PHYTOCHEMICAL AND ANTIMALARIAL STUDIES OF THE LEAVES OF *UVARIA CHAMAE* P.BEAUV. (ANNONACEAE)

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BY

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DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY AHMADU BELLO UNIVERSITY, ZARIA-NIGERIA

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DECLARATION

I declare that the work in this dissertation entitled "PHYTOCHEMICAL AND ANTIMALARIAL STUDIES OF THE LEAVES OF *UVARIA CHAMAE* P.BEAUV. (ANNONACEAE)"has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Hassan Ali Bila

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This Dissertation "PHYTOCHEMICAL AND ANTIMALARIAL STUDIES OF THE LEAVES OF *UVARIA CHAMAE* P.BEAUV. (ANNONACEAE)" by Hassan Ali Bila meets the regulations government the award of the degree of Master of Science in Pharmaceutical and Medicinal Chemistry of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research is dedicated to the Almighty God. For He has been the source of my strength.

ACKNOWLEDGEMENT

First and foremost I am eternally grateful to Almighty God for making it possible for me to carry out this research work. He has been my anchor.

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God bless you.

ABSTRACT

Uvaria chamae P.Beauv. belong to the Annonaceae family of flowering plants. It is distributed in the savannah and secondary forest. The plant is used in ethnomedicine for the treatment of malaria, inflammation, gonorrhea, dysentery; pile and fever. The ethyl alcohol leaves extract of the plant was subjected to phytochemical as well as antimalarial studies. Phytochemical studies were carried out using techniques including preliminary phytochemical tests, thin layer chromatographic analysis (TLC), column chromatography and gel filtration. The antimalarial activity of the crude ethyl alcohol leaves extract was evaluated using two models, suppressive and curative tests. The result of the preliminary phytochemical screening revealed the presence of saponins, flavonoids, carbohydrates, tannins, alkaloids and terpenoids. A flavonoids was isolated from the extract. The structure of the compound isolated was elucidated using UV, IR, 1D NMR and ESI-MS. The compound was found to be an epicatechin (3, 3, 4, 5, 7-pentahydroxyflavan). The leaf extract caused no lethality in mice at oral LD_{50} value of greater than 5000 mg/kg body weight. This indicated that the extract is safe for oral use. In the suppressive test the extract exhibited good antimalarial property that was dose dependent. At doses of 25, 50 and 100 mg/kgbody weight the extract produced a significant ($P< 0.05$) chemosuppression of 48, 53.3 and 65% respectively. Chloroquine the positive control drug produced the highest parasite chemosuppression at 79%. In the curative test the extract at doses of 25, 50 and 100 mg/kg body weight produced significant ($P< 0.05$) chemosuppression at 68, 70 and 73% respectively. Chloroquine the positive control drug produced the highest parasite chemosuppression of 98%. The result of this studies has established the rationale for the use of this plant in ethnomedicine. The isolated compound might be responsible for the observed biological activity.

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LIST OF ABBREVIATIONS

NMR	nuclear magnetic resonance
1D-NMR	1-dimensional nuclear magnetic resonanace spectroscopy
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
COSY	correlation spectroscopy
IR	infrared spectroscopy
g	gramme
kg	kilogramme
LD_{50}	Median lethal dose
mg	milligramme
nm	nanometer
ppm	parts per million
TLC	Thin layer chromatography
UV	Ultraviolet
δ_H	Proton chemical shift
CEE	Crude ethanol extract
EE	ethyl alcohol extract
N/S	Normal saline
EA	Ethyl acetate
R_f	Retardation factor
WHO	world health organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Natural Product

A natural product is a chemical compound or substance produced by a living organism. In the broadest sense, natural products include any substance produced by life (Samuelson, 1999). Natural products can also be prepared by chemical synthesis and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets (Natural Products Foundation, 2013). Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources (Hanson, 2003). Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites (Williams and Lemke, 2002). Secondary metabolites are not essential for survival, but nevertheless provide organisms that produce them with an evolutionary advantage (Maplestone *et al.*, 1992). Many secondary metabolites are cytotoxic and have been selected and optimized through evolution for use as chemical warfare agents against prey, predators, and competing organisms (Hunter, 2008). Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are the active components of drugs not only for most traditional medicines but also for many modern medicines (Samuelson, 1999).

1.2 Traditional Medicine

Traditional medicine has a long history and has been defined as the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (WHO, 2008). The WHO notes however that inappropriate use of traditional medicines or practices can have negative or dangerous effects and that further research is needed to ascertain the efficacy and safety of several of the practices (WHO, 2008).

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 the plant against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated; a number estimated to be less than 10% of the total (Tapsell *et al.,* 2006). Most of these compounds have been reported to have particular activity. Plant components like terpenes and alkaloids as well as xanthones and flavonoids, were reported to have antiplasmodial effect (Phillipson and Wright, 1990; Christensen and Kharazmi, 2001; Go, 2003). This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects (Lai and Roy2004). The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care (WHO, 2008). Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more common in recent years as

scientific evidence about the effectiveness of herbal medicine has become more widely available (Lai and Roy, 2004). Previous studies have shown that 61% of some 877 drugs introduced worldwide can be traced or were inspired by natural products (Cseke *et al.*, 2004).

1.3.0 Malaria

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan (a group of single-celled microorganism) belonging to the genus *Plasmodium* (WHO 2014). Malaria causes symptoms that typically include fever, fatigue, vomiting and headaches, in severe cases it can cause yellow skin, seizures, coma or death (Caraballo, 2014). The disease is transmitted most commonly by an infected female *Anopheles* mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood (WHO, 2014). The parasites travel to the liver where they mature and reproduce. Five species of *Plasmodium* can infect and be spread by humans with most death caused by *P. falciparum* (WHO, 2014). The disease is widespread in the tropical and subtropical regions that exist in a broad band around the equator. This includes much of Sub-Saharan Africa, Asia, and Latin America (Caraballo, 2014). Malaria is commonly associated with poverty and has a major negative effect on economic development (Gollin and Zimmermann, 2007). In Africa it is estimated to result in losses of US$12 billion a year due to increased healthcare costs, loss of ability to work, and effects on tourism (Greenwood *et al.*, 2005) The World Health Organization reports in 2013 that there were 198 million cases of malaria worldwide. The majority of which occurred in Africa (WHO, 2014).

1.3.1 Resistance to Malaria Chemotherapy

Resistance to anti-malarial drugs has been defined as: "the ability of a parasite to survive and multiply despite the administration of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject (White, 2004). The drug in question must gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action (Bloland, 2001). Resistance to anti-malarial drugs has posed a serious problem in the war against malaria and health intervention measures for malaria control need to be region specific due to diversity of resistance types (White, 2004).

Resistance of *P.falciparum* to old generation anti-malarial drugs such as chloroquine and pyrimethamine became widespread in the 1970s and1980s (WHO, 2014). In recent years, parasite resistance to artemisinin has been detected in some countries including Nigeria (Lim *et al.*, 2009).

Several factors can cause treatment failure, including problems with non-compliance and adherence, poor drug quality, interactions with other pharmaceuticals, poor absorption, misdiagnosis and administration of incorrect doses. The majority of these factors also contribute to the development of drug resistance (Bloland, 2001).

The generation of resistance can be complicated and varies between plasmodium species. It is generally accepted to be initiated primarily through an evolutionary benefit provided by a spontaneous mutation, thus giving anti-malarial drugs in use a reduced level of sensitivity. Resistance can become firmly established within a parasite population and exist for a long period of time (Murray and Bennett, 2009).The first type of resistance to be acknowledged was to chloroquine in Thailand in 1957. The biological mechanism behind this resistance was

subsequently discovered to be related to the development of an efflux mechanism that expels chloroquine from the parasite before the level required to effectively inhibit the process of haem polymerization (White, 2004).

1.4 Medicinal plants use for malaria treatment

Traditional herbal medicines have been used to treat malaria for thousands of years in various parts of the world. The first anti-malarial preparation used was extracted from the bark of the Cinchona species (Rubiaceae). Years later quinine (I) was isolated and characterized from the cinchona bark (Saxena *et al.*, 2003). Thus becoming the oldest and most important antimalarial drug. Due to the toxicity and developments of resistance to quinine, other derivatives were synthesied through structure activity relationship based on the structure of quinine. These include: chloroquine (II), amodiaquine (III), among others. Other drugs obtain from medicinal plant includes pyramethamine (IV) and proguanil (V)

Another ancient medicinal plant of millennium use is *Artemisia annua* rediscovered in China in the seventies as an important source of anti-malarial artemisinin (VI) (Bruce-Chwatt, 1982; Klayman, 1985).

Artemisinin based compounds in current use are either extracted as the parent compound found in the *Artemisia annua* plant, or are semi-synthetic derivatives like dihydroartemisinin (VII), artemether (VIII) and artesunate (IX) (Woodrow *et al.,* 2005).

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Artemisinin combined therapies (ACT) were formally adopted as first line treatment of uncomplicated malaria in Nigeria from 2005 onwards (Mokuolu *et al.,* 2007). However, ACT use is limited due to its high costs, limited production and toxicity (Haynes, 2001; Malomo *et al.,* 2001; Borstnik *et al.,* 2002; Adebayo and Malomo, 2002; Afonso *et al.,* 2006; Boareto *et al.,* 2008). Neither the Cinchona nor *Artemisia annua* plants, from which the most potent drugs (quinine and artemisinin) were isolated, are indigenous to sub-Saharan Africa. Savannah and secondary forest plants are known to have higher concentrations of natural chemical defenses and a greater diversity, thus they are potential sources of new medicines (Balick *et al.,* 1996). It seems logical then to encourage studies on plants from these regions with claimed antimalarial activity. Among such plants are a*lstonia boonei* (Apocynaceae), *Azadirachta indica* (Meliaceae) and *Uvaria chamae* (annonaceae).

1.5 Sources of Drugs

Drug is any substance or products that is used or intended to modify or explore the physiological system or pathological states for the benefit of the recipient (WHO, 2014).

Drugs are obtained from many sources. Many inorganic materials, such as metals, are chemotherapeutic; hormones, alkaloids, vaccines, and antibiotics come from living organisms; and other drugs are synthetic or semi-synthetic. The techniques of genetic engineering are being applied to the production of drugs, and genetically engineered livestock that incorporate human genes are being developed for the production of scarce human enzymes and other proteins. (The Columbia Electronic Encyclopedia, 2012).

1.5.1 Natural Source

Natural drugs are gotten from compounds found in nature. The most prevalent natural drug sources are plants. other natural drug sources include animals, microbes, and minerals. (Lahlou, 2014). There is no doubt that plants are among the most perfect natural laboratories for the synthesis of various molecules ranging from simple skeleton to highly complex chemical structures (Shen *et al.*, 2003). Present drug discovery from medicinal plants has mainly relied on biological activity guided isolation methods, which, for example, have led to the isolation, identification and the discovery of important drugs (Lahlou, 2012, Fabricant and farnsworth, 2001). Examples of such drugs are quinine (I) and artemesinin (VI)

1.5.2 Synthetic Drugs

Synthetic drugs come from starting materials that are not found in nature. Instead, they are produced by man from smaller chemical building blocks (Lahlou, 2014). An example of synthetic medicine is the experimental anti-malaria drug, arterolane (X) (Valecha *et al.*, 2010).

1.5.3 Biosynthetic Sources

Biosynthetic engineering of the producing host organism offers an important tool for the modification of complex natural products (Frank, 2012).

1.6 Statement of Research Problem

Malaria is a major public health problem in the world. It has been established that malaria impedes economic growth and keeps households in poverty (Teklehaimanot and Mejia, 2008). out of the 500 million cases of malaria and 2 to 3 million death every year in Africa, Nigeria accounts for 100 million malaria cases and 300,000 deaths annually Mostly children under five years and pregnant women account for most of these death (WHO, 2008: MIS, 2010).

The cost of treatment, Evolution of resistance and its quick widespread against available drugs incurs substantial socio-economic cost of fighting the disease.

1.7 Justification of the Study

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female mosquitoes. About 3.2 billion people – almost half of the world's population – are at risk of malaria. According to the world health organization 2015 there are currently no licensed vaccines against malaria (WHO, 2014). Traditionally herbal medicines have been used to treat malaria for thousands of years in various parts of the world (Saxena *et al*., 2003). Among such plant is *Uvaria chamae* which is use in ethnomedicine for the treatment of malaria. It is therefore important that the claimed antimalarial property of this plant *Uvaria chamae* is investigated in order to establish the rationale behind the use of the plant in the treatment of malaria and to isolate some of the bioactive compounds in it.

1.8 Aim of the Study

To scientifically validate the ethno-medicinal claim for the use of the plant in the treatment of malaria to and isolate some of the constituents that may be responsible for the activity.

1.9 Specific Objectives

1. To establish the phytochemical constituent of the leaves of *Uvaria chamae*.

2. To establish chromatographic profile of the extract and fractions of the leaves of *Uvaria chamae*.

3. To isolate and characterise some of the bioactive constituents from the leaves extract of *Uvaria chamae*.

4. To establish the acute toxicity profile and evaluate the antimalarial activity of the leaves extract of *Uvaria chamae*.

1.10 Research Hypothesis

The ethyl alcohol leaves extract of *Uvaria chamae* contain phytochemical constituents with antimalarial activity.

CHAPTER TWO

3.0 LITERATURE REVIEW

2.1 The plants

The plant *Uvaria chamae* (Annonaceae) belongs to the Uvaria genus of flowering plants consisting of trees, shrubs, or rarely lianas with 2106 accepted species and more than 130 genera, (Bridg, 2001). The family is concentrated in the tropics, with few species found in temperate regions (Chatrou, 2005).

2.2 Habitat

Uvaria chamae P.beauv is distributed in Savannah and secondary forest from Tropical Africa in a belt from Senegal to the Central African Republic (Burkill, 2004).



Plate I: *Uvaria chamae* in it natural habitat

2.3 Taxonomy of the plant

According to the international plant name index, the plant *Uvaria chamae* P.Beauv was classified under the following:

Kingdom: *Plantae*

Phylum: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Magnoliales*

Family: *Annonacea*

Genus: *Uvaria*

Epithet: *chamae* P. Beauvois

2.3.1 Common and local Names

Uvaria chamae is commonly known as finger root or bush banana It is locally called *Kaskaifi* or *Lukuki* by the Hausas, *mmịmị ohea* by the Igbos, and *Oko-oja* by the Yorubas in Nigeria (Burkill, 1985).

2.4 Botanical Description

Uvaria chamae, commonly known as finger root or bush banana is a climbing large shrub or small tree native to tropical West and Central Africa where it grows in wet and dry forests and coastal scrublands (Burkill, 2004).

2.5 Ethnomedicinal uses

The root bark of *Uvaria chamae* is used as an astringent and galactagogue. It is taken internally in the treatment of inflammation of the mucous membranes; bronchitis and gonorrhoea. It is also used in the treatment of dysentery, piles, epistaxis, haematuria, haematemesis and haemoptysis. It is boiled with spices and the decoction drunk in the treatment of fevers that are classed locally as 'yellow-fever.

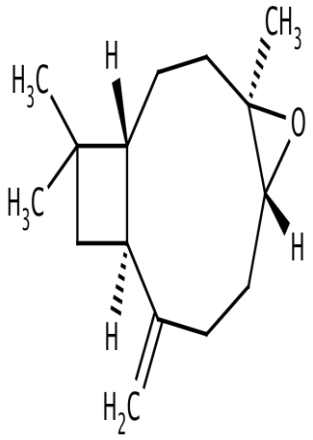
The root is use in the treatment of fever, purgative, stomachic, vermifuge, and amenorrhoea. It is also used to prevent miscarriage; and to relieve the pains of child birth. The sap of the leaves, roots and stems is widely used on wounds and sores and is said to promote rapid healing. A leaf-infusion is used as eyewash and a leaf-decoction as a febrifuge (Burkil, 2004). The leaves are used for the treatment of malaria (Koudouvo *et al*., 2011). Other species of Uvaria have also found use in folklore medicine. This includes *U. doeringii* the leaf decoction of which is taken for piles, palpitations and pains (Burkill 1985). *U. tortilis* is used in the treatment of amenorrhoea (Borquet and Debray, 1974). *U. scabrida* is used in the treatment of insanity while *U. thomasii is* used in the form of a leaf decoction for catarrh and colic (Kerharo and Adam, 1974).

2.6 Pharmacological action of the plants from the genus Uvaria

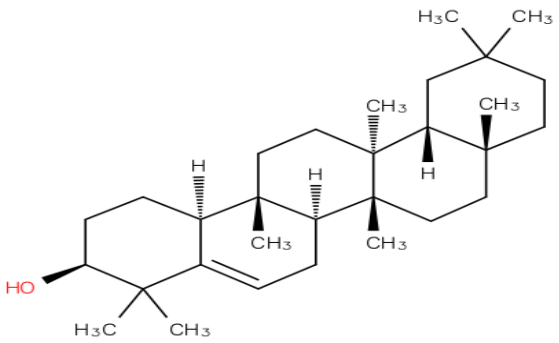
The pharmacological actions of Uvaria species are numerous. *Uvaria chamae* has been reported to have antibacterial and antifungal activities (Oluremi *et al.,* 2010; Okwuosa *et al.,* 2012). A related specie *Uvaria afzelli* has been reported to have anti-parasitic activity (Okpekon *et al,* 2004).

2.7 Chemical Constituents

Previous phytochemical screening of *Uvaria chamae* leaves revealed the presence of alkaloids, saponin, tannins, flavonoids, terpenoids, phenol and steroid (Osuagwu and Ihenwosu, 2014). A large number of flavonoids and other chemical compounds have been isolated from the seeds, leaves, bark and root of plant from Annonaceae family (Hairin *et al.*, 2013). Uvaretin (xi) and isouvaretin (xii) have been isolated from the stem bark of *Uvaria chamae* (Hufford and Lasswell, 1976). linalool (xiii), 1-nitro-2-phenylethane (xiv) and germacrene D (xv) were isolated from the leaf oil of the plant (Moses *et al.*, 2013; Oguntime in *et al.*, 1989). Benzyl benzoate (xvi), caryophyllene oxide (xvii), glutinol (xviii), 5-hydroxy-7-methoxyflavone (xix), 5-hydrox-6,7-dimethoxyflavone (xx) have been isolated from *Uvaria rufa* (Rosandy *et al.*, 2013).



(xvii)



(xviii)

2.7.1 Flavonoids

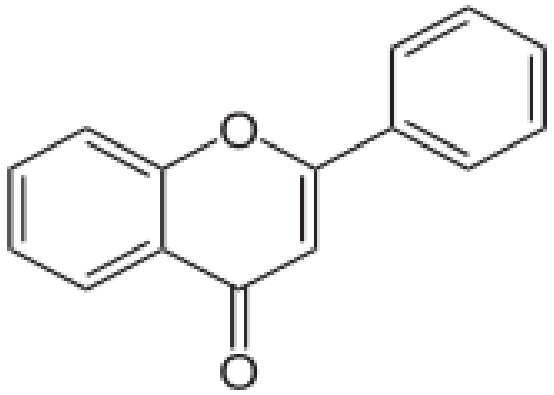
Flavonoids are a large family of compounds synthesized by plants. Chemically they have the general structure of 15-carbon skeleton, which consists of two phenyl rings(A and B) and heterocyclic ring (C); In most cases, B ring is attached to position 2 of C ring, but it can also bind in position 3 or 4; this, together with the structural features of the ring B and the patterns of glycosylation and hydroxylation of the three rings, makes the flavonoids one of the larger and more diversified groups of phytochemicals, so not only of polyphenols, in nature. Their biological activities, for example they are potent antioxidants, depend both on the structural characteristics and the pattern of glycosylation.

They can be subdivided into different subgroups depending on the carbon of the C ring on which B ring is attached, and the degree of unsaturation and oxidation of the C ring. Flavonoids in which B ring is linked in position 3 of the ring C are called isoflavones; those in which B ring is linked in position 4, neoflavonoids, while those in which the B ring is linked in position 2 can be further subdivided into several subgroups on the basis of the structural features of the C ring. These subgroup are: flavones, flavonols, flavanones, flavanonols, flavanols or

catechins and anthocyanins. Finally, flavonoids with open C ring are called chalcones (Middleton, 1998)

2.7.1.1 Flavones

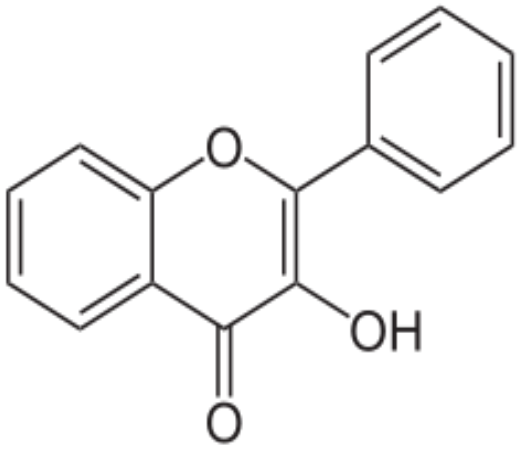
they have a double bond between positions 2 and 3 and a ketone in position 4 of the C ring. Most flavones of vegetables and fruits has a hydroxyl group in position 5 of the A ring, while the hydroxylation in other positions, for the most part in position 7 of the A ring or 3′ and 4′ of the B ring may vary according to the taxonomic classifcation of the particular vegetable or fruit. Glycosylation occurs primarily on position 5 and 7, methylation and acylation on the hydroxyl groups of the B ring. Some flavones, such as nobiletin and tangeretin, are polymethoxylated.



xxi

2.7.1.2 Flavonols

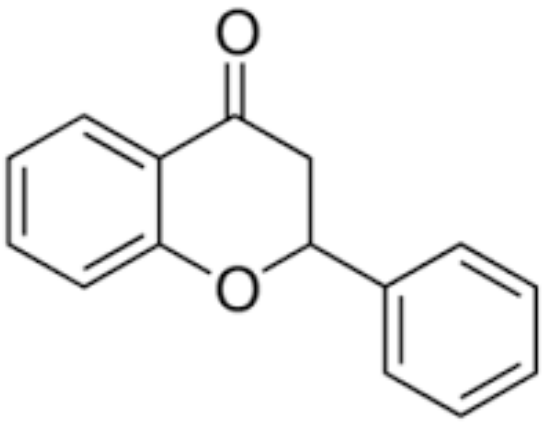
Compared to flavones, they have a hydroxyl group in position 3 of the C ring, which may also be glycosylated. Again, like flavones, flavonols are very diverse in methylation and hydroxylation patterns as well, and, considering the different glycosylation patterns, they are perhaps the most common and largest subgroup of flavonoids in fruits and vegetables. For example, quercetin is present in many plant foods.



xxii

2.7.1.3 Flavanones

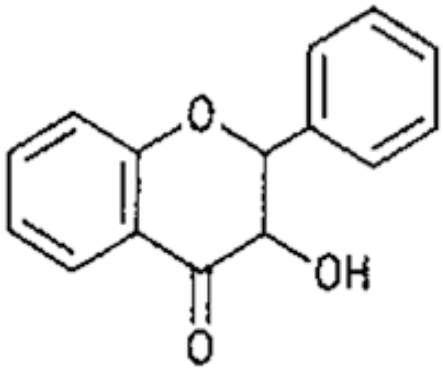
Flavanones, also called dihydroflavones, have the C ring saturated; therefore, unlike flavones, the double bond between positions 2 and 3 is saturated and this is the only structural difference between the two subgroups of flavonoids. The flavanones can be multi-hydroxylated, and several hydroxyl groups can be glycosylated and/or methylated. Some have unique patterns of substitution, for example, furanoflavanones, prenylated flavanones, pyranoflavanones or benzylated flavanones, giving a great number of substituted derivatives.Over the past 15 years, the number of flavanones discovered is significantly increased (Nibbs and Scheidt, 2012).



xxiii

2.7.1.4 Flavanonols

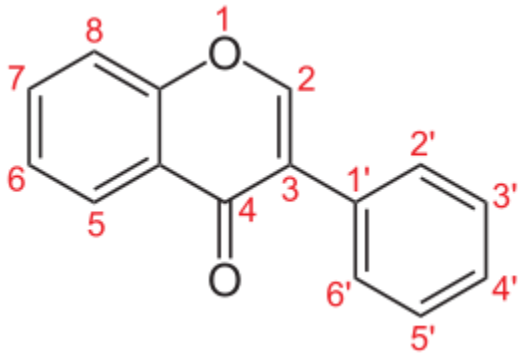
Flavanonols, also called dihydroflavonols, are the 3-hydroxy derivatives of flavanones; they are an highly diversified and multisubstituted subgroup.



xxiv

2.7.1.5 Isoflavones

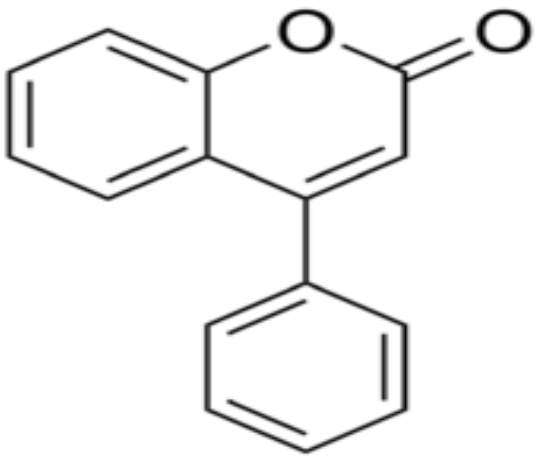
As anticipated, isoflavones are a subgroup of flavonoids in which the B ring is attached to position 3 of the C ring. They have structural similarities to estrogens, such as estradiol, and for this reason they are also called phytoestrogens.



xxv

2.7.1.6 Neoflavonoids

They have the B ring attached to position 4 of the C ring.

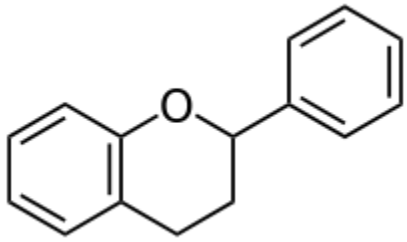


xxvi

2.7.1.7 Flavanols

Flavanols are also referred to flavan-3-ols as the hydroxyl group is almost always bound to position 3 of C ring; they are called catechins as well. Unlike many flavonoids, there is no double bond between positions 2 and 3. Another distinctive features, e.g. compared to flavanonols, with which they share a hydroxyl group in position 3, is the lack of a carbonyl group, that is, a keto group, in position 4. This particular chemical structure allows flavanols to have two chiral centers in the molecule, on positions 2 and 3, then four possible diastereoisomers. Epicatechin is the isomer with the *cis* configuration and catechin is the one with the *trans* configuration. Each of these configurations has two stereoisomers, namely, (+)-epicatechin and (-)-epicatechin, (+)-catechin and (-)-catechin. (+)-Catechin and (-)-epicatechin are the two isomers most often present in edible plants. Another important feature of flavanols,

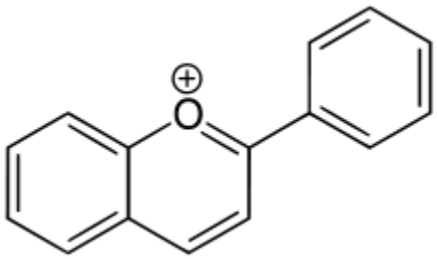
particularly of catechin and epicatechin, is the ability to form polymers, called proanthocyanidins or condensed tannins.



xxvii

2.7.1.8 Anthocyanidins

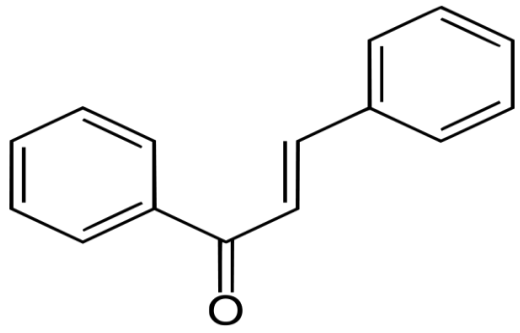
Chemically, anthocyanidins are flavylium cations and are generally present as chloride salts.They are the only group of flavonoids that gives plants colors (all other flavonoids are colorless).Anthocyanins are glycosides of anthocyanidins. Sugar units are bound mostly to position 3 of the C ring and they are often conjugated with phenolic acids. The color of the anthocyanins depends on the pH and also by methylation or acylation at the hydroxyl groups on the A and B rings.



xxviii

2.7.1.9 Chalcones

Chalcones and dihydrochalcones are flavonoids with open structure; they are classified as flavonoids because they have similar synthetic pathways.



xxix

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Solvents/Reagents

Solvents used were of analytical grade and they included; methyl alcohol, ethyl alcohol, hexane, chloroform, ethyl acetate and n-butyl alcohol. Reagents used were freshly prepared and include those for phytochemical screening such as; Molisch's reagent, Meyer's reagent, and Borntrager's reagent. Chromatographic materials used are pre-coated TLC plates (Alminium), Silica gel (60-120 mesh) and Sephadex LH 20.

3.1.2 Equipment

Thermoelectron UV machine at the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, for UV spectroscopy. Ohaus digital weighing balance (Champ 11 CH15R, Ohaus Corporation, Pinebrook NJ, USA), Metler balance (Model P162 supplied by Gallenhamp), Syringes and needles, Mortar and pestle, Sample bottles, Beakers, separating funnel and conical flask. Bruka AVANCE III NMR spectrometer (500MHz) at the School of Pharmacy University of London for 1D NMR spectroscopy.

3.1.3 Experimental animals

Locally bred adult Swiss albino mice of either sex (19-25 g body weight) were acquired from Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello

University, Zaria, Nigeria. The animals were fed with laboratory diet and water *ad libitum* and maintained under standard conditions in cages at room temperature.

3.1.4 Malaria parasite

Mouse-infected chloroquine sensitive strain of *Plasmodium berghei* NK-65 was obtained from National Institute of Medical Research, Lagos. The parasite was kept alive by continuous intra-peritoneal passage in mice at the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

3.3 Methods

3.2.1 Collection and identification of plant material

The plant sample was collected from Zaria metropolis, it was identified and authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria by Mallam Namadi Sanusi. And the voucher specimen number 3129 deposited for future reference. The leaves were air dried under shade and pounded to coarse powder using mortar and pestle.

3.2.2 Extraction and Partitioning

The powdered leaves (2 kg) were extracted with ethyl alcohol (80%) using maceration method for 12 days. The solvent was removed using rotary evaporator. The extract (242.15g) was suspended in distilled water and filtered using a filter paper to obtain water soluble and water insoluble portions. Both the water soluble and insoluble portion was partitioned with n-Hexane, Chloroform, Ethyl acetate and n-butanol to give the n-Hexane, Chloroform, Ethyl acetate and n-Butanol fractions respectively.

3.2.3 Preliminary phytochemical screening.

Standard procedures were used to conduct chemical tests on the crude ethyl alcohol extract for the presence of various phytochemical constituents.

3.2.3.1 Test for Anthraquinones

Bontrager's Test

A small portion of the extract was dissolved in 5ml chloroform,shaken and filtered. To the filtrate, an equal volume of 10% ammonia solution was added with continuous shaking, bright pink colour in the aqueous upper layer indicates the presence of anthraquinone(Trease and Evans, 1996).

3.2.3.2 Test for Alkaloids

Extraction of Alkaloids

10 g of the powdered plant material was soaked with 50ml of methanol in a beaker with gentle shaking at intervals and left over night. The extract was filtered into another beaker, then placed on the water bath and evaporated to dryness. To the residue, 20ml of 5% HCl was added. This residue was heated on water bath with scratching and shaking. The extract was then filtered and was extracted in a separating funnel with 30ml of chloroform where two layers were obtained. The lower (chloroform) layer was drained off and rejected. The upper (aqueous) layer was made alkaline with ammonium hydroxide solution until it was basic to pH of 10. The base was further extracted with chloroform. The chloroform layer was washed with distilled water. The purified chloroform portion was kept with anhydrous sodium sulphate for 24 hours to removed any

residue water. The extract was then filtered and evaporated to dryness on water bath. The concentrated extract was used for preliminary test for alkaloids

a. Dragendoff's Test

The extract (0.2 g) was dissolved in 2 ml of 1% aqueous hydrochloric acid with continuous stirring in a water bath. The mixture was filtered and few drops of Dragendoff's reagent was added, rose red precipitate indicates the presence of alkaloids (Trease and Evans, 1996).

b. Mayer's Test

To 2ml acidic solution of the extract in a test tube, few drops of Mayer's reagent was added, a cream precipitate indicates the presence of alkaloids (Silva *et al.*, 1998).

3.2.3.3 Test for Carbohydrates

Molisch Test

To a small aqueous portion of the extract in a test tube, few drops of freshly prepared Molisch reagent followed by concentrated sulphuric acid were added down the test tube in a slanting position, formation of redish colored ring at the interface indicates the presence of carbohydrate (Trease and Evans, 1996).

3.2.3.4 Test for Cardiac Glycosides

Keller-Kiliani Test

A small portion of the extract was dissolved in 1ml glacial acetic acid containing traces of ferric chloride solution. The solution was then transferred into a dry test tube to which an equal volume

of sulphuric acid was added, a brown ring obtained at the interface indicates the presence of a deoxy sugar.(Trease and Evans, 1996).

3.2.3.5 Test for Saponins

Frothing Test

To a portion of the extract in a test tube,10ml of distilled water was added and shaken vigorously for 30seconds. The tube was allowed to stand for 30 minutes, frothing which persist was taken as an evidence for the presence of saponins(Trease and Evans, 1996).

3.2.3.6 Test for Flavonoids

a. Sodium Hydroxide Test

To the extract,10% sodium hydroxide solution was added, yellow coloration indicate the presence of flavonoids (Silva *et al.*, 1998).

b. Shinoda Test

A small quantity of the extract was dissolved in methyl alcohol. Few pieces of magnesium chips were added followed by few drops of concentrated hydrochloric acid, a pink, orange, or red to purple coloration indicates the presence of flavonoid(Silva *et al.*, 1998).

3.2.3.7 Test for Tannins

a. Ferric chloride test

An aqueous solution of the extract was prepared. few drops of ferric chloride solution was added to the extract in a test tube, a blue-black, green or blue-green precipitate indicates the presence of saponins (Trease and Evans, 1983).

b. Lead acetate test

A solution of 1% lead acetate solution was added to 5ml solution of the extract in a test tube, a cream colored precipitate indicates the presence of tannins (Trease and Evans, 1996).

3.*2.3.8 Test for Steroids/Triterpenes*

Liebermann-Burchard's Test

To the portion of the extract equal voloume of acetic anhydride was added and mixed gently. 1ml concentrated sulphuric acid was added down the test tube. This was observed for instant colour changes and over a period of one hour. Blue to blue-green color in the upper layer and a redish,pink or purple color at the junction of the two layers indicates the presence of triterpene(Trease and Evans, 1996).

Salkowski test

A small portion of the extract was dissolve in 1ml of chloroform, 2-3 drops of concentrated sulphuric acid was added at the side of the test tube. The appearance of red color indicates the presence of steroids (Sofowora, 1980)

3.2.4 Chromatographic procedures

3.2.4.1 Thin Layer Chromatographic Analysis.

Pre-coated TLC plates were used to carry out thin layer chromatography by ascending technique. Capillary tubes were used to apply the spots and the chromatogram was developed in an air-tight chromatographic tank at room temperature employing different solvent system.Visualizationwas done using ultra violet light (254 nm & 366 nm) and by use of a spraying reagent (10% sulphuric acid) followed by heating in an oven at 105^{o}C for 3-5 minutes. The TLC solvent systems used includes:

Ethyl acetate	100%
Ethyl acetate:Chloroform:methyl alcohol:water	15:4:4:1
Ethyl acetate:Chloroform	7:1, 9:1 and 7:3

3.2.4.2 Column chromatography of ethyl acetate fraction

The ethyl acetate fraction (18 g) was subjected to column chromatography. The column was packed using wet slurry method. Slurry of silica gel was poured into column and allowed to settle. The sample was first adsorbed on silica gel by dissolving it in a minimum amount of ethyl acetate; follow by adding small quantity of silica gel to form a paste. The paste was dried, triturated and loaded on to the previously packed column. The column was eluted by gradient elution with chloroform 100% followed by 95:5 chloroform: ethyl acetate and increasing the polarity gradually by 5 up to 100% ethyl acetate, finally the column was eluted with methanol.
The different column fractions collected were pooled together based on similarity in their TLC profile to give 9 major column fractions, coded F1-F7

3.2.4.3 Gel Filtration Chromatography of F4

F4 was subjected to gel filtration based on compound of interest and TLC profile. The elution was done using methanol 100%, 20(2ml) collection were obtained and pooled into 4 fraction coded A, B, C and D. fraction C was further purified using Sephadex and 15 collections were obtained and pooled into 4 fraction based on similarities coded cd1, cd2, cd3 and cd4. Cd4 was further subjected to repeated gel filtration which yielded a brownish amorphous compound coded HB. HB was subjected to physicochemical test and spectroscopic analysis including UV, IR, NMR and ESI-MS.

3.2.4.4 Melting Point determination

The melting point of the isolated compound was determined using gallenkamp melting point apparatus at the Department of Pharmaceutical and Medicinal Chemistry, ABU, Zaria.

3.2.4.5 Test for catechin

Catechin test is the modification of the well-known phloroglucinol test for lignin. Matchtick contains lignin. A matchstick was dip into dilute extract of the drug, it was dried, moisten with concentrated hydrochloric acid, and it was warm near a flame. A wood pink or red colour indicates the presence of catechins (Vinod, 2007).

3.2.5 Pharmacological studies

3.2.5.1 Acute toxicity studies

Nine mice were divided randomly into 3 groups of 3 mice each. In the first phase, varying doses of the extract (10, 100 and 1000 mg/kg body weight) were administered orally to groups 1, 2 and 3 respectively and observed for 24 hours for any sign of toxicity and mortality. In the second phase, doses of 1,200, 1,600 and 2,900 mg/kg were administered base on the result of the phase one to 3 fresh mice through the same route and observed also for 24 hours (Lorke, 1983). The median lethal dose was calculated using the formula:

$$LD_{50} = \sqrt{minimum\ lethal\ dose \times maximum\ tolerated\ dose}$$

3.2.5.2 Antimalarial Studies

Suppressive Test

This study was done according to the method of (Peters, 1965). Twenty-five (25) swiss albino mice (16-20g) were inoculated with standard inoculums containing approximately 10^7 infected erythrocytes through the intra-peritoneal route, two (2) hours post inoculation; the mice were grouped into five (5) group of five mice each. Different doses of the extract at 25, 50 and 100 mg/kg were administered orally to groups 1, 2 and 3 respectively once daily as treatment for 4 days (day 0 to day 3). group 4 and 5 were taken as positive and negative controls and were treated with 5 mg/kg standard chloroquine and 0.2 ml/kg of body weight normal saline respectively. On day 4, thin blood smears were made from the tail. The slides were fixed with methanol, stained with 10% Giemsa for 15 minutes and the parasite count examined under microscope. Average suppression was calculated using the formula:

% suppression = $\frac{\text{\% parasitaemia in control-\% parasitaemia in treated group}}{\text{\% parasitaemia in control}}$

Curative Test

This study was done according to the method of (Ryler and Peters, 1970). Twenty-five (25) mice were inoculated with standard inoculums containing approximately 10^7 infected erythrocytes through the intra-peritoneal route; seventy-two (72) hours post inoculation; the mice were grouped into 5 groups of 5 mice each. Different dose levels (25, 50 and 100 mg/kg of body weight) of the extract were administered to groups 1, 2 and 3 respectively. Groups 4 and 5 were taken as positive and negative controls and were treated with 5 mg/kg standard chloroquine and 0.2 ml/kg of body weight normal saline respectively. Administrations were done orally once daily for four (4) days. On day seven (7) i.e. a day after last treatment, thin blood smears from the tail blood were taken and analyzed for parasite suppression after fixing and staining the slides. Average suppression was calculated using the formula:

% suppression = $\frac{\text{\% parasitaemia in control-\% parasitaemia in treated group}}{\text{\% parasitaemia in control}}$

CHAPTER FOUR

4.0 RESULT

4.1 Extraction Yield

The yield of the crude ethyl alcohol extract and the solvent fractions are as shown in Table 4.1 below

Table 4.1: Extraction of plant material

Solvent	Weight (g)	Yield (%)	Colour
Crude extract	200	10	Dark green
Hexane	39.35	1.97	oily green
Chloroform	33.09	1.65	dark green
Ethyl acetate	23.0	1.15	Lightbrown
N-butyl alcohol	27.62	1.38	dark brown

4.2 Phytochemical constituent of the leave extract of *Uvaria chamae*.

The preliminary phytochemical screening of the ethyl alcohol leaves extract of *Uvaria chamae* revealed the presence of carbohydrates, saponins, flavanoids, glycosides, tannins, steroids/terpenes and alkaloids. as shown in Table 4.2 below

Table 4.2: Phytochemical constituent of the crude extract

Constituent	Test	Observation
Carbohydrates	Molisch	+
Anthraquinones	Bontrager	-
Steroids/Terpenes	Liebermann-Burchard	+
Glycosides	Keller-Kiliani	+
Saponin	Frothing	+
Tannins	Ferric Chloride	+
	Lead acetate	+
Flavonoids	Sodium Hydroxide	+
	Shinoda	+
Alkaloids	Dragendoff	+
	Mayer	+

+ = present, - = absent

4.3 Thin Layer Chromatography

4.3.1 Thin-layer Chromatography of the crude extracts and partitioned fractions

TLC profile of the crude ethyl alcohol extract and various solvent fractions using ethyl acetate: chloroform: methyl alcohol: water (15:4:4:1) as solvent system is summarized in Table 4.3 below



Plate ii: TLC profile of crude extract (EE)

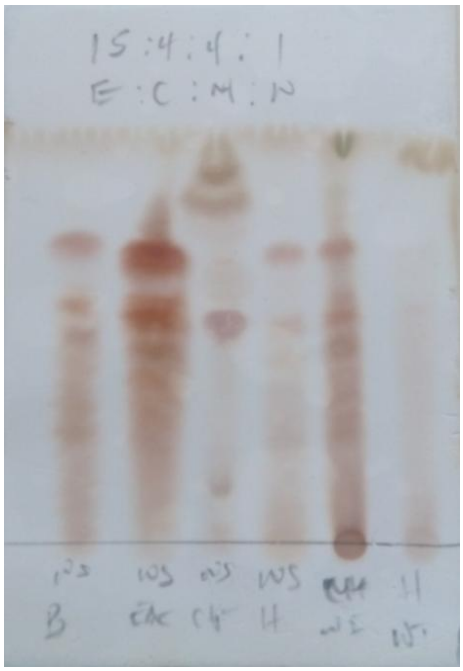


plate iii: TLC profile of the partitioned fractions (n-butanol, ethyl acetate, chloroform, hexane)

Table 4.3: Summary of TLC Profiles of the crude extract and the partitioned fractions (hexane, chloroform, ethyl acetate and n-butanol)

Extract	No. of spots	R_f values
Crude	5	0.22, 0.49, 0.63, 0.71, 0.78
Hexane	3	0.43, 0.52,0.69
Chloroform	4	0.26, 0.55, 0.60, 0.71
Ethyl acetate	4	0.45,0.55, 0.57, 0.69
n-Butanol	4	0.31, 0.50, 0.55, 0.74

4.4 Column Chromatography of Ethyl acetate Fraction

Column chromatographic separation of ethyl acetate fraction yielded seven (7) pooled fractions as shown on the TLC below



Plate iv: TLC of column fraction 11-17 hexane:ethyl acetate (7:3)

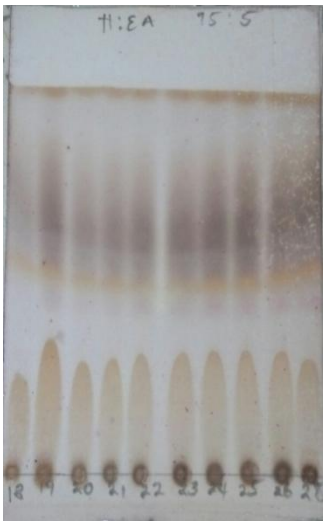


plate v: TLC of column fraction 18-28 hexane:ethyl acetate (95:5)



Plate vi: TLC of column fraction 29-35 hexane:ethyl acetate (95:5)

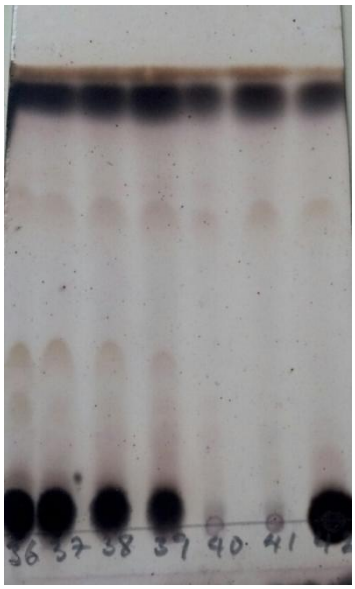


Plate vii: TLC of column fraction 36 42Solvent hexane:ethyl acetate (95:5)

The solvent system used to developed plate viii-x is ethyl acetate: chloroform: methanol: water (15:4:4:1)

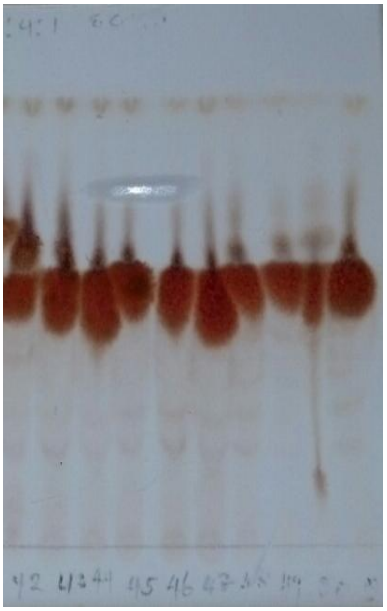


Plate viii:TLC of column fraction 42-51

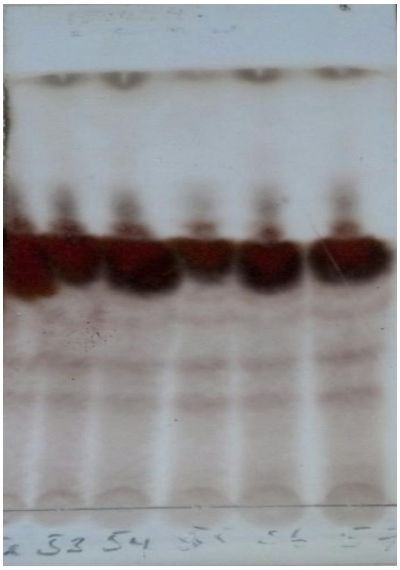


Plate ix:TLC of column fraction 52-57

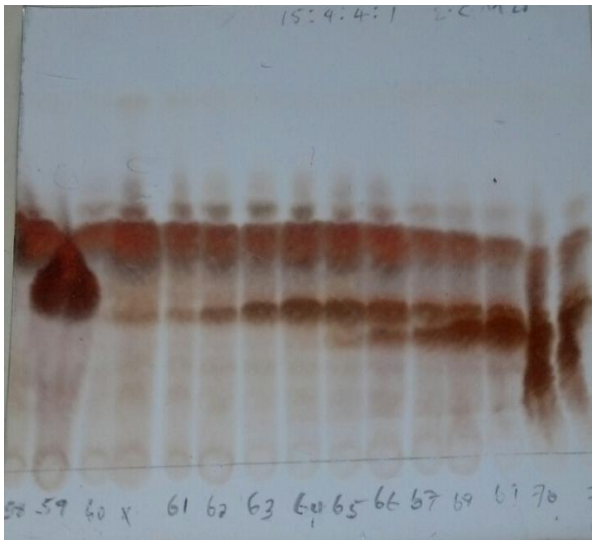


Plate x: TLC of column fraction 58-70

Table 4.4: **Summary of the pooled column fractions**

Fractions	Code	Number of spots
11-17	F1	3
18-35	F2	4
36-41	F3	3
42-52	F4	4
53-60	F5	6
61-64	F6	4
65-70	F7	5

4.5 Gel- Filtration of column fraction F4

Repeated gel filtration chromatography (3 times) of column fraction coded F4 led to the isolation of compound coded HB (3.5 mg) as shown in Plates xi and xii. Compound HB was isolated as brown solid, it gave a single spot on TLC using E.A:CH 7:1 and 100% EA as solvent systems with R_f values of 0.60 and 0.76

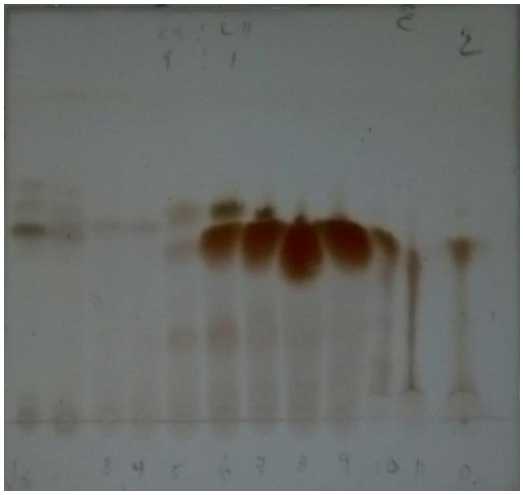


Plate xi: TLC profile of sephadex E 17-20

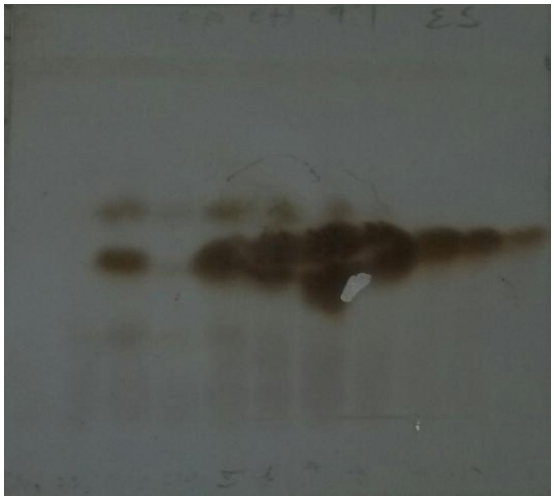


Plate xii: TLC profile of sephadex ES1-12

4.5.1 TLC Profile of HB developed in different solvent system

HB gave single spot when chromatographed using100% EA and EA: $CHCl_3$ 7:1 as solvent system with R_f value of 0.76 and 0.60 respectively (Plate xiii and xiv)





Plate xiii (ethyl acetate 100%) **Plate xiv (ethyl acetate:chloroform 7:1)**

4.6 Solubility Profile of HB

Compound HB was found to be sparingly soluble in ethylacetate and completely soluble in methanol.

4.7 Melting Point of HB

HB was found to melt in the temperature range of 257-259^{o}C

4.8 Chemical Tests on HB

- HB gave green colour when subjected to ferric chloride test (Silva *et al.*, 1998)
- HB gave pink colour when subjected to the modified phloroglucinol test for catechin

4.9 UV Spectra of Compound HB

The UV spectra revealed peak at λmax of 222 nm and 281 nm (figure 4.1).

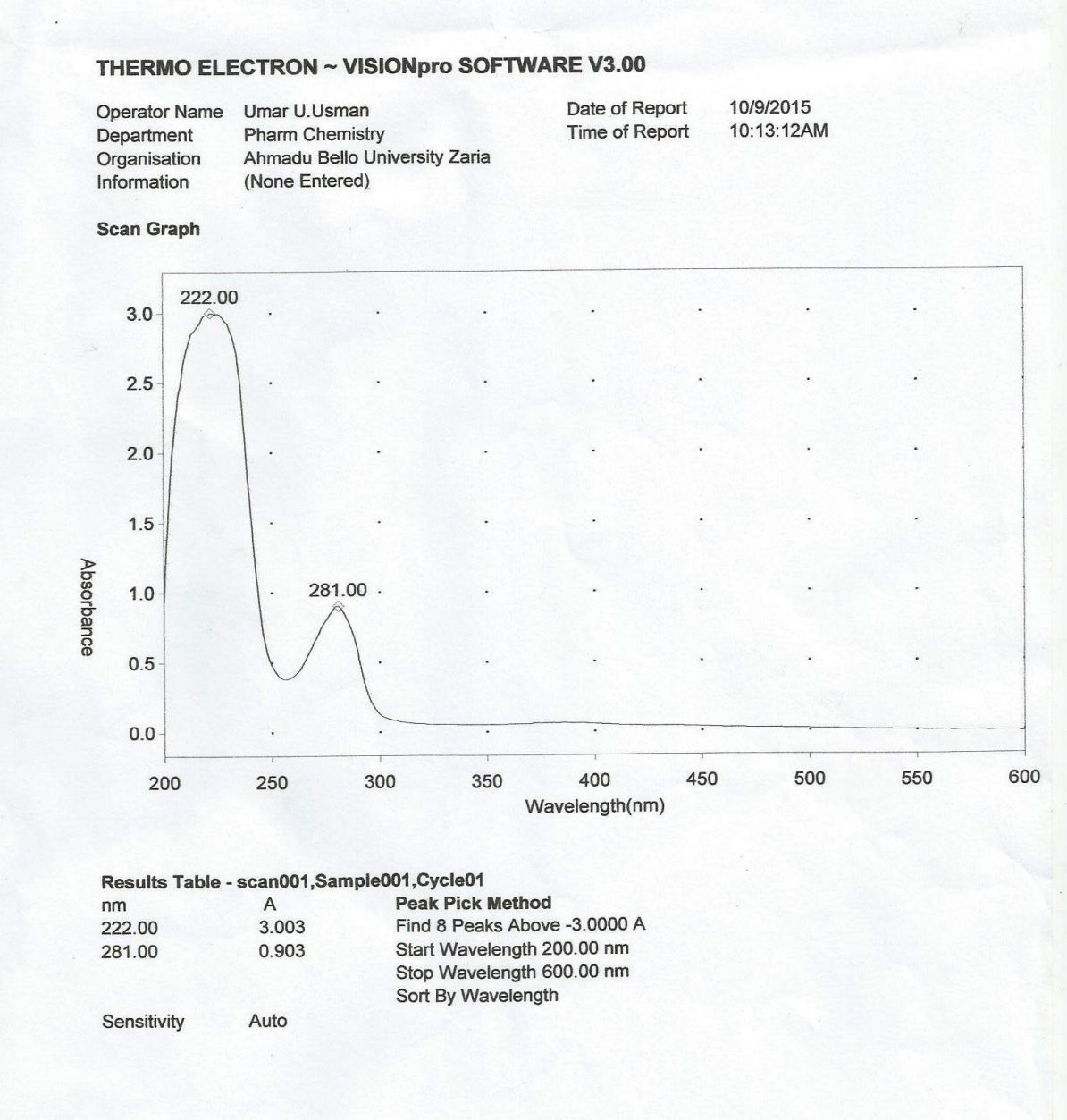


FIGURE 4.1 UV Spectrum of compound HB

4.10 IR Spectra of Compound HB

The IR Spectra of compound HB shows vibration including 3454cm^{-1}, 2930.67cm^{-1}, 1624cm^{-1} and 1045cm^{-1} (figure 4.2)

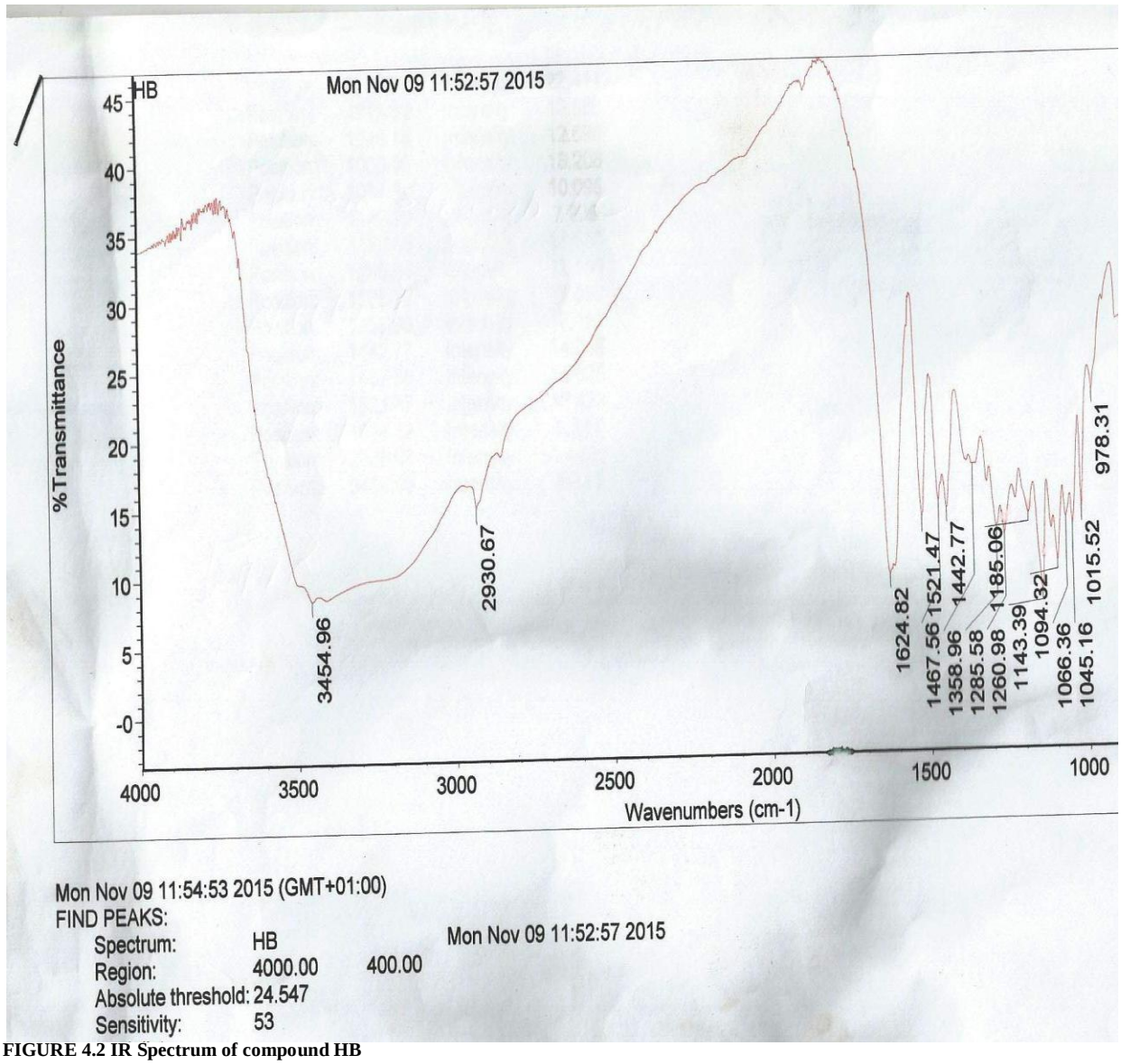


FIGURE 4.2 IR Spectrum of compound HB

4.11 Proton NMR Spectrum of HB

The [1]HNMR spectrum of HB in MeOD revealed signals at δ 2.75, 2.89, 4.18, 4.60, 5.92, 5.94, 6.76, 6.81 and 6.97 (Figures 4.3-4.4).

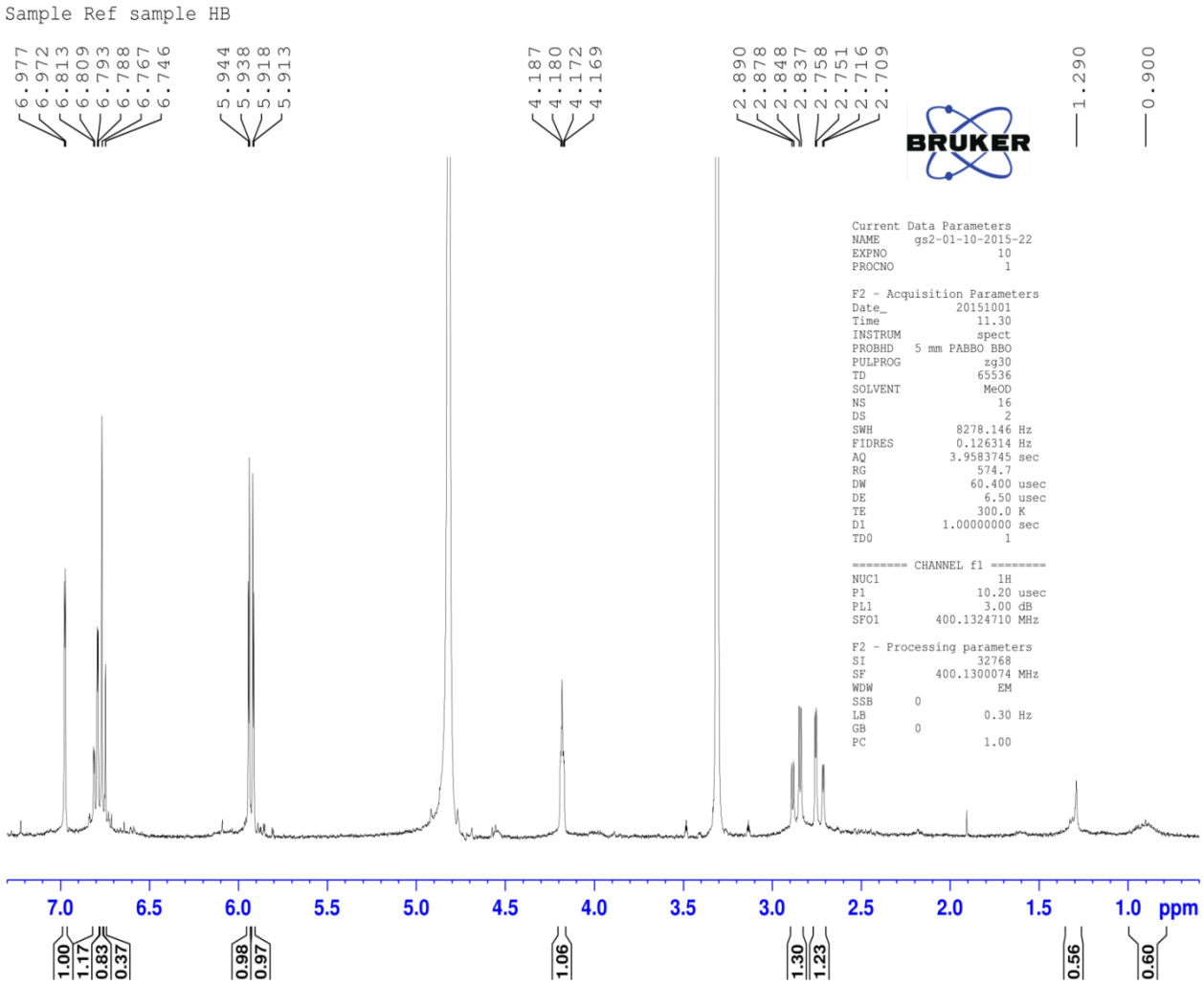


Figure 4.3: Proton NMR Spectrum of HB

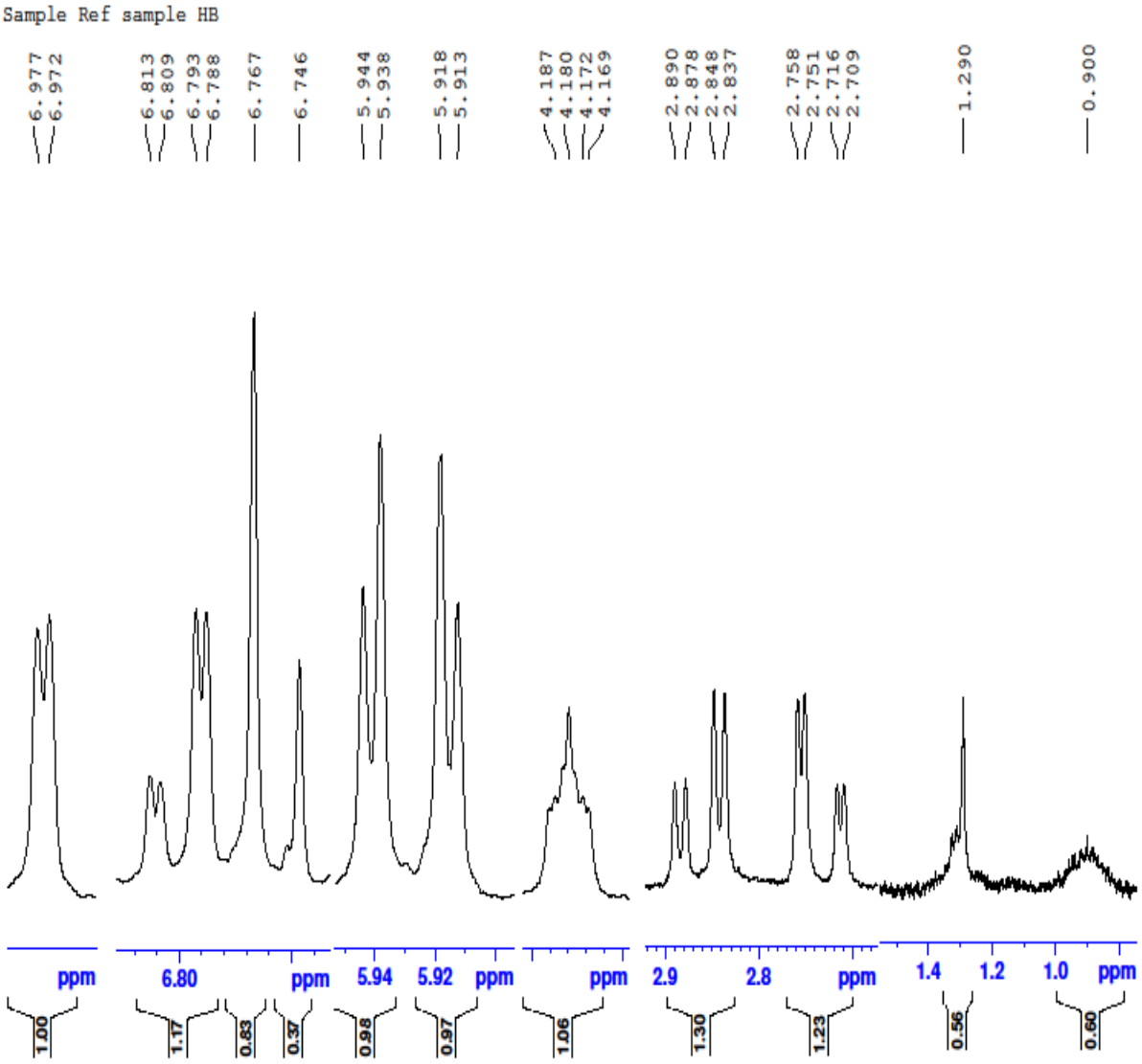


Figure 4.4: Proton NMR Spectrum of HB (expanded)

4.12 ESI-MS of Compound HB (negative mode)

In the negative mode the MS revealed molecular ion peak at m/z 289.1 and other fragment at m/z 113.0, 121.1, 226.8 and 248.8. (figure 4.5)

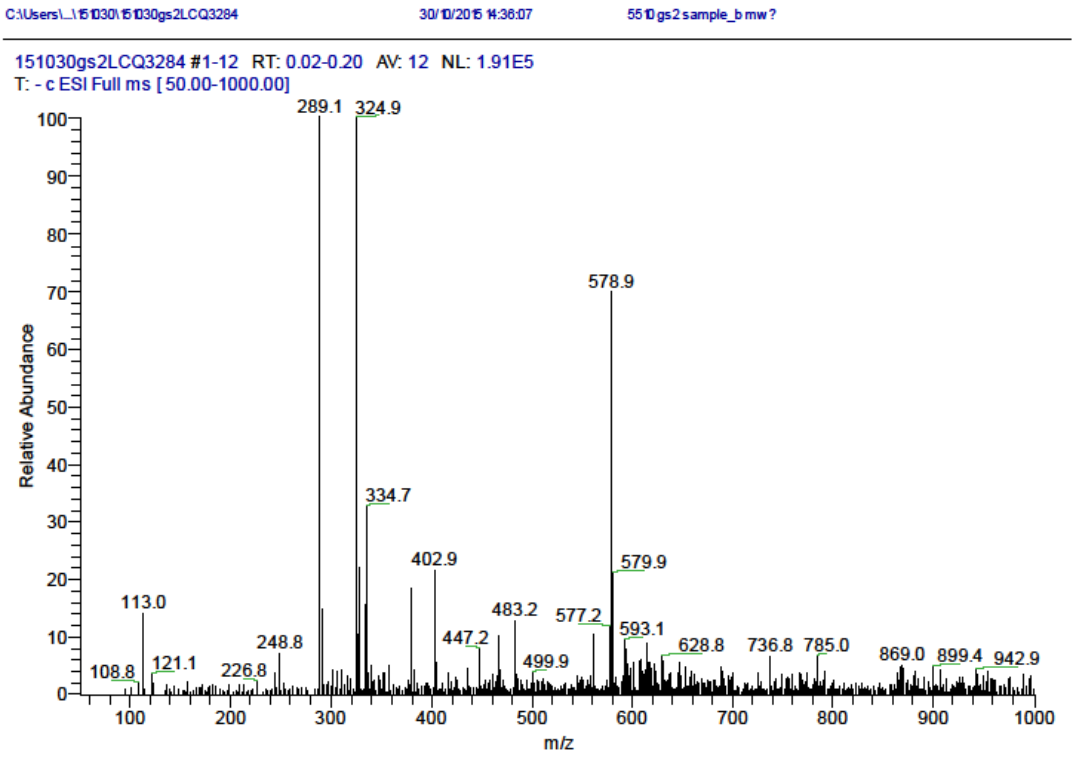


FIGURE 4.5: ESI-MS of Compound HB (negative mode)

4.13 ESI-MS of Compound HB (positive mode)

In the positive mode the MS revealed molecular ion peak at m/z 291 and other fragment at 96.7, 142.6, 145.4 and 273.2.n (figure 4.6)

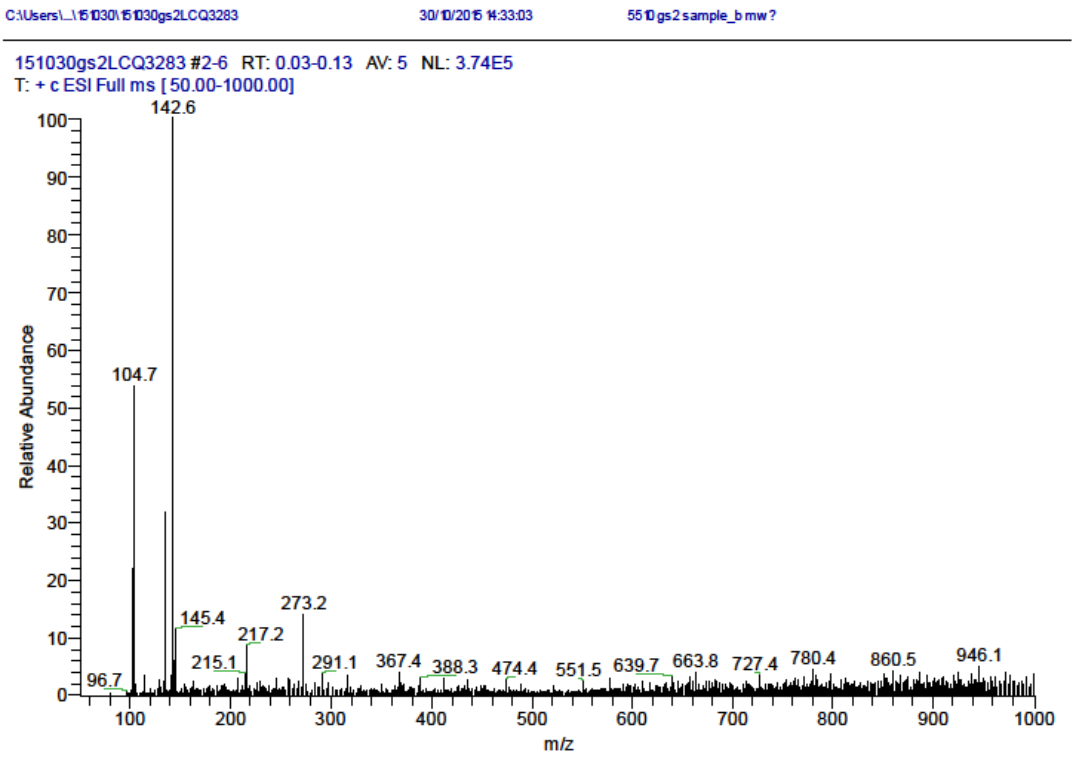


FIGURE 4.6: ESI-MS of Compound HB (positive mode)

4.14 Pharmacological Studies

4.14.1 Toxicity Study

The median oral lethal dose LD_{50} was found to be greater than 5000 mg/kg

4.14.2 Antimalarial studies

Suppressive test

In the suppressive test the crude ethyl alcohol leaves extract of *Uvaria chamae* showed a dose dependent and significant ($p< 0.05$) decrease in parasitaemia level to 48%, 53.3% and 65% at doses of 25, 50 and 100 mg/kg body weight. The extract at the highest dose 100mg/kg body weight had higher efficacy compared to the lowest dose 25mg/kg body weight while chloroquine (5 mg/kg) shows 79.8% suppression.

Table 4.5: Anti-plasmodial activity of crude ethyl alcohol leaf extract of *Uvaria chamae* in early infection (suppresive Test)

Treatment (mg/kg)	SEM	Percentage suppression (%)
CEE (25)	9.58 ± 1.22**	48.06
CEE (50)	12.78 ±2.25*	53.32
CEE (100)	14.22 ± 1.44*	65.00
CQ 5	5.52 ±2.04**	79.84
N/S	27.38 ± 5.99	

Data presented as mean ±SEM, n=5, P<0.01 = **, P<0.05 = *, Dunnet post hoc test

N/S:normal saline, CEE: Crude ethanol extract, CQ: Chloroquin

Curative test

In the curative test, the extract caused a dose dependent significant ($p< 0.05$) decrease in parasitaemia levels of groups treated with different doses of the extract. There was a 73% cure at a dose of 100 mg/kg, 70% cure at a dose of 50 mg/kg and (68%) was observed in the group treated with 25 mg/kg body weight while chloroquine shows 98% suppression.

Table 4.6: Anti-plasmodial activity of crude ethanol leaf extracts of *Uvaria chamae* p.beauv in established infection (Curative Test)

Treatment (mg/kg)	SEM	Percentage suppression (%)
CEE 25	8.74±0.93**	69.00
CEE 50	8.44±1.55**	70.00
CEE 100	7.40±1.06**	73.00
CQ 5	0.68±0.18**	98.00
N/S	28.32±2.42	

Data presented as mean ±SEM, n=5, P<0.01 = **, Dunnet post hoc test

N/S: normal saline, CEE: Crude Ethyl Alcohol, CQ: Chloroquine

CHAPTER FIVE

5.0 DISCUSSIONS

The results of the preliminary phytochemical test of the ethyl alcohol leaf extract of *Uvaria chamae* revealed the presence of flavanoid, alkaloid, saponin, terpenoids and carbohydrate. These phytochemical constituent have been reported to have varying degree of pharmacological activity (Cowan, 1999; Badam *et al.*, 2002; Gupta and Tandon, 2004). Antimalarial activity has been associated with some isolated flavonoids from plants (Ichino *et al.,* 2006). Column chromatographic separation followed by gel filtration led to the isolation of compound HB. HB gave a single homogeneous spot on TLC using two different solvent systems showing that the compound was obtain pure. The colour change observed when sample was treated with $FeCl_3$ solution is an indication of phenolic OH (Silva *et al.,* 1998). The melting point of HB was found to be 257-259^{o}C. The UV spectra of HB recorded in methanol showed absorption maxima at 222 nm and 281 nm. Which is typical of flavan-3-ols (Mabry *et al.*,1970; Harborne 1994;). The IR frequencies at 3454 cm^{-1}, 2930.67 cm^{-1}, 1624 cm^{-1} and 1045 cm^{-1} suggested vibrations for OH, C-H, C=C and C-O respectively.The ^{1}HNMR spectrum of compound HB revealed the presence of five aromatic protons at δH 6.97 (1H, d, J=2.0Hz), δH 6.76 (1H d J=8.4Hz)and δH 6.80 (1H dd J= 1.6Hz and 6.4Hz) assignable to the tri-subtituted benzene ring. Meta coupled doublet at δH 5.92 (1H, d J=2.0Hz) and δH 5.94 (1H, d, J=2.4Hz) assignable to the tetra subtituted benzene ring. The aromatic proton signals at δH 6.97, δH 6.76 and δH 6.80 were assigned to H-2', H-5' and H-6' of ring B respectively (Samaraweera *et al.,* 1983; Saini and Ghosal 1984;). The ^{1}HNMR spectrum exhibited signals attributable to the ring C methylene proton at δH 2.76 (1H, dd J= 2.8Hz, 16.8Hz) and δH 2.89 (1H, dd, J= 4.8Hz, 16.8Hz) representing H-4_{axial} and H-$4_{eqeutorial}$ respectively. Hydroxylated methine proton at δH 4.60 (1H, s) and δH4.18 (1H, m,

J=2.8Hz, 6.0Hz), representing H-2 and H-3 respectively. The spectral data for compound HB agreed with the one reported previously for an epicatechin (Antonelli *et al.,* 2007)

The mass spectrometer of the isolated compound shows molecular ion peak in the positive and negative ion mode at m/z 291.1 and m/z 289.1 respectively. And other fragments were observed at 113, 121.1, 142, 145, 245, 248 and 273. They are found to be in agreement to the work of (Wolf *et al.,* 2010) which shows molecular ion peak at m/z 291.1 and other fragment at 123, 140, 147, 155, 240 and 273. Which is typical of epicatechin?

The median oral LD_{50} of the extract in mice was greater than 5000 mg/kg suggesting that the extract is practically non-toxic when administered orally (Lorke, 1983).

Standard antimalarial drugs like chloroquine, halofantrin, mefloquine and artemisinin have been tested using animal model. The *in vivo* antiplasmodial activity of the ethyl alcohol leaf extract of *Uvaria chamae* was investigated by evaluating the chemosuppression during the early infection (suppressive test) and established infection (curative test) using standard animal model. The suppressive test is a standard test commonly used for antimalarial screening and the determination of percentage inhibition of parasitaemia is the most reliable parameter (Peter and Anatoli, 1998; Madara *et al.*, 2010). Antiplasmodial effect of plant derived natural product may be through inhibition of protein synthesis (Salawu *et al*., 2010).

In the suppressive test the crude ethanol leaves extract of *Uvaria chamae* showed a dose dependent and significant ($p< 0.05$) decrease in parasitaemia level to 48%, 53.3% and 65% at doses of 25, 50 and 100 mg/kg body weight. The extract at the highest dose 100mg/kg body weight had higher efficacy compared to the lowest dose 25mg/kg body weight while chloroquine (5 mg/kg) shows 79.8% suppression.

In the curative test, the extract caused a dose dependent significant ($p< 0.05$) decrease in parasitaemia levels of groups treated with different doses of the extract. There was a 73% cure at a dose of 100 mg/kg, 70% cure at a dose of 50 mg/kg and (68%) was observed in the group treated with 25 mg/kg body weight while chloroquine shows 98% suppression.

This observation showed that the extract was active against the malaria parasite used in this study and is consistent with the ethnomedicinal uses of *Uvaria chamae* reported in Nigeria and Togo (Koudouvo *et al*., 2011). The mechanism of antiplasmodial action of the plant extract has

not been elucidated. However, antiplasmodial effect of natural products has been attributed to some of their active phytochemical component (Ayoola *et al.,* 2008; Sofowora, 1980). Some of these phytochemical constituents such as terpenes and flavonoids were reported to have antiplasmodial activity (Philipson and Wright, 1990; Christensen and Kharrami, 2001; Go, 2003). Increase oxidation has also been shown to create an intracellular environment that is unfavorable to plasmodial growth (Boris and Schaeffer, 1992; Lavender and Agar, 1993). However, the lack of oxidizing action in some plant does not rule out antiplasmodial activity since they may be active through other biochemical mechanism (Alli *et al.,* 2011). The results of this work suggested that the extract could be safe and this partly explains the use of the plant by the local people who have been using the plant in management of malaria in Nigeria and Togo.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECCOMENDATION

6.1 Summary

Preliminary phytochemical screening of the ethyl alcohol leaf extract revealed the presence of flavonoid, saponin, alkaloid, terpenoids, tannin and carbohydrate. Chromatographic studies of the ethyl acetate fraction led to the isolation of HB a flavan-3-ol (Epicatechin). Antiplasmodial evaluation of the crude ethyl alcohol extract showed that the extract possesses significant antimalarial activity *invivo*.

6.2 Conclusion

To the best of my search, this is the first report on the isolation of Flavan-3-ol (epicatechin) from the plant. The plant has exhibited significant ant malaria activity in Swiss albino mice, thus lend credence to the ethnomedicinal claim for the use of plant to treat malaria.

6.3 Recommendations

There is need to carry out bioassay guided isolation with a view of isolating the compounds responsible for the observed biological activity.

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APPENDIX

Appendix I: Determination of median lethal dose (LD_{50}) of the Ethyl alcohol leaves extract of *Uvaria chamae*

First Phase

Dose ($mgkg^{-1}$)	Number of mice used	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3

Second Phase

Doses ($mgkg^{-1}$)	Number of mice used	Mortality
1200	1	0/1
1600	1	0/1
2900	1	0/1
5000	1	0/1

Appendix II: Comparison of 1D NMR data of compound HB with literature data

	Compound HB (MeOD)	Antonelli *et al.*, 2007 (CD_3OD)
Position	δ^1H, J (Hz)	δ^1H, J (Hz)
1		
2	4.60 [s,]	4.80 [s,]
3	4.18 [m, J=2.8, 6.0]	4.16 [m, J= 2.7 and 4.5]
4_{ax}	2.75 [dd, J=2.8,16.8]	2.72 [dd, J= 2.7 and 16.8]
4_{eq}	2.89 [dd, J= 4.8, and 16.8]	2.86 [dd, J= 4.5 and 16.8]
5		
6	5.92 [dd, J=2.0]	5.90 [d, J=2.4]
7		
8	5.94 [d, J= 2.4]	5.93 [d, J= 2.4]
9		
10		
1``		
2’	6.98 [d, J=2.0]	6.97 [d, J= 1.8]
3’		
4’		
5’	6.76 [d, J= 8.4]	6.75 [d J=8.1]
6’	6. 8 [dd, J= 1.6, 6.4]	6.79 [dd, J= 1.8 and 8.1]