PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE METHANOL EXTRACT OF THE ROOT OF *NAPOLEONAEA HEUDELOTTI (*A.JUSS*)*

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AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

JUNE, 2015

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BY

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JUNE, 2015

DECLARATION

I declare that the work in this dissertation entitled: "PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE METHANOL EXTRACT OF THE ROOT OF *NAPOLEONAEA HEUDELOTTI''* has been carried out by me in the Department of Chemistry, Ahmadu Bello University, Zaria under the supervision of Professor A.O. Oyewale and Dr (Mrs) R.G. Ayo. The information obtained from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for the award of another degree or diploma at this or any other institution.

Musa Abubakar Shafii

_____	_____	_____
Name of Student	**Signature**	**Date**

CERTIFICATION

This dissertation entitled: "PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE METHANOL EXTRACT OF THE ROOT OF *NAPOLEONAEA HEUDELOTTI''* by Musa ABUBAKAR SHAFII, meet the regulation governing the award of the degree of Master of Science in Organic Chemistry of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. A.O. Oyewale	**--————**	**----------————**
Chairman, Supervisory committee	Signature	Date
DR. R.G. Ayo	**————**	**----------————**
Member, Supervisory Committee	Signature	Date
Prof. V.O. Ajibola	**————**	**----------————**
Head of Department	Signature	Date
Prof. A. Z. Hassan	**————**	**---------————**
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This research work is dedicated to Allah for His infinite mercies, blessings, guidance and protection over me.

To my late mother, Mallama Asabe Shafii, my father, Mr Shafii Abubakar Ndajiya, my wife, Zainab Mohammad, and to my beloved child, Ridhwaan Musa Shafii Abubakar.

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Finally, my deep gratitude and appreciation goes to my parents, my uncle, my brothers and sisters for their support financially and morally. May Allah reward you all in many ways.

ABSTRACT

The extracts of the root part of *Napoleonaea heudelotii* were subjected to phytochemical and anti-microbial studies. Extraction was done by continuous Soxhlet extraction using methanol. The phytochemical screening of the crude methanol extract, chloroform and ethyl acetate fractions revealed the presence of carbohydrate, cardiac glycosides, saponins, steroids, triterpenes, flavanoids and tannins. The result of the antimicrobial screening of the crude methanol extract, ethyl acetate and chloroform fractions showed activity against *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris,* and *Candida albicans.* However, the chloroform fraction was the most active fraction against the test microoganisms. The zone of inhibition of the methanol extract ranged between 16 mm and 21 mm, the chloroform fraction ranged between 17 mm and 25 mm while the ethyl acetate fraction ranged between 15 mm and 21 mm. The MIC results of methanol extract, ranged between 12.5 mg/ml and 1.562 mg/ml, chloroform fraction ranged between 12.5 mg/ml and 1.562 mg/ml, while ethyl acetate ranged between 6.25 mg/ml and 1.625 mg/ml. The MBC of methanol extract and chloroform fraction ranged between 12.5 mg/ml and 1.562 mg/ml, while that of ethyl acetate fraction ranged between 6.2 mg/ml and 1.562 mg/ml. The chloroform fraction being the most active fraction was subjected to extensive chromatographic purification; white crystalline solid labelled N_{HPE} were isolated. The structures of the isolated compounds were determined to be a mixture α-amyrin and β-amyrin using 1D and 2D NMR.

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List of Abbreviations

PPM	Part per million
NMR	Nuclear magnetic resonance
TLC	Thin layer chromatography
DEPT	Distortionless Enhancement by Polarization Transfer
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
N_{HPE}	*Napoleonaea heudelotti* plant extract

CHAPTER ONE

1.0 INTRODUCTION

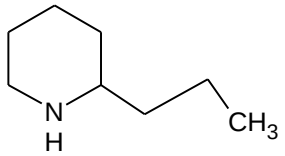
Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals (Babalola, 2009). Chemical compounds in plant mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects. Ethnobotany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from ethnomedical plant sources (Babalola, 2009). Many of the pharmaceuticals currently available to physicians have a long history of use, as herbal remedies, including aspirin, digitalis, quinine, and opium. Treatment of diseases is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals (Beltrame *et al.,* 2002). The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care (Beltrame *et al.,* 2002). Studies in the United States and Europe have shown that the use of herbal madicine is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about it effectiveness has become more widely available. The annual global export value

of pharmaceutical plants in 2011 accounted for over US$ 2.2 billion. Plants have continued to be major source of medicine either in the form of traditional medicine preparations or as pure active principles (Hill, 2011). This has made it important to identify plants with useful therapeutic actions for possible isolation and characterization of their active constituents. About 80 % of the world population relies on the use of traditional medicine which is predominantly based on plant materials (Brunton *et al*., 2006). Plant have been part of our lives since beginning of time, we get numerous products from plants, most of them, not only good and beneficial but also crucial to our existence. The use of plant to heal or combats illness is probably as old as human kind. Out of these simple beginning came the pharmaceutical industry. Yet the current view of plant is very different from how it all started. The acceptance of traditional medicine as an alternate form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial herbal extract (WHO, 1993). In Africa, particularly Nigeria is rich in plants which are used in herbal medicine to cure diseases and to heal injuries. Some of these plants exhibit a wide range of biological and pharmacological activities such as antihelmenthics, oxytoxic laxative (Hostettmann *et al.,* 2012). The secondary metabolites of plant provide human with numerous biological active components which have been used extensively as drugs, foods, additives, flavours, insecticides and chemicals. They exhibited remarkable biological activities, which include inhibitory effects on enzymes, modulatory effects on some cell types, protect against allergies antioxidants (Dongmo *et al.*, 2001).

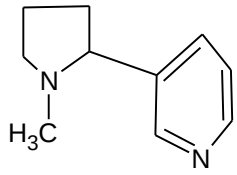
1.2 SECONDARY METABOLITES

1.2.1 Alkaloid

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms e.g Coniine **(1)** and Quinine **(2**). This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also attributed to alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulphur and more rarely other elements such as chlorine, bromine, and phosphorus. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms (Kumar *et al.,* 2010). They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals (Kumar *et al.,* 2010).



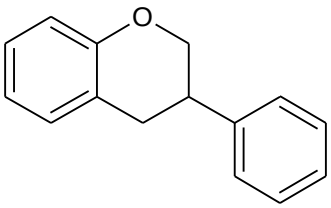
Coniine (1)



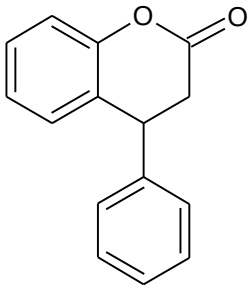
Quinine (2)

1.2.2 Flavonoid

Flavonoids are a class of plant secondary metabolites. Flavonoids are also described as non-ketone polyhydroxy polyphenol compounds which are more specifically termed as flavanoids e.g Isoflavan **(3)** and Neoflavonoid **(4).** The three cycle or heterocycles in the flavonoid backbone are generally called ring A, B and C. Ring A usually shows a phloroglucinol substitution pattern. Flavonoids are widely distributed in plants, fulfilling many functions (Dongmo *et al.,* 2001). Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors (Kumar *et al.,* 2010).



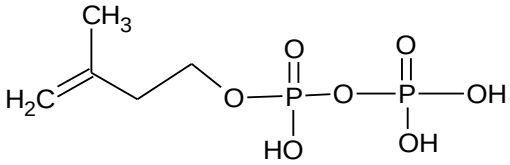
Isoflavan (3)



Neoflavonoid (4)

1.2.3 Terpene

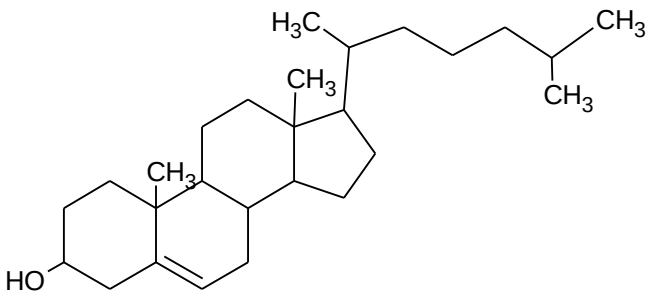
Compounds classified as terpenes constitute what is arguably the largest and most diverse class of natural products. A majority of these compounds are found only in plants, but some of the larger and more complex terpenes occur in animals, e.g Isopentenyl pyrophosphate **(5)**, is the basic unit in which terpene exist in natural organism. Instead, the number and structural organization of carbons is a definitive characteristic. Terpenes may be considered to be made up of isoprene (more accurately isopentane) units, an empirical feature known as the isoprene rule (Dongmo *et al.*, 2001).



Isopentenyl pyrophosphate (5)

1.2.4 Steroid

The important classes of lipids called steroids are actually metabolic derivatives of terpenes, but they are customarily treated as a separate group. Steroids may be recognized by their tetracyclic skeleton, consisting of three fused six-membered and one five-membered ring. These rings are synthesized by biochemical processes from cyclization of a thirty-carbon chain. Hundreds of steroids are found in animals, fungi and plants e.g cholesterol **(6),** the sex homones, estradiol and testosterone (Kaisar *et al.,* 2011).

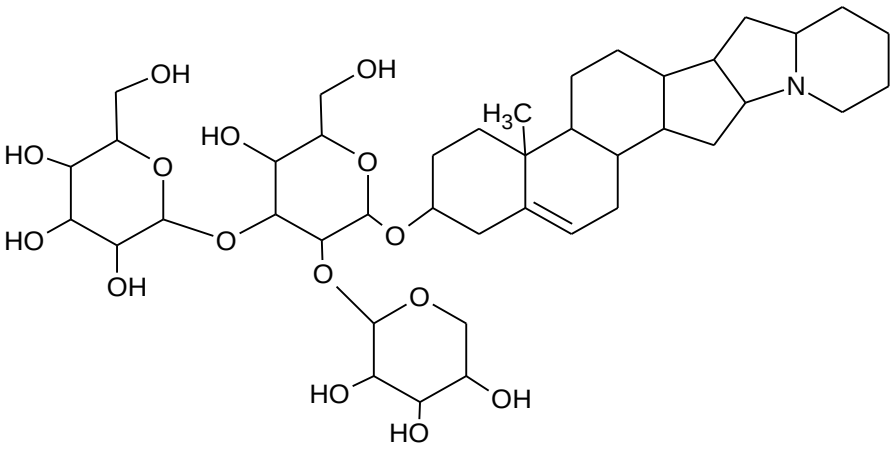


Cholesterol (6)

1.2.5 Saponins

Saponins are a class of chemical compounds found in abundance in various plant species. More specifically, they are amphipathic glycosides grouped by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (Kaisar *et al.,* 2011).

Saponins have historically been understood to be plant-derived, but they have also been isolated from marine organisms. Saponins are indeed found in many plants, and derive their name from the soapwort plant (genus *Saponaria*, family Caryophyllaceae), the root of which was used historically as a soap. Saponins are also found in the botanical family *Sapindaceae*, with its defining genus *Sapindus* (soapberry or soapnut), and in the closely related families Aceraceae (maples) and Hippocastanaceae. An example of saponin is solanine **(7).** It is also found heavily in *Gynostemma pentaphyllum* (*Gynostemma*, Cucurbitaceae) in a form called gypenosides, and ginseng or red ginseng (*Panax*, Araliaceae) in a form called ginsenosides. Within these families, this class of chemical compounds is found in various parts of the plant: leaves, stems, roots, bulbs, blossom and fruit. Commercial formulations of plant-derived saponins, e.g., from the soap bark (or soapbark) tree, *Quillaja saponaria*, and those from other sources are available via controlled manufacturing processes, which make them of use as chemical and biomedical reagents. Saponins are used widely for their effects on ammonia emissions in animal feeding**.** The mode of action seems to be an inhibition of the urease enzyme, which splits up excreted urea in feces into ammonia and carbon dioxide (Kaisar *et al.,* 2011).



Solanine (7)

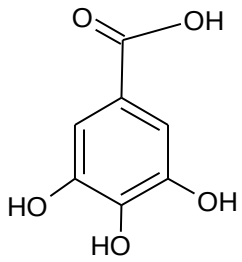
1.2.6 Tannins

A tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids.

The term tannin (from *tanna*, an Old High German word for oak or fir tree, as in Tannenbaum) refers to the use of wood tannins from oak in tanning animal hides into leather; hence the words "tan" and "tanning" for the treatment of leather. However, the term "tannin" by extension is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with various macro molecules (Kaisar *et al.*, 2011).

The tannin compounds (e.g Gallic acid **(8)**) are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red

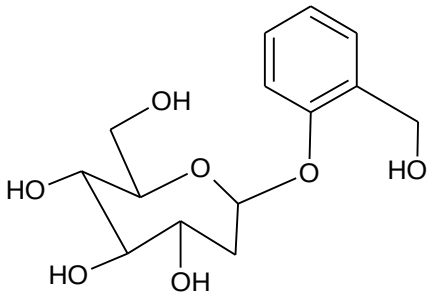
wine. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine (Kaisar *et al.,* 2011). .



Gallic acid (8)

1.2.7 Glycosides

Glycosides are compounds of sugar bonded to non carbohydrate group, they are used in the treatment of congestive heart failure and cardiac arrhythmia.The example of glycosides is salicin **(9).** These glycosides are found as secondary metabolites in several plants, and also in some insects (Kaisal *et al.,* 2011).



Salicin (9)

1.3 Statement of the Research problem

There are different types of active compounds in use for the treatment of various types of infectious diseases, but only few are known by the researchers. Therefore there is need to know more of these compounds which are active against these diseases.

1.4 Justification of the Research

Base on the ethnomedicinal claims of *Napoleonaea heudelotti* there is need for scientific study to ascertain the active components responsible for these actions.

1.5 Aim

To isolate and characterise bioactive compounds likely to be present in the root part of *Napoleonaea heudelotti* and determine its activity against common microorganisms

1.6 Objectives

The aim of this research work would be achieved through the following objectives;

a. Establish the phytochemical constituents present in the root part of the plant.
b. Isolate some of the compounds present in root part of the plant
c. Establish the antimicrobial activities of the root part of the plant and the isolated compounds
d. Elucidate the structure of the isolated compound(s) using spectroscopic techniques.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 SCIENTIFIC CLASSIFICATION OF *NAPOLEONAEA HEUDELOTTI*

Kingdom: Plantae

Phylum: Angiosperm

Class: Equisetopsid*a* C. Agard

Sub class: Magnoliidae Novak

Order: Ericales, Bercht. j. press

Sub-order: Asloranae

Family: Lecythidaceae

Sub-family: *Napoleonae* Qideae

Genus: *Napoleonaea* P. Beauu

Species: *Napoleonaea heudelotii*.

Scientific name author: A. Juss

Tiv: *enamokprichi*

2.2 BOTANICAL DESCRIPTION OF *NAPOLEONAEA* SPECIES

Napoleonaea (Family: Lecythidaceae) is a tropical plant which is commonly found in West Africa. It is found in Northern parts of Nigeria. It is a small tree with large pale reddish flower, greenish leaf about 12-15 cm and 8 m tall. The stem bark is light brown. The young fruits are blue and ripen fruit usually brown (Mabberley, 1997). The fruit is usually depressed globose and lobed often orange-yellow to red-brown when ripe, the shape of the fruit are usually pomegranate, sometimes warty outside, crowned by the calyx, 5-6 celled, with one kidney-shaped seed in each cell (Tulikk, 2003). *Napoleonaea* comprises of about 4-5 species which occur in South West Africa, North West Africa, America, and in Australia.

2.2.1 Taxonomy of *Napoleonaea* species

Name	Family	Genus	Order	Species	Kingdom
N. *heudelotii*	Lecythideceae	*Napoleonaea*	Ericals	*Heudelotii*	*Plantae*
N. i*mperialis*	Lecythideceae	*Napoleonaea*	Asteroids	*Imperialis*	*Plantae*
N. *egertomi*	Lecythideceae	*Napoleonaea*	Magnolidge	*Egertonii*	*Plantae*
N. *vogetii*	Lecythideceae	*Napoleonaea*	Vogetti	*Asloranaea*	*Plantae*

2.3 ORIGIN AND GEOGRAPHICAL DISTRIBUTION

Napoleonaea heudelotii is dominantly distributed in forest zones in West Africa, e.g. Sierra Leone, Nigeria, Guinea and Ghana (Kone *et al.,* 2004).

2.4 MEDICINAL USES

The purple coloured fruit is said to be edible. It was reported that people use the macerated fruit of different species of *Napoleonaea* to cure diseases. The bark and fruit are used as a cough medicine, and the raw bark is usually chewed as remedy for asthma and also as a pain reliver. The fruits are used as mucilage. Other traditional uses involve treatment of stomach ache, typhoid fever, and urinary track infections e.g siphillis, gonorrhoea and candidiasis (Burkill, 1999).

2.5 PHYTOCHEMICAL CONSTITUENT OF *NAPOLEONAEA* SPECIES

The phytochemical screening of Lecythideceae revealed the presence of alkaloid, saponins, cardiac glycoside, tannins and terpenes (Neuwinger, 2000). The pharmacological and phytochemical properties of *Napoleonaea asteroids* confirmed that the plant has numerous bioactive compounds such as flavonoids, vitamins, enzymes, nicotinic acid, and tyrosine. The plant exhibit high pharmacological activity against the tested organisms which is attributed to the present of bioactive components. The morphological study of *Napoleonaea vogetii* also confirmed that it is a thorny tree growing to 30 feet tall, while the phytochemical study showed the presence of chemical compounds such as alkaloids, flavonoids, triterpenoid and lectin (Neuwinger, 2000).

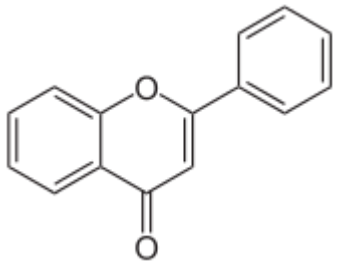
Plate 1: The photograph of leave and fruit part of ***Napoleonaea heudelotti***

2.6 PHARMACOLOGICAL ACTIVITIES OF *NAPOLEONAEA* SPECIES

The aqueous leaf extract of *Napoleonaea imperialis* showed the toxicity effect at 2000-2800 mg Kg^{-1} in albino rat which is attributed to the combined effect of phytochemical constituents (Musa *et al.,* 2008). The anti-inflammatory and analgesic property of the root-bark of *Napoleonaea asteroids* was studed and the result showed that the plant is rich in monomeric and diagomeric procyanidin. These findings confirmed that the extract posseses' potent anti-inflammatory and analgesic properties and the procyamidin which lead to the observable pharmacological effect (Dongmo *et al.,* 2001). The ethyl acetate fraction of *N. asteroids* was also studied, and the fraction tested positive to secondary metabolites such as tannins, flavonoids which are responsible for activities such as antispasmodic, antihelmintic, anti-inflammatory, hepatoprotective, antidiabetics and antibacterials (Musa *et al.,* 2008). The compound isolated from the fraction was evaluated for it antimicrobial activity was found to be inactive against the *S. typhi* and *C. albicans,* while it showed moderate activity against *P. vulgari* (Jonathan *et al.,* 2000). The diterpenoid alkaloid 7 β-*hydroxy-7-deoxo-6-oxonorcassaide* was also obtained from stem-bark extract of *Napoloenaea egertomii.* The compound also showed wide range of antimicrobial activity against gram-positive and gram-negative bacteria (Cowan, 1999). The alkaloids present in *Napoleonaea vogetii* were reported to be responsible for its anti-inflammatory and analgesic activity while the isoflavonoid is responsible for its antibacterial and antihelminthic effect (Harbone, 1998). The aqueous extract also shows pharmacological effects such as neuromuscular blocking, smooth muscle relaxant.

2.5.1 Structure of some of the compounds isolated from *Napoleonaea* species

The structures of the two compounds isolated from *Napoleonaea* species include *2-phenuylchromen-4-one s (2-phenyl-1-benzopyran-4-one)* **(10)** from *Napoleonaea vogetti* and *3, 4, 5-trihydroxylbenzoic acid* (Gallic acid **(8)**) (Justin, 2011).



2-phenuylchromen-4-one s (2-phenyl-1-benzopyran-4-one)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials use for extraction

3.1.1 Solvents

i Methanol

ii Ethyl acetate

ii Chloroform

iv Petroleum ether (60-80)

3.1.2 Apparatus

I . Vacuum pump

ii. Rotavapour

iii Soxhlet extractor apparatus

iv Water bath

v Heating mantle

vi Washing bottle

vii Separatory funnel

vii Beaker

3.1.3 Reagents for phytochemical screening

i NaOH (10 %)

II HCl acid (Concentrated and 10 %)

iii Dilute H_2SO_4

iv Acetone

v Ammonia solution (28 %)

vi Acetic anhydride

vii Wagner's reagent (prepared from iodine (2 g) + potassium iodide (3 g) in water)

viii Picric acid

ix Mayer's reagent (prepared from potassium iodide (5 g) + mercuric iodide (4 g) in H_2O)

x Fehling solution A (CuSO4 (7 g) in a distilled H_2O (10 ml))

xi Fehling solution B (potassium tartarate (32.5 g) in NaOH (15.4 g)

xii Wagner's reagent (2g of iodine +3g of possium iodide in water)

xii Dragendoff's reagent (potassium iodide (4 g) + bismuth iodide (3 g) + acetic acid 5 ml in H_2O ml)

xii Molisch reagent (solution of α-naphthol 10 % in methanol (15 ml)

xiv Lieberman-burchard reagent (sulphuric acid (5 ml) + nitrous acid (5 ml))

All these reagents were prepeared as described by Vogel, (2009)

3.1.4 Test microorganisms used

Gram-positive and gram-negative micro-organisms such as *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris, Candida albicans, Candida krusei* and *Candida tropicali*s were used for the antimicrobial studies. The microorganisms were obtained from the Department of Microbiology, A.B.U. Zaria.

Standard commercial drug for antimicrobial screening: Ciprofloxacin

3.2 Collections of Plant Materials

The root part of *Napoleonaea heudelotii* was collected from Makurdi, Benue State Nigeria in June, 2012. The plant was properly identified and authenticated by Mallam U.S Galla at the Herbarium unit of Biological Science Department, Ahmadu Bello University, Zaria-Nigeria; where a sample of it was assigned the voucher specimen number 4299. The root material was collected and air-dried under shade for 21 days and ground to coarse powder using wooden mortar and pestle.

3.3 Extraction of Plant Materials

The dried powder (500 g) of root material was extracted in methanol using Soxhlet extractor, round bottomed flask, anti-bumping chips and rotary evaporator at 40 ^{o}C. The crude extract was air-dried and partitioned with chloroform and ethyl acetate to obtain chloroform and ethyl acetate fractions respectively. The solvent were removed *in-vacuo* to yield 30 g of methanol extract, 15 g of ethyl acetate and 12 g of chloroform fraction.

3.4 Phytochemical Screening

The methanol extract, ethyl acetate and chloroform fractions were subjected to phytochemical screening using standard procedures. The secondary metabolites that were tested for include saponins, glycosides, tannins, flavonoids, cardiac glycosides and alkaloids (Harbone, 1998; Sofowora, 1993; Silva *et al.*, 2009; Trease and Evans, 2009).

3.4.1 Test for glycosides

Dilute hydrochloric acid (5 ml) was added to small portion of each sample and boiled for 5 minutes and then neutralized with 10 % sodium hydroxide (5 ml). A mixture of Fehling's solution A and B (5 ml) were added to the cold solutions. Brick red precipitate confirmed the presence of glycoside.

3.4.2 Test for Cardiac Glycosides (Keller – killiani test)

Glacial acetic acid (1 ml) containing trace of ferric chloride solution was added to the aqueous solution of the methanol extract followed by concentrated sulphuric acid (1 ml) at an angle 45 ^{0}C to the wall of the test tube and allowed to stand. A purple colour was formed at the interphase which indicates the presence of saponin.

3.4.3 Test for tannin

a Ferric chloride test

To each sample (1.0 g), distilled water (10 ml) was added, boiled and filtered. Few drops of ferric chloride solution was added to the filtrate, a blue black precipitate was observed which confirmed the presence of tannins.

b Lead sub-acetate Test

Lead sub-acetate solution (3 drops) was added to the aqueous solution of each sample. A green precipitate confirmed the presence of tannins.

3.4.4 Test for saponin

Distilled water (5 ml) was added to each portion of the sample (0.3 g) in the test tube and shaken vigorously for about 30 seconds, and then allowed to stand for 45 minutes. A honey-comb froth was formed which indicates the presence of saponins.

3.4.5 Test for flavonoids (Shinoda test)

A small quantity of magnesium powder and a few drop of conc. HCl were added to an alcoholic solution of each sample. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoid.

Sodium hydroxide test

A sodium hydroxide solution (10 %, 3 drops) was added to the aqueous solution of each sample. A yellow colored solution which became colourless on addition of dilute hydrochloric acid was observed which indicates the presence of flavonoid.

3.4.6 Test for carbohydrates

Molisch's test

Each sample (0.5 g) was transfered into a test tube and a few drop of Molisch'sreagent was added. Concentrated H_2SO_4 (1 ml) was allowed to flow down the side of the inclined tube so that the acid form a layer beneath the aqueous solution without mixing with it. Red ring was formed at the interphase with a dull-violet precipitate which indicates the presence of carbohydrate.

Fehling's test

A mixture of equal volumes of Fehling's A and B was added to (0.5 g) of each sample and boiled for five minutes. A brick-red precipitate confirmed the presence of carbohydrate.

3.4.7 Test for combined reducing sugar

Dilute hydrochloric acid (5 ml) was added to (0.5 g) of each sample and boiled for 5 minutes and then were neutralized with sodium hydroxide (10 %, 5 ml). Equal volumes of Fehling's solution A and B were added to the cold solutions. A reddish brown precipitate was confirmed.

3.4.8 Test for Steroids/Triterpenes

Lieberman-Buchard test

Each sample was dissolved in chloroform and a few drop of acetic anhydride were added followed by concentrated sulphuric acid. The mixture was carefully mixed and the resulting solution was observed for blue, green, red or orange colour that changes with time.

Salkowski test

Small quantity of each sample was dissolved in (1 ml) chloroform (1 ml) concentrated sulphuric acid (1 ml) which was added down the test tube to form two phases. Fomation of red or yellow colouration was taken as an indication for the presence of steroids/triterpenes.

3.4.9 Test for alkaloids

To each sample (0.5 g) with 1 % aqueous hydrochloric acid (5 ml) was added and heated on a water bath, it was allowed to cool and filtered. The filtrates (3 ml) were divided into three portions. To the first portion few drop of freshly prepared Dragendoff's reagent was added and observed for formation of orange to brownish precipitate. To the second portion 1 drop of Mayer's reagent was added and observed for formation of white to yellowish or cream color precipitate. To the third portion 1 drop of Wagner's reagent was added to give a brown or reddish or reddish-brown precipitate (Silva *et al.*, 2009).

3.5 Anti-microbial Screening.

3.5.1 Culture media

The culture media used were Mueller Hinton agar (MHA) Mueller Hinton broth (MHB) Nutrient agar (NA), and potato dextrose agar. All the media were prepared according to manufacturer's specifications. The standard used was ciprofloxacin 10µg.

3.5.2 Preparations of different concentrations of the extracts

Various concentrations of the extracts ranging from 100 mg/ml to 6.25 mg/ml were prepared by first dissolving each extract (1 g) with sterile distilled water (10 ml) to obtained 100 mg/ml concentration. Serial dilutions of the extracts were carried out into four different bottles to obtain concentration of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively.

3.5.3 Preparation of standard inoculums of test organisms

The inoculum of the test organisms were prepared by first streaking the organisms on the prepared nutrient agar holes to obtain discrete colonies of the bacteria. A colony was picked and subculture unto sterile nutrient broth and incubated at 37 ^{o}C for 24 hours. From the broth culture a loopful of each bacterial suspension were transferred into bottles containing sterile distilled water to obtain a bacterial density of 1.5 ×108 cfu/ml as determined by the McFarland scale No 1 (Vollekova *et al.,* 2001).

3.5.4 Sensitivity test of the extracts using agar well diffusion method

The standardised bacterial isolates were uniformly streaked unto freshly prepared Mueller Hinton agar (MHA) using sterilized swab stick. Five wells were punched on the agar holes

using sterile cork borer (8 mm) in diameter. The wells were properly labelled according to different concentrations prepared. The wells were then filled with 0.2 ml of different concentration of the extracts. The plates were left on the bench for about one hour for the extracts to diffuse into the agar and then, they were incubated at 37 ^{o}C for 12 hours. After the incubation period, the plates were observed for any evidence of inhibition, which will appear as a clear zone that was completely devoid of growth around the wells. The diameters of the zones were measured using a transparent ruler calibrated in millimetre (Vollekova *et al.,* 2001).

3.5.5 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined using the tube dilution methods. First serial dilutions of the extracts were carried out in well labelled test tubes using Mueller Hinton broth as a diluent. The lowest concentrations showing inhibition for each organism when the extracts were tested using agar well diffusion methods were serially diluted in test tubes containing Mueller Hinton broth. Each organism was inoculated into each test tube containing the diluted extract. A tube containing sterile Mueller Hinton broth without any organism is used as a control. The tubes were then incubated at 37 ^{O}C and examined for the presense or absence of growth using turbidity as criterion. The lowest concentrations in the serial dilution without visible signs of growth after 12 hrs were considered to be the minimum inhibitory concentration (MIC) (Vollekova *et al.,* 2001).

3.5.6 Determination of minimum bactericidal concentration (MBC)

The results from the minimum inhibitory concentration (MIC) were used to determine the minimum batericidal concentration (MBC). A sterilized wire loop was dipped into the test tubes that did not show turbidity in the (MIC) test, it was then streaked on fresh nutrient agar plates. The plates were incubated at 37 ^{o}C for 24 hours. After incubation period the plates were examined for the presence or absence of growth. This was done to determine whether the antibacterial effect of the samples is bactericidal or bacteristatic (Vollekova *et al.,* 2001).

3.6 Purification of Chloroform fraction

3.6.1 Thin layer chromatography

This technique was used in order to determine the possible numbers of compounds present in the fraction, as well as suitable solvent system and solvent mixture. Small portion of chloroform fraction dissolved in chloroform was spotted on a pre-coated plate (4 x 4 cm), and developed in a chromatographic tank containing solvent system of petroleum ether and ethyl acetate at different ratios (9:1, 8:2, and 7:3). The plate was removed, the solvent front was marked and the plate was dried. The plate was viewed under UV lamp and the R_F value of spot was measured and calculated. Similar R_F values were collected together and determined.

3.6.2 Preparation of Preparative Thin Layer Chromatography plates

Preparative TLC plate (20 x 20 cm) was prepared by dissolving 50 g of silica gel in 100 ml of distilled water and shaken vigorously for 1 minute. The slurry mixture was spread

on the plates, and was air-dried for 30 minutes and activated in an oven for 1 hour 30 minutes. The plate was allowed to cool for another 30 minutes.

3.6.3 Isolation of pure compound from chloroform fraction

The chloroform fraction (5.0 g) was dissolved in chloroform, which was then spotted on the preparative TLC plates (20 x 20 cm). The solvent system (petroleum ether/ethyl acetate (80:20 v/v) was allowed to move up the plates (developed) for 15-20 minutes in a saturated chamber (tank). The solvent front of each plate was marked immediately it was removed from the tank. The plates were dried rapidly to avoid diffusion of spots, and the dried plates were viewed under UV lamp (366 nm). The position of the spots on the plates were marked and R_F values of each spot was measured and calculated. Fractions with similar R_F values were collectively combined together. The combined fractions were then washed with methanol and filtered, the filtrates were evaporated and lebelled A, B and C. The fraction B was purified further using preparative TLC technique, and a white crystal compound named N_{HPE} was obtained.

3.6.4 Melting Point Determination

The melting point of the isolated compound, N_{HPE} was determined using the Gallenkamp melting point apparatus.

3.6.5 Spectra Analysis:

The ^{1}H, ^{13}C, DEPT NMR and GC/MS of the isolated compound N_{HPE} spectra were obtained at the School of Chemistry and Physics, University of Kwazulu-Natal Durban South Africa on Bruker AVANCE spectrometer (600 MHz for ^{1}H and 400 MHz for ^{13}C), using the TMS peaks as standard Chemical shift values which were reported in parts per million (ppm) relative to TMS standard and coupling constants are given in Hz. The solvent used for these measurements is deuterated chloroform.

CHAPTER FOUR

4.0 RESULTS

4.1 % Recovery on Extraction

Table 4.1: Weight of the various extrats of *Napoleonaea heudelotti*

Extracts	**Mass of extract (g/500g)**	**(%) Recovery**
Methanol	30	6
Chloroform	12	3
Ethyl acetate	15	2.3

The percentage recovery of the extract and fractions were shown in Table 4.1. Methanol gave the highest recovery of 30 g (6 %). Ethyl acetate gave 15 g (3 %) while chloroform gave the least yield of 12 g (2.3 %).

4.2 Phytochemical Screening

Table 4.2.Phytochemical Screening of *Napoleonaea heudelotti*

Secondary metabolites	**Tests**	**Methanol extract**	**Chloroform fraction**	**Ethyl acetate fraction**
Carbohydrates	Molisch test	+	+	+
	Fehling test	+	+	+
Glycosides	Fehling test	+	+	+
Cardiac glycosides	Kelle killiani test	+	+	+
Saponins	Froth test	+	+	+
Steroid / triterpene	Lieberman-Burchard Test	+	+	+
	Salkwoski test	+	+	+
Flavonoid	Shinoda test	+	+	+
	NaOH test	+	+	+
Tannin	Lead acetate	+	+	+
	Ferric chloride	+	+	+
Alkaloid	Mayers test	-	-	-
	Wagner's test	-	-	-
	Dragendoff's test	-	-	-

KEY + = present - = absent

The methanol extract and the two fractions showed the presence of carbohydrates, glycosides, and cardiac glycoside (Table 4.2). The extract and the fractions also showed the presence of saponins, steroids/triterpene, flavonoids and tannins. Alkaloids are absent in all extracts.

4.3 Antimicrobial Susceptibility Test

Table 4.3: Antimicrobial Susceptibility Test of the extract and the fractions

Test organisms	**Chloroform fraction**	**Ethylacetate fraction**	**Methanol extract**
Staphylococcus aureus	S	S	R
Bacillus subtilis	S	S	S
Escherichia coli	S	S	S
Salmonella typhi	S	R	S
Pseudominas aeruginosa	R	R	S
Proteus vulgaris	S	R	S
Candida albicans	S	S	R
Candida krusei	S	S	S
Candida tropicalis	S	R	S

Keys S= *sensitive R= resistant.*

The activity of methanol extract and other fractions (ethyl acetate and chloroform), were tested against both gram positive and gram negative microbes, which are *Staphyloccucos aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgari, Candida albicans, C. krusei* and *Candida tropicalis.* The results (Table 4.3) indicate that the microorganisms are all sensitive to chloroform fraction except *Pseudomonas aeruginosa*. Four pathogens showed resistance to ethyl acetate namely *Salmonella typhi, Proteus vulgaris, Candida tropicali*s and *Pseudomonas aeruginosa,* while two microorganisms showed resistance to methanol extract which are; and *Staphylococcus aureus* and *Candida albicans*

4.4 Zone of Inhibition (mm)

Table 4.4: The Zone of Inhibition (mm) of the Extract and Fractions

Test organism	Diameter of Zone of inhibition (mm)			
	Methanol Extract	Ethyl acetate Fraction	Chloroform fraction	Ciprofloxacin 10µg/disc
S. aureus	-	20	24	40
B. subtilis	20	14	23	35
E. coli	16	15	25	32
S. typhi	17	-	21	34
P. vulgaris	21	-	19	37
C. albicans	-	16	18	33
C. krusei	18	14	18	30
C. tropicalis	18	-	17	31

The zone of inhibition of the extract and the two fractions in Table 4.4 showed that the chloroform fraction inhibits all the tested microorganisms, methanol extract showed no inhibition against *S. aureus and C. albicans,* While the ethyl acetate fraction showed no inhibition to three microorganisms which are: *S. typhi, P. vulgari* and *C. tropicalis.* The methanol extract ranged between 16 mm and 21 mm, chloroform fraction ranged between 17 mm and 25 mm, while the ethyl acetate fraction ranged between 14 mm and 20 mm respectively.

4.5 Minimum Inhibitory Concentration (MIC)

Table 4.5: Minimum Inhibitory Concentration (MIC) of the Extract and Fractions

Test organism	Methanol extract (mg/ml)	Chloroform (mg/ml)	Ethyl acetate (mg/ml)
S.aureus	-	12.5	6.25
B. subtilis	6.25	6.25	-
E.coli	3.123	3.123	3.123
S.typhi	12.5	12.5	-
P.vulgaris	1.562	6.25	-
C.albicans	-	1.562	6.25
C. krusei	1.562	6.25	3.123
C.tropicalis	3.123	3.123	-

Key: - = No activity

The minimum inhibitory concentration (MIC) confirmed that the chloroform fraction inhibit growth in all of the tested microorganisms (Table 4.5). The (MIC) of methanol extract was inactive against *S. aureus, and C. albicans,* while the ethyl acetate fraction was not active against *B. subtilis, S. typhi*, *P. vulgari*, and *C. tropicalis.* The MIC of methanol extract ranged between 1.562 mg/ml and 12.5 mg/ml, chloroform fraction ranged between 1.562 mg/ml and 12.5 mg/ml, while that of ethyl acetate is between 3.123 mg/ml and 6.25 mg/ml.

4.6 Minimum Bactericidal Concentration (MBC)

Table 4.6: Minimum Bactericidal Concentration (MBC) of Methanol extract Ethyl acetate and Chloroform fractions.

Test organism	Methanol extract (mg/ml)	Chloroform fraction (mg/ml)	Ethyl acetate fraction (mg/ml)
S.aureus	-	6.25	6.25
B. subtilis	6.25	12.5	-
E. coli	3.123	3.123	3.123
S. typhi	12.5	1,562	-
P. vulgaris	1.562	6.25	-
C. albicans	-	12.5	1.625
C. krusei	6.25	1.562	12.5
C. tropicalis	3.123	3.123	-

Key:- = No growth

The minimum bactericidal concentration (MBC) confirmed that the chloroform fraction inhibit growth in all of the tested microbes (Table 4.6). The (MBC) of methanol extract was inactive against *S. aureus* and *C. albicans*, while the ethyl acetate fraction showed resistance to four microbes, namely, *B. subtilis, P. vulgaris, C. albicans*, and *C. tropicalis.* The methanol extract ranged between 1.562 mg/ml and 12.5 mg/ml; chloroform fraction ranged between 1.562 mg/ml and 12.5 mg/ml; while the ethyl acetate fraction ranged between 1.625 mg/ml and 6.25 mg/ml respectively.

4.7 Preparative Thin Layer Chromatography

Table 4.7: Preparative Thin Layer Chromatography of Chloroform Fraction with Solvent mixture of petroleum ether /ethyl acetate ratio (8:2).

Spots	**R_F values**
1	0.10
2	0.40
3	0.90

The chloroform fraction gave three spots with R_F values of 0.10, 0.40 and 0.90 with solvent system of petroleum ether/ethyl acetate, ratio 80:20 (Table 4.7).

4.8: Chemical test of the isolated compound

Table 4.8: Chemical test of the isolated compound.

Secondary metabolites	**Tests**	**N_{HPE}**
Steroid/Triterpene	Lieberman-Buchard test.	+

+ = positive

Lieberman-Buchard test was carried out on the isolated compound and it showed the presence of triterpene. This test confirmed that the isolated compound is pentacyclic triterpene.

4.9 Melting Point of the Isolated Compound

The melting point of the isolated compound N_{HPE} is between 182 -183 ^{o}C

4.9 Spetroscopic Analysis of Sample N_{HPE}

These analyses are ^{1}H NMR, ^{13}C NMR, DEPT NMR and GC/MS.

4.9.1 Gas chromatography and Mass spectroscopy

The gas chromatogram (Fig 4.1) of the isolated compound N_{HPE} showed 5 small peaks at different R_T values of 10,11,12 13 and 14 min. The intensity of the major peak is 6000000 times than the small peaks at R_T value of 28.2 min. The mass spectrum showed the molecular ion of the compound to be 426.4, and the base peak at 218.3.

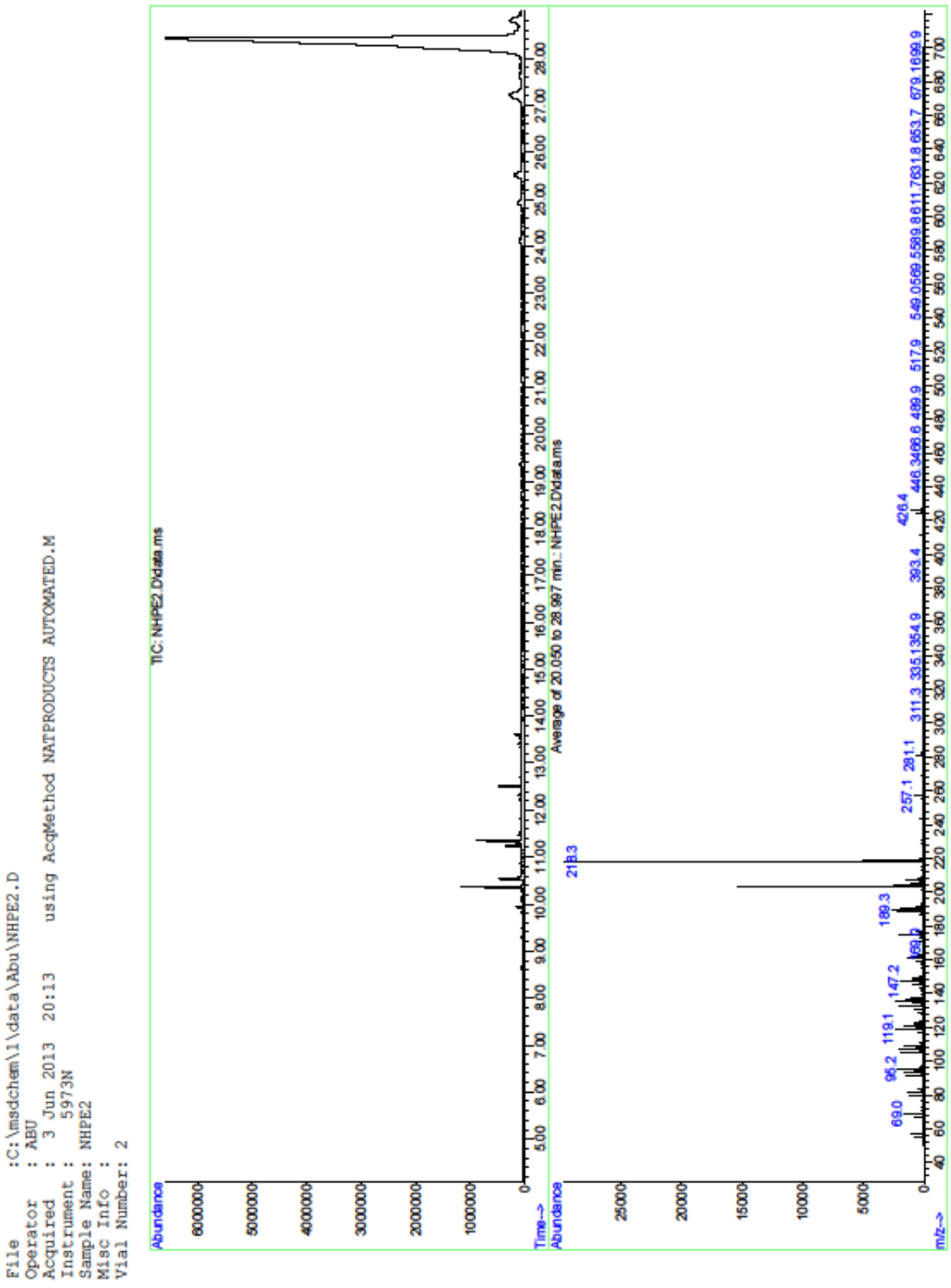


Fig 4.1: Gas chromatogram and Mass spectrum of N_{HPE}

4.10 Nuclear Magnetic Resonance Spectrum

^{1}H NMR ^{13}C NMR and 2D NMR of the isolated compound, N_{HPE} were run.

(A) ^{1}H NMR specturum

The ^{1}H NMR spectrum (Fig 4.2) showed four chemical shift region, ranging from δ_H 0.5 ppm to 5.3 ppm. According to the report by Kuo *et al.* (2007), the signals from δ_H 0.5 ppm to 2.3 ppm may be due to overlapping of methyl (-CH_3), methylene (-CH_2), and methine (-CH) protons. The signals at δ_H 3.2 ppm may be due to the oxygenated methane proton O-C-OH,while signals at δ_H 5.1 ppm and 5.3 ppm may be due to olefinic protons (-CH=CH-).

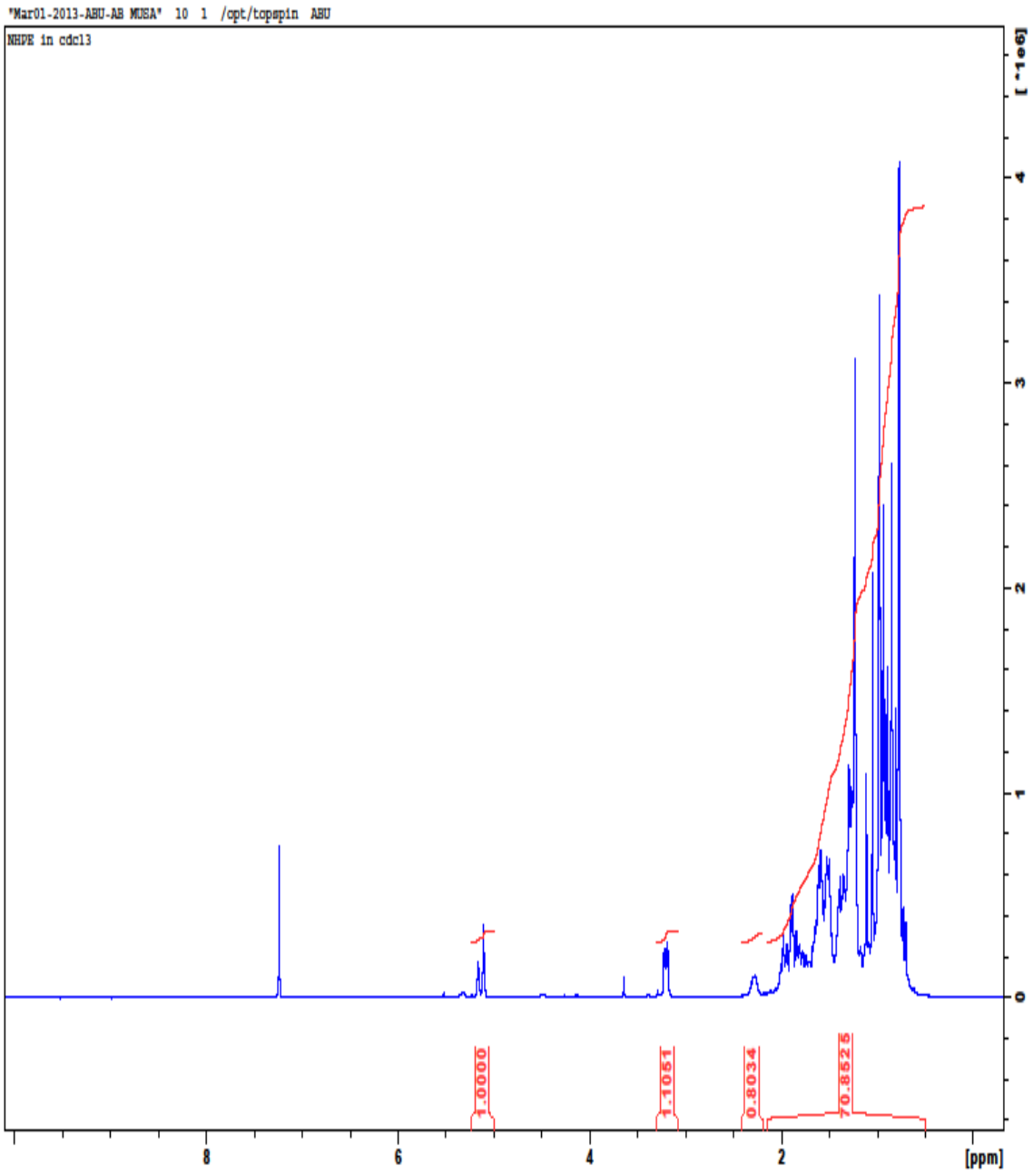


Fig 4.2: ^{1}H NMR spectrum of N_{HPE}

(B) ^{13}C NMR spectrum

The ^{13}C NMR spectrum (Fig 4.3a) showed sixty signals. The signals ranging from δ_C 14.33 ppm to 145.2 ppm. This confirms the presence of sixty carbon atoms, which are CH_3, CH_2, -CH=CH-, CH, and C respectively.

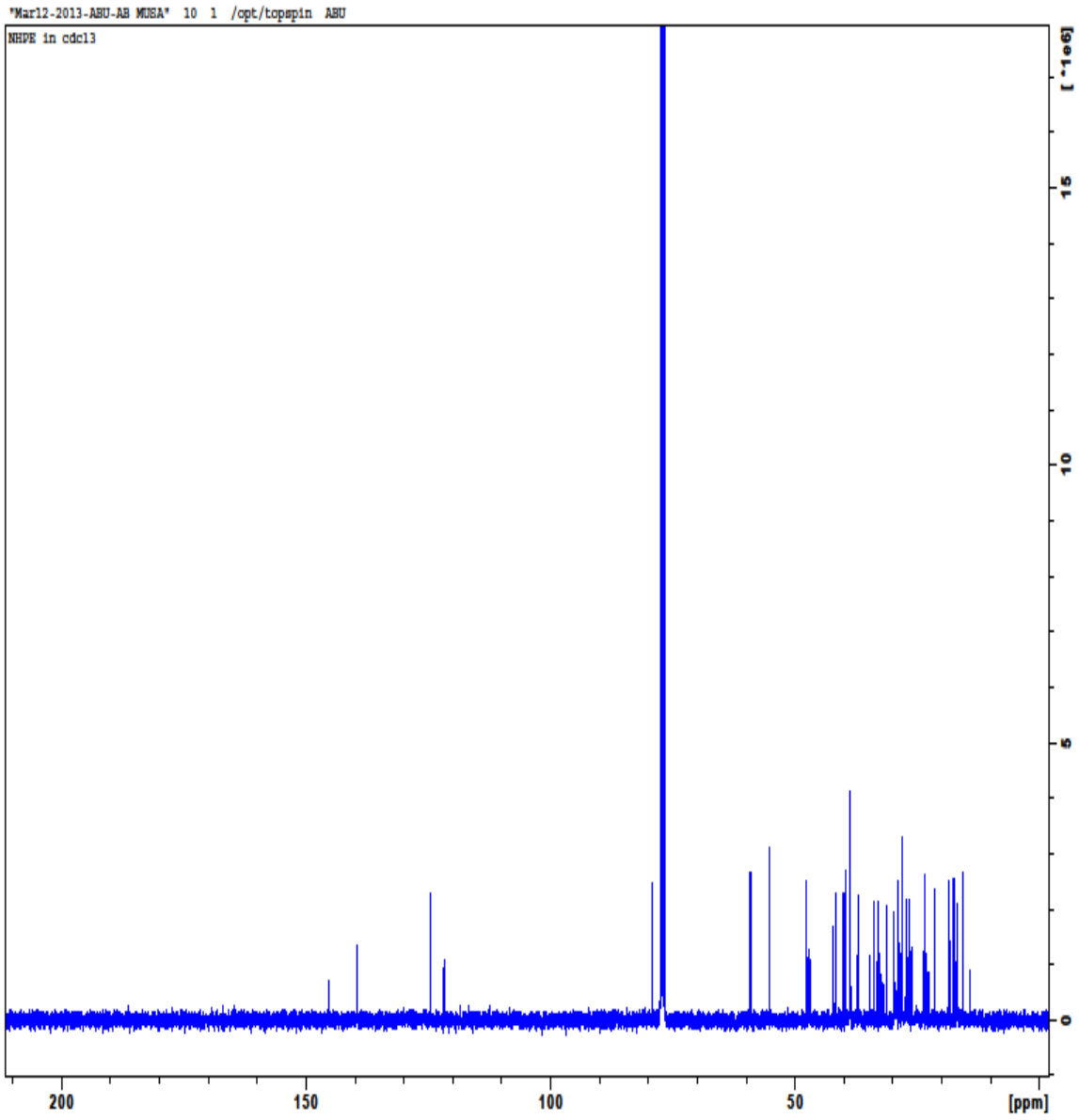


Fig 4.3a: ^{13}C NMR spectrum of N_{HPE}

First expansion: The ^{13}C NMR spectrum (Fig 4.3b) showed 16 peaks with chemical shift ranging from 45 ppm to 145 ppm. The carbons between these ranges are $CH_2=CH_2$, from 122 ppm to 125 ppm, C at 80 ppm and CH-OH from 140 ppm to 145 ppm.

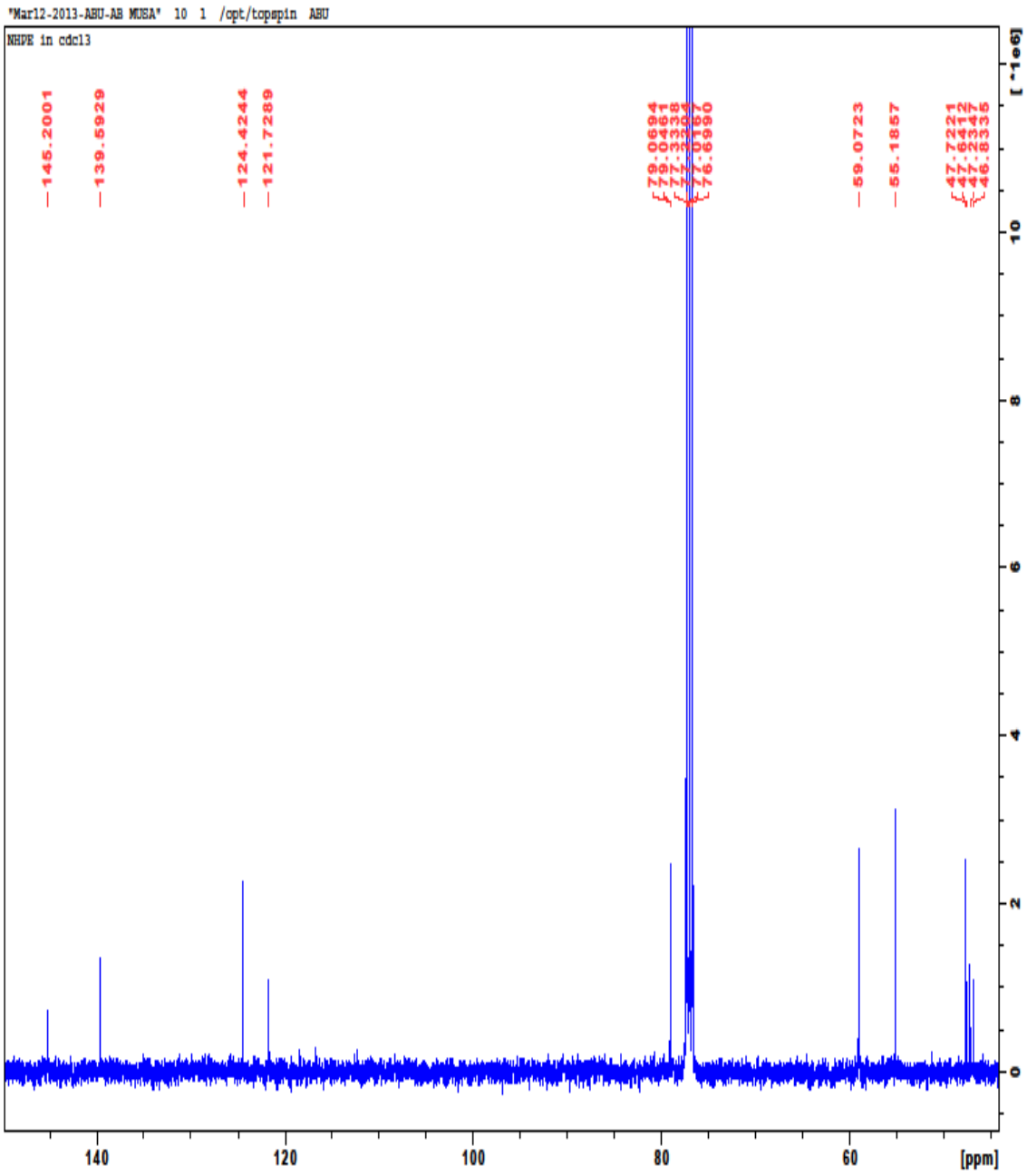


Fig 4.3b: ^{13}C NMR spectrum from 45 ppm - 150 ppm of N_{HPE}

Second expansion: The ^{13}C NMR spectrum (Fig 4.3c) showed 44 peaks with chemical shift ranging from 14.5 ppm to 45 ppm. The carbons between these ranges represent CH_3, CH_2, CH, and C carbon atoms.

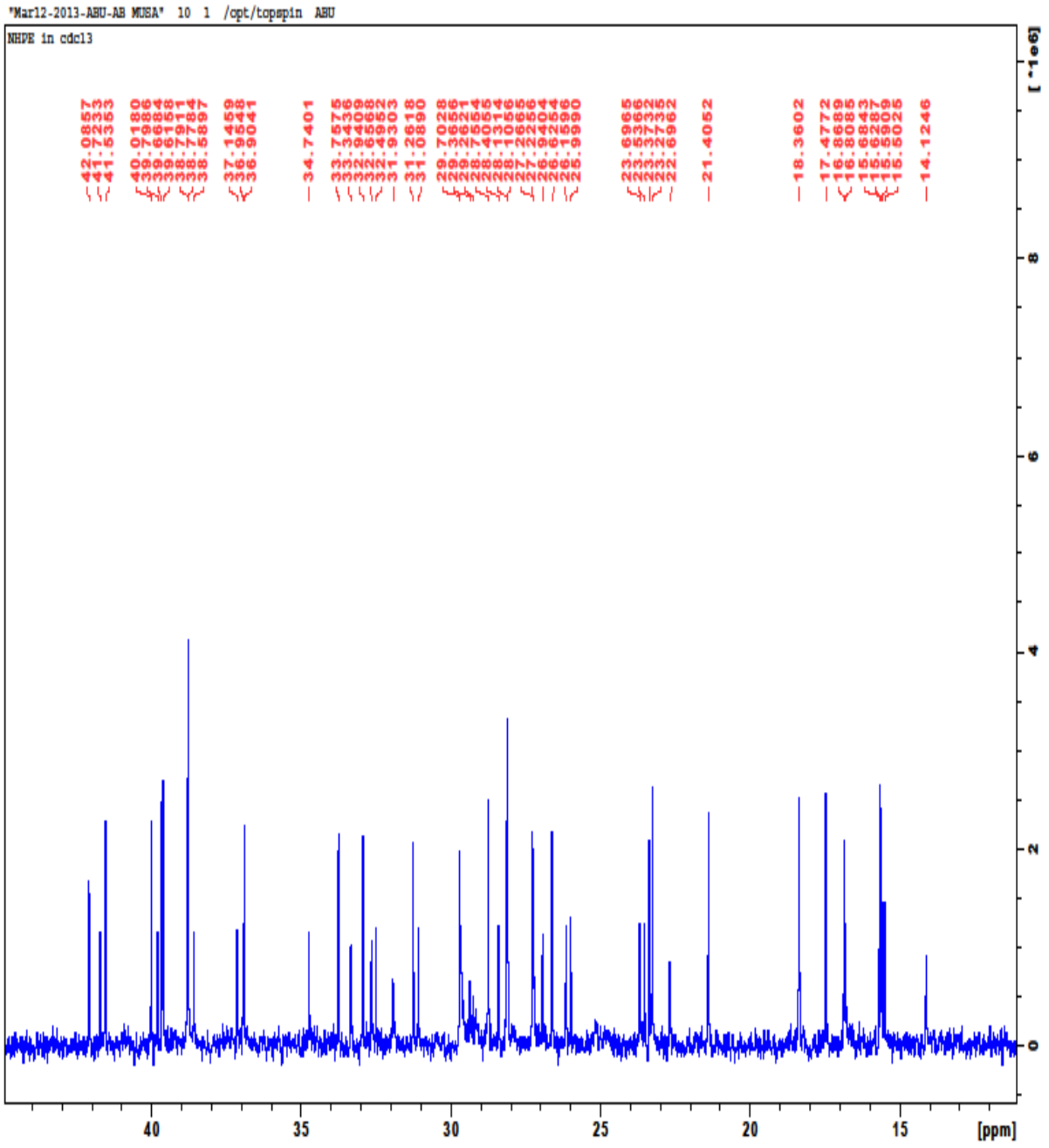


Fig 4.3c: ^{13}C NMR spectrum from 1 ppm - 45 ppm of N_{HPE}

4.10.1 (C) DEPT SPECTRUM: The DEPT spectrum (Fig 4.4) showed three layers of signals which include upper layer, middle layer and lower layer. The lower layer consists of all numbers and types of carbon atoms present (CH_3, CH_2, CH and C), in the compound. The middle layers consist of only the total number of CH_3, while the upper layer consist of CH_2 at the lower part and CH_3 at the upper part only. Therefore, the spectrum consists of sixteen CH_3 carbons, sixteen CH_2 carbons, twelve CH and sixteen C carbon atoms.

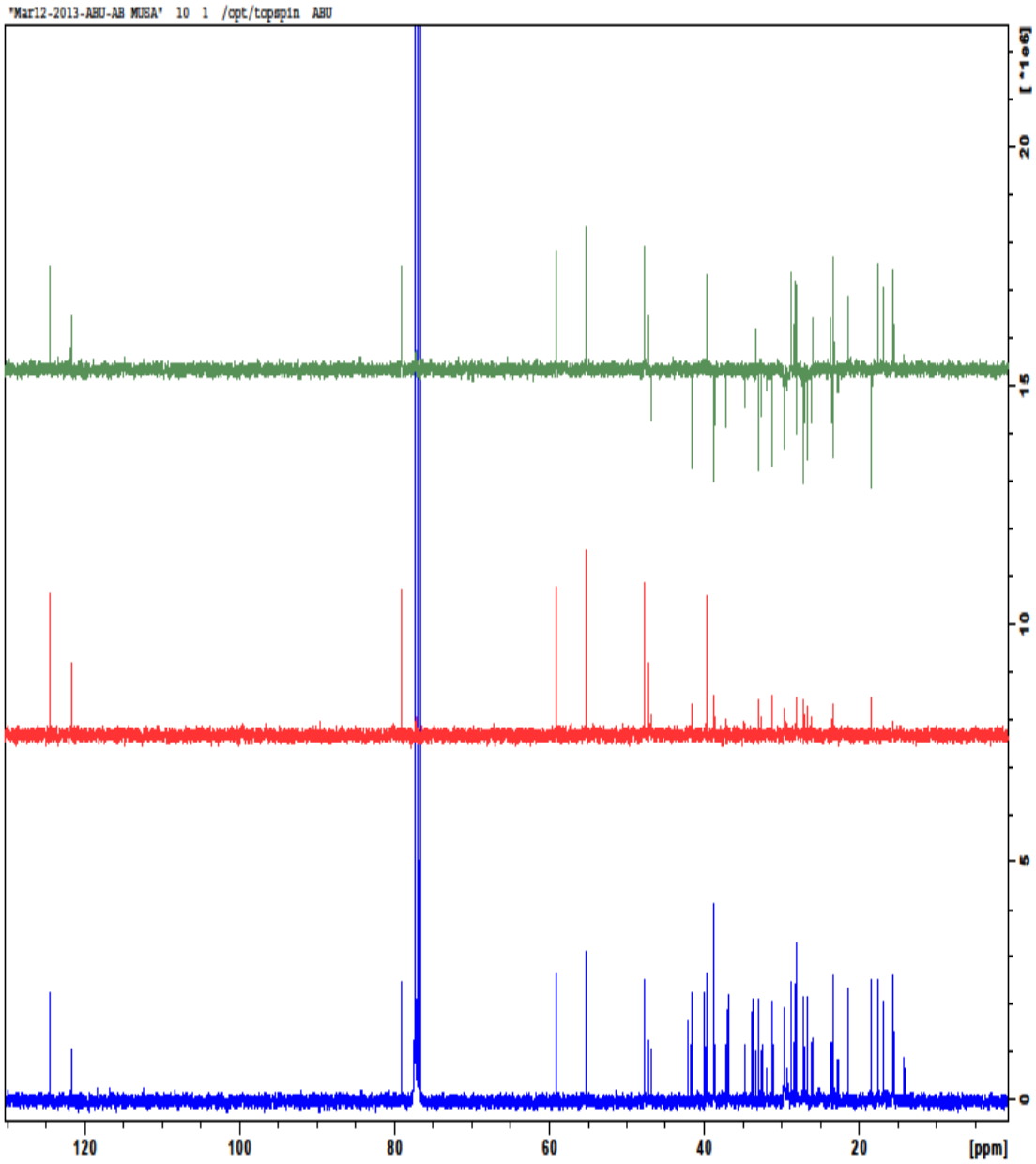


Fig 4.4: DEPT ^{13}C NMR spectrum of N_{HPE}

CHAPTER FIVE

5.0 DISCUSSION OF RESULTS

The extract and the fractions confirmed the presence of saponin, steroids/triterpene, flavonoids, tannins, carbohydrate and cardiac glycosides. This confirmed that the root part of *Napoleonaea heudelotti* posseses antimicrobial properties as such, they can be used as drugs traditionally and also for drug synthesis. The secondary metabolites were also reported to be present in the ethyl acetate fraction of the stem bark of *Napoleonaea vogetii* studied by Neuwenger, (2000). According to the report by the traditional medical practitionals and researches conducted on *Napoleonaea* speices, shows that the plant *Napoleonaea heudelotti* has maximum ability to synthesize secondary metabolites which serve as defensive mechanisms against microorganisms. The presence of terpenoids and glycosides show the ability of the plant *Napoleonaea heudelotti* to exhibit antifungal activities on some of the fungi parasites. The confirmation of saponins also demonstrates several biological and pharmacological importances such as antipyretic, analgetic, anticancer and anti-inflammentory activities (Mathia, 2010).

The result of the zone of inhibition confirmed that *Napoleonaea heudelotti* extract and the fractions were active against the tested gram positive and gram negative microorganism*s (S. aureus, P. vulgari E. coli e.t.c).* This shows that *Napoleonaea heudelotti* can be used in the treatment of different types of bacteria infections. Other species of *Napoleonaea (e.g N. vogetti and N. imperialis)* were also been reported to have shown high activity aganist gram positive and gram negative microbes like *Escherichia coli, Salmonella typhi,*

Pseudomonas aeruginosa and Candida tropicalis (Tulikk, 2003). The lack of activity of the fractions to some of the microorganisms may be due to the nature of the microorganism which pose strong mechanisms that prevent the effect of the active components on them (Kanwal *et al.*, 2009).

The MIC and MBC results of the extract and the fractions confirmed that the claim made by the traditional doctors that the plant is used for treatment of typhoid fever, sexually transmited diseases like *Staphylococcus aureus*, Siphillis and gonorrhoea is confirmed. The isolated compounds N_{HPE} which are confirmed by the chemical test to be triterpene could be further developed as a drug for the treatment of infectious diseases.

The chemical shift signals with the number of protons in ^{1}H NMR spectrum Fig 4.1. (α and β amyrins). α Amyrin include δ_H C_3 3.16 (dd, J= 5.1; 11.2 H), C_{12} 5.06 (t, J = 3.2 H), C_{15} 1.89 (td, J = 4.5; 13.5 H), C_{16} 1.76 (td, J = 5.0; 13.5 H), C_{22} 1.85 (dt, J = 3.0; 7.0 H), from C_{23} to C_{30} include, 0.93s, 0.74s, 0.73s, 0.89s, 1.01s, 0.94s, 0.85(d, J = 6.0 H), and 0.73 (d, J = 7.0). β amyrin include, δ_H C_3 3.15 (dd, J = 4.4; 10.8 H), C_{12} 5.12 (t, J = 3.2 H), C_{15} 1.94 (td, J = 4.0; 14.0 H), C_{19} 1.93 (dd, J = 4.0;13.17 H), from C_{23} to C_{30} include, 0.77s, 0.90s, 0.73s, 0.93s, 1.19s, 1.07s, 0.87s, and 0.80s. The peaks and signal agreed with the literature.

Table 4.9: Comparism of ^{13}C NMR spectrum of isolate with that of α-amyrin

Position of Carbon	Experimental Chemical Shift α Amyrin	Chemical Shift in the Literature α Amyrin
1	38.7	38.7
2	28.40	28.7
3	79.06	79.6
4	38.79	38.7
5	55.18	55.1
6	18.36	18.4
7	32.66	32.2
8	40.01	40.7
9	47.72	47.7
10	36.96	36.6
11	23.37	23.3
12	124.4	124.4
13	135.59	139.5
14	42.08	42.0
15	27.26	27.2
16	26.23	26.6
17	33.76	33.7
18	59.07	59.0
19	39.76	39.6
20	39.61	39.9
21	31.93	31.2
22	41.63	41.5
23	28.16	28.1
24	15.68	15.6
25	15.62	15.6
26	16.80	16.8
27	23.27	23.2
28	28.23	28.1
29	17.27	17.4
30	21.40	21.4

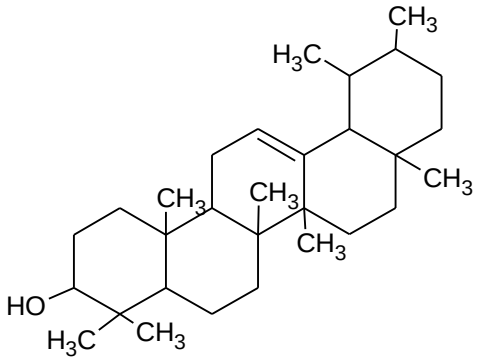
(Liliana *et al.*, 2005)

Table 4.10 : comparism of 13C NMR spectrum of isolate with that of β-amyrin

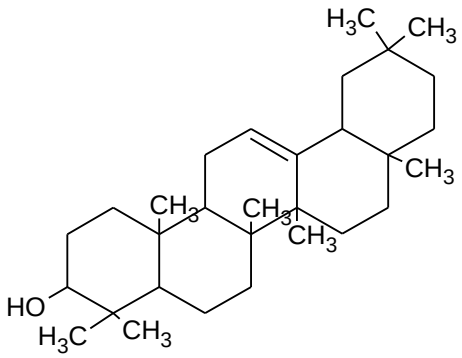
Position of Carbon	Experimental chemical shift β amyrin	Chemical shift in the literature β amyrin
1	38.62	38.7
2	27.22	27.2
3	79.04	79.3
4	38.77	38.5
5	59.07	55.1
6	18.50	18.6
7	32.49	32.4
8	39.66	39.8
9	47.64	47.6
10	37.14	36.9
11	23.69	23.6
12	121.72	121.7
13	145.20	145.2
14	41.72	41.7
15	26.16	26.2
16	26.0	26.1
17	32.49	32.6
18	47.23	47.2
19	31.29	31.0
20	46.50	46.8
21	34.74	34.7
22	36.69	37.1
23	28.10	28.0
24	15.59	15.4
25	15.50	15.4
26	16.80	16.8
27	25.99	25.9
28	28.32	28.4
29	33.76	33.8
30	23.62	23.7

(Liliana *et al.*, 2005)

The experimental data of ^{13}C NMR spectrum of the isolated compounds N_{HPE} (Table 4.13) correlate with the literature report (Kuo *et al.*, 2007). The observed melting point of the isolated compounds were also the same with what were reported in the literature.Therefore the compound is confirmed to be a mixture of α-amyrin **(11)** and β-amyrin **(12)**. The isolated compounds N_{HPE} are white crystalline solid compounds with melting point ranging from 182-183 ^{O}C. It was confirmed to be triterpene in Salkowski and Lieberman-Burchard test.



α –Amyrin (11)



β-Amyrin (12)

α -amyrin is a triterpene of natural origin isolated from various sources, most notably plant resins. Considerable amounts of this triterpene are available in the resins of *Bursera* and *Protium* species of the *Burseraceae* family (Scalbert *et al.,* 2005). Other known sources of ***α*** amyrin include Mexican copal (5 g/kg) *Cassia obtusifolia* (140 mg/kg) and the resin of *Commiphora holtziana* (syn. *Commiphora erythraea*) (200 mg/kg) (Mogbaddam, 2009). The most important sources of β-amyrin include lotus (*Nelumbo nucifera* Gaertn) bee pollen (3 g/kg) (Xu *et al.,* 2011), bark of "cuachalalate" (*Amphipterygium adstringens*) (2.4 g/kg), semi-preparative isolation from resin of *Protium* (***α*** -amyrin 1g /kg and β -amyrin 1.7 g/Kg) (Dongmo *et al.,* 2001). *Eucalyptus globulus* biomass residues from the pulping industry (326 mg/kg) *Ficus carica* latex (1.2 g/kg), root bark of *Ficus cordata* (20 mg/kg), stem bark *Ficus cordata* (200 mg/kg) and leaves and bark of *Byrsonima crassa* Niedenzu (IK) (1.3 g/kg) . Mixtures of ***α***- and β- amyrin were obtained from stem bark residues of *Byrsonima crassifolia* (Nance) (9 g/kg) leaves of *Byrsonima fagifolia* Niedenzu (2.3 g/Kg), and leaves of *Pouteria gardnerii* (Mart & Miq) Bahemi gave ***α*** -, β -amyrin and other triterpenes (Silva *et al.,* 2009). ***α***- and β- amyrin

are bioactive compounds commonly found in leaves, barks and resins. Such plant material is an interesting source of these triterpenoids, as it allows for easy extraction (Kon *et al.*, 2004). Extensive research over the last four years has identified ***α-*** and β- amyrin in several plants and the pure compounds have been shown to exhibit anti-inflammatory and other interesting biological activities. Amyrins are also involved in the biosynthetic pathways of other biologically active compounds such as avenacine, centellosides, glycyrrhizin or ginsenosides. The development of biotransformation systems to convert amyrins into these or other compounds would open new ways for using ***α-*** and β- amyrins as a source of bioactive plant secondary metabolites more scarcely distributed in the plant kingdom (Dongmo *et al.*, 2001).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY.

The whole plant of *Napoleonaea heudelotti* was collected from Makurdi, Benue State; it was properly identified at the Herberium unit of the Department of Biological Sciences, A.B.U. Zaria, with the voucher number 4299. The root part of the plant was air- dried and pulverized. The powdered root was extracted using methanol. The crude methanol extract as well as chloroform and ethyl acetate fractions that were subjected to preliminary phytochemical screening revealed the presence of saponins tannins flavonoids steroids and triterpenes. The antimicrobial screening of the methanol extract with the chloroform and ethyl acetate fractions showed a broad spectrum of antibacterial activity against tested micro-organisms. The chloroform fraction being the most active fraction was subjected to chromatographic techniques leading to the isolation of a mixture of pure compounds coded N_{HPE}. The isolated compounds were confirmed by spectroscopic analysis to be a mixture of pentacyclic triterpene, α and β amyrin.

6.2 CONCLUSION

This research work has proved scientically the uses of *Napoleonaea heudelotti* for the treatment of various infectious diseases. To the best of our knowledge, this is first report of the isolation of alpha and beta amyrin from the root of this plant species. This work will therefore add to the global database of the natural products. Base on the findings in this research work, it can be stated that the use of *Napoleonaea heudelotti* in the treatment of infectious diseases is confirmed.

6.3 RECOMMENDATIONS

This study only covered the root part of *Napoleonaea heudelotti*, since the aim is to validate the use of the plant for the treatment of certain diseases and to identify those chemical compounds responsible for such claims, therefore it is recommended that further phytochemical studies and biological activities tests should be conducted on the remaining parts of the plant organs.

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