Values (%w/w)*
Total Ash Value	18.33±0.16
Acid Insoluble Ash Value	8.16±0.08
Water Soluble Ash Value	2.53±0.03
Alcohol soluble extractive	3.29±0.04
Water soluble extractives	9.33±0.08
Moisture Content (loss on Drying)	13.99±0.01

*Average of three determinations

4.2 Phytochemical Studies on aqueous ethanol extract of *G. boveanum*

This section constitutes the results of extract and its fractions preparation, TLC, column chromatography, isolation and the results for structural characterization of the isolated compounds.

4.2.1 Extraction Yield of *G. boveanum* whole plant with 70% ethanol

The extract obtained is solid, dark brown, gummy, having a weight of 119g and percentage yield of 17%^w/_w as compared with the weight of powdered plant material used (700g).

4.2.2 Extraction Yield of the Various Fractions of Ethanol Extract

The results of solvent-solvent partitioning of aqueous ethanol extract of are as shown on table 4.3.

Table 4.3 Fractions of aqueous ethanol extract of *G. boveanum* whole plant

Fraction	Nature of the fraction	weight(g)	%yield *
n-Hexane	gummy dark green solid	15.6	19.5
Chloroform	gummy dark green solid	2.08	2.60
Ethylacetate	gummy dark brown solid	1.32	1.65
n-butanol	gummy reddish-brown solid	11	13.75

Aqueous	gummy reddish-brown solid	31	38.75
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* percentage yield of the various solvent fractions

4.2.3 Preliminary Phytochemical Studies on *G. boveanum* by Thin Layer Chromatography

In this section, Tab.4.4 shows the result of phytochemical screening carried out using specific detecting reagents base on the chromatograms in the appendix G, while the various chromatographic profiles of the crude extract and its different solvent fractions are presented as the chromatograms on plate V- VII.

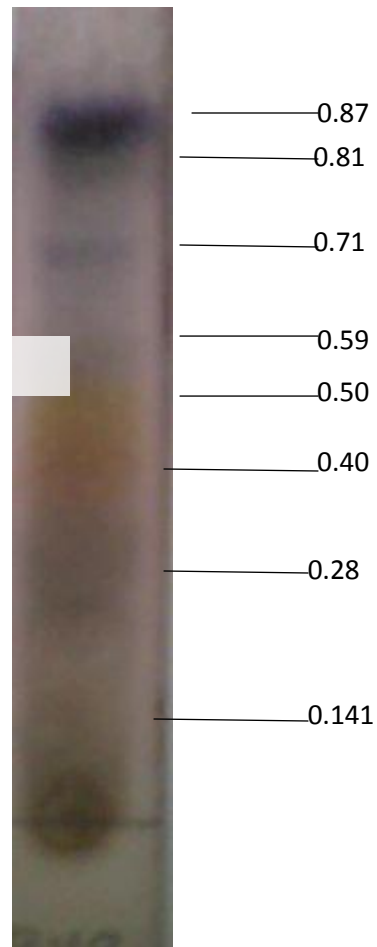


Plate V. The chromatogram of aqueous ethanol extract of *G. boveanum* developed at room temperature (26^oC) using n-butanol/acetic acid/water (6:1:1) solvent system and detected using p-anisaldehyde/sulphuric acid and heated at 110^oC.

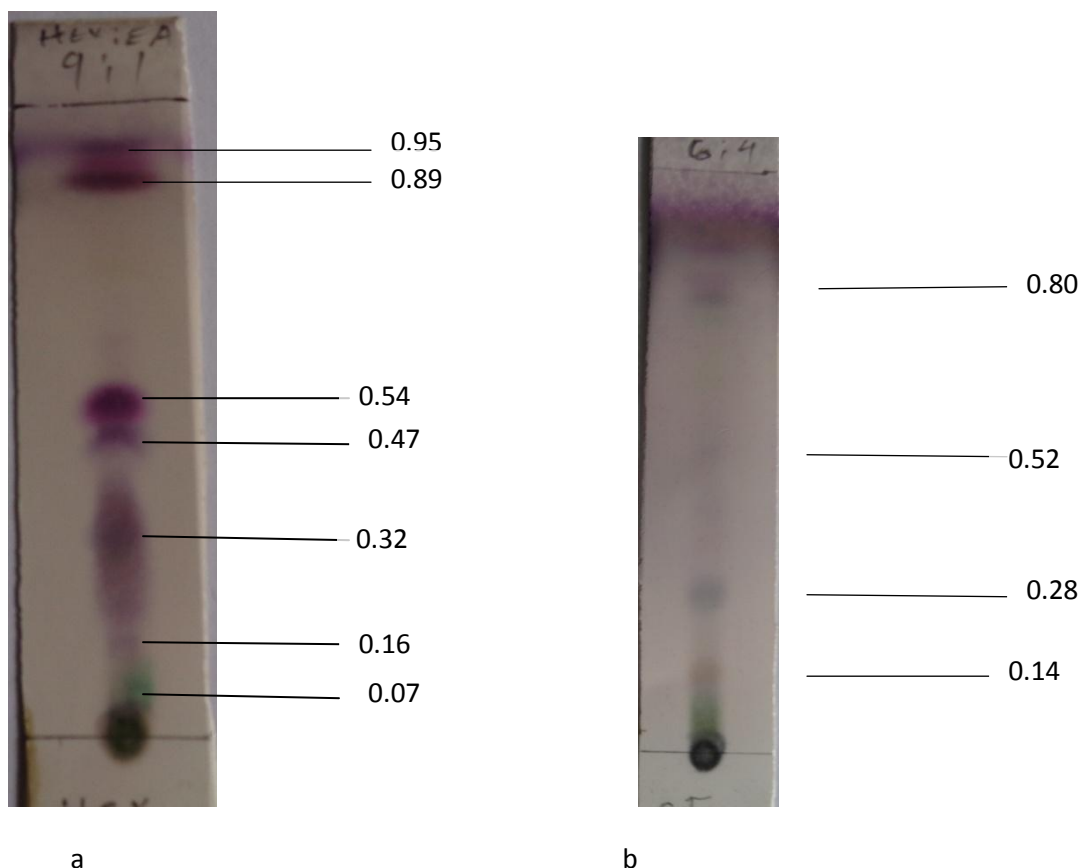


Plate VI. a and b

a The chromatogram of n-hexane fraction developed at room temperature (26°C) using hexane/Ethylacetate(9:1) solvent system and detected using p-anisaldehyde/sulphuric acid after heating to 110°C

b The chromatogram of chloroform fraction developed at room temperature (26°C) using hexane/ ethyl acetate (6:4) solvent system and detected using p-anisaldehyde/sulphuric acid after heating to 110°C

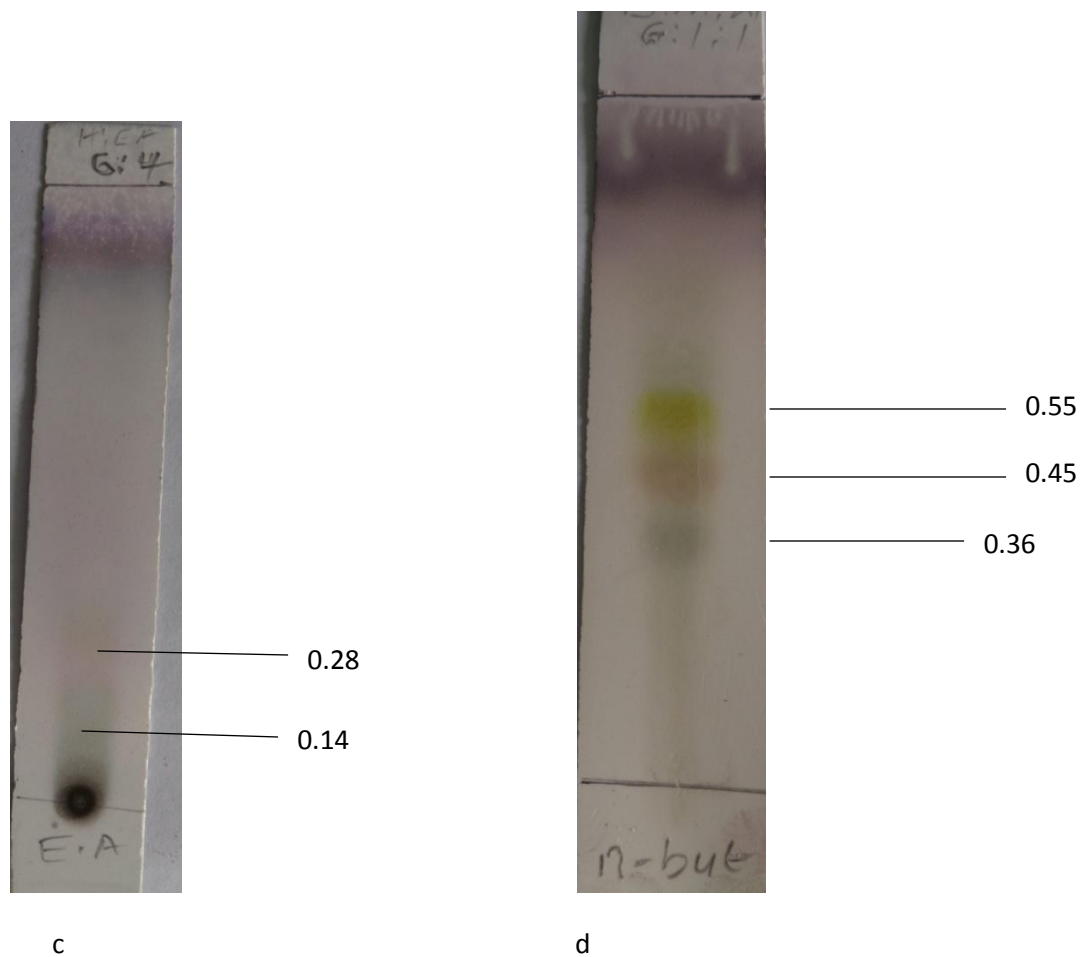


Plate VII. c and d

c The chromatogram of Ethylacetate fraction developed at room temperature (26°C) using hex/Ethylacetate (6:4) and detected using p-anisaldehyde/sulphuric acid after heating to 110°C.

d The chromatogram of n-butanol fraction developed at room temperature (26°C) using n-butanol/acetic acid/water (6:1:1) and detected using p-anisaldehyde/sulphuric acid after heating to 110°C

Table 4.4 Phytochemical Screening of the Various fractions of *G. boveanum* Aqueous

Ethanol Extract

Detecting Reagent	Fraction of the Extract	Observation	Inference
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Liebermann Buchard	n-Hexane	Dark brown spots	Steroids and triterpenes present
	Chloroform	Dark brown spots	Steroids and triterpenes present
	Ethyl acetate	-	-
	n- butanol	Dark yellow and brown spots	Steroids and triterpenes present
Ferric chloride	n-Hexane	Blue-black spots	-
	Chloroform	-	-
	Ethylacetate	Blue- black spots	Phenolics present
ALCL ₃ under UV	n-butanol	Blue- black spots	Phenolics present
	n-Hexane	-	-
Borntragger's reagent	n-butanol	Yellow fluorescence	Flavonoids present
	Ethylacetate	Yellow fluorescence	Flavonoids present
	n-hexane	-	-
	Chloroform	-	-
Dragendorff's reagent	Ethylacetate	Brown spot	Anthracenes present
	n-butanol	Brown spot	Anthracenes present
	n-Hexane	-	-
	Chloroform	-	-
	Ethyl acetate	-	-
	n-Butanol	-	-

4.3 Column Chromatography of n-Hexane Fraction of ethanol extract of *G. boveanum*

This chromatographic procedure led to separation of 80 column fractions (40ml each), which were concentrated and screened by Thin layer chromatographic technique. Based on the TLC profiles, column fraction CF30 to CF33 having similar profile were pooled together and labelled as CLF1 and column fraction CF48 to CF51 pooled together and labelled as CLF2.

The various column fractions and the solvent polarity at which they were collected are as in Tab.4.5.

Tab.4.5 Column fractions of n-Hexane Fraction of *G. boveanum* ethanol extract

S/N	Column fraction	Eluting solvent (HEX/CF)
1.	CF (1 – 5)	100: 00
2.	CF (6 –15)	95: 50
3.	CF (16 – 25)	90 : 10
4.	CF (26 –35)	85 : 15
5.	CF (36 – 45)	80 : 20
6.	CF (46 – 60)	70 :30
7.	CF (61 – 70)	60: 40
8.	CF (71 – 80)	50: 50

4.3.1 Chromatograms of the Column fractions of the n-hexane Portion of *G. boveanum*

Ethanol Extract

The TLC profile of the column fractions from which the major spots were identified, the fractions were separately pooled together and coded as CLF1 and CLF2 are as shown in the chromatograms on plate VIII.

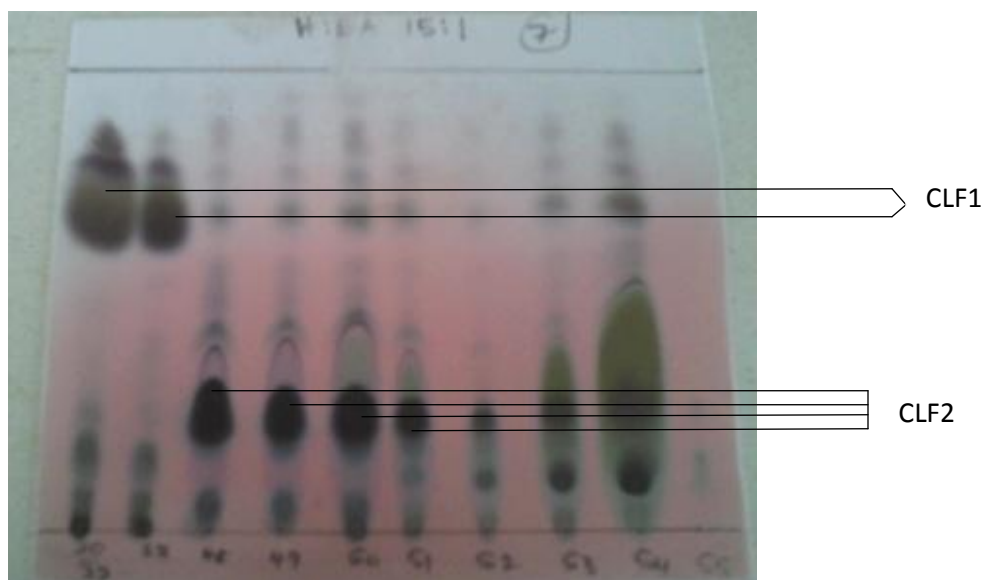


Plate VIII. Chromatograms of Column Fraction (CF₃₀-CF₅₄).

Developed with hexane/ethylacetate mixture (15:1)

4.3.2 The Chromatogram of CLF1 Column fractions of *G. boveanum*

The TLC profile of CLF1 column fractions from which the major spots were identified and the fractions subsequently pooled together and coded as CLF3 is as shown in the chromatograms on plate IX.

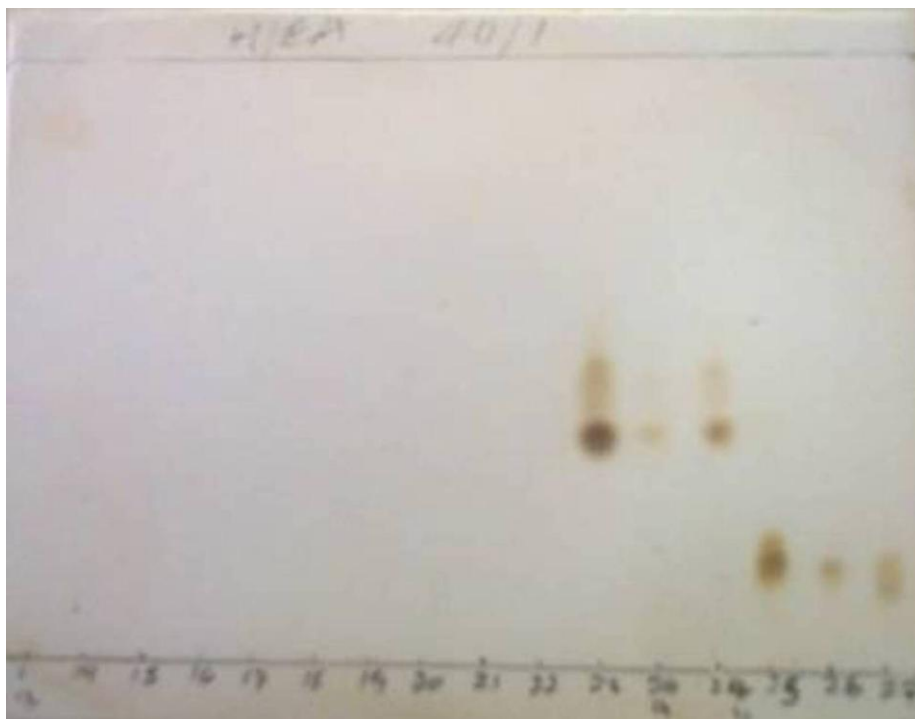


Plate IX. Chromatogram of CLF1 Column fractions (cf1-cf30) of *G. boveanum*

Developed with hexane ethyl acetate mixture (40:1)

4.3.3 Isolation of compound SAL₁ from CLF3 of *G. boveanum* by Preparative TLC

After the column fraction CLF3 was subjected to preparative thin layer chromatography, compound SAL₁ was isolated. This is as shown in the chromatogram on plate X.

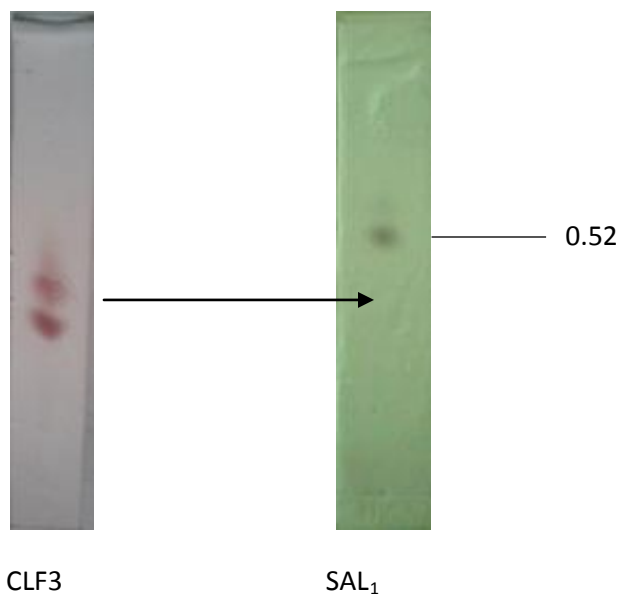


Plate X. Isolation of compound SAL₁ from CLF3 of *G. boveanum* by Preparative TLC

CLF3 Solvent system : Hexane/ ethylacetate 50:1, detection with 10% sulphuric acid

SAL₁ Sovent sytem: Hexane/ ethylacetate 40:1, detection with 10% sulphuric acid

4.3.4 Chromatogram of CLF2 Column fractions of *G. boveanum*

The TLC profile of CLF2 column fractions from which the major spots were identified and the fractions subsequently pooled together and coded as CLF4 is as shown in the chromatograms on plate XI.

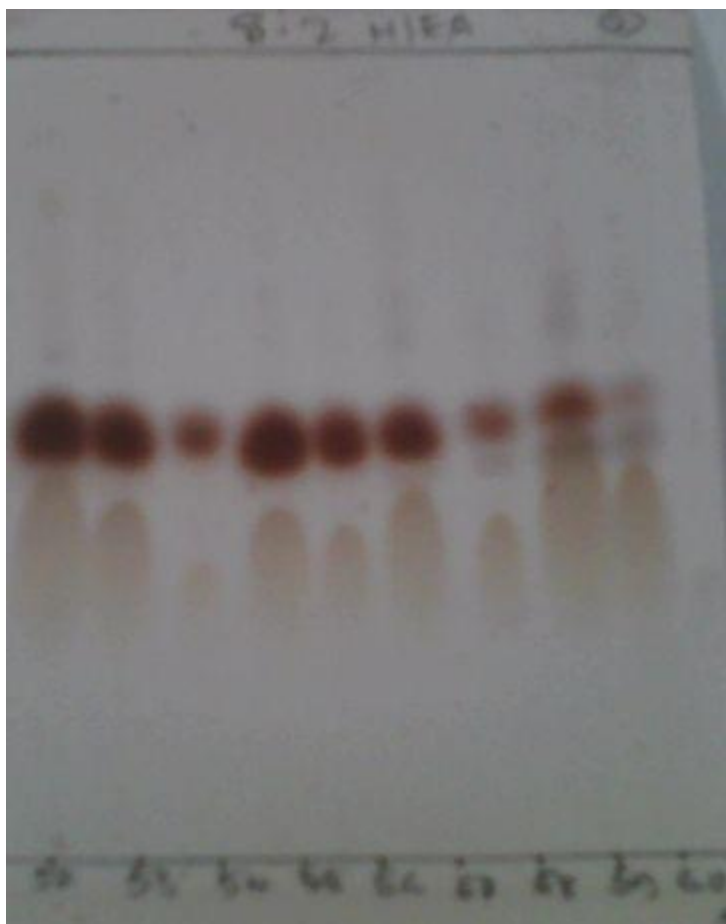


Plate X. Chromatogram of CLF2 fractions (cf52-cf57) of *G. boveanum*

Developed with hexane ethylacetate mixture (8:2)

4.3.5 Isolation of compound SAL₂ from CLF4 of *G. boveanum* by Preparative TLC

After the column fraction CLF4 was subjected to preparative thin layer chromatography, compound SAL₂ was isolated. This is as shown in the chromatograms on plate XII.

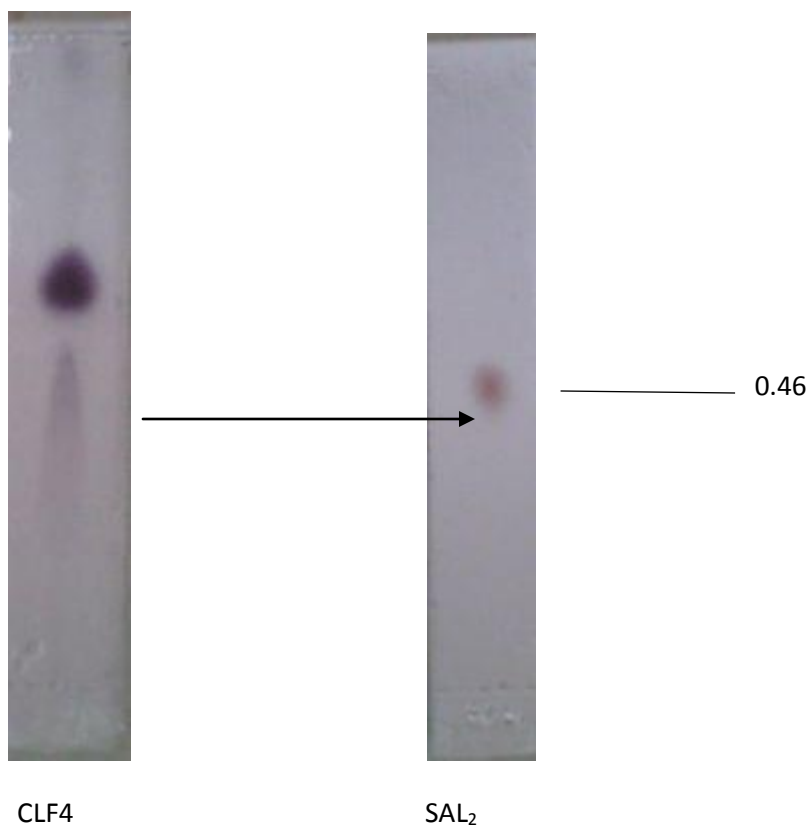


Plate XII. Isolation of compound SAL₂ from CLF₄ of *G. boveanum* by Preparative TLC

CLF₄ Solvent system: Hexane/ethylacetate 8:2, detection with 10% sulphuric acid

SAL₂ Solvent system: Hexane/ ethylacetate 9:1, detection with 10% sulphuric acid

4.3.6 Physical Properties of SAL₁ and SAL₂ from *G. boveanum*

Compound SAL₁ was obtained as white powder, 22mg weight, with melting point of 223-227⁰C while compound SAL₂ has an amorphous white powder, 26mg weight with melting point of 210-213⁰C.

4.3.7 Result of the Chemical test

Both compound SAL₁ and SAL₂ reacted positively with Libermann buchard test showing reddish brown ring and purple colouration of the upper layer.

4.4 Nuclear Magnetic Resonance Spectroscopy of SAL₁ and SAL₂ from *G. boveanum*

The results of Nuclear magnetic resonance spectroscopic studies carried out on the isolated compounds SAL₁ and SAL₂ for structure elucidation are as follows.

4.4.1 ¹H NMR spectrum of Compound SAL₁ Isolated from *G. boveanum*

The signals observed in the ¹H NMR spectrum of compound SAL₁ include; δ 5.12 (1H), δ 4.51 (1H,m), δ 2.05 (3H,s). and δ 0.88 (3H,s), 0.79 (3H,s), 1.02 (3H,s), 1.02 (3H,s), 1.00 (3H,s), 1.13 (3H,s), 0.79(3H,s) 0.92 (3H,s). Some overlapping signals were also observed between 0.85 to 1.92 in the spectrum. These observations are as shown in the proton spectrum on figure 3 and the expanded proton spectrum on figure 4. These results are as tabulated on table 4.6

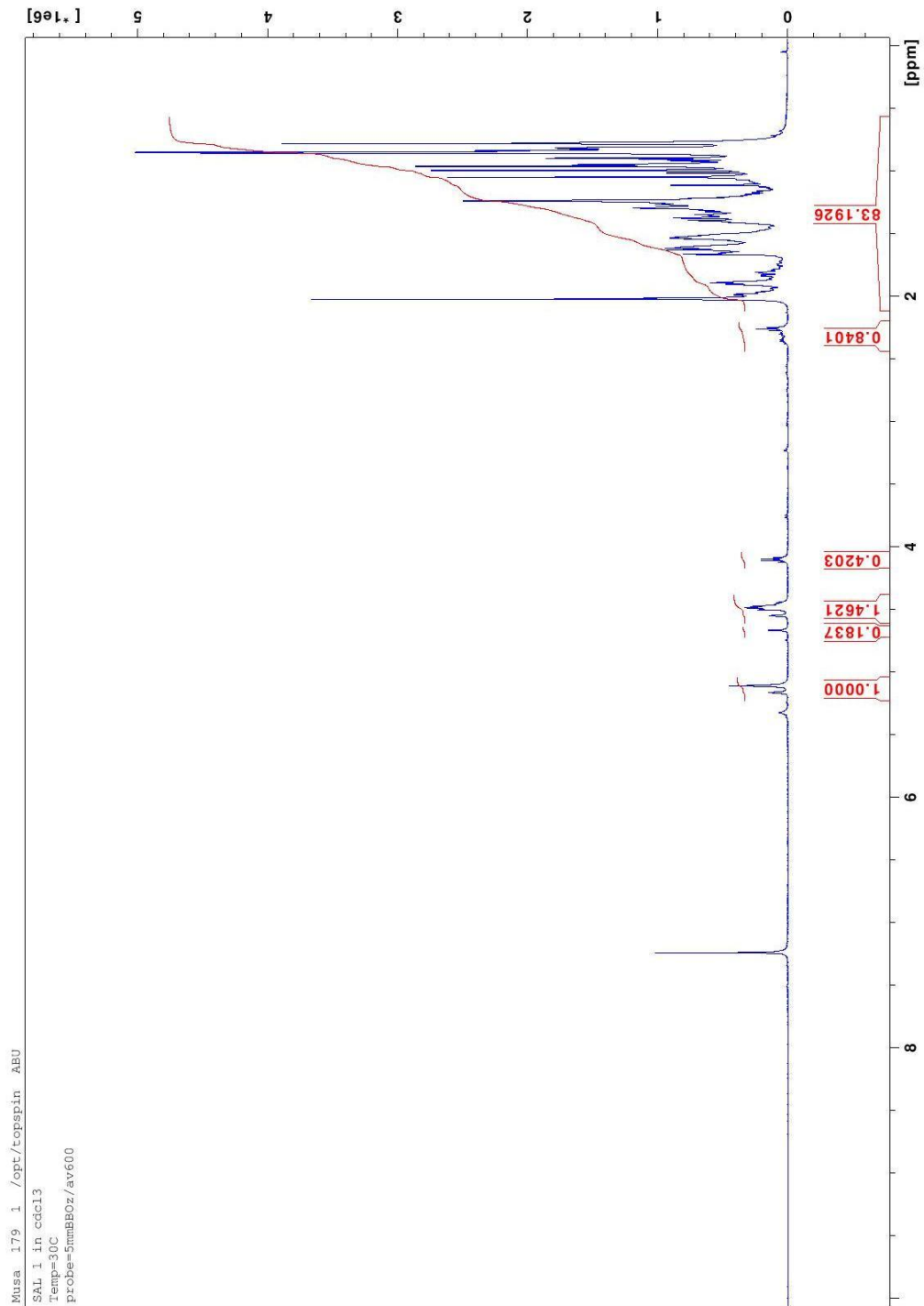


Fig. 3: Proton NMR Spectra of SAL₁ Isolated from *G. boveanum*

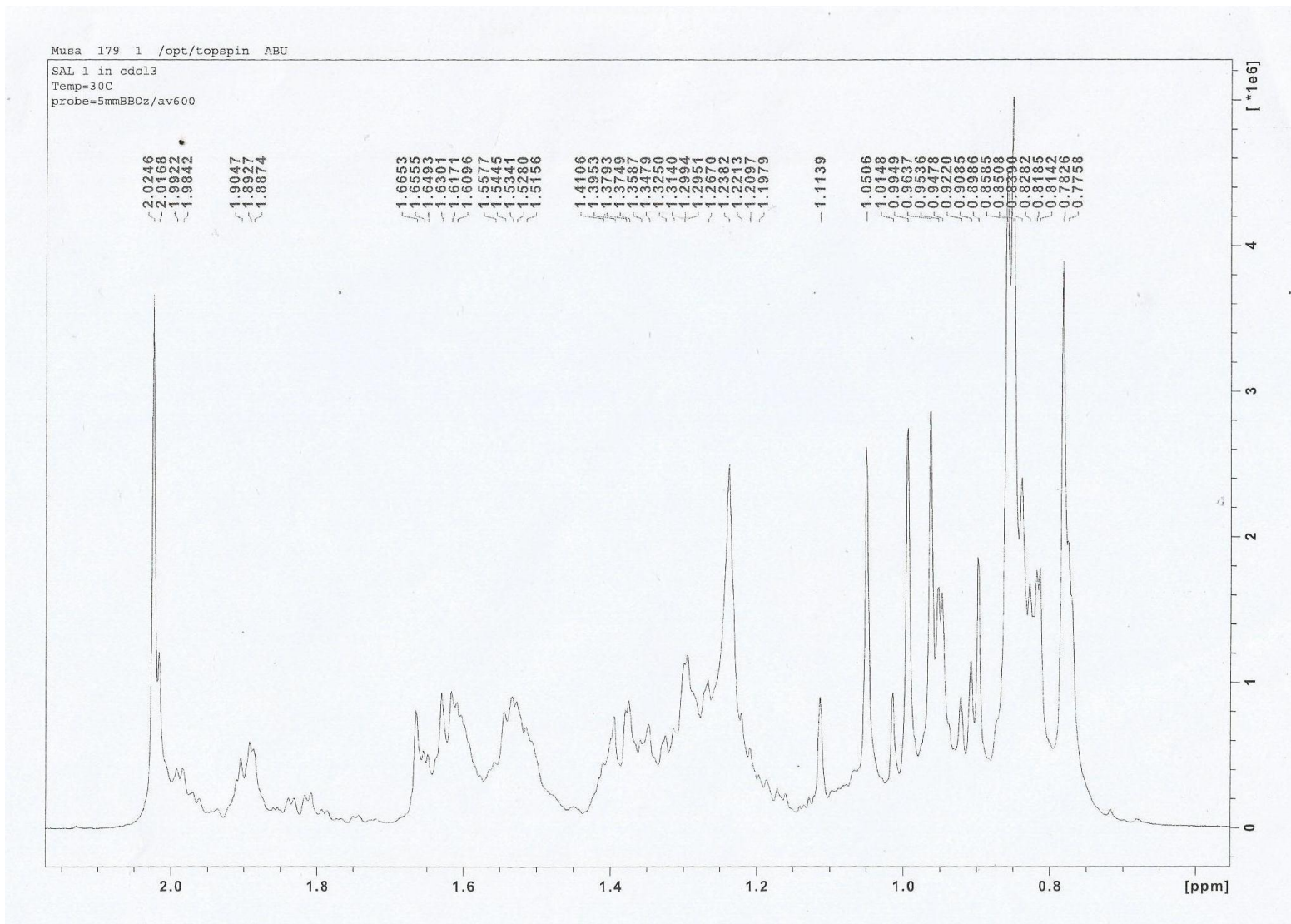


Figure 4. Expanded proton spectrum of Compound SAL₁ Isolated from *G. boveanum*

4.4.2 ¹³C NMR spectrum of Compound SAL₁ Isolated from *G. boveanum*

The ¹³C NMR spectrum showed the carbon resonances of compound SAL₁. Some of the diagnostic signals include; δc 170.86, δc 81.66, δc 125.55 and δc 139.65. These are as shown in figure 5.

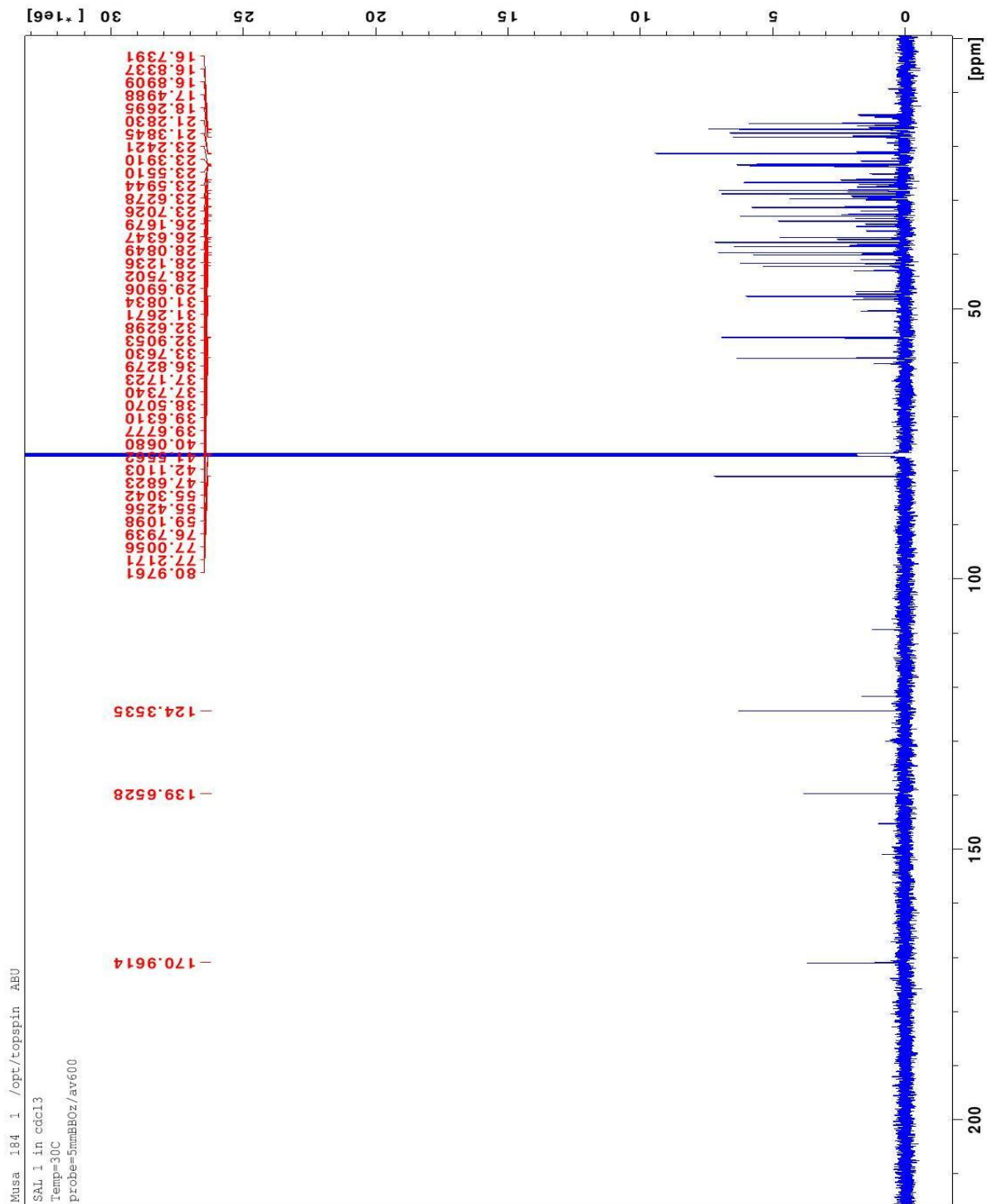


Figure 5. ^{13}C -NMR Spectra of Compound SAL₁ Isolated from *G. boveanum*

4.4.3 DEPT spectra of compound SAL₁ from *G. boveanum*

From the DEPT spectra of compound SAL₁ the following type of carbons were observed; 9 methyl, 9 methylene, 7 methine and 7 quaternary carbon atoms in the compound. This is as shown on figure 6 and table 4.6.

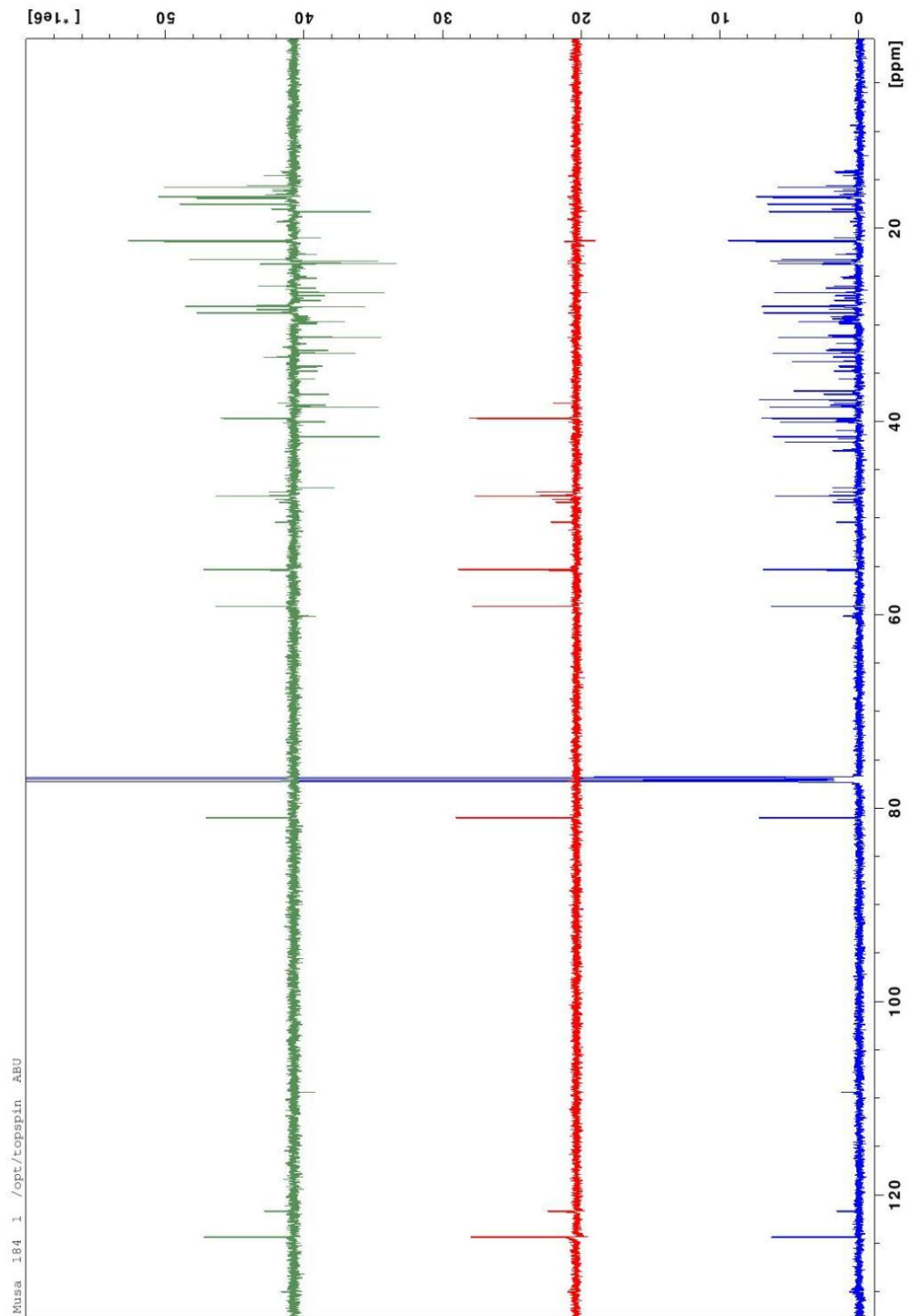


Figure 6. DEPT Spectra of Compound SAL₁ Isolated from *G. boveanum*

4.4.4 COSY Spectrum of compound SAL₁ from *G. boveanum*

In the COSY spectrum, cross peaks were observed between δ H 5.12, and δ H 1.92, and between δ H 4.51 and δ H 1.62. Details and other COSY correlations is as in the figure 7.

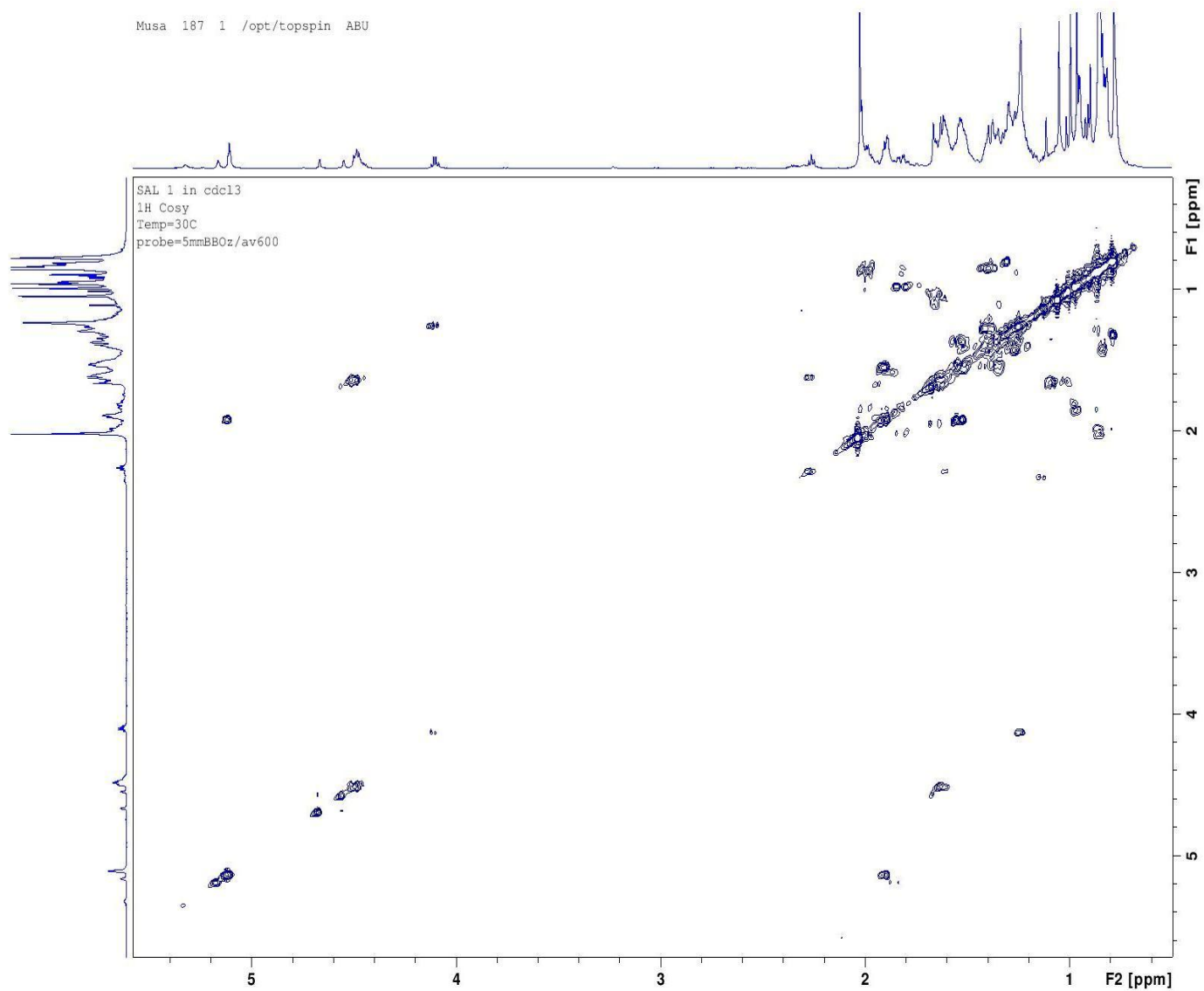


Figure 7. H-H COSY of Compound SAL₁ Isolated from *G. boveanum*

4.4.5 HSQC spectrum of Compound SAL₁ from *G. boveanum*

It showed H-C correlations (ie which proton attached to which carbon) in compound SAL₁ such as between δ_H 5.12 and δ_C 125.55, between δ_H 4.51 and δ_C 81.66, between δ_H 2.05 and δ_C 22.22.

Details are as shown in HSQC spectrum in figure 8 and the expanded HSQC in figure 9. The results are as tabulated on table 4.6.

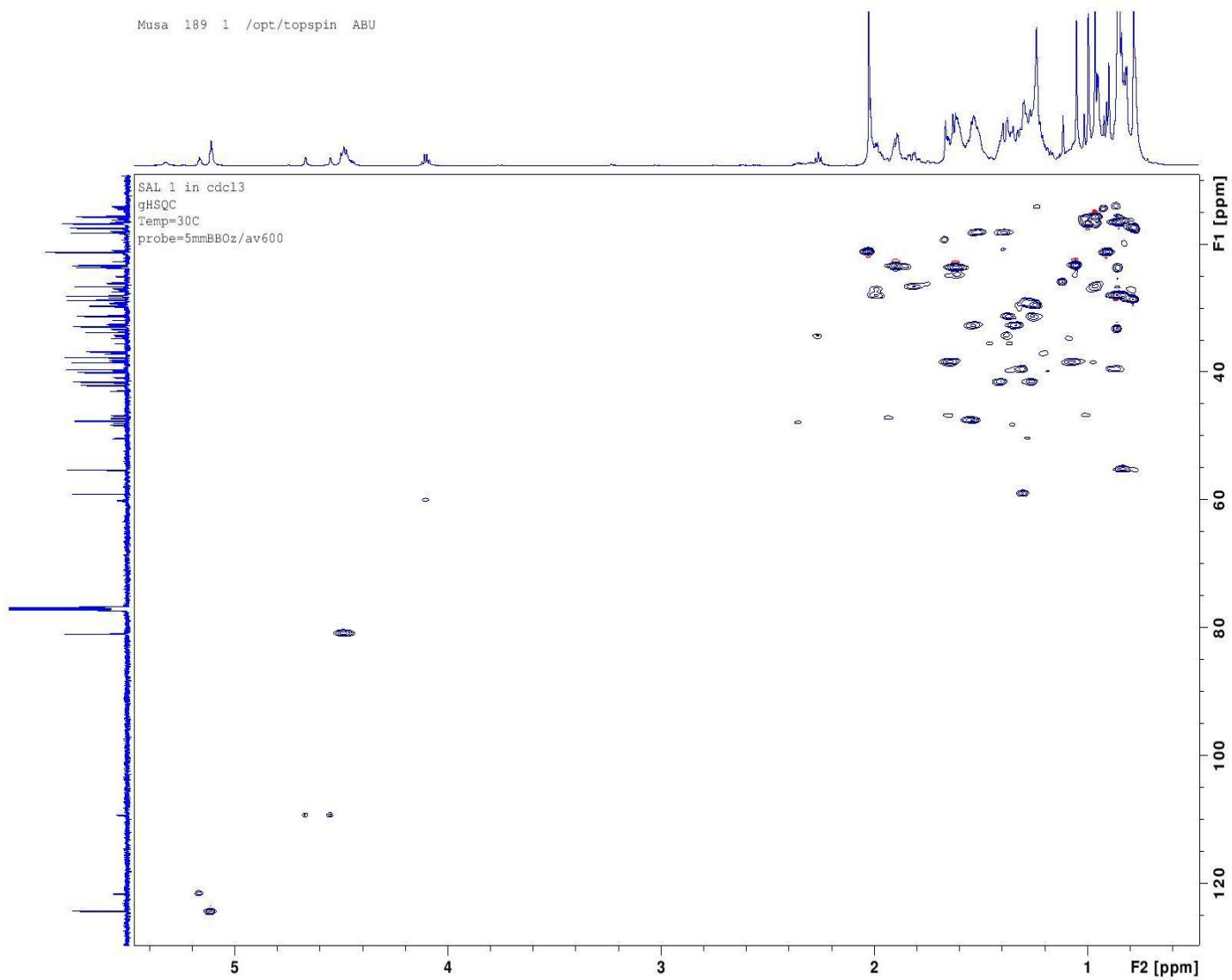


Figure 8. HSQC Spectra of Compound SAL₁ Isolated from *G. boveanum*

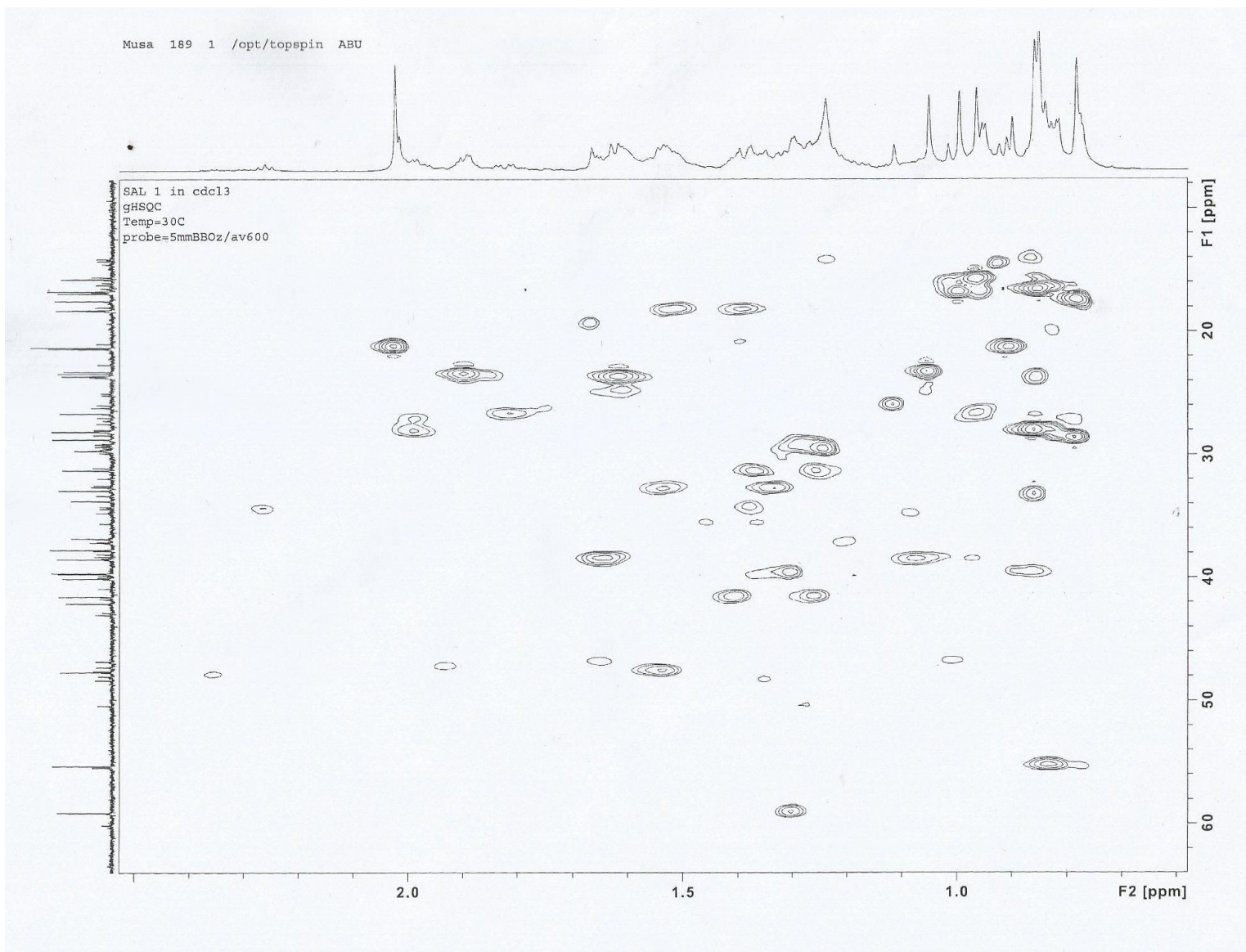


Figure 9. Expanded HSQC spectrum of Compound SAL₁ Isolated from *G. boveanum*

4.4.6 HMBC Spectrum of compound SAL₁ from *G. boveanum*

HMBC shows long range connectivity between different fragments. Cross peaks were observed between signal at δ H 2.05 and δ c 170.96, signal at δ H 4.51 and δ c 15.44, δ c 28.88, signal at δ H 5.12 and δ c 23.61,

δ_c 47.77, and between proton signal δ_H 1.13, and δ_c 139.65. Details and with other correlations are as shown in the HMBC spectrum in figure 10.

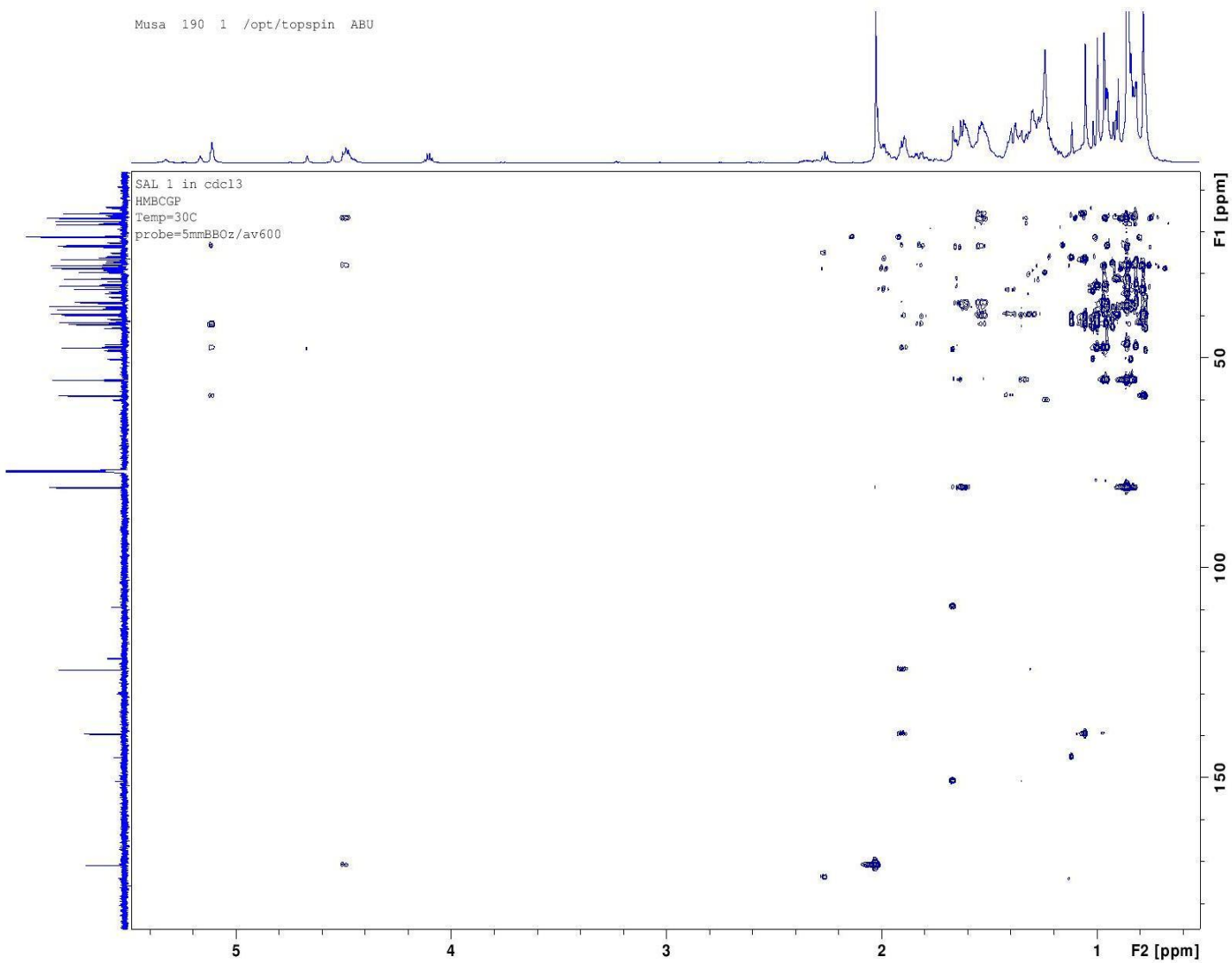


Figure 10. HMBC Spectra of Compound SAL₁ Isolated from *G. boveanum*

4.4.7 Carbon-13 and Proton (¹³C and ¹H) NMR Data of SAL₁ Isolated from *G. boveanum*

Table 4.6 shows the carbon-13 (^{13}C) and proton (^1H) chemical shift values of SAL₁ as deduced from the spectra on figure 3-10.

Tab.4.6 ^{13}C and ^1H Chemical Shift Data, DEPT and HSQC of Compound SAL₁ from

G. boveanum

C	Type of Carbon	^{13}C -shift value(Hz)	^1H -shift value(Hz)
1	CH ₂	38.61	1.06m,1.67m
2	CH ₂	23.64	1.62m
3	CH	81.66	4.51dd
4	C	37.77	-
5	CH	55.55	0.85m
6	CH ₂	18.22	1.50m
7	CH ₂	33.05	0.87
8	C	40.27	-
9	CH	47.77	1.53m
10	C	37.16	-
11	CH ₂	23.61	1.92m
12	CH	125.55	5.12t
13	C	139.65	-
14	C	42.22	-
15	CH ₂	26.66	1.8, 0.98m
16	CH ₂	28.05	1.98m
17	C	33.88	-
18	CH	59.44	1.33m
19	CH	40.00	1.33m
20	CH	40.00	1.33m
21	CH ₂	31.11	1.22, 1.37m
22	CH ₂	41.66	1.40m
23	CH ₃	15.44	0.88s
24	CH ₃	28.88	0.79s
25	CH ₃	16.38	1.02s
26	CH ₃	16.38	1.02s
27	CH ₃	23.05	1.13s
28	CH ₃	17.22	0.79s
29	CH ₃	17.77	1.00s
30	CH ₃	22.22	0.92s
1'	C	170.96	-
2'	CH ₃	22.22	2.05s

Tab. 4.7 Comparison between Chemical shift Data of SAL₁ from *G. boveanum* and that of α -amyrin acetate as obtained from Literature

C	Compound SAL ₁		α-amyrin acetate (A)		α-amyrin acetate(B)	
	¹³ C-shift value(Hz)	¹ H-shift value(Hz)	¹³ C-shift value(Hz)	¹ H-shift value(Hz)	¹³ C-shift value(Hz)	¹ H-shift value(Hz)
1	38.61	1.06m,1.67m	39.4	1.06 m,1.7m	38.5	
2	23.64	1.62m	24.3	1.60 m	23.4	
3	81.66	4.51dd	82.3	4.47 dd	80.9	4.50 dd
4	37.77	-	38.4	-	37.7	-
5	55.55	0.85 m	56.4	0.87 m	55.3	
6	18.22	1.50 m	19.4	1.50 m	18.3	
7	33.05	0.87	33.7		32.9	
8	40.27	-	41.0	-	39.7	-
9	47.77	1.53m	48.8	1.62 m	47.7	
10	37.16	-	37.7	-	36.8	-
11	23.61	1.92m	24.2	1.95 m, 1.35 m	22.8	
12	125.55	5.12t	125.5	5.17 t	124.2	5.12 t
13	139.65	-	141.1	-	139.5	-
14	42.22	-	42.9	-	42.1	-
15	26.66	1.8, 0.98m	27.3	1.63 m, 1.03m	28.2	
16	28.05	1.98m	28.9	2.06 m	26.7	
17	33.88	-	34.5	-	33.8	-
18	59.44	1.33m	60.3	1.36 m	59.0	
19	40.00	1.33m	40.6	1.36 m	39.7	
20	40.00	1.33m	40.6		39.7	
21	31.11	1.22, 1.37m	32	0.80 m	31.3	
22	41.66	1.40m	42.3	1.39 m	41.6	
23	15.44	0.88 s	28.4	0.89 s	28.1	0.88 s
24	28.88	0.79 s	16.9	0.90 s	15.8	0.88 s
25	16.38	1.02s	16.0	1.03 s	14.2	
26	16.38	1.02s	17.2	1.06 s	16.8	
27	23.05	1.13 s	23.3	1.13 s	17.6	
28	17.22	0.79s	29.0	0.82 s	28.8	0.79 s
29	17.77	1.00s	17.8	0.83 s	23.3	0.88 s
30	22.22	0.92s	21.5	0.93 s	21.4	0.88 s
3/1 ¹	170.96	-	172.7	-	170.8	-
3/2 ¹	22.22	2.05s	20.8	2.03 s	21.5	2.05 s

A=Rhourri-Frih *et. al* 2013, B=Niaz, 2013

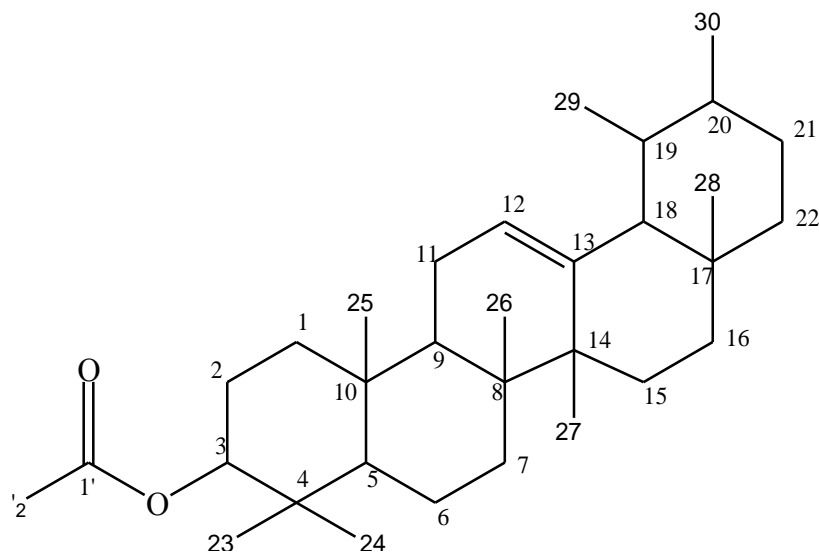


Figure 11: Proposed Chemical Structure of Compound SAL₁ (α -amyrin acetate) Isolated from *G. boveanum*

4.4.8 ¹H NMR of Compound SAL₂ Isolated from *G. boveanum*

The signals observed in the ¹H NMR spectrum of compound SAL₂ include; doublets of 2 protons each at δ 4.56 and δ 4.67 (1H), H δ 3.19 (1H,m), δ H 2.36 (1H m) and δ 0.99 (3H s), 0.75(3H s), 0.80 (3H s), 1.02 (3H s) 0.91 (3H s), 0.75(3H s) and 1.68 (3H s). Some overlapping signals were also observed between 0.93 and 1.45 in the spectrum. These observations are as shown in the proton spectrum in figure 12 and the expanded proton spectrum on figure 13. These results are as tabulated in table 4.8

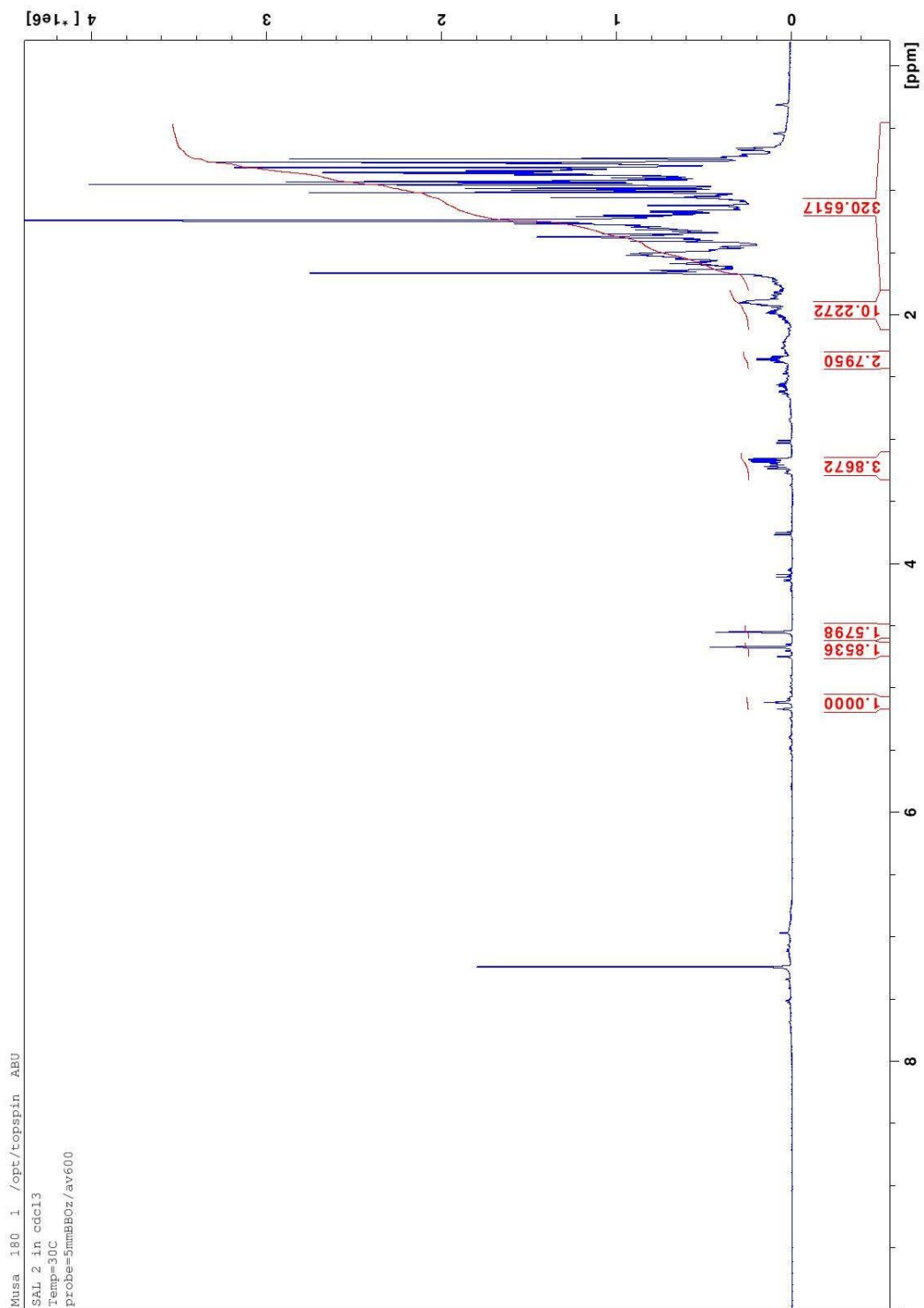


Figure 12. Proton NMR Spectra of Compound SAL₂ Isolated from *G. boveanum*

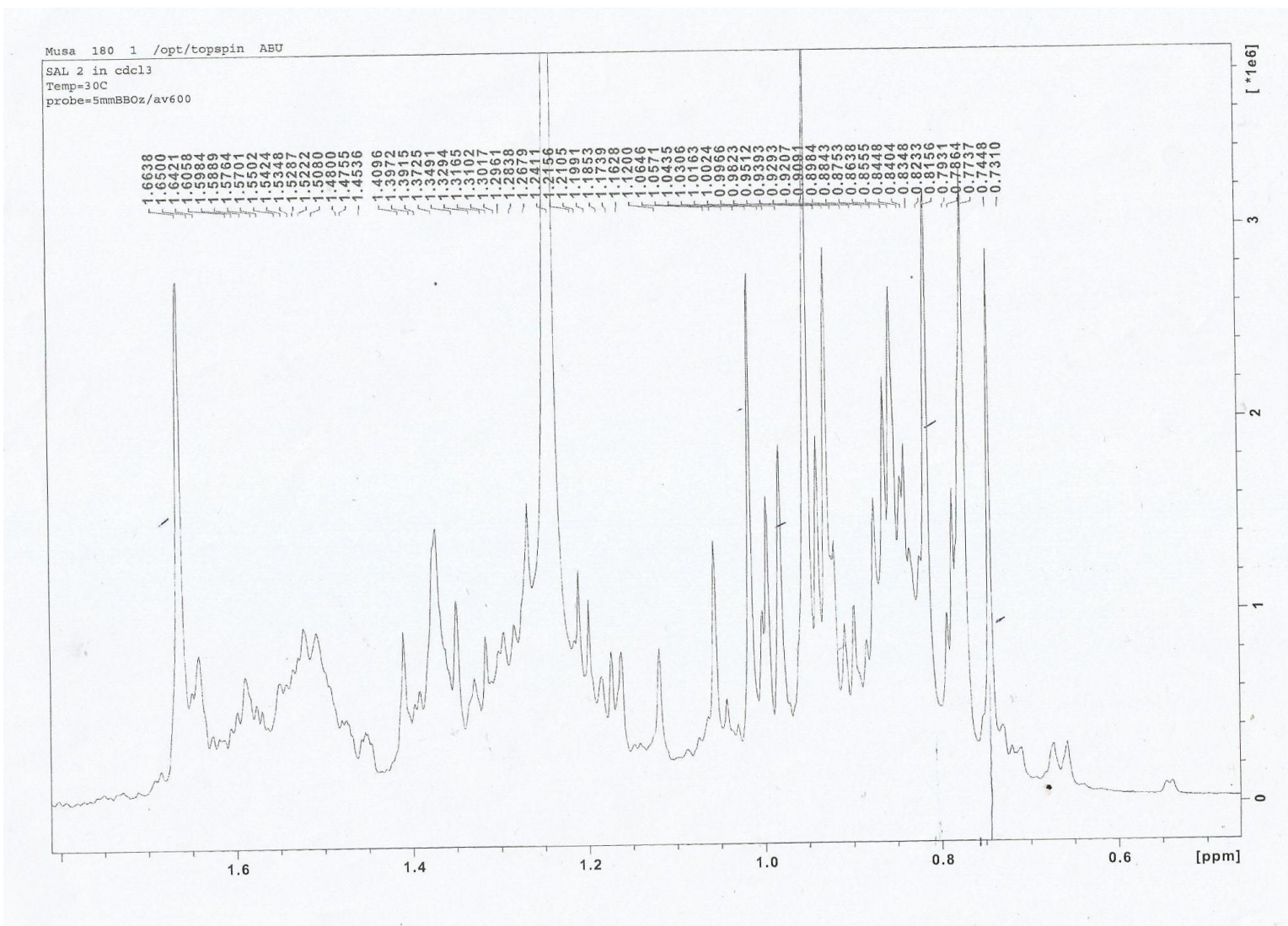


Figure 13. The expanded Proton NMR Spectra of Compound SAL₂ Isolated from *G. boveanum*

4.4.9 ¹³C NMR Spectrum of Compound SAL₂ Isolated from *G. boveanum*

The ¹³C NMR spectrum showed the carbon resonances of compound SAL₂. Some of the diagnostic signals include; δc 150.87, δc 109.47 and carbon signal at δc 79.29. Detail is as shown on figure 14.

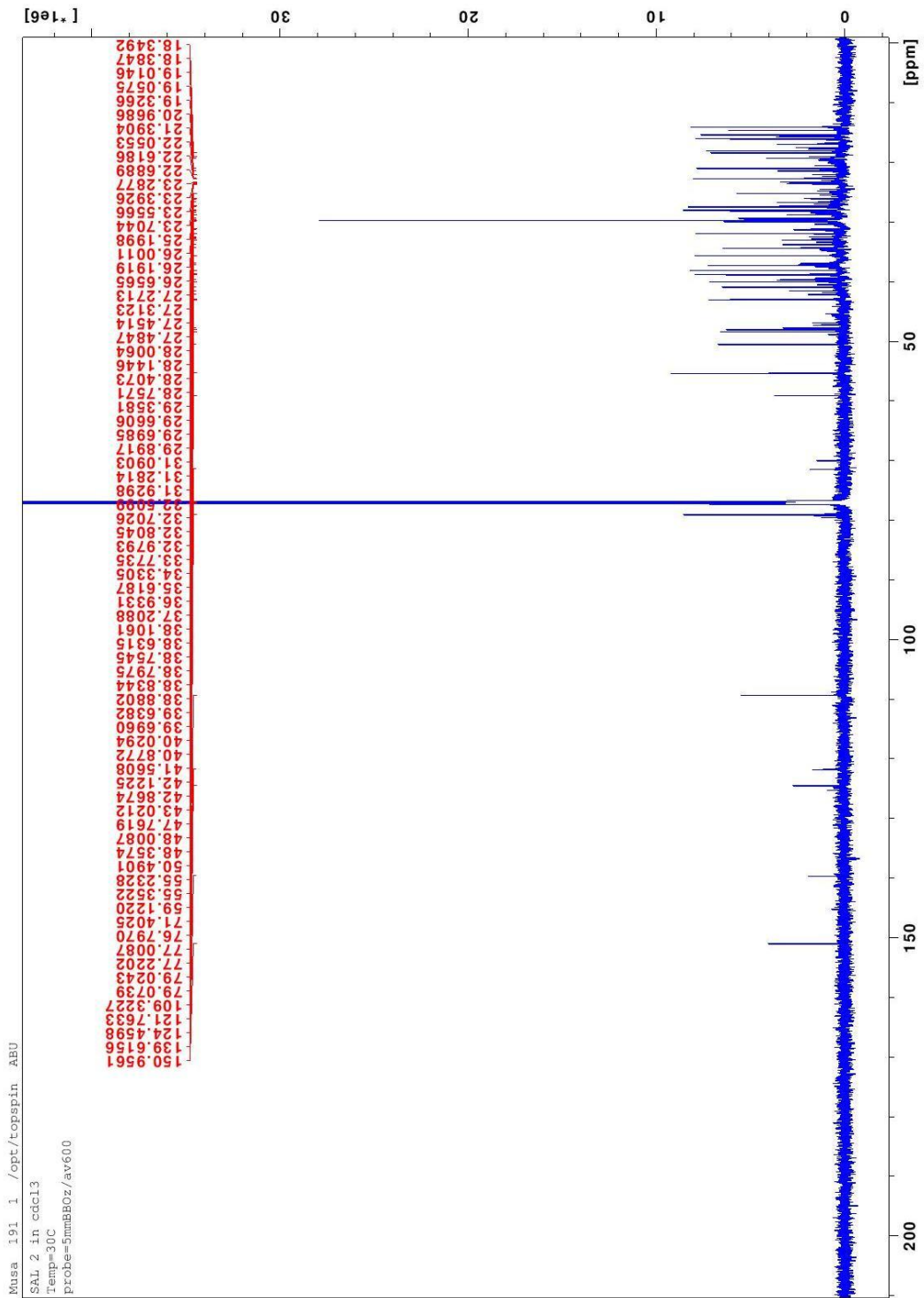


Figure 14. ^{13}C - NMR Spectra of Compound SAL₂ Isolated from *G. boveanum*

4.4.10 DEPT Spectra of Compound SAL₂ Isolated from *G. boveanum*

From the DEPT spectra of compound SAL₂ the following type of carbons were observed; 7 methyl, 11 methylene, 6 methine and 6 quaternary carbon atoms in the compound. This is as shown on figure 15 and table 4.8.

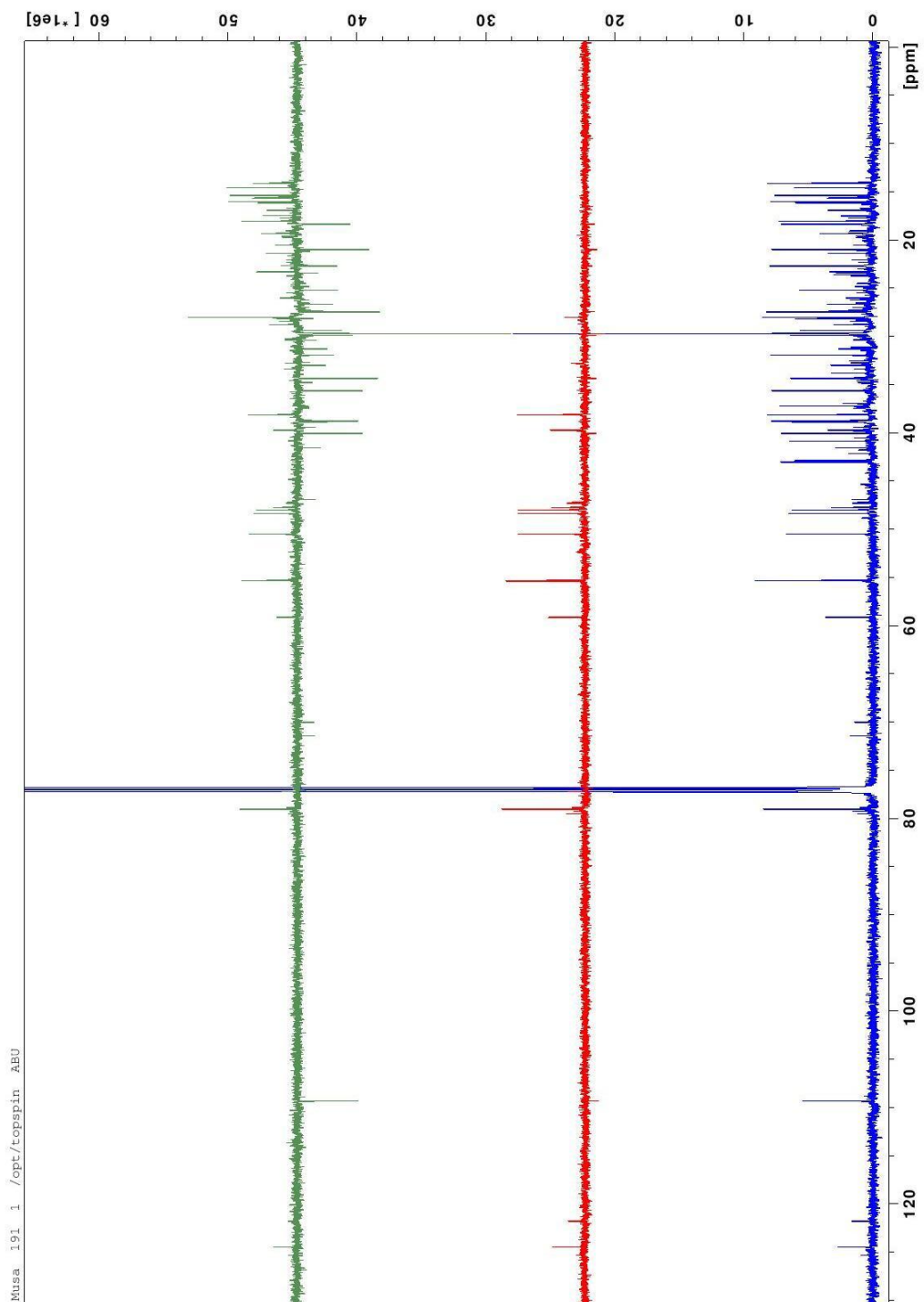


Figure 15. DEPT Spectra of Compound SAL₂ Isolated from *G. boveanum*

4.4.11 COSY Spectrum of Compound SAL₂ Isolated from *G. boveanum*

In the COSY spectrum, cross peaks were observed between those between δ H 2.36, and δ H 1.28, δ H 1.38, Between proton signal δ H 3.19, and δ H 1.60, There also exist a cross peak between δ H 4.67, and δ H 4.56. Details of other COSY correlations are as shown in the figure 16.

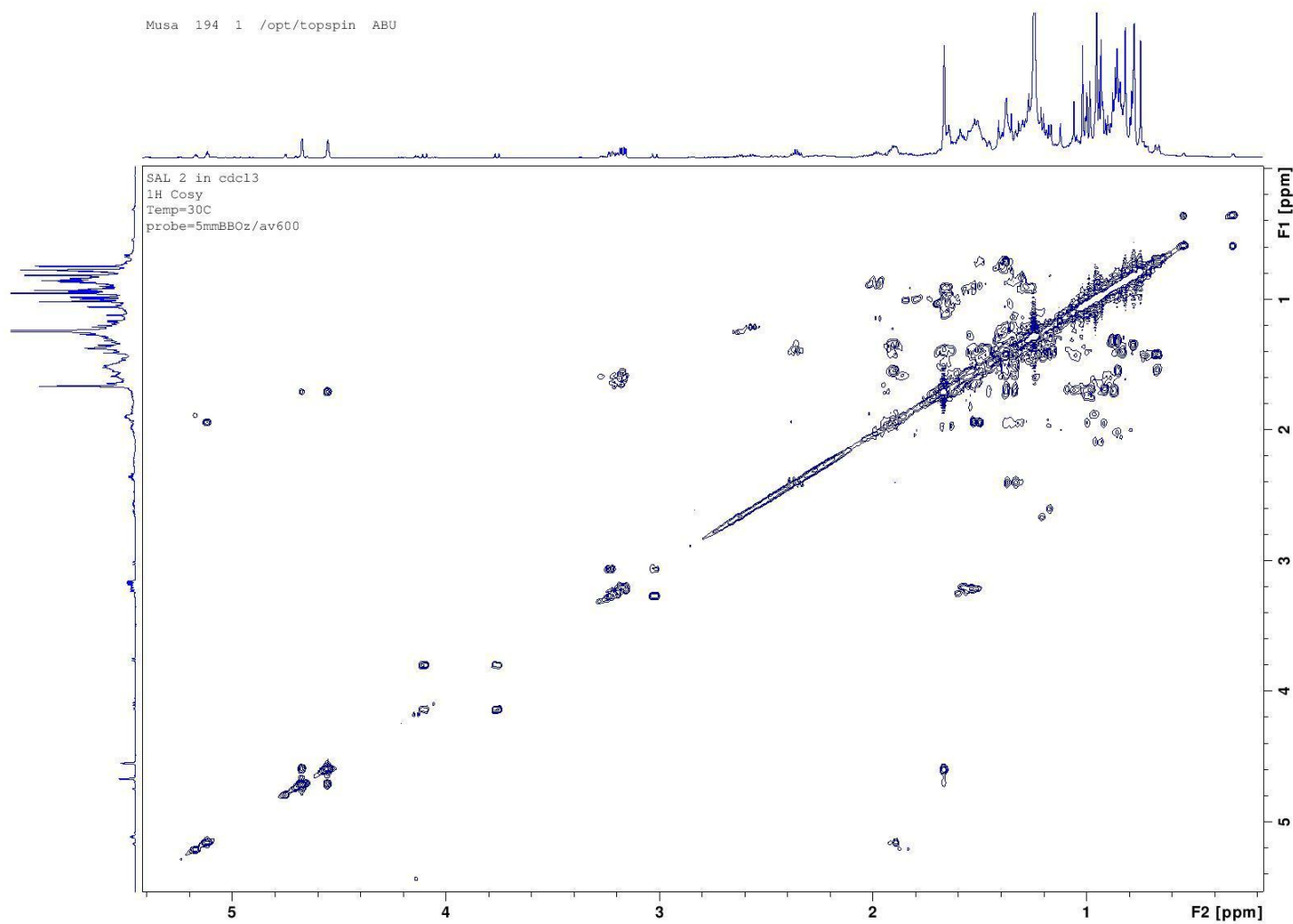


Figure 16. H-H COSY of Compound SAL₂ Isolated from *G. boveanum*

4.4.12 HSQC Spectrum of Compound SAL₂ Isolated from *G. boveanum*

The HSQC spectrum showed H-C correlations (ie which proton is attached to which carbon) in compound SAL₂ such as between δ H 4.67, 4.56 and δ c 109.47, δ H 3.19 and δ c 79.29, (δ H 2.36 and δ c

48.10), (δ_H 1.17 and δ_C 40.5). Details are as shown in HSQC spectrum in figure 17 and the expanded HSQC in figure 18. The results are as tabulated on table 4.8.

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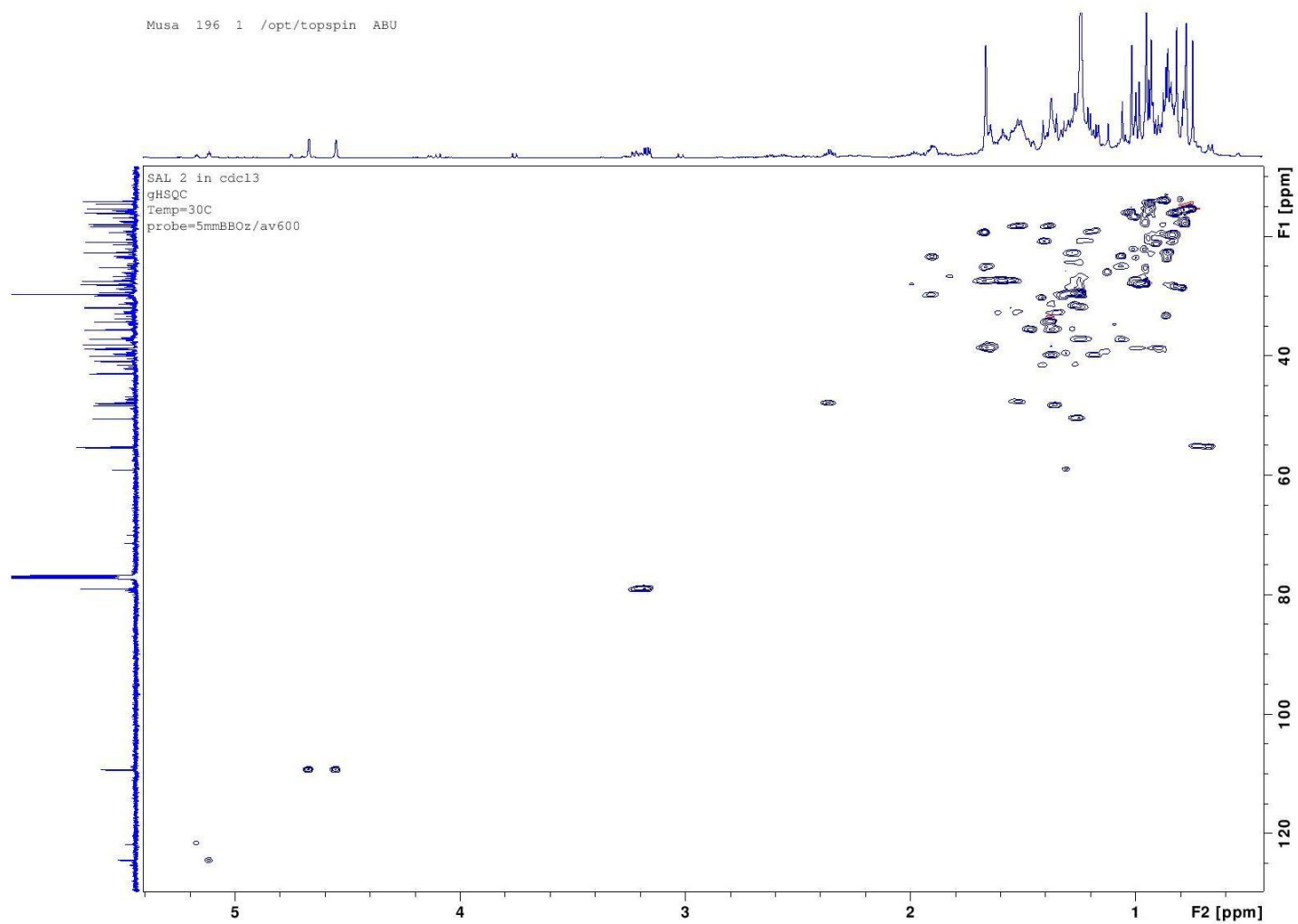


Figure 17. HSQC Spectra of Compound SAL₂ Isolated from *G. boveanum*

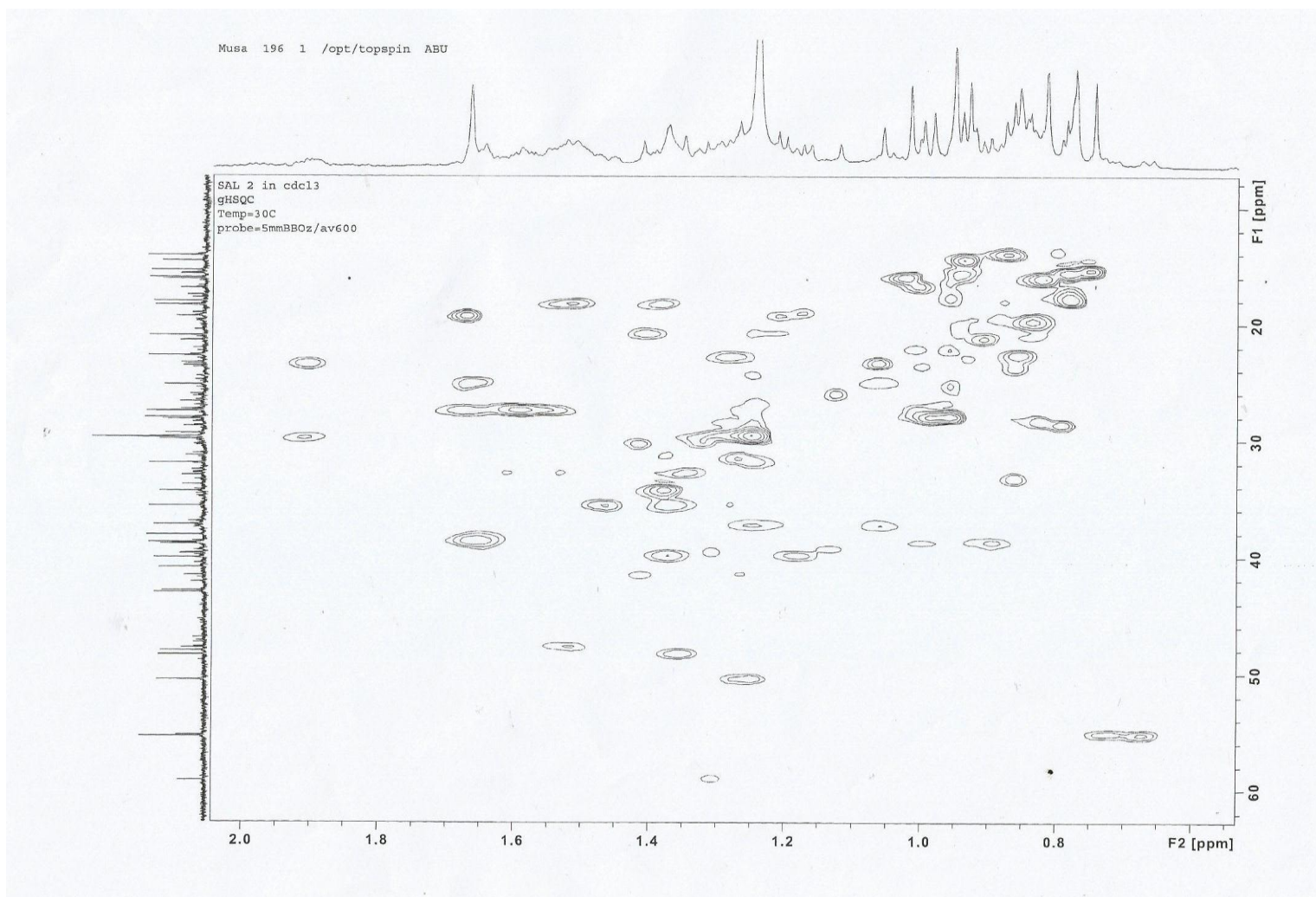


Figure 18. Expanded HSQC Spectrum of Compound SAL₂ Isolated from *G. boveanum*

4.4.13 HMBC Spectrum of Compound SAL₂ Isolated from *G. boveanum*

There are cross peaks between proton signal at δ H 3.19 and δ c 28.0, δ c 15.5, δ c 18.33. The multiplet proton signal at δ H 2.36 (H-19) also showed cross peaks with carbon signals δ c 30.0, δ c 109.47, δ c 48.25, and δ c 19. Also, proton signal at δ H 4.56 and 4.67 showed HMBC correlations with carbon signal δ c 48.10 and δ c 19.44. Details are as shown on figure 19.

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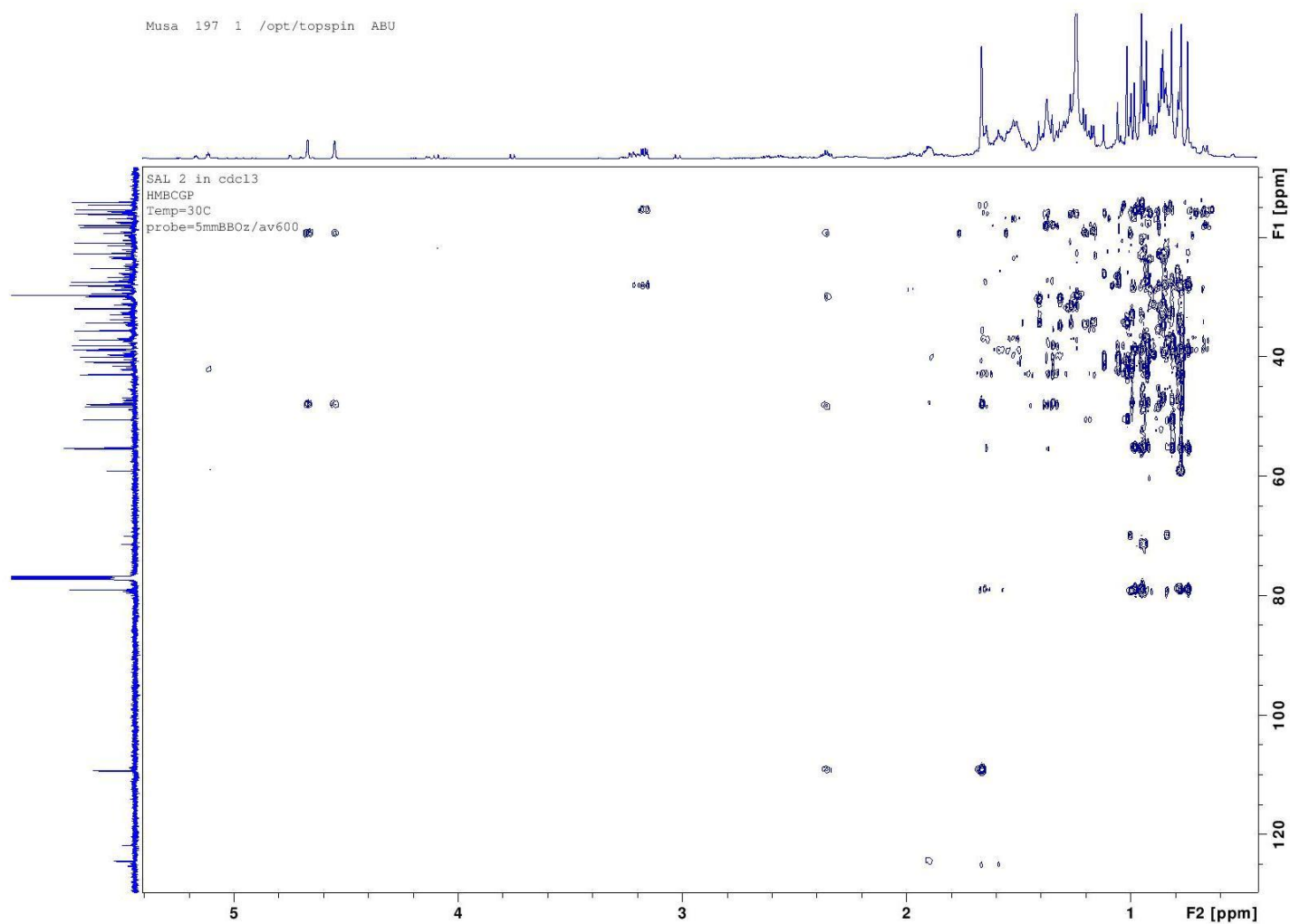


Figure 19 . HMBC Spectra of Compound SAL₂ Isolated from *G. boveanum*

4.4.14 ^{13}C and ^1H NMR Data of Compound SAL₂ Isolated from *G. boveanum*

Table 4.8 below shows the carbon-13 (^{13}C) and proton (^1H) chemical shift values of SAL₂ as deduced from the spectra on figure 12 to 19.

Tab.4.8 ^{13}C and ^1H Chemical Shift Data, DEPT and HSQC of compound SAL₂ from

G. boveanum

C	Type of Carbon	^{13}C -shift value(Hz)	^1H -Shift value(Hz)
1	CH ₂	38.78	0.93 m
2	CH ₂	27.19	1.60 m
3	CH	79.29	3.19 dd
4	C	39.10	-
5	CH	55.30	0.68 m
6	CH ₂	18.33	1.40 m, 1.19m
7	CH ₂	34.21	1.38 m
8	C	41.10	-
9	CH	50.60	1.28 m
10	C	37.20	-
11	CH ₂	21.05	1.4 m
12	CH ₂	25.43	1.45 m, 1.68 m
13	CH	38.30	1.63 t
14	C	43.22	-
15	CH ₂	28.05	0.97 m, 1.60 m
16	CH ₂	35.20	1.45 m
17	C	43.33	-
18	CH	48.25	1.38 m
19	CH	48.10	2.36 m
20	C	150.87	-
21	CH ₂	30.00	1.28 m
22	CH ₂	40.50	1.17 m
23	CH ₃	28.00	0.99 s
24	CH ₃	15.55	0.75 s
25	CH ₃	16.20	0.80 s
26	CH ₃	16.00	1.02 s
27	CH ₃	14.77	0.91 s
28	CH ₃	18.20	0.75 s
29	CH ₂	109.47	4.67 s, 4.56s
30	CH ₃	19.44	1.68 s

Tab. 4.9 Comparison between chemical shift Data of SAL₂ from *G. boveanum* and that of

Lupeol as obtained from Literature

C	SAL ₂		lupeol (a)		Lupeol (b)	
	¹³ C-shift value(Hz)	¹ H-Shift value(Hz)	¹³ C-shift value (Hz)	¹ H-shift value(Hz)	¹³ C-shift value (Hz)	¹ H-shift value(Hz)
1	38.78	0.93 m	38.7	0.94 m, 1.64 m	38.7	
2	27.19	1.60 m	27.5	1.61 m	27.4	
3	79.29	3.19 dd	79.3	3.18 dd	79.0	3.17 dd
4	39.10	-	39.8	-	38.9	-
5	55.30	0.68 m	55.55	0.69 m	55.3	0.66d
6	18.33	1.40 m, 1.19m	19.0	1.39 m, 1.52m	18.3	
7	34.21	1.38 m	34.2	1.38 m	34.3	
8	41.10	-	41.1	-	40.8	-
9	50.60	1.28 m	50.9	1.3 m	50.4	
10	37.20	-	37.2	-	37.2	-
11	21.05	1.4 m	21.2	1.43 m	20.9	
12	25.43	1.45 m, 1.68 m	25.3	1.1 m, 1.7 m	25.1	
13	38.30	1.63 t	38.5	1.62 t	38	
14	43.22	-	42.8	-	42.8	-
15	28.05	0.97 m, 1.60 m	27.2	1.61, 0.96d	27.4	
16	35.20	1.45 m	35.9	1.48 m	35.6	
17	43.33	-	43.2	-	43.0	-
18	48.25	1.38 m	48.5	1.39 t	48.3	
19	48.10	2.36 m	47.8	2.38 m	48.0	2.36 m
20	150.87	-	151.2	-	151.0	-
21	30.00	1.28 m	30.10	1.27 m	30.0	
22	40.50	1.17 m	40.3	1.19 m	40.0	
23	28.00	0.99 s	28.4	0.97 s	28.0	0.94 s
24	15.55	0.75 s	15.6	0.77 s	15.4	0.74 s
25	16.20	0.80 s	16.2	0.84 s	16.1	0.81 s
26	16.00	1.02 s	16.1	1.04 s	16.0	1.01 s
27	14.77	0.91 s	14.8	0.96 s	14.5	0.92 s
28	18.20	0.75 s	18.1	0.80 s	18.0	0.76 s
29	109.47	4.67 s, 4.56s	109.5	4.68, 4.56 br	109.3	4.55,4.67br
30	19.44	1.68 s	19.8	1.7 s	19.3	1.66 s

a=Soumia *et. al.*, 2012, b=Thanakijcharoenpath and Theanphong 2007.

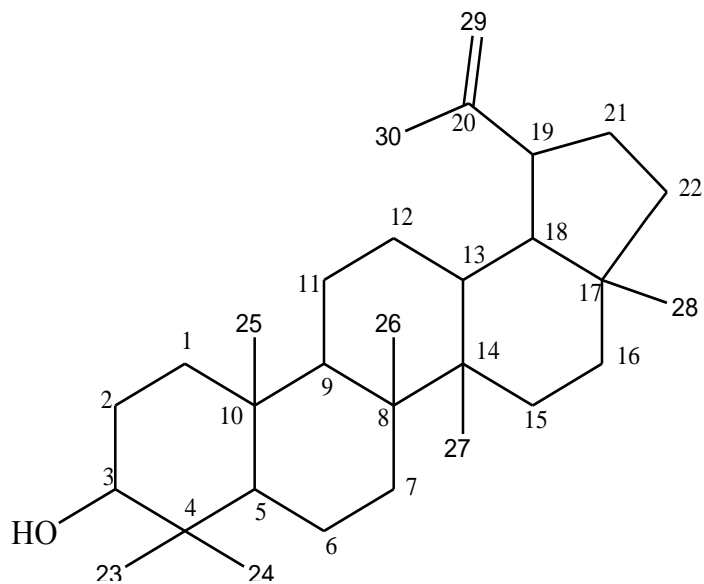


Figure 20. Proposed Chemical Structure of Compound SAL₂ (Lupeol) Isolated from *G. boveanum*

4.5 Acute Toxicity Studies on *Glossonema boveanum*

The major sign of toxicity considered in this study is death of the animal and the result computed from the two phases of the study is as follows:

4.5.1 LD₅₀ of Aqueous Ethanol Extract of *Glossonema boveanum*

The intra-peritoneal median lethal doses (LD₅₀) of aqueous ethanol extract of *G. boveanum* in mice and in chicks were 1264 mg/kg and 471 mg/kg respectively.

4.6 Anticonvulsant Studies on the Aqueous Ethanol Extract of *G. boveanum*

The results of anticonvulsant studies carried out on the aqueous ethanol extract of the plant are as follows:

4.6.1 Anticonvulsant Activity of Aqueous Ethanol extract of *G. boveanum* (EGB) against

Maximal Electroshock-induced Seizure (MES)

The aqueous ethanol extract of *G. boveanum* (EGB) conferred 40% protection against maximal electroshock-induced seizure at a dose of 140mg/kg. It also conferred 20% protection against MES at 35 and 70mg/kg doses. It did not show any significant increase in mean onset of seizure or decrease in mean recovery time at all the three doses (35, 70, and 140mg/kg) of the extract. Phenytoin 20mg/kg, the standard drug employed conferred 90% protection against maximal electroshock-induced seizure as in Table 4.10.

Table 4.10.: Effect of Aqueous Ethanol Extract of *G. boveanum* (EGB) and Phenytoin on Maximal Electroshock-induced Seizure in Chicks

Treatment mg/kg	Mean Onset (sec)	Mean recovery (min)	% Protection
Control	5.05±0.73	3.7±0.58	0
EGB (35)	3.98±0.39	3.87±0.44	20
EGB (70)	3.52±0.18	5.25±0.77	20
EGB (140)	4.13±0.39	6.5±1.28	40
Phenytoin (20)	5.21	3.00	90

4.6.2 Anticonvulsant Activity of Aqueous Ethanol extract of *G. boveanum* (EGB) against Pentylenetetrazole-induced Seizure in Mice

The aqueous ethanol extract did not exhibit any protection against Pentylenetetrazole-induced convulsion. There were no significant differences in the onset of seizure in the extract treated groups compared to the saline control group. There was no significant difference in the mean mortality period between the extract treated group and saline control group. Valproic acid (200 mg/kg) the standard anticonvulsant used conferred 100% protection and with no mortality as in Table 4.11

Table 4.11: Effect of Aqueous Ethanol extract of *G. boveanum* (EGB) and Valproic acid on PTZ-induced Seizure in Mice

Treatment mg/kg ⁻¹	Mean Onset (min)	Mean Mortality(mn)	%Protection
Control	7.4±2.0	15.66±1.45	16.67
EGB (90)	7.5±2.0	20.75±3.0	0
EGB (180)	6.0±2.2	9.0 ±2.0	16.67
EGB (360)	9.5±1.54	12.16 ±1.0	0
VA (200)	-	-	100

CHAPTER FIVE

5.0 DISCUSSION

The powdered *G. boveanum* was observed to be greenish in colour, with aromatic odour, wool-like in appearance with characteristic taste. Some of these sensory features are characteristic and could be serve useful toward identification of the plant material. Microscopic examination of the powder revealed the presence of straight wall polygonal epidermal cells, stomatal cells (anisocytic), unicellular covering trichomes with cystolith around their bases and fragment of fibres. Apart from these cellular structures identified, the chemo microscopy of the powder showed the presence of cellulosic cell walls, lignified tissues, suberins, and aleurone grains. Under the microscope, proteins are observed in form of aleurone grains and commonly found in seeds. Its presence showed that the powdered herb may consist of seeds. All these cellular and ergastic substances may serve as useful diagnostic characters for identification of powdered *G. boveanum*.

The physicochemical parameters of powdered plant were determined based on the methods recommended by World Health Organization (WHO). As apparent from Table 4.2, percent weight loss on drying or moisture content value was found to be $13.99 \pm 0.01\%^{w/w}$. The less value of moisture content of drugs could prevent content bacterial, fungal or yeast growth through storage (Pandey *et al.*, 2012). The general requirement for moisture content in crude drugs was that, it should not be more than 14% (Pandy *et al.*, 2012). Since it is still within the range, it implies that the powder of this plant material can be stored for a longer period with lower chances of microbial attack and some other adverse chemical reactions. The ash values; total ash, acid insoluble ash and water soluble ash values, which were found to be $18.33 \pm 0.16\%$, $8.16 \pm 0.08\%$ and $2.53 \pm 0.03\%$ respectively (Table 4.2), serve as important quantitative standards that gives an idea on quality, authenticity and purity of crude drugs (Swamy and Kamil., 2010) and could serve useful in setting the monograph of *Glossonema boveanum*. The solubility percentage of *G. boveanum* in aqueous cold extraction is higher ($9.33 \pm 0.08\%$), when compared with ethanol cold extraction ($3.29 \pm 0.04\%$). This signifies that the plant material may likely contain highly polar constituents more than the non-polar or less polar constituents. These extractive values are valuable tools that could help in estimating the chemical constituents, gives an idea on the nature of the constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent (Shwetajain *et al.*, 2011).

Base on the results of the chromatograms in the appendix G, and table 4.4, the fractions of the extract; n-hexane, chloroform and n-butanol were found to contain steroids and triterpenes, Phenolic compounds were shown to be present in the ethylacetate and n-butanol fractions. Anthracene derivatives were found to be present in both Ethylacetate and n-butanol fractions. The yellow fluorescence observed under UV (366nm) after spraying with Aluminium chloride from the

chromatogram of the Ethylacetate and n-butanol fraction of the extract revealed the presence of flavonoids. Previously, Flavonol glycosides have been reported to be present in *Glossonema boveanum* (Henedeik *et al.*, 2006). These groups of phytochemical compounds present in *G. boveanum* may be responsible for some of its biological activities.

Compound SAL₁ was obtained as white powder (22mg), readily soluble in chloroform. It gave a positive reaction with Libermann buchard test, suggesting the presence of a steroidal or a triterpenoidal nucleus. It showed a melting point of 223-227⁰C, which was found to be relatively close to melting point of alpha-amyrin acetate (222-223⁰C) (Renuka *et al.*, 2013). Its chromatogram (developed using 40:1, hexane/ethylacetate) gave a pink spot, an R_F value of 0.52 when sprayed with 10% sulphuric acid, as shown on plate XI. The ¹H NMR spectrum showed the presence of a triplet proton signal indicative of an olefinic proton (H-12) at δH 5.12 ppm. Also in the downfield of the spectrum, a multiplet at δH 4.51 ppm referring to the oxymethine proton (H-3) was observed. An intense proton signal (a singlet) ascribable to methyl protons of the acetate group attached to C-3 of a triterpenoidal nucleus is shown at δH 2.05ppm. The de-shielding of this methyl proton was as a result of the carbonyl functional group, it is attached to. The spectrum also revealed the presence of eight tertiary methyl protons at δH 0.88, 0.79, 1.02, 1.02, 1.00, 1.13, 0.79 and 0.92ppm. Other proton signals observed between 0.85 to 1.92 in the spectrum are due to several methylene and methine protons in the compound as shown in table 4.5. These assignments are in good agreement for the structure of alpha-amyrin acetate (Rhourr-Frih *et al.*, 2013, Niaz, 2013).

The structural assignments of SAL₁ was further substantiated by ¹³C NMR experiment which showed thirty two prominent carbon resonances indicative of 32 carbon atoms in the compound. DEPT spectra revealed the nature of these carbon atoms. It showed the presence of 9 methyl, 9 methylene, 7 methine and 7 quaternary carbon atoms in the compound. These, with their respective resonances as deduced from the spectra are as in table 4.5. A highly de-shielded ¹³C signal at δc 170.86 ppm indicates the carbonyl carbon of an ester attached to C-3 of the alpha amyrin moiety (Rhourr-Frih *et al.*, 2013) while the signal at δc 81.66ppm indicated the oxymethine carbon (C-3). The carbon signals at δc 125.55 ascribed to C-12 and δc 139.65 ascribed to C-13 were highly de-shielded due to the presence of an olefinic bond between them. And this conformed to the data obtained from literature for alpha-amyrin acetate (Rhourr-Frih *et al.*, 2013, Niaz 2013). All the protons were carefully assigned to their respective carbon atoms using the HSQC spectra in figures 8 and 9.

The confirmation of the structure of SAL₁ was accomplished through the 2D NMR experiments (COSY and HMBC). The COSY spectrum of SAL₁ exhibited some cross peaks such as between δH 5.12, (H-12) and δH 1.92, (H-11) methine and methylene protons respectively. Cross peaks are also observed between δH 4.51 an oxymethine proton signal (H-3) and one methylene proton signal (δH 1.62, H-2). In the HMBC spectrum, methyl proton signal at δH 2.05 (H-2¹) of the acetate showed cross peaks with carbonyl carbon (δC 170.96, C-1¹) by J₂ correlation. Also, there was an observed cross peak between oxymethine proton signal δH 4.51 (H-3) and methyl carbon (δc 15.44, C-23) and with another methyl carbon (δc 28.88, C-24) by J₃ correlation. Likewise, cross peaks were also observed between δH 5.12 methine proton signal (H-12) and methylene carbon signal (δc 23.61, C-11) by J₂ correlation, and with another methine carbon signal (δc 47.77, C-9) by J₃ correlation. Methyl proton δH 1.13, H-27 is also

found to exhibit cross peaks with quaternary carbon signal (δ_c 139.65, C-13) by J_3 correlation. Analysis of the results of 1D and 2D NMR and comparing the spectra data suggests that; compound SAL₁ is alpha-amyrin acetate, an ursane type of pentacyclic triterpenoid, previously isolated from *Tylophora hirsuta* of *Asclepiadaceae* sub-family, where it was demonstrated to have antispasmodic activity on rabbits' jejunum (Niaz, 2013).

Compound SAL₂ was obtained as amorphous white powder (26mg), readily soluble in chloroform, It gave positive reaction with Libermann buchard test suggesting the presence of a steroidal or a triterpenoidal nucleus. It showed a melting point of 210-213⁰C, which was found close to 212-213⁰C, the melting point of lupeol ((Soumia *et al.*, 2012). Its chromatogram (developed using 8:2, hexane/ethylacetate) gave a pink spot, an R_f value of 0.46 when sprayed with 10% sulphuric acid, as shown on plate XII. The ¹H NMR of SAL₂ displayed a pair of broad singlet at δ 4.56 and δ 4.67 (1H each) an important characteristic of olefinic protons (H-29 and b) typical of lupeol (Abdullahi *et al.*, 2013). It displayed a multiplet proton signal H δ 3.19 ascribed to H-3 which was de-shielded because of the hydroxyl group attached to C-3. The spectrum also showed a multiplet of one proton at δ H 2.36 ascribed to the 19 β -H of lupeol. The up-field region of the spectrum revealed the presence of seven tertiary methyl proton signals at δ 0.99,0.75,0.80, 1.02,0.91,0.75 and 1.68. These also serve as some distinguishing features of lupeol. Other proton signals observed between 0.93 and 1.45 are due to methylene and methine protons in the compound. The assignments and the data are in good agreement for the structure of lupeol as obtained in the literature (Soumia *et al.*, 2012, Abdullahi *et al.*, 2013).

The structural assignments of SAL₂ was further supported by ¹³C NMR experiment which showed thirty prominent carbon resonances indicative of 30 carbon atoms in the compound. DEPT spectra revealed the nature of these carbon atoms. It showed 7 tertiary methyls, 11 methylenes, 6 methines and 6 quaternary carbon atoms as deduced from the spectra, are as shown in table 4.7. Carbon signals δ_c 150.87 ascribed to C-20 and carbon signal δ_c 109.47 assigned to C-29 were strongly de-shielded due to the olefinic bond between them. Likewise, carbon signal at δ_c 79.29 (C-3) was de-shielded because of the hydroxyl group attached to it. All the protons were carefully assigned to their respective carbon atoms using the HSQC spectra on figure 17 and 18.

The confirmation of the structure of SAL₂ was accomplished through the 2D NMR experiments (COSY and HMBC). The COSY spectrum of SAL₂ exhibited some cross peaks such as those between δ H 2.36, H-19 and one methylene proton signal (δ H 1.28, H-21) and another methine proton signal (δ H 1.38, H-18); and between oxygenated methine proton signal (δ H 3.19, H- 3) and methylene signal (δ H 1.60, H-2). There also exist a cross peak between δ H 4.67, H-29a and one methylene proton signal (δ H 4.56, H-29b).

In the HMBC spectrum, the methine proton signal at δ H 3.19 (H-3) showed cross peaks with a methyl carbon signal δ_c 28.0, C-23 and δ_c 15.5, C-24 by ³J correlation. The multiplet, methine proton signal at δ H 2.36 (H-19) showed cross peaks with two methylene carbon signals δ_c 30.0 (C-21) by ²J and δ_c 109.47 (C-29) by ³J correlation, a methine carbon signal δ_c 48.25 (C- 18) by ²J correlation and a methyl carbon signal δ_c 19.44 (C-30) by ³J correlation . The pair of broad singlets of olefinic proton at δ H 4.56 and 4.67 showed cross peaks with a methylene carbon signal δ_c 48.10 (C-19) and δ_c 19.44 (C-30) by ³J correlation.

All these correlations confirmed the assignments of these carbons and protons to their respective positions. Careful spectral analysis and comparison with reported data, (Thanakijcharoenpath and Theanphong 2007, Soumia *et al.*, 2012) led to the suggestion that; compound SAL₂ is lupeol. This pentacyclic triterpene, have been previously isolated from some members of *Asclepiadaceae* such as; *Oxystelma esculentum* (Pandya and Anand, 2011), and *Calotropis gigantean* (Saratha *et al.*, 2011). Some important pharmacological activities of lupeol includes; anti-inflammatory, anti-microbial, anti-protozoal, anti-proliferative and cholesterol lowering properties (Siddique and Saleem, 2011).

The intraperitoneal median lethal dose of aqueous ethanol extract of *G. boveanum* in mice determined as 1264mg/kg; was the geometric mean of 1000mg/kg the highest dose that causes no mortality and 1600mg/kg the lowest dose that caused mortality base on the results of the second phase. Similar acute toxicity studies carried out on the extract in chicks showed that; the intraperitoneal median lethal dose was 471mg/kg and this significantly varied from the value obtained in mice. This variation might be due to physiological and anatomical differences between the two species of the animals. Base on the LD₅₀ value determined in mice, the extract was noted to be slightly toxic according to (Corbett, 1984 and Matsumura, 1975). The two LD₅₀ values determined gave an idea in selecting the suitable doses used for the anticonvulsant studies (which is 30% of the LD₅₀ for each of the highest dose used).

The aqueous ethanol extract of *G. boveanum* conferred 40% protection at 140mg/kg (the highest dose of the extract used) against Maximal electroshock-induced seizure (MES) but did not significantly decrease the recovery time. The ability of the extract to inhibit tonic hind limb extension by 40% in MES as compared to Phenytoin (90% protection) in the model suggests anticonvulsant activity which could be exploited for the management of generalized tonic-clonic and partial seizures.

Current available antiepileptic drugs that are clinically effective in the management of generalized tonic-clonic and partial seizures such as carbamazepine, phenytoin, primidone, phenobarbital, valproate and lamotrigine all suppress tonic hind limb extension in Maximal electroshock- induce seizure (Browning, 1992, Rho and Sankar, 1999). The anticonvulsant protection against tonic hind limb extension indicates the ability of the testing material to inhibit or prevent seizures discharge within the brainstem seizure substrate (Browning, 1992). The moderate anticonvulsant activity of the extract may be attributed to the presence of some phytochemicals in the plant. Triterpenes, steroids and flavonoids which were shown to be present in the plant have been reported to possess anticonvulsant activity in some experimental seizure models (Chauhan *et al.*, 1988). Previously, an acetylated isomeric mixture of alpha- and beta-amyrin isolated from *Protium heptaphyllum* administered intraperitoneally or orally was shown to exhibit significant protection against Pentylene-tetrazole induced seizure in mice (Gislei *et. al.*, 2009).

The extract did not protect against Pentylene-tetrazole-induced seizure in mice. It did not significantly increase the time of onset of the seizure. This suggests that the extract exhibits its anticonvulsant activity via different path ways other than γ -aminobutyric acid (GABA) potentiation in the brain. Clinically effective anticonvulsants used for treatment of absence seizure prevent PTZ-induced seizure in mice and rats (Tripathi, 2003).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

In the study conducted, the organoleptic evaluation of the powdered whole plant of *G. boveanum* showed that it is green in colour, aromatic in odour, wool-like in appearance with a characteristic taste. Microscopical examination of the powdered *G. boveanum* identified the presence of polygonal epidermal cells, anisocytic stomatal cells, covering trichomes with cystolith incrustated in them. Starch grains, suberins, lignins, aleurone grains and silica were also found to be present. The powdered plant was found to have a moisture content of 13.99%, Total ash of 18.33%, acid insoluble ash of 8.16%, water soluble ash of 2.53%, water soluble extractive of 9.33% and ethanol extractive value of 3.29%.

Thin layer chromatographic analysis on the various fractions of the extract revealed the presence of triterpenes, steroids, anthracenes and flavonoids in the plant. Column and preparative thin layer chromatography on the n-Hexane fraction of its aqueous ethanol extract led to the isolation of two pentacyclic triterpenoids, labeled; as SAL₁ and SAL₂ which were identified to be alpha-amyrin acetate and lupeol respectively. The identification was based on their respective NMR spectroscopic (1D and 2D) data and by comparison with spectroscopic values obtained in the literature.

The crude ethanol extract of the plant protected chicks against maximal electroshock induced seizure by 40% compared to the saline control group and this could serve as the basis for its use in the treatment

of epilepsy. The intraperitoneal median lethal dose of the extract in mice and chicks was found to be 1264 and 471mg per kg body weight respectively.

6.2 CONCLUSION

The present studies have established:

- i) Some of the pharmacognostic features of powdered *G. boveanum*, which could serve useful towards its standardization.
- ii) The various classes of Phytochemical compounds of the plant and the identity of two pentacyclic triterpenoids; alpha-amyrin acetate and lupeol isolated and characterized from the n-Hexane fraction of its aqueous ethanol extract.
- iii) A moderate anticonvulsant activity (40% protection) of the aqueous ethanol extract of the plant against Maximal electroshock-induced seizure (MES) in chicks at the highest dose (140 mg/kg).

6.3 RECOMMENDATIONS

It is recommended that; activity guided isolation need to be carried out with the aim of isolating and characterizing the bioactive compound(s) that is/are responsible for the anticonvulsant activity of the plant. More anticonvulsant study models need to be used to study the plant, as some bioactive compounds were shown to exert their anticonvulsant activity only or better via some specific seizure models. Other ethno-medicinal uses of the plant need to be validated scientifically.

Detailed standardization studies need to be carried out to set parameters for its monograph.

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APPENDIX A

Determination of Ash Value of powdered whole plant of *G. boveanum*

Description	1	2	3
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Constant weight of crucible (g)	38.390	37.470	38.100
Weight of crucible and content (g)	40.390	39.470	40.100
Weight of crucible and Ash (g)	38.760	37.820	38.460
Weight of Ash (g)	0.370	0.370	0.365
Ash Value (%)	18.50	18.50	18.00
Average mean (%)		18.33	

Sample calculation

$$\text{Ash value} = \frac{\text{weight of Ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Ash Value} = \frac{0.37 \times 100}{2 \text{ g}} = 18.5 \% \text{w/w}$$

APPENDIX B

b) Determination of Acid insoluble Ash of powdered whole plant of *G. boveanum*

Description	1	2	3
Constant weight of crucible (g)	38.390	37.470	38.100
Weight of crucible and Acid insoluble ash (g)	38.550	37.635	38.265
Weight of Acid insoluble ash (g)	0.160	0.165	0.165
Acid Insoluble Ash Value (%)	8.00	8.25	8.25
Average mean (%)	8.16		

Sample calculation

$$\text{Acid Insoluble Ash value} = \frac{\text{weight of acid insoluble ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Acid insoluble ash} = 0.16/2g = 8 \%w/w$$

APPENDIX C

Determination of water soluble Ash of powdered whole plant of *G. boveanum*

Description	1	2	3
Constant weight of crucible (g)	38.390	37.470	38.10
Weight of crucible and Ash (g)	38.760	37.840	38.46
Weight of Ash (g)	0.370	0.370	0.36
Weight of water Insoluble ash (g)	0.320	0.320	0.308
Weight of water soluble ash (g)	0.050	0.050	0.052
Water soluble Ash Value (%)	2.500	2.500	2.600
Average mean (%)	2.53		

Sample calculation

$$\text{Water soluble Ash value} = \frac{\text{Wt of total ash} - \text{Wt of Water Insoluble Ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Water soluble ash Value} = \frac{(0.37 - 0.32) \times 100}{2 \text{ g}} = 2.5 \% \text{w/w}$$

APPENDIX D

Water –Soluble Extractive value of *G. boveanum*

4 g of the powder was used in 100 ml of water.

Description	1	2	3
Constant weight of dish (g)	239.84	230.0	200.0
Weight of crucible and content after heating (g)	240.21	230.38	200.37
Water extractive content (g)	0.37	0.38	0.37
water extractive Value (%)	9.25	9.50	9.25
Average mean (%)	9.33		

Sample calculation

Water extractive value = Wt of dish & content after heat (g) - Const wt. of dish (g)/Initial weight of Drug ×100

$$=(0.37 \div 4) \times 100 = 9.25\%_{w/w}$$

APPENDIX E

Alcohol – Soluble Extractive value

4 g of the powdered whole plant of *G. boveanum* was used in 100 ml of 90% ethanol

Description	1	2	3
Constant weight of dish (g)	123.18	123.97	123.175
Weight of dish and content after heating (g)	123.31	124.10	123.31
Alcohol extractive content (g)	0.13	0.13	0.135
Alcohol extractive Value (%)	3.25	3.25	3.37
Average mean (%)	3.29		

Sample calculation

Alcohol extractive value = {Wt of dish & content after heat (g) - Constant wt. of dish.(g)} ÷ initial weight of drug X 100

$$= [(123.31-123.18) \div 4] \times 100 = 3.255\% /_w$$

APPENDIX F

Determination of moisture content of powdered whole plant of *G. boveanum*

3g of the powdered plant material was used

Description	1	2	3
Constant weight of crucible (g)	50.710	46.730	50.30
Weight of crucible and powder before heating(g)	53.710	49.730	53.30
Wt of crucible and content after heating to constant weight (g)	53.290	49.3097	52.8807
Loss in weight (g)	0.420	0.4203	0.4193
Loss on drying (%)	14.00	14.010	13.980
Average mean (%)		13.99	

Sample calculation

% Loss on drying = [(wt of crucible and content before heating - wt of crucible and content after heating) ÷ 3g] × 100

$$= [(53.71-53.29) \div 3] \times 100$$

$$= 14.0\% /_w$$

APPENDIX G

Thin layer chromatography of the various fractions of ethanol extract of *G. boveanum*

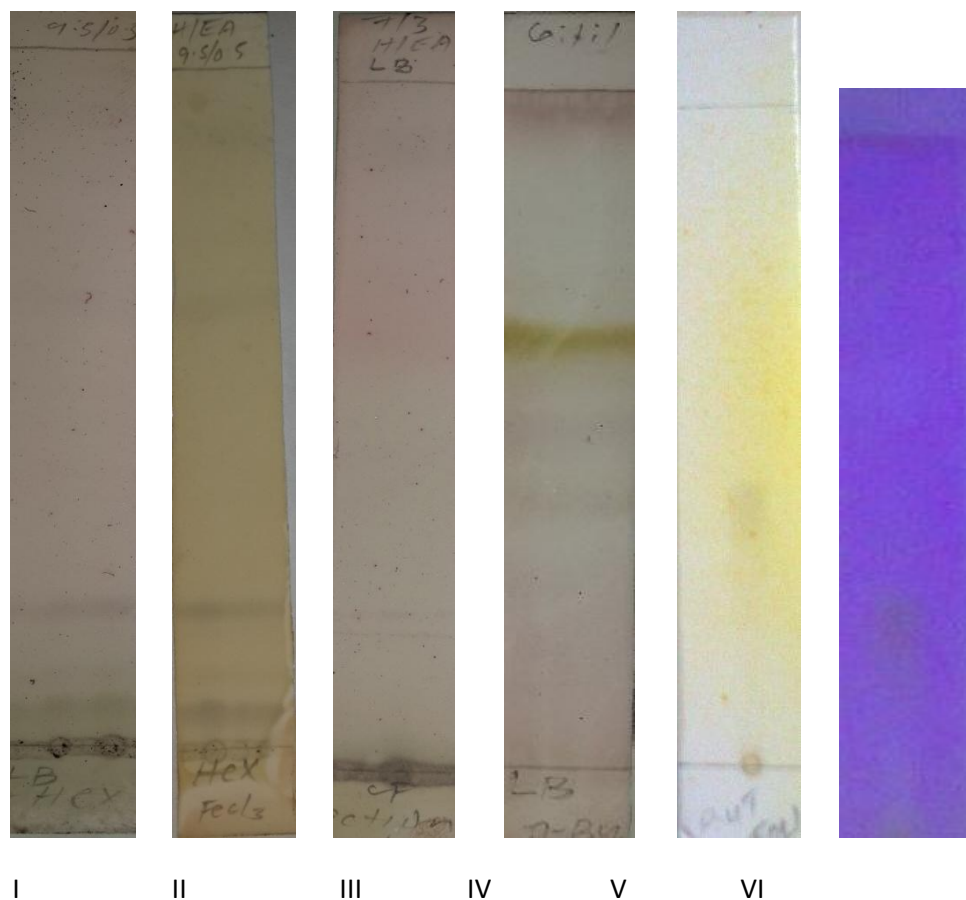


Plate I : TLC plate of n-hexane fraction sprayed with Libbermann Buchard reagent

Plate II: TLC plate of n-Hexane fraction sprayed with Ferric chloride solution

Plate III: TLC plate of chloroform fraction sprayed with Libbermann Buchard's reagent

Plate IV : TLC plate of n-butanol fraction sprayed with Libbermann Buchards reagent

Plate V: TLC plate of n- butanol fraction sprayed with Ferric chloride solution

Plate VI: TLC plate of n-butanol fraction sprayed with Aluminium chloride solution

viewed under UV (366nm)

APPENDIX G Cont'd



VII

VIII

IX

X

Plate VII: TLC plate of Ethylacetate fraction sprayed with ferric chloride solution

Plate VIII: TLC plate of Ethylacetate fraction sprayed with Acl_3 and viewed under UV (366nm)

Plate IX : TLC plate of n-butanol fraction sprayed with Borntragger's reagent

Plate X: TLC plate of ethylacetate fraction sprayed with Borntragger's reagent

APPENDIX H

Determination of Acute toxicity of extract of *G. boveanum* using Lorke's Method in mice

Phase I

Group	Dose mgKg^{-1}	No. Of death/ No. Of Survival
1	10	0/3
2	100	0/3
3	1000	1/3

Phase II

Group	Dose mgKg ⁻¹	No. Of death/ No. Of Survival
1	2900	1/1
2	1600	1/1
3	1000	0/1
4	600	0/1

$$LD_{50} = \sqrt{\text{Minimum toxic dose} \times \text{Maximum tolerated dose}}$$

$$= \sqrt{1000 \times 1600}$$

$$= 1264 \text{ mg Kg}^{-1}$$

APPENDIX I

Determination of Acute toxicity of extract of *G. boveanum* using Lorke's Method in chicks

Phase I

Group	Dose mgKg ⁻¹	No. Of death/ No. Of Survival
1	10	0/3
2	100	0/3
3	1000	3/3

Phase II

Group	Dose mgKg ⁻¹	No. Of death/ No. Of Survival
1	600	1/1
2	370	0/1
3	225	0/1
4	140	1/1

$$LD_{50} = \sqrt{\text{Minimum toxic dose} \times \text{Maximum tolerated dose}}$$

$$= \sqrt{370 \times 600}$$

$$= 471 \text{ mg Kg}^{-1}$$

APPENDIX J

TLC Spraying Reagents

Sulphuric acid spray reagent: 10ml of concentrated sulphuric acid was added in 90ml of ethanol to obtain a 10%^v/_v of acid in ethanol.

Liebermann-Buchard reagent: 5ml of acetic anhydride was placed in an ice bath, 5ml of concentrated sulphuric acid was added, the mixture was added to 50ml ice cold absolute ethanol.

Dragendorffs reagent: 0.11g of potassium iodide and 0.18g of bismuth sub-nitrate were dissolved in 20ml acetic acid and the volume was made up to 100ml with water.

Ferric chloride solution: 2.7g of ferric chloride salt was dissolved in 100ml of 2M hydrochloric acid.

P-Anisaldehyde/ Sulphuric acid: 0.5ml of p-anisaldehyde was dissolved in 50ml of glacial acetic acid and thereafter 1ml of concentrated sulphuric acid was carefully added and the mixture stirred.