PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON THE LEAF EXTRACT OF *STEREOSPERMUM KUNTHIANUM* (BIGNONIACEAE)

BY

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MARCH, 2008

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BY

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B.Sc (BUK 1995)
M.Sc/Pharm.Sci./39592/04-05

A Thesis Submitted to the Postgraduate School, Ahmadu Bello University, Zaria in Partial Fulfillment of the Requirements for the Award of TheDegree of Master of Science in Pharmaceutical Chemistry

Department of Pharmaceutical and Medicinal Chemistry,
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MARCH, 2008

DECLARATION

I declare that the work in the project report entitled phytochemical and pharmacological studies on the leaf extract of *Stereospernum kunthianum* (*Bignoniaceae)* has been performed by me in the Department of Pharmaceutical and Medicinal Chemistry under the supervision of Dr. M.I Sule and Dr. U.U. Pateh. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project report was previously presented for another degree or diploma at any University.

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______________ Sign

__________ Date

CERTIFICATION

This Project report entitled "Phytochemical and pharmacological studies on the leaf extract of *Stereospernum kunthianum* (*Bignoniaceae*)." By Hanwa, Umar Aliyu meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENT

My sincere gratitude goes to my supervisor, Dr. M.I. Sule. Dean of the faculty and head of Department of Pharmaceutical and Medicinal chemistry for his patience, constructive criticism, and immense guidance through out this research work. I am also indebted to my co-supervisor Dr. U.U. Pateh for his words of encouragement, advice and support. My appreciation goes to Drs. A.A Ahmadu, Ibrahim I, and Mal. Aliyu Musa Mention must be made of my principal Dr. Y. K. Kajuru and my vice-principal Mrs. P.K. Ityokyaa (DSS, ABU) for their moral support and encouragement. I must mention Mr.A. Ejila of CHELTECH Zaria, for interpreting the Spectrum.

My appreciation also goes to my wife and the children for their understanding, prayers and support during the course of this study. I also wish to extend my gratitude to all the technical and administrative staff of department of pharmaceutical and medicinal chemistry especially Alh. I M. Salisu, Mustapha and Mahmud, I am also grateful to the HOD Pharmacognosy and DD Dr. K.Y Musa and technical staff of his department. Thanks are also due to technical staff of department of pharmacology particularly Mal. Ibrahim Adamu. To Malam Musa Abdullahi, I thank you very much.

Finally, I thank God for his mercy and guidance that saw me through this research work.

ABSTRACT

Stereospermum kunthianum (Bignoniacea) herb is used in Hausa ethnomedicine in treating bronchitis, veneral diseases, diarrheoa and dysentery. It is also used in the treatment of ulcers, leprosy, skin eruptions, respiratory ailments and gastritis. The plant is common in Northern Nigeria where it is used as abortifacient and as antihypertensive agent.

General phytochemical investigation of both petroleum ether and ethanolic extracts of the leaves of *Stereospermum, kunthianum* revealed the presence of sterol/triterpenes, coumarin, fatty acids while flavones and alkaloids were absent. In the ethanolic extract, sterols/triterpenes saponins, tannins, coumarins and free carboxylic acids were found to be present.

Extensive phytochemical, chromatographic and physiochemical and spectroscopic investigation of the petroleum ether extract of the leaves of *Stereospermum kunthianum* using column chromatography on silica-gel with gradient elution afforded methyl-1-undecyl-2, 3, 3a, 5a, 6, 7, 8, 9, 9a 10, 11, 11a- dodecahydro-IH-cyclopenta[a] phenanthrene -4-carboxy. The structure was established by elemental analysis and the use of ACD/ NMR Assistant (a computer software). Library search and computer interpretation of the ^{1}HNMR spectrum obtained support the molecula formular of the compound as C_{30} $H_{48}O_2$.

Pharmacological studies of the n-butanol extract on isolated rabbit jejunum showed a dose-dependent relaxation of the tissue. The acute toxicity test for the extract in mice established an intraperitioneal LD_{50} of 3,807.9mg/kg. In castor oil-induced diarrhoea, 60% protection was observed at doses of 500mg/kg and 1000mg/kg respectively. The plant extract exhibited anti-diarrheoal activity that was comparable to that of Loperamide 5mg/kg. The result revealed that the extract have pharmacological activity against diarrheoa, this suggests some traditional claims of the plant.

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ABBREVIATIONS

^{1}H-NMR: Proton nuclear magnetic resonance

^{13}C-NMR: Carbon-13 nuclear magnetic resonance

GC-MS: Gas chromatography - Mass spectroscopy

M/Z: Mass change ratio

TLC: Thin layer chromatopraphy

NH_4OH: Ammonium hydroxide

C-C: Carbon-Carbon linkage

EcV: endotheliod cell

Uv: Ultraviolet spectroscopy

FTIR: Fourrier transform inra-red spectrometer

IR: Infra-red spectroscopy

PE: Petroleum ether Extract

Hcl: Hydrochloric acid

EE: Ethanolic Extract

PI: Unsaponifiable matter

P2: Saponifiable mather

CHAPTER ONE

1.0 INTRODUCTION

Throughout the development of human culture, the relationship between man and his ambient vegetation has been intimate and vital. Man truly has lived with and depended on his green plants. The medicine man usually an accomplished botanist represents probably the oldest professional man in social evolution.

Most of the medicines as well as foods we eat use from plant kingdom were discovered by trial and error over millennia in unlettered culture. Man probably put most plants into his mouth, many were innocuous, a few nourished him, a number made him ill or killed him. Some, however, relieved symptoms of discomfort or sickness and a very few though hallucinations, took him from this mundane existence to the realms of ethereal wonder. The plants in the two categories became his medicines.

Primitive societies believed in healing by Similarity. A red resin, for example meant that the plant was good for the blood; a heart-shaped leaf signaled its cardiac properties, while a liver-shaped leaf was a sign of efficacy against jaundice [William *et al.*, 1978]

There were no accurate data available to assess the value and extent of the use of plants or active principles derived from countries. Traditional medicine has passed through developmental stages. That is, by passing through civilizations, from a less developed civilized to a higher one. This is the reason why tracing the exact dates of starting the use of traditional medicines is difficult.

But, records indicating the use of traditional medicines by the ancients civilization of Chinese, Hindus, Babylonians and the ancient Greek exist. The ancient Egyptians were in the possession of vast knowledge of traditional medicines. They have recognized the medicinal values of dates, honey, garlic and castor beans etc. a fossil remains of *lephophora williamsii* used by Amerindians was found useful in the earliest time (Plowman, 1984).

The Chinese emperor Shen Nung published a book on herbs around 370 B.C., and published compendium uses of nearly 500 medicinal plants. Plants have undisputedly played a major role in the treatment of human traumas and diseases. The use of herbal medicines in India dates back to over 5,000 years and has become codified in the Ayurveda, which contains over 8,000 herbal remedies. However, this same ancient system of treatment is still used in over 14,000 dispenaries [Huxley, 1984].

Also earliest records revealed the use of a specie of *Hydrocarpus geartn,* for leprosy treatment. Ginseng, a Chinese herbal drug for Vitality and male potency (Sofowora, 1982).

The importance of plants in folkloric medicine may be attributed to interesting historical discoveries and subsequent development of many biologically active compounds from such claims. This include quinine, reserpine, hyoscyamine, emetine, morphine, strophanthidine digitoxis, physostigmine, and of recent, artemisinin, the active constituent of the Chinese antimalarial medicinal herb *Artemisia annua* L. These discoveries led to the intensive search for more medicinal agents that are responsible for medicinal activity, that is, primary and secondary metabolites example carbolydrates and amino acids, alkaloids, phenolic groups (Flavonoids, tannins), glycoside and anthraquinones. [Trease and Evans, 1997].

It is noteworthy that, apart from traditional uses, natural products, may also be used as building blocks for the synthesis of "semi-synthetic" drug. This is the case for plant saponins which can be extracted and easily altered chemically to produce sapogenin for the manufacture of steroidal drugs. Also a very important medicinal plant that led to the development and synthetic drugs is the cocaine from *Erythroxyllum coca*. It provided the

chemical structure for the synthesis of procaine and other related local enaesthetic. [Ole Hamann, 1988].

In present times, due to the economic condition of the people, there is a greater patronage of the traditional medicine. Traditional medicines are by far cheaper and accessible than the orthodox and modern medicines, which in most cases are synthetic or semi synthetic with the accompanying serious adverse effects, for instance, Loperamide hydrochloride (imodium) is effective against a wide range of secretory stimuli and can be utilized in the control and symptomatic relief of acute diarrheoa that is not secondary to bacterial infection. Adverse effects associated with its use include abdominal pain and distention, constipation, dry mouth, hypersensitivity, nausea and vomiting (Charles *et al.,* 1997). In the developing nations especially Africa, some people in the rural areas have the belief that some diseases such as epilepsy, and other mental illness, jaundice and cancer have no cure in orthodox medicine, they resorted in using traditional medicines for such diseases.

In the developing nations, traditional medicines has become part of the primary health care, due to this development, scientists are making efforts to confirm the efficacy of the traditional medicines and also standardized them.

It is obvious that the drug bill for many countries still represents a sizeable proportion of their total health expenditure. These countries have to purchase the drugs at exorbitant and often inflated cost from multinational companies, which spend a disproportionately large amount on advertising in developing countries [Sofowora, 1982].

In the developed counties, plant derived drugs are still used. For example, in the United state of America (USA), 25% of all prescriptions dispensed from the community pharmacies from 1959 to 1980 contained plant extract or active principle prepared from higher plants [Farnsworth, 1975]

1.1 STATEMENT OF THE PROBLEM

Diarrheoa is still one of the major health threats to tropical and subtropical countries (Heinrich, 2005). In Nigeria, it remains the number one killer disease among children under 5 years, while babies between the ages of 7-12 months remain the most susceptible (Audu, *et al*, 2002). The WHO has estimates that 3-5 billion cases occur each year with one billion in children below the age of 5 and 5 million deaths result from diarrhea annually with 50% in children below the age of 5 (Abdullahi *et al*, 2000). Despite the effective and simple cheap treatment of oral rehydration theraphy, majority of the local populace still rely on the herb to treat diarrheoa.

In Hausa ethnomedicine of Northern Nigeria, some medicinal plants are used frequently for treating diarrheoa diseases and these include: *Stereospermum kunthianum* (Bignoniaceae). In Northern Nigeria, local superstitions, as well as the medicinal uses, prevent *Stereospermum kunthianum* being cut for firewood. The bark is valued both by Hausas and Fulanis as a remedy for diarrheoa and dysentery, also given to horses. It is also used for veneral disease, a decoction boiled with natron, or, as in Sokoto, the bark mixed with a white variety of Guinea-corn to which red natron is added in boiling, being used for gonorrhea. The root, along with other roots, including that of the palm *Hyphaene thebaica* is a remedy for the disease called 'rana' with symptoms of haematuria (Dalziel, 1955). As part of our efforts to screen some ethnomedicinal plants of Northern Nigeria for antidiarrheoa activity the leave of *Stereopermum kunthianum* was investigated

1.2.2. AIM AND OBJECTIVE OF THE PRESENT STUDY

1. *Stereospermun kunthianum* has been in use as a remedy for diarrheoa, dysentery, venereal disease and as a cure for gornorrhea for long in the Hausa land. A thorough perusal for the chemical abstract has shown that not much work has been conducted on the plant. The present study/work hopes to be a basis for establishing the identity of

the chemical Compound present in the plant, the pharmacological basis for the observed action and also to determine how safe is the plant.

2. The plant is commonly found in dry areas of deciduous forest, wood land, bush rocky outcrops, termite mounds and margin of evergreen forests. The species is well spread all over the sahel region and is often found near streams, it is expected that this research will uncover certain compounds that would be used in the treatment of certain illness that were not reported by the traditional healers.
3. The use of synthetic antidiarrheoa and antimicrobial agents are but with an attendant fear for their side effects. As such naturally occurring treatment of the diseases will go a long way in eliminating the unwanted side-effects.
4. The synthetic drugs used as antidiarrheoal drugs are usually costly and beyond the purchasing power of earning Africans. Unfortunately, this class of people constitutes the majority of African population. Therefore, the abundance of *Stereospermum kunthianum* all over Africa will serve as a very cheap substitute.

5. Tropical African is blessed with vegetation Consisting of vast number of plant species. This may help facilitate the growth of our economy and provide the much needed foreign exchange.

1.1.3. SCOPE OF THE STUDY

The plant *Stereospermum kunthianun* is widely used by traditional medicine practitioners in Northern Nigeria in the treatment of diseases such as diarrhea, dysentery, Venereal, gornorrhea and a disease called 'rana' with symptoms of haematuria it is also used locally as an abortifacient and an antidiarrheoal, hence the need to carry out extensive investigation on the plant is important.

Diarrheoa a water related diseases is the passage of loose or liquid stools more frequently than is normal for the individual. It is primarily a symptom of gastrointestinal infection. Depending on the type of infection, the diarrheoa may be watery (for example in cholera) or passed with blood (in dysentery for example). Diarrheoa is a symptom of infection caused by a host of bacterial, viral and parasitic organisms most of which can be spread by contaminated water (WHO, 2000). Other causes include irritable bowel syndrome, infectious disorder, malabsorption or maldigestion, and laxative abuse. Medications used to treat other disorders also may induce diarrhea.

For example. Xanthines such as theophlline preparations cause diarrhea secondary to alteration of mucosal cyclic adenosine monophasphate (cAMP). Antihypertensive drugs, such as reserpine and guanethidine, may induce diarrhea by changing gut neuronal input and reducing noradrenergic-mediated relaxation (Craig *et al.*, 1997).

Phytochemical Screening will be carried out to determine the various chemical constituents. Chromatography and other separative techniques will also be employed for possible isolation of constituents. Appropriate Biological evaluation would also be employed to investigate ethnomedicinal uses.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DESCRIPTION OF *STEREOSPERMUM KUNTHIANUM*

Taxonomy:

Current Name:	*Stereospermum kunthianum*
Family:	Bignoniaceae
Common Names:	
Hausa:	Sansami or Jiri
Fulfulde:	Golombi
English:	Pink Jakaranda

2.1.2 BOTANICAL DESCRIPTION

Stereospermum kunthianum is a deciduous shrub or tree, 3-15m high, with a stem diameter of 25cm; leaves imparipinnately compound, 25cm long, alternate with 2-4 opposite pairs of leaflets and one terminal leaflet. Flowers precocious, fragrant, bisexual, showy, more usually pinkish, with red streaks on the lower corolla lobes. Fruits slender, flat capsules or paired pods (Dalziel, 1955).

2.1.3 ECOLOGICAL DISTRIBUTION

Natural Habitat

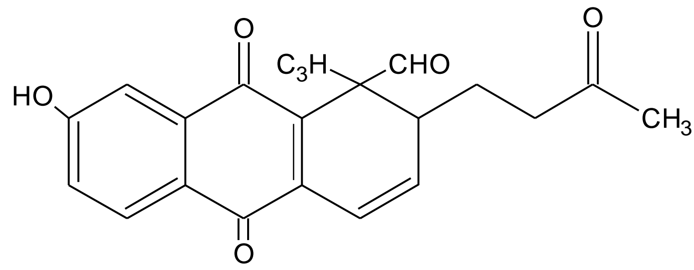
Stereospermum kunthianum is found in dry areas of deciduous forest, woodland bush, rocky outcrops, termite mounds and margin of evergreen forests. The specie is well spread all over the sahel region and is often found near streams.

Geographic distribution

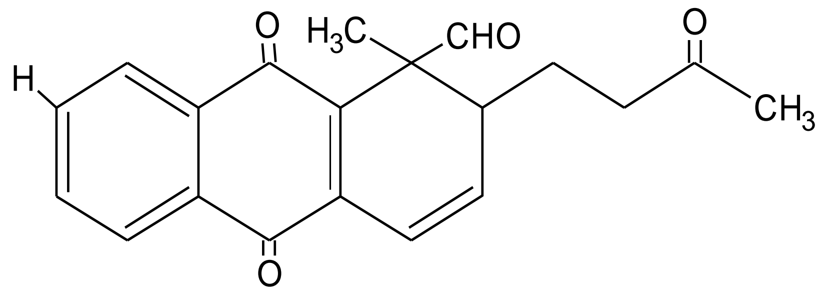
The geographical distribution of *Stereospermum kunthianum* cut across countries of the Sudan-guinea Savanna up to an altitude of 500-2400m, with the mean annual rainfall 450-900mm and mean annual temperature up to 40^0c. The soil type can be silty and sandy (Keay, 1989)

2.1.4 UPDATE ON CHEMICAL CONSTITUENTS OF *STEREOSPERMUM KUNTHIANUM* AND OTHER PLANTS IN THE FAMILY BIGNONIACEAE

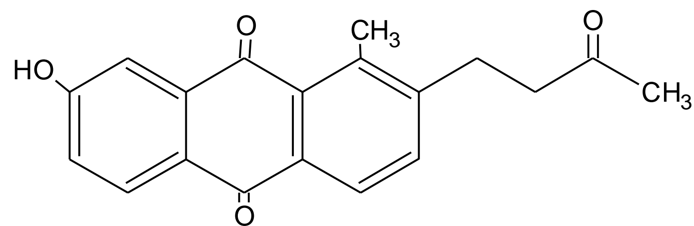
The bioassay-guided fractionation of root bark of extract of *Stereospermum kunthianum* led to the isolation of four novel naphthoquinones (*Sterekunthals* A and B, pyranokunthones A and B) together with the known naphthoquinone pinnatal (Onegi *et al.,* 2002).



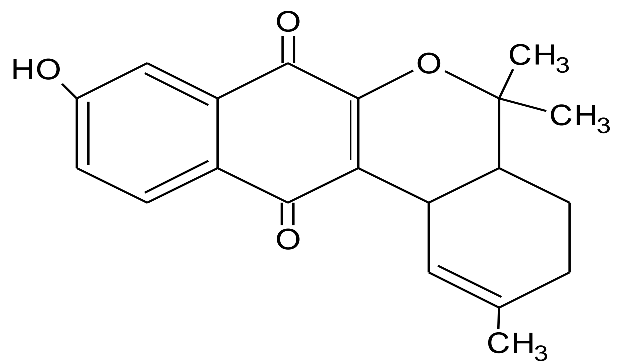
Sterekunthal A



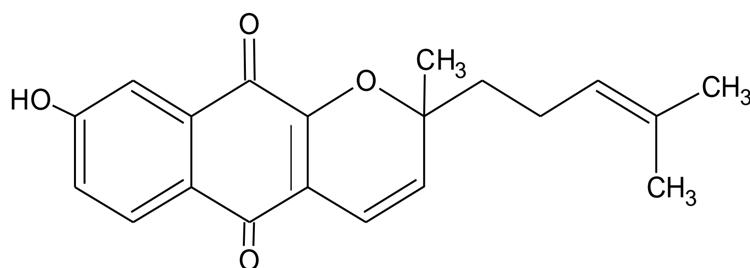
Sterekunthal B



Anthrakunthone



Pyranokunthone A



Pyranokunthone B

The bark of *Kigelia pinnata* is used in traditional medicine, it contains naphthoquinoids and iridoids and has been investigated for antibacterial activity.

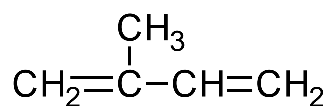
Tecoma stan is a shrub with bright yellow funnel-shaped flowers, it is found in British greenhouse; it contains monoterpene alkaloids.

Mussata hyacinthiana, contains iridoids and iridoid glycoside, saponins, phenylpropanoids, tannins and quinines, alkaloids are rare (Trease and Evans, 1996). Generally, the chemical constituents of Bignoniacea are variable and include the following

2.2 TERPENOIDS

These are most diverse and chemically interesting groups of natural products (Hansel, 1972). Terperoids or terpene are a group of compound that are made up of one or more units called isoprene (I). They are widely distributed through out the plant kingdom. Essential oils constitute the simpler mono and sesquiterpenoids. The di- and triterpenoids are found in tree gums and resin while the tetraterpenoids exemplified to as carotenoids and polyterpenoids exemplified by rubber are also found commonly in plants.

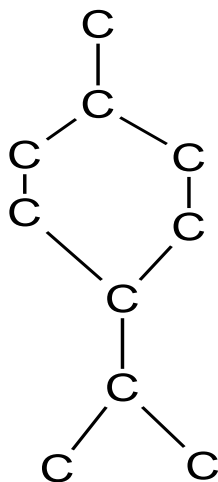
Terpenoidal compounds are classified based on the isoprene units present. The molecular formula employed for the classification is $(C_5H_8)n$, where n is used as the basis for classification.



(I)

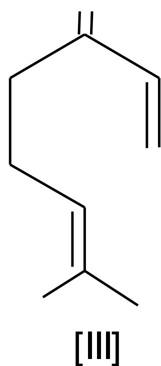
Terpenoidal compounds include monoterpene, triterpene and polyterperoid chiefly found in the latex. Monoterpenes are compounds made up of two isoprene units that are joined head to tail. The monoterpenes can either be

monocyclic or bicyclic, but most monoterpenes are derivatives of P-cymene structure (II).

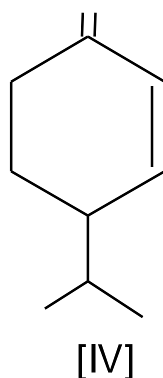


(II)

Monoterpenes are exemplified by myrcene [iii], β- phellandrene [iv] and carvone [v]. In general, there are four methods of extraction and isolation of monoterpenes. Viz:- expression; steam distillating extraction by using volatile solvents and adsorption in purified fats (enflurage). (Finar, 2003)



[III]



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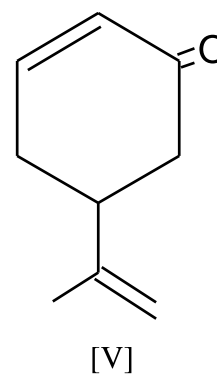
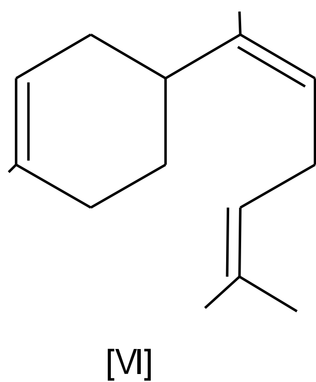
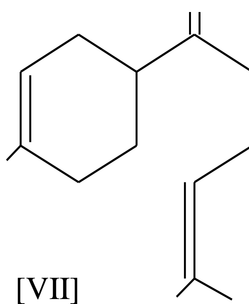


[V]

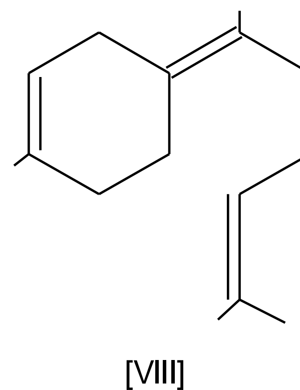
Sesquitepenes are made up of three isoprene Units. They can be acyclic, monocyclic, bicyclic or tricyclic. Examples of sesquiterpene are α – bisabolene [vi], β – bisabolene [vii] and λ- bisabolene [viii]. (Finar, 2003).



[VI]



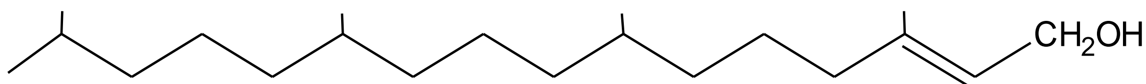
[VII]



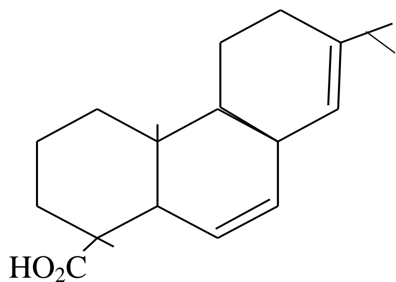
[VIII]

Diterpenoids contain four isoprene units and are mostly found in plants. They are produced from the hydrolysis of chlorophyll and it also form part of the molecules of vitamins E and K. Diterpenes can be classified based on the number of rings present. Although, the acyclic diterpenenoid occurs, the

cyclic are found as monocyclic, tricyclic and tetracyclic in nature. Examples of diterpenes includes phytol [IX]; abietic acid [X] and phythocladene [XI]



[IX]



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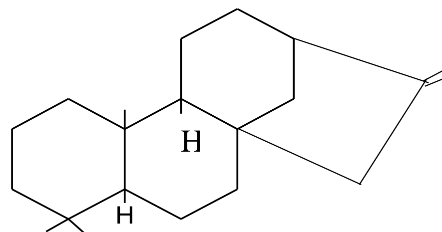
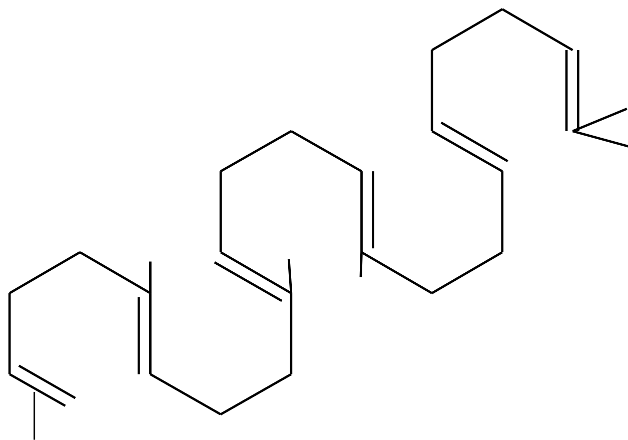


[XI]

Triterpenoid compounds are made up of six isoprene Units and can be acyclic or cyclic. The cyclic triterpenoids are usually tricyclic, tetracyclic or pentacyclic. Examples among others are squalene [xii] and Ambrein [xiii].



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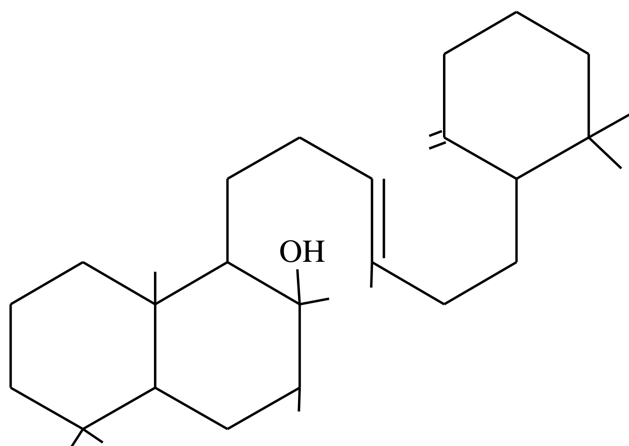


[XIII]

Polyterpenoids are compound with more than 8-Isoprene Units contained in a linear pattern.

2.2.1 EXTRACTION OF TERPENES

A large number of terpenes are usually non-polar compounds and may therefore be separated from polar plant constituents by extraction with solvents like benzene or ether. Associated matters like lipids, esters and waxes may be removed by saponification in alcoholic alkali, followed by extraction with ether (Robinson, 1969). Terpenoid glycosides however are usually insoluble in non-polar solvents, they are most conveniently extracted from plants with 70-95% ethanol. Extraneous lipids are removed from the alcolohic solution by partition with benzene. Acidic terpenes when present as free acids are soluble in non-polar solvents, but on saponification will pass into alkaline phase.

2.2.2 SPECTRAL PROPERTIES OF TERPENES

[1] Infra-red (IR) spectroscopy

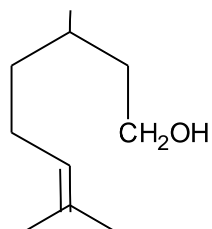
Infra-red spectroscopy is of importance in terpenoid chemistry, the two major regions of the spectrum that are importance are:

i. O-H stretching (3650-2650cm^{-1}). This region may be used to determine the presence of hydroxy group and related species.

ii. Carbonyl stretching (1820-1640cm^{-1}). This is a strong absorption region, and the region is further divided into two (a) saturated carbonyl (1750-1700cm^{-1}) and (b) unsaturated α, β- Carbonyl (1700-1600cm^{-1}).

Infra-red spectra is particularly useful for detecting the presence of isopropenyl group in terpenes usually between 895-885cm^{-1}. For example, the spectrum of citronenollol [xiv] show a maximum at 890cm^{-1} which corresponds to the presence of the Isopropenyl group while that of isopropylidene is 850-790cm^{-1}.

Myrcene [iii] with λmax 224 had no band at 890cm^{-1}. This shows the complete absence of the isopropenyl group.



[XIV]

Heteroannular dienes and α,β- unsaturated Ketones can be distinguished by the infra-red spectra.

[ii] Ultraviolet spectroseopy (UV)

Conjugation can be detected by ultraviolet spectroscopy technique in terpenoid chemistry. For simple acyclic dienes, λmax is 217-218nm, while in heteroannular dienes λmax is 230-240nm, and for homoannular dienes λmax is 250-265nm (Finar, 2003).

The absorption of the diene system is affected by substitution and woodward (1942) prososed empirical rules, which was later modified by Fieser (1948) for calculating the λmax from the molecular structure of a compound. A monoterpene myrcene [iii] based on the rules have a λmax of 219nm while the observed λmax is 224nm. Similarly, carvone [v] has a calculated λmax of 237nm, while the observed λmax is 237nm.

[iii] Nucleaar magnetic Resonance (NMR) Spectoscopy

(a). ^{1}H-NMR

^{1}H-NMR has been used to detect and identify double bonds, to determine the nature of end groups, the member of rings present and to ascertain the orientation of methyl groups present. For example, Abscisin, a plant hormone and a monocyclic sesquiterpenoid has a ^{1}H-NMR spectrum as

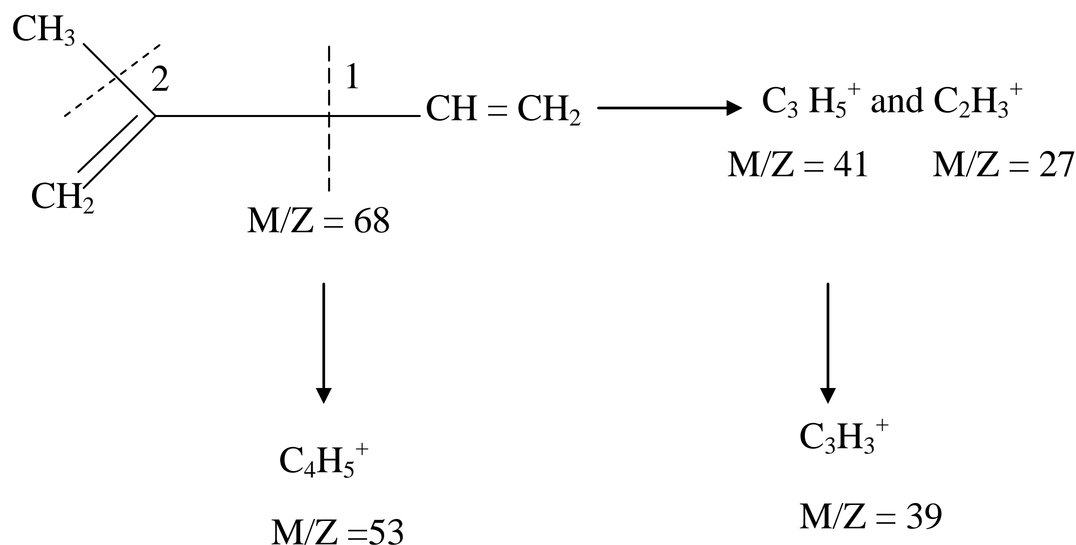
follow: methyl singlets at δ=1.1 and δ=1.1.7; two vinyllic methyl groups singlets at δ = 1.1, and 2.1; and a methylenic group adjacent to the carbonyl group at δ 2.41.

(b). ^{13}C-NMR

The ^{13}C-NMR is a more useful tool than ^{1}H-NMR for the elucidation of carbon skeleton of a compound (stothers, 1972). This is because most of the carbon compounds of moderate complexity give rise to separate signals, while the proton spectrum on the other hand permits the identifications of only a few positions in the compound.

[iv] Mass spectrometry (MS)

Mass spectrometry is being increasingly used as a means of elucidating the structure of terpenoids, thus, it is possible to determine the molecular weight, molecular formular, the nature of various founctional groups and the relative position of double bonds. Since isoprene is the building unit of terpenoids, the following include the peaks which are usually observed in the spectra of terpenoids in general: 68($C_5H_8^+$), 67 ($C_5H_7^+$), 53($C_4H_5^+$), 51($C_4H_3^+$), 41 ($C_3H_5^+$), 39 ($C_3H_3^+$), 29 ($C_2H_5^+$) and 27 ($C_3H_3^+$). Paths that account for some of these fragments are:



2.2.3 PHARMACEUTICAL IMPORTANCE OF TERPENES

The volatile terpenes especially the monoterpenes have wide application in perfumery industries in production of perfumes; the sesquiterpenes have use in pharmaceutical practice especially with the introduction of the Chinese antimalaria artemisinin which is an unusual sesquiterpene lactone, it is used in the treatment of Plasmodium falciparum resistant malaria and also in cerebral malaria. The diterpenoids are also of current interest because they are future drugs either as isolated from plant or as modified derivatives eg Forskohlin (coleanol) a diterpene isolated by Indian workers from *Coleus forskohlii.* Forskohlin has been shown to have hypotensive spasmolytic and cardiotonic effect. A preparation of the species have long been used in

Hindu and Ayurvedic traditional medicine for treatment of heart diseases, abdominal colic (Ammon and Muller, 1985).

2.3 SAPONINS

The group of natural products which have in common the property of foaming when shaken with water are called saponins. They are widely distributed in the vegetable kingdom and have been reported to be present in at least 500 general of plants. Chemically, saponins are glycosides which yield on hydrolysis (a) and one or more sugar units and (b) sugar-free aglycones which are derived from polycylic ring systems and are commonly referred to as sapogenins (Basu, 1967).

This group share in varying degrees two common characteristics.

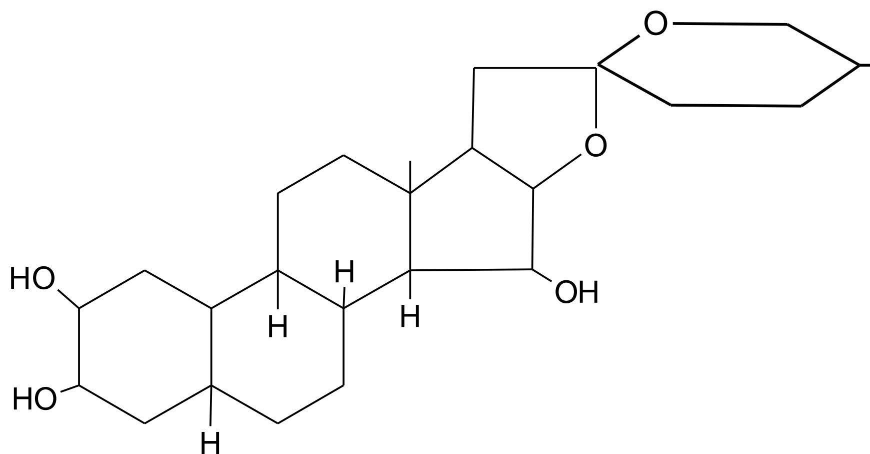
a. They foam in aqueous solution; that is have the property of foaming in water

b. Intravenous injection of their aqueous solution into animals results in haemolysis (Olaniyi *et al.,* 1993).

Classification of saponins into two broad groups is based on the chemical nature of the sapogenins, these are:

1. Saponins of cholane group. As in sterols, bile acids and cardiac aglycones, saponins of cholane group are derived from the same tetracyclic

ring system (Fieser, 1960). Some example includes digitogenin *(Digitalis purpure and D. lanata)* [xv] Tigogenin [xvi]; sarsasapogenin [xvii]



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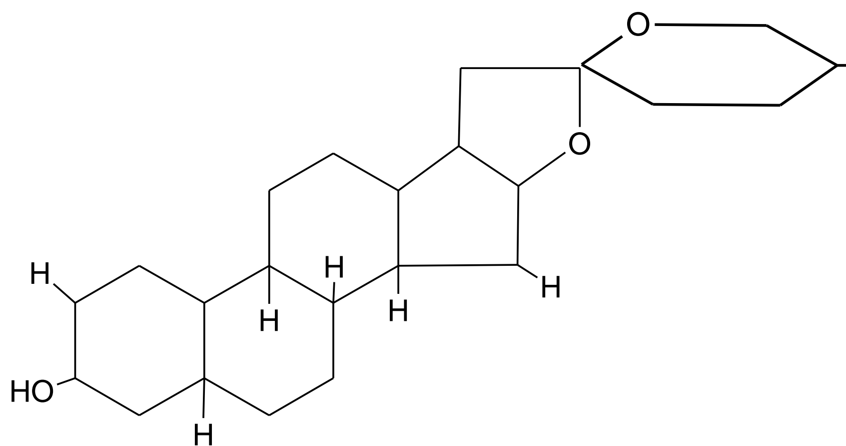
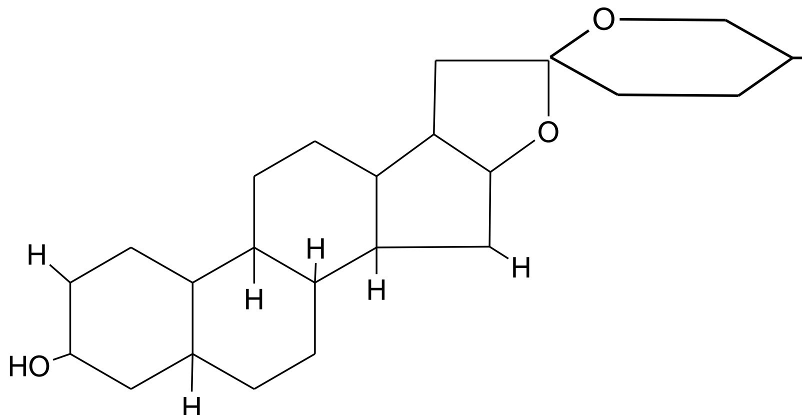


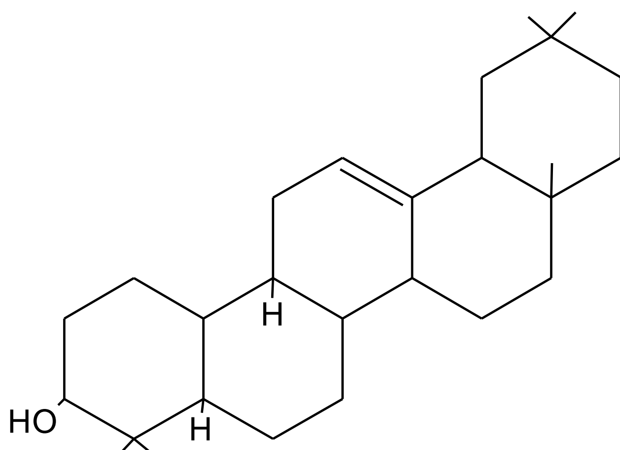
[XVI]



(XVII)

These groups are C-27 steroid carrying a spiroketal side chain which exhibits several characteristics strong I.R. bands in the regions of 1350 – 875cm^{-1} (Basu, 1967).

2. Saponins of triterpenoids group. The groups are extensively distributed in the plant kingdom and constitute the majority of the saponins found in nature. A number of these saponins differ only in the number and the type of units in the carbohydrate moiety linked to a particular sapogenin. This group, with few exceptions, belong to β-amyrin[xviii] group and are usually simple alcohols and acids. But, sapogenins can be aldehydic and ketone (lactone) functions occasionally (Basu, 1967).



(XVIII)

Triterpenoid saponins have been known for over 100 years and a few efforts have been made more than a decade ago to review their chemical and biological properties. However, in the recent years a noteworthy progress has been made in this field as a result of the development of improved techniques for isolation and detection of natural products, and the elucidation of the finer details of triterpenoid chemistry (Basu, 1977)

Saponins and their genins are usually detected by means of the characteristic colour they produce with various reagents, eg Liebermann- Buchard, thionylchloride, phosphomolybdic acid, silico-tungstic acid etc. and the haemolysis of blood. For the purpose of detection on paper chromatograms, some of the above reagents have been modified. A quantitative and highly

sensitive micro method in which filter paper disc wetted with saponin are embedded in blood gelatin has been devised. Other reagents used for the detection are sodium metaperiodate, alkaline potassium permanganate mixture, antimony trichloride, chloroform-chloroform vanillin. Various solvent system have been reported to provide satisfactory separations, viz ethyl acetate: Pyridine: Water (3:1:3); butanol: acetic acid: water (6:1:3) and butanol: 1m NH_4OH: 95% alcolhol (60:30:5:13) etc. chloroform: tetrahydrofuran: pyridine (10:10:2) saturated with formamide and chloroform: methanol: 1m NH_4OH: 95% alcohol (60:30.5:13) saturated with formamide and water (65:35:10) have proved to be satisfactory. For paper Chromotography, TLC as well as separation of saponins on Cellulose and silica gel columns (Basu, 1967).

A comparative study of the saponin content in saponaria, polymomium, primula, etc. has shown that the highest yields are obtained by collecting the plant material just before the flowering time [Stecka, 1963]. The ease with which saponins are hydrolysed into sepogenins and sugars (of which there may be up to 12 units) varies from case to case; usually refluxing with 5 – 10% mineral acid is necessary for a complete breakdown. In some cases, hydrolysis can be carried out with enzymes, eg hydrolysis of alfalfa saponin has been achieved with a fungus preparation (Aspergillus).

The sugar components have been usually identified by the conventional method of paper chromatography in various solvents. The sugar units are linked to the genin by glycosidic linkage; and ester linkage is rarely encountered and only D-glucose has so far been found in such combination. The following eight sugars have almost exclusively been found to be involved in glycosidation: D-glucose, D-galacturonic acid, D-glucoronic acid, D-Xylose, L- arabinose, L-Furanose, L-rhamnose and D-galactose. The structure of the sugar chains in a few cases have been elucidated by the usual method of methylation followed by hydrolysis and the identification of the individual methylated units. It has been found that the sugar moiety is very often linked at C-3 OH of the aglycone, but in some, this linkage is at other positions, eg in Musennin at C-16 OH and in asiaticoside at C-28 COOH (Basu, 1967).

2.3.1 PHARMACEUTICAL IMPORTANCE OF SAPONINS

Saponins occurred widely in nature, this has evoked considerable interest in their uses and there are considerable data accumulated on their physiological action and other properties. They tend to alter the permeability of the wall hence exert a general toxicity in all organized tissues, their haemolytic and anti-lipemic activity and capacity to lower serum chlolesterol level can be

their important characteristics. The ability of saponins to reduce surface tensions have been utilized in making emulsion stabilizer (Basu, 1967)

2.4 TANNINS

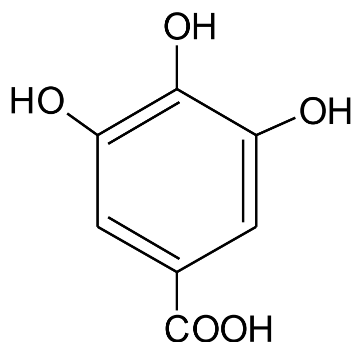
The term "tannin" was first applied by Seguin in 1796 to denote substances present in plant extracts which were able to combine with protein of animal hides, to prevent their putrefaction and convert them into leather. On this note, a tannin is a substance which is detected qualitatively by a tannin test (the goldbeater's skin test) and is determined quantitatively by its adsorption on standard hide powder. This definition excludes simpler phenolic substance, often present with tannins, such as gallic acid, catechins and chlorogenic acid, although, they may under certain conditions give precipitate with gelatin and be partly retained by hide powder. Such substances of relatively low molecular weight are called 'pseudo-tannins'.
Most tannins have molecular weights of from 1000 to 5000. To be effective for tannage, the polyphenol molecule most be neither so large as to be unable to enter the interstices between the collagen fibrils of the animal skin nor so small that it is unable to cross – link between the protein molecules of adjacent fibrils at several points (Trease and Evans, 1996).

The characteristics properties of tannins derive from the accumulation within a moderately sized molecule of a substantial number (1-2 per 100 molecular weight) of phenolic groups many of which are associated with O-dihydroxy and O-trihydroxy orientation within a phenyl ring.

Two main types of groups of tannins are usually recognized, these are the hydrolysable tannins and the condensed tannins (proanthocyanidins).

2.4.1 HYDROLYSABLE TANNINS

These may be hydrolysed by acids or enzymes such as tannase. They are formed from several molecules of phenolic acids such as gallic [xix] and hexahydroxy diphenic acids [xx] which are united by ester linkages to a central glucose molecule. The above examples were formerly known as pyrogallol tannins, because on dry distillation, gallic acid and similar components are convented into pyrogallol.



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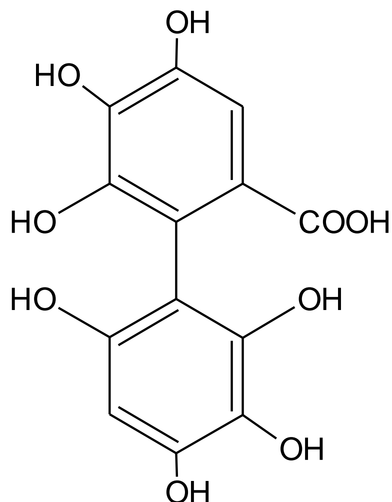


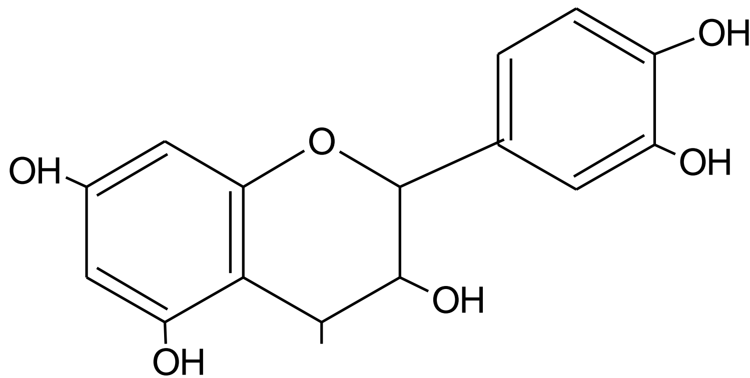
(XX)

The hydrolysable tannins can be hydrolysed by boiling with dilute hydrochloric acid, the alcoholic components of the esters is usually sugar. Hydrolysable tannins are often complex mixture containing several different acids esterified to different positions with sugar molecule and these render it water soluble. They are sometime named after the acid derivatives eg gallitannins for tannin from gallic acid and ellagitannin for tannin from ellagic acid. They can also be hydrolysed by an enzyme called tannase. They are soluble in water, dilute alkalis, acetone, alcohol but insoluble in chloroform and benzene.

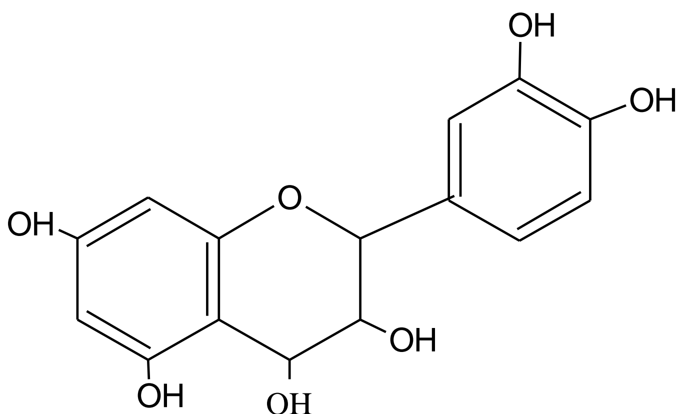
2.4.2 CONDENSED TANNINS (Proanthocyanidins)

Unlike hydrolysable tannins, these are not readily hydrolysed to simpler molecules, and they do not contain a sugar moiety. They are related to the flavonoids pigments and have polymeric flavan-3-ol structures. Catechins, [xxi] which also occur with tannins and flavan-3,4-diols [xxii] (leucoanthocyanidins) are intermediates in the biosynthesis of the polymeric molecules.

Condensed tannins have no sugar linkage molecules, they consist of larger phenolic group chemically linked directly by carbon-carbon (C-C) linkage.



(XXI)



(XXII)

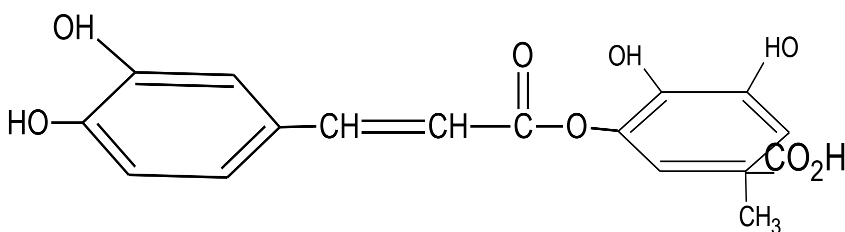
On treatment with acids or enzymes condensed tannins are converted into red insoluble compound known as phlobaphenes. Phlobaphenes give the characteristics red colour to many drugs such as red cinchona bark, which contain these phloba-tannins and their decomposition products on dry distillation, they yield catechol and these tannins like catechol itself, their solutions turn green with ferric chloride (Trease and Evans, 1996)

2.4.3 COMPLEX TANNINS

The term 'complex tannins' is applied to a newly discovered group of tannins which are from both a hydrolysable tannin (mostly a C-glucoside ellagitannin) and a condensed tannin. The union occurs through a C-C bond between the C-1 of the glucose unit of the ellagitannin and the C-8 or C-6 of the flavan – 3-ol devivative.

2.4.4 PSEUDO TANNINS

Pseudo tannins are compounds of lower molecular weight than true tannins and they do not respond to the goldbeater's skin test. Example of pseudotannin is the chlorogenic acid tannins [xxiii]



(XXIII)

2.5 FATTY ACIDS

These acids are important as components of plant oils (acyl lipids) in which they occur as esters with the trihydric alcohol glycerol. They are also components of the resins of convolvulaceae and of waxes in which they are esterified with long chain alcohols. Most are C-10 to C-20. The naturally occurring fatty acids, with a few exception are straight chain unsubstituted acid containing an even number of carbon atoms. Although, only a few are branched chained, hydroxy and acids moreover, a few odd numbered carbon atom from C-7 to C-15 have been detected free or as esters in fatty substances.

Over 200 fatty acids have been isolated from natural sources but relatively few are ubiquitous in their occurrence the vast majority of fatty acids has an unbranched carbon chain and differs from one another in chain length and degree of unsaturation Oleic acid is the most widely spread natural fatty acids occurring in practically every naturally fat mixture. Generally fatly acids may be saturated eg Oleic acids. The double bonds, with a few minor exceptions such as seed oil of pomegranate are cis. Less commonly, they are cyclic compounds such as hydroscapic acid and the prostaglandins. The latter are a group of physiologically active essential fatty acids found in most body tissues and are involved in the platelet-aggregation and inflammatory processes. They promote smooth muscle contraction making them of clinical use as effective abortifacients and for inducing labour (Trease and Evans 1996).

2.5.1 SATURATED FATTY ACIDS

These are commonly found in nature, they belong to the homologous series $CH_3(CH_2)_nCOOH$, in which n is mostly an even number. At present, there is evidence that most of the possible saturated fatty acids between butanoic, $CH_3(CH_2)_2COOH$ and hexacosanoic $CH_3(CH_2)_{24}COOH$ occurs in plants. However, while the even-numbered fatty acids from C-10 to C-18 can

comprise of greater than 50% of the total fatty acids of the plant lipids, the odd-numbered rarely exceed 1% of the total fatty acids.

The major fatty acids in plant are only sparingly soluble in water. Most are hydrophobic and are readily soluble in organic solvents eg Hexane, benzene, chloroform and chloroform methanol mixtures. The solubility of fatty acids decreases with increasing chain length both in aqueous and in organic solvents. Some of the commonly found saturated fatty acids are shown in table 2.1 below.

Table 2.1: some naturally occurring straight chain saturated fatty acids

Number of carbon atom	Common Name	Systematic Name	Structure
12	Lauric	Dodecanoic	$CH_3 (CH_2)_{10} COOH$
14	Myristic	Tetradecanoic	$CH_3(CH_2)_{12} COOH$
16	Palmitic	Hexadecanoic	$CH_3(CH_2)_{14} COOH$
18	Stearic	Octadecanoic	$CH_3 (CH_2)_{16} COOH$
20	Arachidic	Eicosanoic	$CH_3 (CH_2)_{18} COOH$
22	Behemic	Decosanoic	$CH_3(CH_2)_{20} COOH$
24	Lignoceric	Tetracosanoic	$CH_3 (CH_2)_{22} COOH$

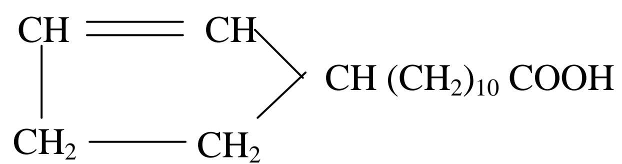
2.5.2 UNSATURATED FATTY ACIDS

These comprises a greater percentage than the saturated fatty acids. The predominant Unsaturated fatty acids are 16 and 18 carbon atoms. The presence of Unsaturated centres in fatty acids chain lead to the possibility of positional isomerism of the double bonds. Thus, α-linolenic systematic name all –cis –Δ 9,12,15 – octadecatrienoic acids, has 18 carbons and leads to the possibility of geometrical isomeric. Example of straight- chain Unsaturated fatty acids are shown in Table 2.2 below.

Table 2.2: some naturally occurring straight-chained Unsaturated fatty acids

No of Carbon aton	Common Name	Systematic Name	Structure
16	Palmitoeic	Cis-9-hexadece noic	$CH_3(CH_2)5\text{-}CH{=}\ CH\ (CH_2)_7\ CO_2H$
18	Oleic	Cis-9-hexadece noic	$CH_3(CH_2)_7\text{-}CH{=}\ CH\ (CH_2)_7\ CO_2H$
22	Erucic	Cis-13-docoseconoic	$CH_3(CH_2)_7\text{-}CH{=}CH\ (CH_2)_{11}\ CO_2H$

Examples of cyclic Unsaturated acids are Hydrocarpic [xxix]; Chaumoogric acid [xxx] and Gorlic acid [xxxi]



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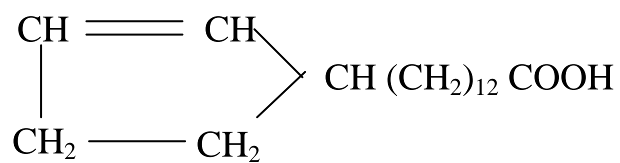

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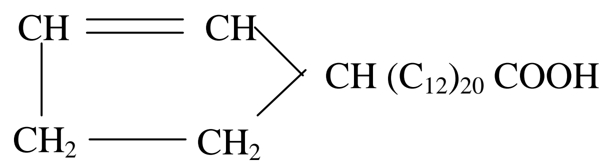


XXXI

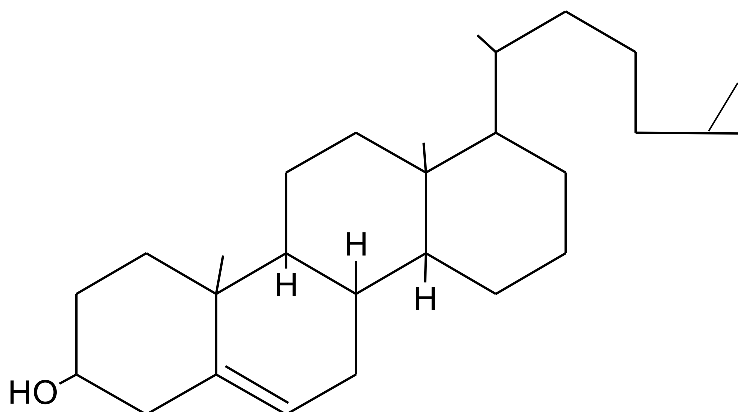
2.6 STEROIDS

The steroids form a group of structurally related compounds which are widely distributed in animals and plants. Included in the steroids are the sterols [from which the name steroids is derived] , Vitamin D, the bile acids, a number of sex hormones, the adrenals cortex hormones, some carcinogeric hydrocarbons, certain sapogenics etc. the structures of the steroids are based on the 1,2- cyclopentenophenanthrene skeleton. All the steroids give, among other products, Diels' hydrocarbon on dehydrogenation with selenium at 360°C. Steroid could be defined as any compound which gives Diels' hydrocarbon when distilled with selenium (Finar, 2003).

2.6.1. STEROLS

Sterols occur in animal and plant oils and fats. They are group of crystalline steroidal alcohol containing between 27 and 30 carbon atoms all possess 3β-hydroxyl group and an exocyclic double bond usually in 5, 6 position, together with a side – chain which exhibits various degrees of branching and unsaturation (Dence, 1980). There are three groups of sterols:-

(a) **The animals sterols**:- Cholesterol [xxxii]; cholestanol [xxxiii]; are the animal sterols. The animal sterols are referred to as zoosterol



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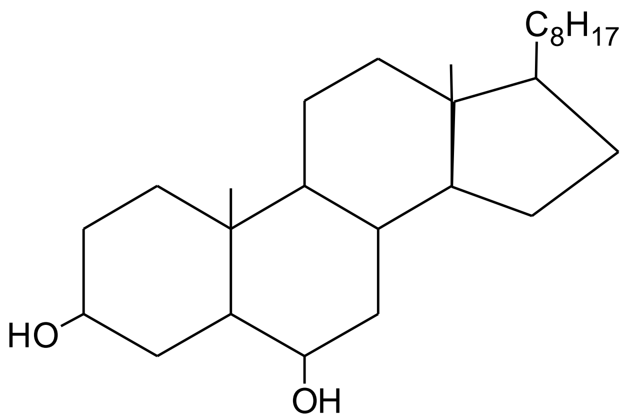
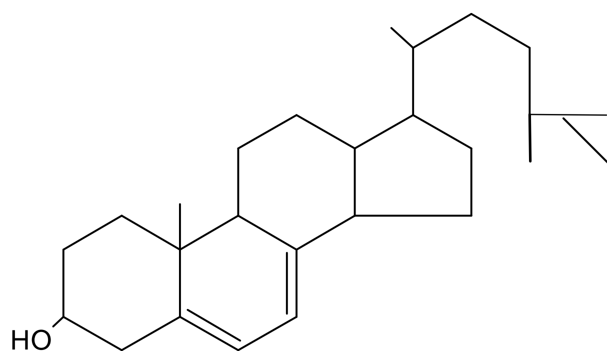


[XXXIII]

(b) **Plant sterols:-** Ergosterol [xxxiv] and stigmasterol [xxxv] are the principal plant sterols. The difference in structure between animal and plants sterol is the presence of an additional alkyl in the C-17 side chain of the plant sterols. This group is referred to as phytosterols.

(c) **Yeast Sterols**:- Yeast sterols is classified under plant sterol sometime. It is an important example of plant steroids. Eg Cardiac ghycosides, digitoxin occur generally largely in a conjugated form with one or more sugar residue. This is referred to as mycosterols.



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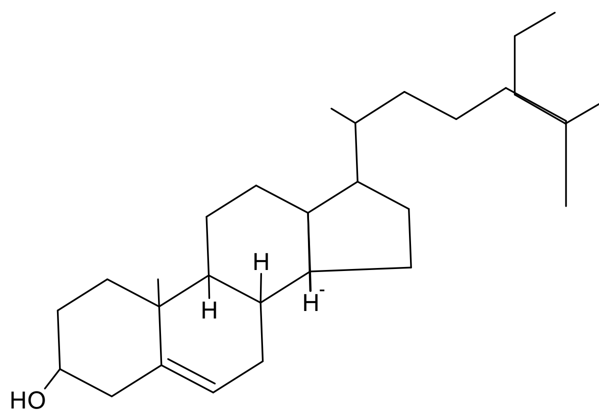


(XXXV)

2.6.2 TEST FOR STEROIDS

There are two chemical test usually conducted to infer the presence of sterols.

a. The Salkowski reaction. When concentrated sulphuric acid is added to a solution of sterol in chloroform, a red colour is produced in the chloroform layer.

b. The Liebermann-burchard reaction

When a solution of sterol in chloroform is treated with concentrated sulphuric acid and acetic anhydride, a greenish colour is development to indicate the presence of sterol.

2.7 ETHNOMEDICINAL USES OF *STEREOSPERMUM KUNTHIANUM*

Locally, *Stereospermum kunthianum* is known to be used**: -**

(a) In Burkina faso, as toxic plant by the local people and the source of bad feelings in household (Dalziel, 1955). However, despite the toxicity of *Stereospermum kunthianum,* it is widely used as a medicinal remedy of many ailments in Africa.

(b) In treating bronchitis (roots and leaves) (Watson *et al.,* 1991).

(c) As a remedy for cough. The pod of *Stereospermum kunthianum* is used by the Ha people in Tanganyika when chewed with salt (Brandwijk, 1962).

(d) A decoction of the plant is used in treating venereal diseases; gonorrhea; diarrhea and dysentery (Dalziel, 1955).

(e) *Stereospermum kunthianum is* used in the treatment of ulcers, leprosy, skin eruptions and pneumonia. The roots and leaves are used for respiratory ailments and gastritis (Dalziel, 1955).

(f) The root including that of the palm *Hypaene thebaica,* is a remedy for the disease called 'rana' with symptoms of haematuria, the root bark is value as remedy for certain tribes in Uganda to treat fever (Onegi *et al.,* 2002).

(g) The root-bark is used in inducing labour. The leave and the stem bark are used in the treatment of hypertension in Northern Nigeria.

2.8 PHARMACOLOGICAL PROPERTIES OF THE PLANT

The compounds found in *Stereospernum kunthianum* showed antiplasmodial activity invitro against endotheliod cell line EcV-304, its activity against Plasmodium falciparum represents them as interesting lead compounds for drugs against malaria (Onegi *et al*., 2002)

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 MATERIALS

Chemical/reagents

All organic solvent used are of analytical grade, all equipment and capacity are quoted accordingly and operated to standard procedures. The silica gel for column chromatography is of mesh size (60 – 120) column size and that of thin layer chromatography is 0.25mm thickness.

3.1.1 INSTRUMENTS

1. NUCLEAR MAGNETIC RESONANCE

Proton nuclear magnetic resonance Spectroscopy was carried out on Bruker DRX at 400MHz the Spectrum obtained was interpreted using ACD/NMR Assistant computer software.

3.2 ANALYTICAL METHODS

[I] Thin layer chromatography (TLC)

Technique: Ascending

Stationary phase: silica gel 60 F_{254} pre-coated plates by MERK of thickness 0.25mm. silica gel G. slurry made by dissolving 40g of silica gel in some of

distilled water and spread unto the plates (2x5cm) using a spreader (Stahl, 1975), and activated at 100°C for 1 hour.

Thickness: 0.25mm for analytical TLC plate and activated at 110°C for I hour before use.

Spotting and development: The spots were applied manually using capillary tubes and the plates developed at room temperature using a shandom chromatotank.

Mobile phase: Details in text

Visualization: The developed chromatogram were dried in a fume cupboard and viewed under

1. U.V. light for any fluorescent spots.
2. Exposed to Iodine Vapour in a closed chamber.
3. Sprayed with suitable chromogenic reagents.

[2] Column chromatography:

Technique: Gradient Elution

Column: Sintered Glass column (2x10cm) packed by wet slurry method

Adsorbent: silica gel. (60 – 120 mesh size)

Eluting solvents: details in text

3.3 SPRAY / VISUALIZATION REAGENTS

(i). Iodine Vapour

(ii). U.V. Light

(iii). Sulphuric acid (spray)

3.4 EXTRACTION PROCEDURES

3.4.1 COLLECTION AND DRYING OF PLANT MATERIAL

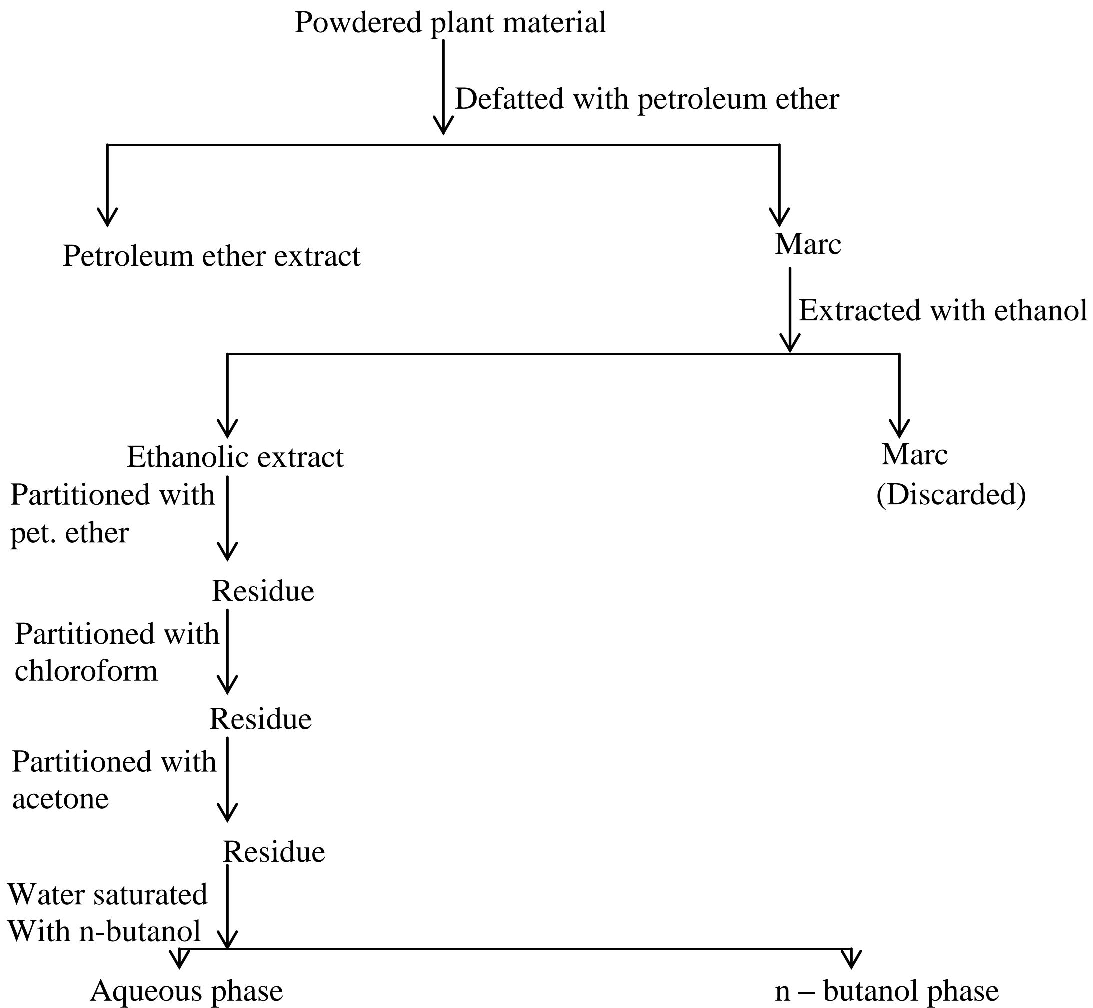
Whole fresh plant material bearing fruits and leaves, growing wild was collected at HANWA Village in the month of December,2005. The plant was authenticated at herbarium unity, Department of Biological Sciences, A.B.U., Zaria. Where a specimen voucher number 1381 has been deposited. The leaves were air – dried for 21 days before powdering.

3.4.2 EXTRACTION OF PLANT MATERIAL

The powdered leaves (165g) of the plant was continuously defatted with petroleum ether (60 – 80°C) using soxhlet extractor until the draining solvent was clear. Solvent used was recovered at reduced pressure to afford a yellowish green waxy material (6.64g) that was there after referred to as the petroleum ether extract coded PE.

The marc after defatting was air – dried at room temperature and then extracted continuously with ethanol using soxhlet extractor. The solvent was evaporated at reduced pressure to afford ethanolic extract (10.60g) that was thereafter referred to as ethanolic extract coded EE. The ethanolic extract was further partitioned using petroleum ether, chloroform, acetone, saturated with water and then n-butanol. The n-butanol extract was kept for further analysis. The extraction procedure is as shown in fig 1.

Fig 1: shows the extraction procedure of the leaves of St*ereospermum kunthianum*



3.5 PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening of the extract was carried out using the standard procedure .

3.5.1 PRELIMINARY PHYTOCHEMICAL SCREENING OF PE

(a) Test for steroids and triterpenes

About 0.2g of the PE was dissolved in equal amount (0.5ml) of acetic anhydride and chloroform. This solution was transferred into a dry test tube by means of pipette. Concentrated sulphuric acid (1 – 2ml) was added to the bottom of the tube.

Liebermann – Burchard's reaction). (Silva *et al*., 1998)

(b) Test for flavone aglycone

Shinoda test

A small portion of the extract was taken in a test tube, (1 – 2ml) of ethanol was added and heated. Magnesium chips (1 – 2 pieces) and (4 – 5) drops of Conc. HCl. Was added (Farnsworth, 1966; Markham, 1982).

(c) Test for coumarins

A small portion of the PE was dissolved in hot water, after cooling, the solution was divided into two test tubes. One contains the reference, the

aqueous solution in the second tube was made alkaline with 0.5ml of ammonia solution (10%). They were observed under the ultraviolet light.

(d) Test for basic alkaloids

A small portion of PE was dissolved in 5ml of 2% HCl. The solution obtained was then divided into three equal portions in three test tubes. To the first tube, two drops of freshly prepared Dragendorff's reagent was added; to the second tube, two drops of Wagner's reagent was added, and to the third tube, two drops of Mayer's reagent was added.

(e) Test for fatty acids

To a small portion of PE, 4ml of chloroform was added; 5ml of alcoholic potassium hydroxide was added and refluxed for 30mins. The mixture was cooled, diluted with purified cold water and extracted with chloroform. The chloroform layer with non-Saponifiable oil and the aqueous layer with saponifiable lipids including fatty acids. This was acidified with dilute hydrochloric acids to pH 3-4, under these conditions, the fatty acids are released for their alkaline salts and are extracted by shaking the aqueous layer with ethyl ether repeatedly. The ether solutions were evaporated to dryness. Residue obtained was spotted on filter paper.

3.5.2 PRELIMINARY PHYTOCHEMICAL SCREENING OF EE

(a) Test for flavonoids glycoside

(i) Sodium hydroxide test.

About 0.2g of EE was dissolved in 2 ml of 10% sodium hydroxide and 4 drops of dilute hydrochloric acid was added. (Trease and Evans, 1996)

(ii) Shinoda test

About 0.2g of EE was dissolved in Ethanol, warmed and filtered, three pieces of magnesium chips were added followed by a few drops of concentrated HCl acid.

(b) Test for sterol and triterpenes

Liebermann – Burchard's Test

1ml of anhydrous acetic acid and 1ml of chloroform were combined together with the extract and cooled in ice, them 1 drop of concentrated sulphuric acid was added down the side of the test tubes.

(c) Test for alkaloidal salt.

A sample portion of EE was dissolved in 3ml of dilute hydrochloric acid and the solution divided into three test tubes and alkaloidal test carried out using the following reagents.

(i) Dragendroff's

(ii) Mayer's

(iii) Wagner's

(d) Test for saponins

Frothing test

About 0.2g of EE in water was shaken in a test tube for 2 mins and allowed to stand for 15mins (Sofowora, (1993); Silva *et al*, (1998)).

(e) Test for tannins

Ferric chloride test.

A small portion of EE was boiled with water, and filtered. Two drops of ferric chloride was added to the filtrate.

(f) Test for free carboxylic group.

(i) ketose test.

1g of resorcinol crystals was dissolved in aqueous solution of EE and shaken, 2ml of concentrated hydrochloric acid was added.

(ii) Pentose test

1g of phloroglucinol crystals was dissolved in aqueous solution of EE and shaken, 2ml of conc. HCl was added.

3.6 INVESTIGATION OF PETROLEUM ETHER EXTRACT (PE)

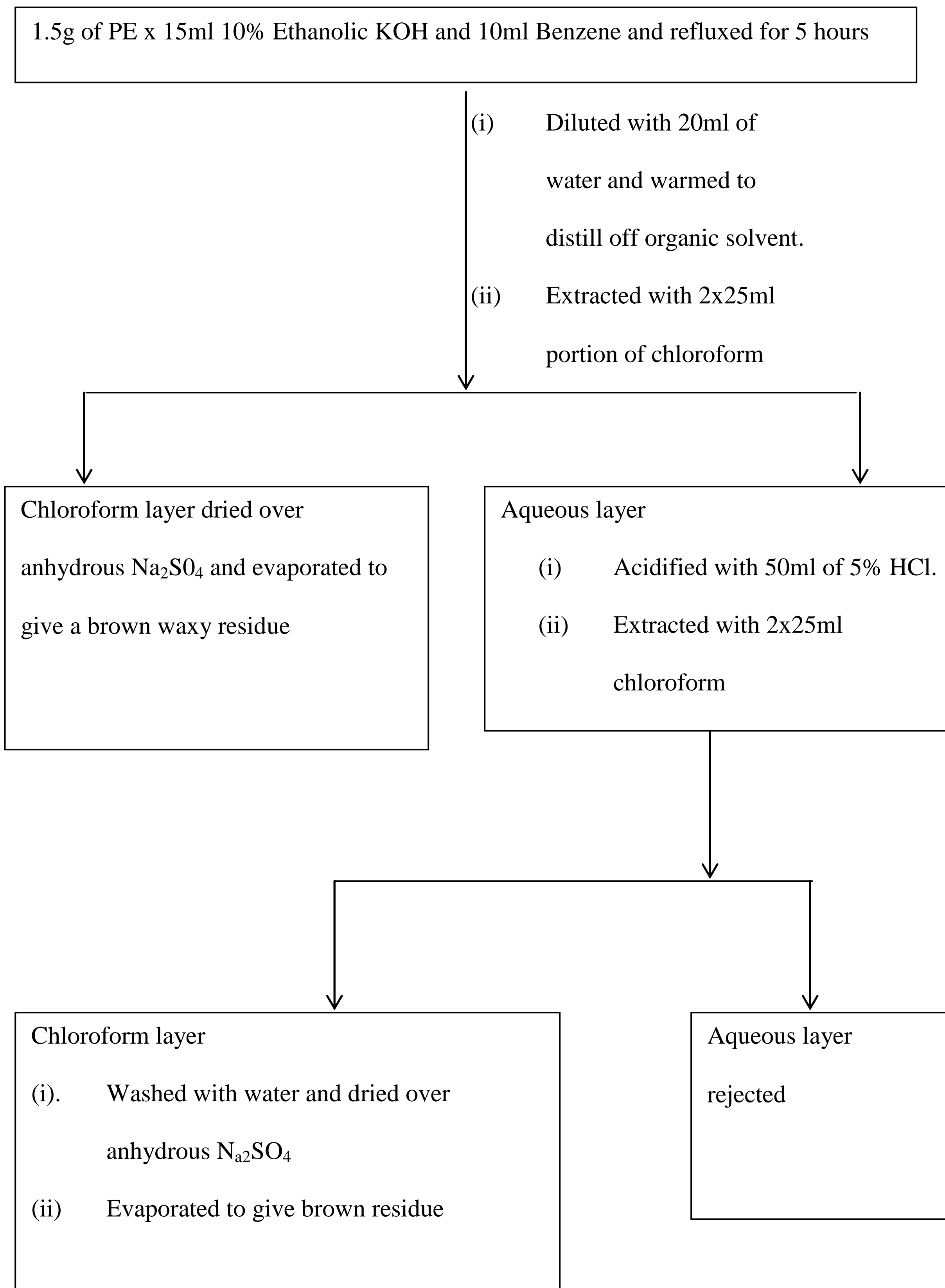
3.6.1 SAPONIFICATION OF PETROLEUM ETHER EXTRACT

petroleum ether extract (PE) was saponified as described by the method of Mahrain *et al*., (1980), where 1.5g of the extract was refluxed for 5 hours

with ethanolic potassium hydroxide (15ml) and benzene (10ml). The resultant mixture was diluted with water (20ml), and concentrated at reduced pressure to distill off the organic solvent present. This was extracted with 2x25ml portion of chloroform in 100ml separating funnel. The chloroform layer was collected, dried over anhydrous sodium sulphate and evaporated to give a light brown waxy material which was referred to as Unsaponifiable matter coded P1.

The aqueous layer which was referred to as saponifiable portion was acidified with 50ml of 5% dilute hydrochloric acid, until acidic to litmus, this was extracted with 2x25ml portion of chloroform. The chloroform layer was washed with water; dried over anhydrous sodium sulphate to give yellowish fraction which was evaporated at reduced pressure to give a brownish residue (21.1mg) with rancid odour. This was coded P2. Fig. 2

Fig 2: Saponification of Petroleum Ether Extract



3.6.2 THIN LAYER CHROMATOGRAPHY OF P1

A little quantity of unsaponifiable matter P1 was dissolved in few drops of chloroform, the resulting solution was spotted on a coated silica gel plates. The spot was developed using the solvent system. Hexane: chloroform (7:1). After the development of the plate with the above solvent system, the plate was allowed to dry, then visualized under:

(i). visible light

(ii) Ultraviolet light

(iii) Exposure to Iodine Vapour in a closed chamber

(iv) Spraying with freshly prepared sulphuric Acid. The R_f for the spot was recorded.

3.7 COLUMN CHROMATOGRAPHY

3.7.1 SAMPLE PREPARATION

The unsapofiable matter was applied over a well – packed silica gel column. After introduction of sample into the column, it was eluted gradually with n-hexane, then the polarity was gradually increased using chloroform as indicated in Table 3.1

Eluents were collected in 10ml aliquots, and the progress of the separation was monitored by thin layer chromatography, using the solvent system Hexane; chloroform (3: 1), similar fraction were pooled.

Table 3.1: column chromatography of Unsaponifiable matter (P1)

Eluent	Eluting Solvent	Fraction	Developing Solvent	Visualisation		N0 of Spots	Remarks
				Daylight	Iodine Vapour		
1	100% Hex	1-4	Hex : $CHCl_3$ (3 : 1)	-		-	
2	100% Hex	5-8	(3 : 1)		Pink	1	Minor
3	100% Hex	9-11	(3 : 1)				
4	100% Hex	12-14	(3 : 1)				
5	100% Hex	15-17	(3 : 1)				
6	100% Hex	18-20	(3 : 1)				
7	Hex: $CHCl_3$ 9 : 1	21-23	(3 : 1)				
8	Hex: $CHCl_3$ 9 : 1	24-26	(3 : 1)				
9	Hex: $CHCl_3$ 9 : 1	27-29	(3 : 1)				
10	Hex: $CHCl_3$ 9 : 1	30-33	(3 : 1)				
11	Hex: $CHCl_3$ 9 : 1	34-36	(3 : 1)				
12	Hex: $CHCl_3$ 9 : 1	37-39	(3 : 1)				
13	Hex: $CHCl_3$ 9 : 1	40-44	(3 : 1)		Blue-black	1	Minor

14	8:2	45-49	(3 : 1)			-	
15	8:2	50-56	(3 : 1)	Greenish	Yellowish	2	
16	8:2	57-62	(3 : 1)	Greenish	Yellowish	2	
17	8:2	63-62	(3 : 1)	Greenish	Yellowish	3	
18	8:2	68-73	(3 : 1)	Greenish	Yellowish	-	
19	8:2	74-82	(3 : 1)	Greenish	Yellowish	2	
20	8:2	83-106	(3 : 1)	Greenish	Yellowish	2	
21	8:2	107-118	(3 : 1)	Purple	Pink	1	Major
22	8:2	119-121	(3 : 1)	Purple	Pink	1	Minor
23	8:2	122-127	(3 : 1)	Violet	Pink	2	
24	8:2	128-139	(3 : 1)			2	
25	8:2	149-170	(3 : 1)			2	

Fractions (107-118) gave a single major spot on TLC using 100% chloroform and Hexane chloroform (3:1) as solvent system, and with R_f value as 0.56. The chromatograms were visualized using (i) UV light (ii) Iodine vapour (iii) sulphuric acid reagent. These fractions were coded F1, on evaporation a white amorphous solid was found to be soluble in chloroform, Insoluble in methanol, acetone and benzene F1 was subjected to Proton Magnetic Resonance analysis.

CHAPTER FOUR

4.0 PHARMACOLOGICAL SCREENING

4.1 RATIONAL FOR THE PROJECT

The aim of this work is to assay pharmacologically the effect of the ethanolic extract of the leaf of *Stereospermum kunthianum* on isolated rabbit jejunum in order to ascertain the purported uses of the plant in relieving gastro intestinal disturbance (Dalziel, 1955). Which include diarrheoa.

4.2 MATERIALS

4.2.1 TEST ANIMALS

New Zealand rabbit weighing 1.5kg and Swiss albino mice 20.0 $\pm$ 0.5g maintained in the animal house of Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria were used for the experiments. The animals were fed with standard laboratory feeds and water *ad libitum*.

4.2.2 APPARATUS

(i) Recording microdynanometer and Transducer model No. 7050, (UGO Basile Italy).

(ii) Thermostatically controlled tissue bath (25ml)

(iii) Dissecting kit

4.2.3 STANDARD DRUGS AND SAMPLE USED

The standard drugs and crude n- butanol extract were prepared by directly weighing and dissolving the known weight of the drug/extract in the required volume of de-ionised water. Stock concentration prepared were as follow.

i. Acetylcholine 10μg/ml

ii. N-butanol extract

iii. Physiological solution

iv. Tyrode solution

v. Castor oil

vi. Loperamide

4.3 EXPERIMENTAL PROCEDURES

4.3.1 TOXICITY STUDY

The method of Lorke (1983) was adopted, n-butanol fraction was used . The study was divided into two phases. A total of fifteen mice were used, in phase one, mice were divided into three groups of three mice each with geometrical doses of 10mg/Kg, 100mg/Kg and 1000mg/Kg of n-butanol fraction administered intraperitoneally, the last group received normal saline as a control. No death was recorded after 24 hours. In phase two, 1600mg/Kg, 2900mg/Kg and 5000mg/Kg were administered. The median lethal dose (LD_{50}) was calculated as the geometric mean of the lowest lethal dose and the highest non lethal dose of which there was 1/1 and 0/1 survival.

4.3.2 EFFECT ON ISOLATED RABBIT JEJUNUM

The rabbit was sacrificed by a blow on the head, dislocating the neck, exsanguinations. Segments of the jejunum, about 3cm long, were removed and disserted free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode's solution of the following composition in millimoles (mM): NaCl, 136.8; KCl, 2.7; $CaCl_2$, 1.3; $NaHCO_3$, 12.0; $MgCl_2$, 0.5; $NaPO_4$, 0.14; glucose, 5.5. The tissue was mounted in a 25ml organ bath containing Tyrode's solution maintained at $35 \pm 1.0\ ^0C$ and aerated with air. A lot of 0.5g was applied. A one hour equilibrium period was allowed

during which the physiological solution was changed in every 15mins. At the end of the equilibrium period, the effect of acetylcholine (2.0×10^{-8}mg/ml – 3.2×10^{-7}mg/ml) an extract of *Stereospermum kunthianum* were investigated non-cumulatively. The contact time for each concentration was one minute, which was followed by washing three times. The tissue was allowed a period of 15mins before the next addition. Responses were recorded isometrically using Ugo Basile recorder 7050 (Amos *et al*., 1998; Agunu *et al*., 2005 and Ahmadu *et al*., 2007).

4.3.3 EFFECT ON CASTOR OIL-INDUCED DIARRHEA IN MICE

The mice were fasted for 12 hours prior to the commencement of the experiment and were randomly divided into 5 groups of five mice each. Mice in the first group received 10ml/Kg (ip) normal saline, the second, third and fourth groups received 2000mg/Kg, 1000mg/Kg and 500mg/Kg of n-butanol extract of *Stereospermum kunthianum* (ip), while the fifth group received Loperamide 5mg/Kg (ip). After 30mins of administration of extract, Castor Oil 0.2ml/mouse was administered intragastrically. The animals were placed on individual cages over clean filter paper. Three hours after the administration of oil, the cages were inspected for the presence of characteristic diarrhea droppings. Their absence was recorded as the

protection from diarrheoa, and the percentage protection calculated (Diurno *et al* , 1996; Akah and Offiah, 1996).

4.3.4 STATISTICAL ANALYSIS

The result on Castor Oil-induced diarrhea were analyzed using the Chi – Square Test and were regarded as significant when $P<0.05$.

CHAPTER FIVE

5.0 RESULTS

5.1 PERCENTAGE YIELD OF EXTRACTS

Percentage yield of petroleum –ether extract = 4.03%

Percentage yield of ethanolic extract = 6.42%

5.2 PRELIMINARY PHYTOCHEMICAL SCREENING OF THE LEAF EXTRACT OF *STEREOSPERMUM KUNTHIANUM*

These are shown on table 5.1 and 5.2. Fatty acids, sterols, triterpenes and coumarins were found to be present. Saponins and tannins were only present in the ethanolic extract, while Flavonoids and alkaloids were found to be absent.

Table 5.1: Results of preliminary phytochemical screening of petroleum ether extract of the leaves of *Stereospermum kunthian*

	Compound/group	Test	Observation	Inference
1	Sterols/triterpenes	Licbermann-burchard test	Greenish coloration	+
2	Flavone aglycone	Shinoda test	No red or orange coloration observed	**-**
3	Coumarins	Aq.extract + NH_3 and observed under U.V.light	Intense fluorescence observed	+
4	Alkaloid	Dragendorff's reagent	No precipitation	**-**
	Alkaloid	Mayer's reagent	No precipitation	**-**
	Alkaloid	Wagner reagent	No precipitation	**-**
5	Higher fatty acids	Alkaline aq. Solution of extract, extracted with ether and acidified with conc. HCl pH3-4	Opalescence of acidic aq. Layer	+

Key: - + = **Present**

- = **Absent**

Table 5.2: Results of preliminary phytochemical screening of ethanolic extract of the leaves of *Stereospermum kunthianum*

	Compound/group	Test	Observation	Inference
1	Sterols/triterpenes	Licbermann-bur chard test	Violet-brown coloration	+
2	Saponins	Frothing test	Foam formation	+
3	Taninins	Ferric chloride test	Green-black coloration	+
4	Coumarins	Aq.extract +NH_3 and observed under uv light	Intense fluorescence observed	+
5	Flavone glycone	Shinoda test	No red or orange coloration	-
6	Alkaloid	Dragendorff's reagent Mayer's reagent Wagner's reagent	No precipitate No precipitate No precipitate	- - -
7	Free carboxylic group	selivanoff's reaction	colourles gas evolved	+

Key: - + = **Present**
- = **Absent**

5.3 THIN LAYER CHROMATOGRAPHY OF PETROLEUM ETHER EXTRACT (PE)

5.4 THIN LAYER CHROMATOGRAPHY OF UNSAPONIFIABLE MATTER (P1)

Solvent System: Hexane: Chloroform (7:1)

Visualization: Sulphuric acid sprayed and heated at 100 for 5 mins. Two spots were observed and the color of the spots observed to be pink with R_f values 0.82 and 0.77 respectively.

5.5 COLUMN CHROMATOGRAPHY OF P1

Elution of P1 in Hexane: Chloroform (8:1) gave fractions (107 - 118) with a single major spot after the TLC using Hexane: Chloroform (3:1). F1 was obtained which was soluble in Chloroform but insoluble in Methanol, acetone and benzene.

5.6 SPECTROSCOPIC ANALYSIS OF F1 ISOLATED FROM PETROLEUM ETHER EXTRACT

5.6.1 PROTON NUCLEAR MAGNETIC RESONANCE (^{1}HNMR) SPECTRA

The white amorphous solid substance F1 was subjected to Proton nuclear magnetic resonance and the spectrum was shown in fig. 5.1.

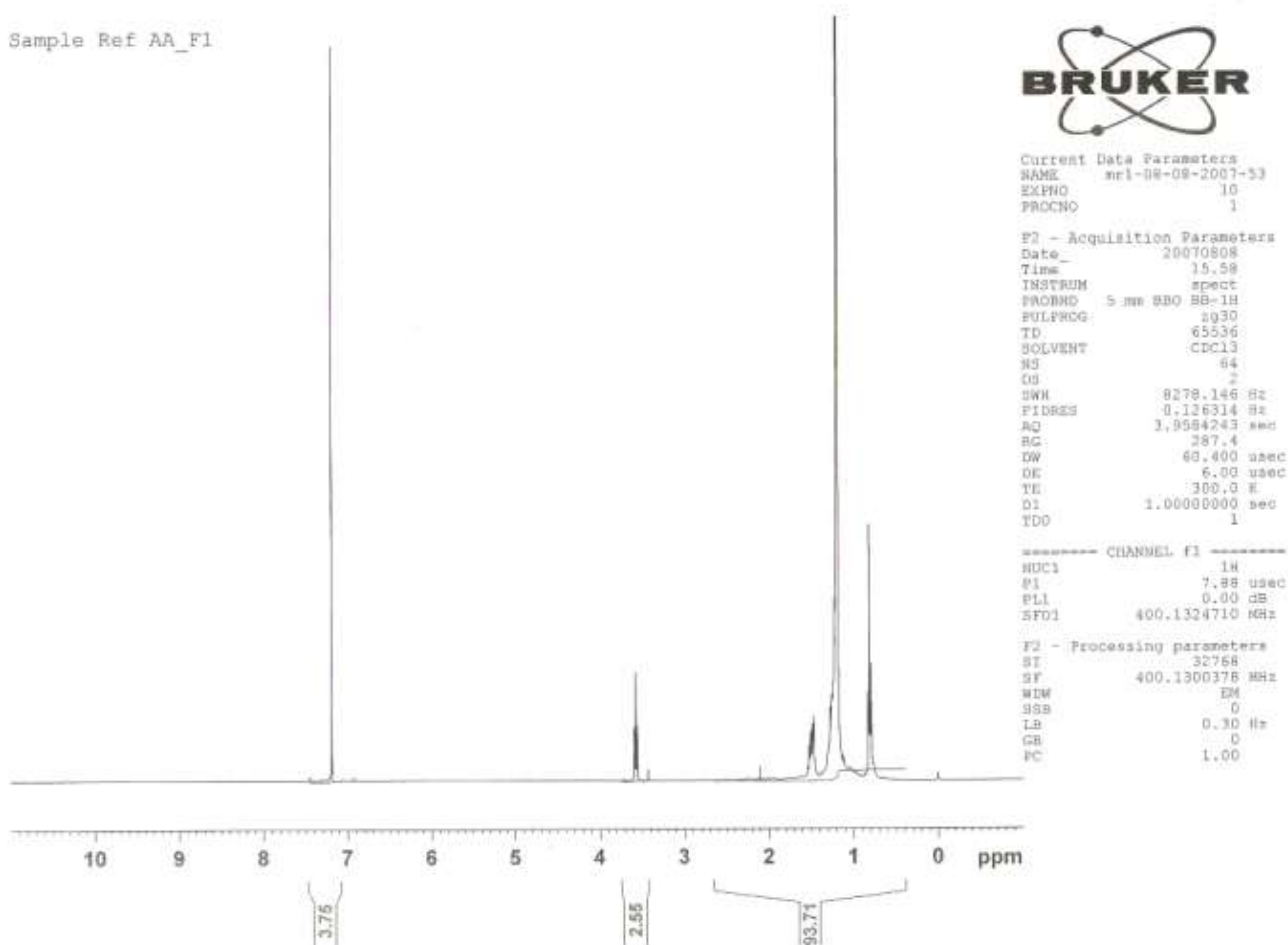


Fig 5.1: ^{1}HNMR of Sample F1

The result of Computer interpretation of F1 spectra revealed that F1 was a compound of sterol with the IUPAC name as Methyl – 1 – undecyl - 2,3,3a,5a,6,7,8,9,9a,10,11,11a – dodecahydro – 1H – cyclopenta[a] phenantherene – 4 – carboxy. The computer interpretation of ^{1}HNMR of F1 is shown in fig 5.2

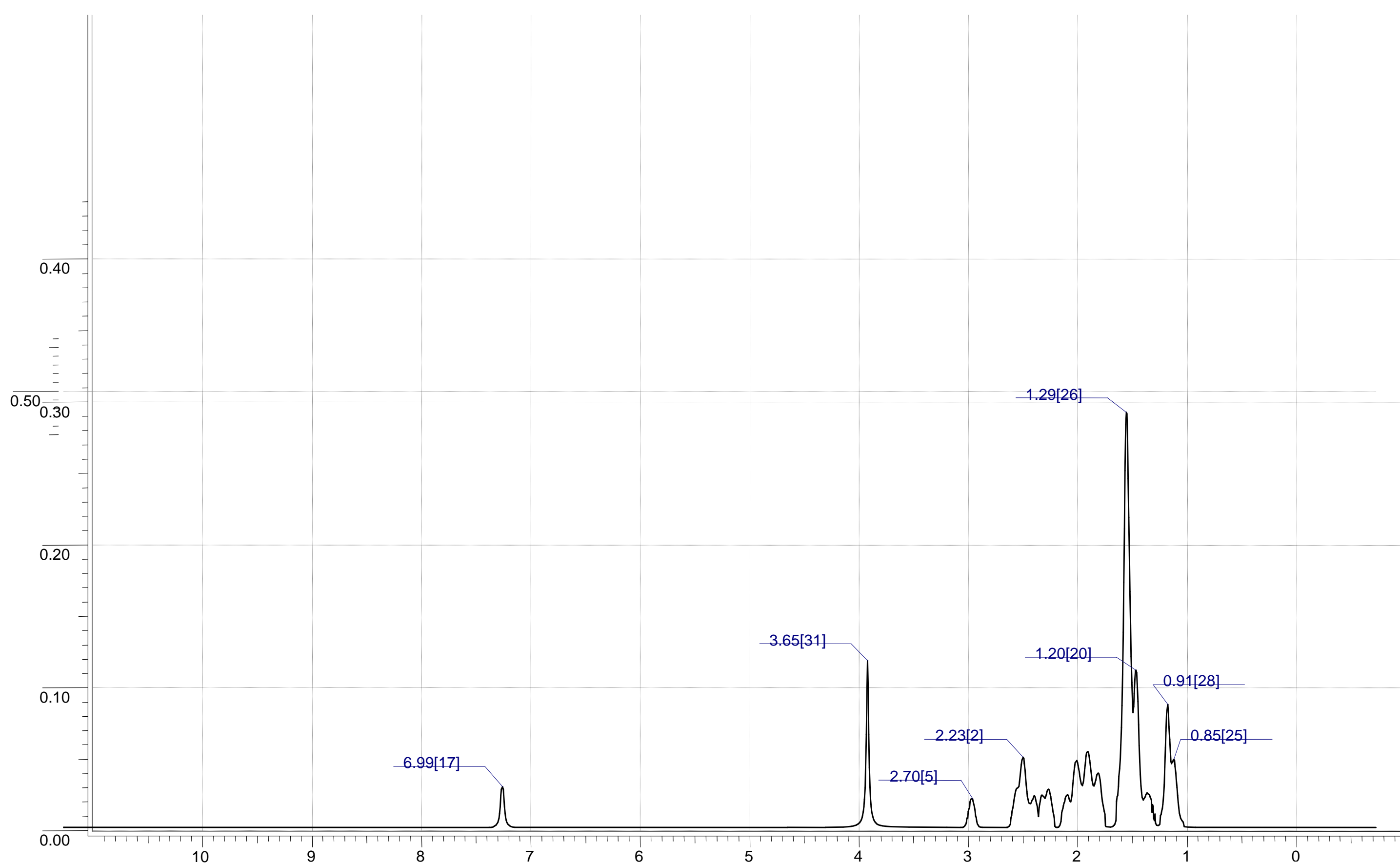


Fig 5.2: Computer Interpretation of ^{1}HNMR of F1 Spectrum

Table 5.3: Showing Chemical Shifts (ppm) from Bruker and Computer Soft Ware

H- proton	δ_ value(Bruker)	δ_ value(computer)
1	0.80	0.90
2	1.26	1.29
3	1.52	1.20
4	2.15	2.23
5	3.45	2.70
6	3.65	3.65

The probable chemical structure of the compound is shown in fig 5.3

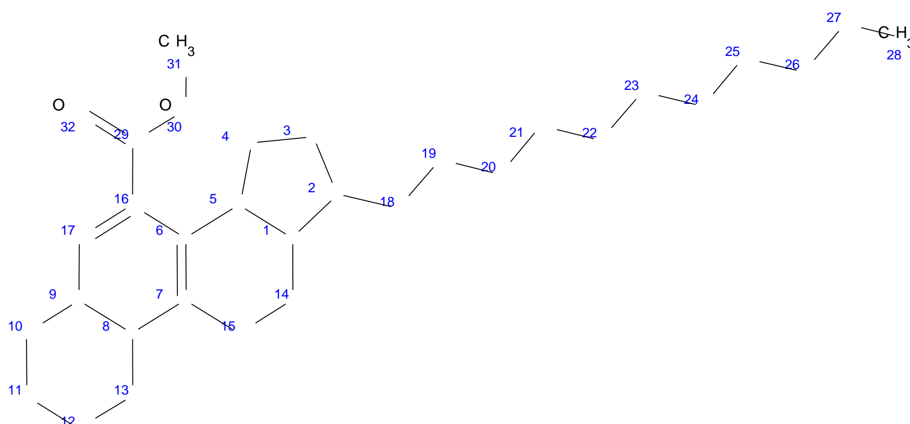


Fig 5.3: Chemical Structure of Steroid F1

The IUPAC name of the structure is Methyl – 1 – undecyl - 2,3,3a,5a,6,7,8,9,9a,10,11,11a – dodecahydro – 1H – cyclopenta[a] phenantherene – 4 – carboxy and the molecula formular is $C_{30}H_{48}O_2$

5.7 RESULTS OF THE PHARMACOLOGICAL STUDIES

5.7.1 ACUTE TOXICITY STUDIES

The acute toxicity studies for n-butanol extract of *Stereopermum kunthianum* in mice (ip) was found to be 3,807.9mg/kg

5.7.2 EFFECTS OF EXTRACT ON ISOLATED RABBIT JEJUNUM

The effects of n-butanol extract of *Stereospermum kunthianum* (1mg/ml – 100mg/ml) on the rabbit jejunum were dose – dependent (fig.5.4).

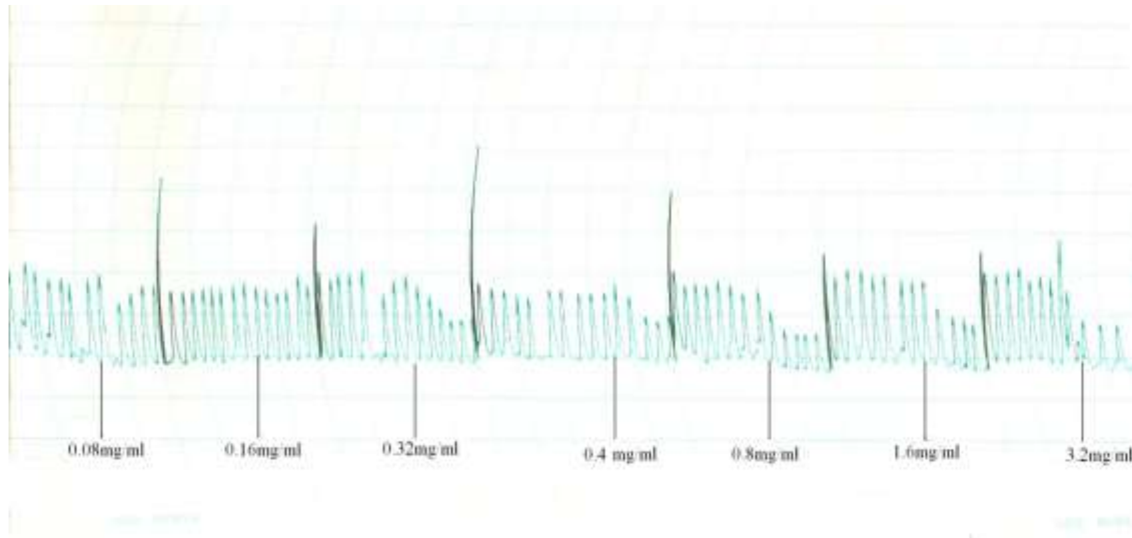


Figure 5.4: Effect of the N-butanol extract of Stereospermum *kumthianum* on isolated rabbit jejunum

5.7.3 EFFECTS OF EXTRACT ON CASTOR OIL-INDUCED DIARRHEOA

The n-butanol extract of *Streospermum kunthianum* inhibited Castor oil – induced diarrhea in mice (Table 5.4)

Table 5. 4 : The effect of n-butanol extract of the leaf of *Stereospermum kunthianum* on Castor Oil-induced diarrheoa in mice.

Treatment n=5	Dose (*i.p*)	No. of mice with diarrhea	Protection %
Castor Oil	0.2ml/mouse	5/5	0
Stereospermum	500mg/Kg	2/5	60
kunthianum	1000mg/Kg	2/5	60
	2000mg/Kg	5/5	0
Loperamide	5mg/Kg	1/5	80

The n-butanol extract of *Stereospermum kunthianum* (500mg/kg and 1000mg/kg) and Lopermide gave significant protection ($P < 0.05$) on mice against Castor oil – induced diarrheoa when compared with the control. The result showed that at high dose a significant protection was observed.

CHAPTER SIX

6.0 DISCUSSION AND CONCLUSION

6.1 PHYTOCHEMICAL SCREENING

6.1.1 PETROLEUM ETHER EXTRACT

The petroleum ether extract was found to contain steroids and triterpenes as inferred from the positive Liebermann-Burchard reaction and fatty acids and Coumarins inferred from the action of alkaline solution of the extract acidified with Conc. HCl and aqueous extract in ammonia respectively. Flavones and Alkaloids were absent.

The petroleum ether extract yield 4.03% of non- polar constituents which shows that only a little constituents can be extracted from the extract,this is due to the penetration of the cell by the solvent.The ethanolic extract yield 6.42% which was higher than that of the petroleum ether extract,the amount of each extract obtained was largely depended on the amount of material used and the method of extactionused.

6.1.2 ETHANOLIC EXTRACT

The preliminary phytochemical screening of the ethanolic extract of the leaves of *Stereospermum kunthianum* has revealed the presence of steroids

and/or triterpenes, saponins, tannins, coumarins and free carboxylic group, also flavones and alkaloid were absent.

6.2. SAMPLE P1

Sample P1 was obtained from saponification of petroleum ether extract. The sample was coded P1 from the unsaponifiable portion.

Sample P1 was subjected to column chromatography in which a sample F1 a white amorphous solid substance was obtained and subjected to proton nuclear magnetic resonance. (^{1}HNMR) fig 5.1. The chemical shifts obtained from the spectrum of F1 was introduced to "ACD/ NMR Assistant", a computer software that interpret a spectra and produces a possible molecular structure of a compound.

The compound interpreted was found to be methyl-1- undecyl-2, 3,3a,5a,6,7,8,9,9a,10,11,11a- dodecahydro- 1H-cyclopenta [a] phenanthrene-4- carboxylate. The structure is as shown in fig 5.3 … The molecular weight of the compound was found to be 440.70. This compound was earlier isolated from *Castanapsis lamontii* (Wari-Haan *et al.,* 1976). The following data were obtained from the computer software. The mp 187-189 while the polarizability α = +93.0. Since hydrogen is present in the vast majority of

compounds, often at strategic position ^{1}HNMR spectroscopy will often yield information about the structure of molecules (Robin *et al.,* 1978)

From the phytochemical screening it can be concluded that the leaves extract of *Stereospermum kunthainum* contain steroid, tritenpenoids, saponins, tannins, coumarins and free carboxylic group, which on separation by column chromatograph it was found that steroid was isolated using spectrum obtained, a computer interpretation revealed that the steroid was methyl-1-undecyl-2,3,3a,5a,6,7,8,9,9a,11,11a- dodecahydro-1H- cyclopenta [a] phenanthrene-4- carboxylate. This work happened to be the first study in which sterol was isolated from the plant.

6.3 PHARMACOLOGICAL SCREENING

The LD_{50} value of the n-butanol extract of the plant *Stereospermum kunthianum* was found to be 3,807.9mg/kg (ip) mice. Castor oil is made up of 90% ricinoleate (Mekeon *et al.,* 1999) which when metabolised is responsible for the observed effect of the oil. The active metabolites recinoleic acid is responsible for its diarrheoa inducing properties, which diminishes Na^+ and Cl^- permeability in the intestine (Gaginella and Phillips, 1975); it is also associated with endogenous stimulation of prostaglandins release (Zavala *et al.,* 1998). Earlier studies showed that anti-dysenteric and

anti-diarrheoal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, steroids and or terperoids and reducing sugars (Anonymous, 1962; Galvez *et al.,* 1991, 1993; Longanga *et al.,* 2000). The antidiarrheoal activity of this extract may be due to the presence of sterol and/or triterpenes. These compounds can present anti-diarrhoeal effect since they can precipitate the proteins of enterocytes, reducing the peristaltic movement and intestinal secretions (Gabriel *et al.,* 1999).

Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any effect on colonic motility (Theoderan *et al.,* 1991). It reduces the daily fecal volume, decreases intestinal fluid and electrolyte loss. Loperamide produces a rapid and sustained inhibition of the peristaltic reflux through depression of longitudinal and circular muscle activity, presumably through an effect on intestinal opiate receptors. Loperamide is effective against a wide range of secretory stimuli and can be utilized in the control and symptomatic relieve of acute diarrhoea that is not secondary to bacterial infection (Charles *et al.,* 1997).

In conclusion the plant extract exhibited anti-diarrhoeal activity. The effect was comparable to loperamide which is presently one of the most widely

used anti-diarrhoeal drug and it elicited its activity by antagonising diarrhoea induced by castor oil (Niemegeers *et al.,* 1974) and prostaglandins (Karim and Adaikum, 1997), its therapeutic effect could also be due to its antimotility and its anti-secretory properties (Couper, 1987). The extract similarly inhibited spontaneous agonist induced contractions of rabbit jejunum. The effect may also contribute to the observed anti-diarrhoeal activity. Steroids have been known to make the intestinal mucosa more resistant and reduce secretion, therefore, inhibit diarrhoea induce by castor oil (Tripathi, 1994). The presence of steroids in the extract could be responsible for the anti-diarrhoeal activity. The results of this investigation suggest that the leaves extract of *Stereospermum kunthianum* possess anti-diarrhoeal activity and justify the ethno-medicinal use of the plant and the LD_{50} further validate its safety.

REFERENCE

Abdullahi, I.A; Azbo, M.O; Amos, S; Gamaliel, K.S; and Wambebe, C. (2006). Anti-diarrheal activity of aqueous extract of *Terminalia avicinnoides* root. Phytother, Res. 51: 431-434

Agunu, A; Sadiq, Y; Gabriel, OA; Zezi, AU and Abdurahman, EM (2005). Evaluation of five Nigerian Medicinal Plants used in treatment of diarrhea in Nigeria. *J. Ethnopharmacol.* 61 : 209 – 213.

Ahmadu, A. A; Zezi, A.K; and Yaro A. H. (2007). Anti-diarrheal activity of the leaf extracts of *Daniellia Oliveri*_ Hutch and Dalz (Fabaceae) and *Ficus sycomorus* Mio (moraceae). *Afr. J. Trad. CAM* 4(4) : 524 – 528.

Akah, P. A; Offiah, V. N., (1996). Gastrointestinal effects of *Allamanda cathartica* leaf extracts *International Journal of harmacognosy*, 30 : 213 – 217.

Ammmun, H, P, and Muller, A.B. (1985): in Text book of Pharmacognosy, 13th ed edited by Evans, W.C. (1988). A Balliere Tindall publication. pp 42.

Amos, S; Okwusaba, F. K; Gamaniel, K; Akah, P. A. and Wambebe, C. (1998). Inhibitory effects of Gastrointestinal and extracts of *Parefta crassipes* leaves on Gastrointestinal and uterine smooth muscles

preparations isolated from Rabbit, Guinea Pigs and Rats. *J. Ethnopharmacol*, 61: 209 – 213.

Anonymous (1962. The wealth of India (Raw material). CSIR, New Delhi: vol 6: 280-281

Audu, R., Umilag, S.A; Renner, J. K; Awodiji (2000). Diarrhea Management. *J. Nigeria Infection Control Association*. 3 : 15

Basu N; and Rastogi, R.P. (1967) Triterpenoid saponins and sapogenins. phytochemistry vol 6: pp 1249-1270

Couper.I.M. (1987). Opiod action on the intestine, the importance of the intestinal mucosa. Life Sci. 41: 917 - 925.

Charles R. Craig and Robert E. Stitzel., (1997). *Modern Pharmacology with Clinical Applications* 5th Edition, Little Brown and Company – Boston: 504 – 506.

Dalziel, J.M. (1955), The useful plants of west Tropical Africa; An appendix to the flora of west Tropical Africa. Watmongh's Limited. Bradford. Pp 442 - 446

Dence, J. (1980) Stereoids and *peptides:* A Wiley- Inter science publication , New York pp 99-101

Diurno, M. U., Izzo, A. A; Mazzoni, B; Bologgnese, A., Capasso, F; (1996). Anti- diarrheal activity of New thiazolidinones related to Loperamide. *Journal of Pharmacy and Pharmacology* 45; 1054 – 1059.

Farnsworth, N.R. (1966) Biological and Phytochemical Screening of Plant, Journ. of Pharm. Sci. 55. Pp 225-276

Farnsworth, N.R. and Morriz; R.W. (1975) Higher Plants The Yiants of drug development, Am. J. Pharm 148, 46-52

Finar, I.L., (2003): Organic Chemistry of Natural Products. 5th edition. ELBS with Longman. Pp 390 - 435

Gabriel, S.E., Davenport, S.E., Steagale, R.J., Vimal., V., Calson, T., Rozham, T.(1999) novel plant derived inhibitor of CAMP mediated fliud and electrolyte Secretion. American journal of physiology, 39: G58-G68.

Galvaz, J. Zarzuelo, A. Crespo, M.E., Jimenez, J. (1993). Antidirrhoeic activity of *Euphobiahirta* extract and isolatibu of an active flavonoids constituents. Planta medica, 59: 333-336.

Galvaz, J. Zarzuelo, A., Crespo, M.E., (1991) Antidiarrhoeic activity of *Sclerocarya birrea* bark extract and extract and its tannins constituent in rats. Phytother Res; 5: 276-278

Galvez, J. Zarzuelo A. Crespo, M. E; Lorente, M.D; Ocets, M. A.; Jiménez J; (1993). Anti-diarrheal activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. *Planta Medica,* 59 :333 – 336.

Hansel, J.R. (1972): Medicinal plants and Empirical Drug Research in plants in the Development of modern medicines. Edited by Swain, T. pp. 86-87

Heinrich, M; Heneka, B; Ankli, A; Rimple, H; Sticher, O; Kostiza, T. (2005): Spasmolytic and antidiarrheal properties of the Yucatee Mayan medicinal plant *Casimora tetrameria* J. Pharm Pharmacol. 57(9): 1081 - 1085

Huxley A: (1984) "Green inheritance", The World Wild Life Fund (WWF) Book of Plants. London,

Keay, R.W.J. (1989), Trees of Nigeria: Clarendo Press. Oxford. Pp432 - 435

Longanga, Otshudi A., vercruysse, A., Forriers, A. (2000). Contribution to the ethnobotamical, phytochemical and pharmacological studies of traditional used medicinal plants in the treatment of dysentery and diarrhoea in Lomera area, Democratic Republic of Congo (DRC). J. Ethnopharmacol. 71 (3): 411-423

Lorke, D. (1983). A new approach to acute toxicity testing. Arch Toxicol. 54 : 275 – 287

Mahrain. GH; El-Hosary, G. A; and Mansour, W.M. (1980): Phytochemical Study of *Nyphae lotus linn* growing in Egypt. Journ. of African med. Plants vol 3,pp. 88-92

Mekeon, T. A; Lin, J. J; Stafford, A.E; (1999). Biosynthesis of ricinoleate in Castor Oil. *Adv.Exp.Med. Biol*. 46437 – 46447

Olaniyi, A.A; Ayim, J.S.K; ogundaini-A.O and Olugbade, I.A. (1993) chemistry of Natural products in Text book of Essential inorganic and organic pharmaceutical chemistry._A Shaneson C-I. Publication Ibadan pp. 555-583

Ole Hamman, (1988) The Joint IUCN-WWF Plants Conservation Programme and its Interest in Medicinal Plants, Ed. By O.Akerde, V. Heywood and Synge. Cambridge University. Press. Pg13-22

Onegi; B; Kraft, C; Kohler, I; Feid, M; Jenethsiems, K; Beyer, G; Melzig, M.F; Bienzle U; Eich, E. (2002): Antiplanmodial activity of Naphthoquinones and one anthraquinone from *Stereospermum kunthianum* phytochemistry: 60 (1) 39-44

Plowman, T. (1984). The Origin, Evolution and diffusion of coca, *Erythroxylum* specie in south and central America in Pre- Columbian Plant Migration ed. by D. Stone. pg 125-165

Robinson, T. (1972): Saponifiable Lipids. in the organic constituents of Higher plants, pp 77-79.

Silva, G.L Chai, H; Farns worth, N.R. and Gupta, M.P. (1998). In Natural Products Isolation. edited by Richard Carnell-A Humana Press publication New Jersey pp. 349,-356-359

Sofowora, A; (1982). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Limited Ibadan.Pp.34-42

Stothers, J. B. (1972) ^{13}C-NMR spectroscopy structural and Stereo chemic Application An Academic Press publication New York pp 439- 441; 445-449

Trease, K; and Evans, W.C. (1996): Pharmacognosy Text Book, Fourteent edition, Harcourt Brace and company, Asia, PTE Limited, India pp 504-507

Tripathi K. D. (1994). *Essentials of Medical Pharmacology*. Jay Pee Brothers Medicals Publishers (P), New Delhi P. 775

Watt, J.M. and Brayer – Bradwijk, M.L.(1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa. An E&S Livingstone publication, London.pp.899 – 990

WHO, (2000): *Global Water supply and Sanitation Assessment.* World Health Organisation Geneva. Pg 1342-1346

WHO,(1985). *The Management of Diarrhea and the use of Oral Dehydration Therapy*:A joint WHO/UNICEF Statement second edition Geneva

William, A.R.Thompson, M.D. (1978), Healing plants Modern Herbal. McGraw-Hill Co-publication, United Kingdom

Zavala, M. A; Perez, C., Vargal, R; Perez, R. M. (1998). Anti-diarrheal activity of Waltheria Americana, Commelins , Cuelestris and alternanthra repens; *J. Ethnopharmacol*. 61 : 41 – 47

APPENDIX 1

A paper published from the research work in Biological and Environmental Sciences Journal for the Tropics: Volume 4 Number 2 December, 2007

ANTI–DIARRHEAL ACTIVITY OF THE LEAF EXTRACT OF *STEREOPERMUM KUNTHIANUM* (BIGNONIACEAE)

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Abstract

Preliminary phytochemical screening of the leaves extract of *Sterospermum kunthianum* revealed the presence of sterols/triterpenes, saponins, tannins, coumarins, and free carboxylic group. The leaves of the plant *Stereospermum kunthianum* (Bignoniaceae) used in diarrhea treatment in Hanwa Village, Sabon gari, Kaduna State, Nigeria were investigated. The study was carried out on perfused isolated rabbit jejunum and castor oil-induced diarrhea in mice. The ethanolic extract (0.8 – 3.2mg/ml) causes a dose – dependent relaxation of isolated rabbit jejunum. The acute toxicity test for the extract in mice established intraperitoneal LD_{50} of 3807.9mg/kg. In castor oil-induced diarrhea, 60% protection was observed at doses of 500mg/kg and 1000mg/kg respectively. The anti-diarrheal activity was comparable to Loperamide 5mg/kg. The result revealed that the extract have pharmacological activity against diarrhea.

Key words: *Stereospermum kunthianum*, anti – diarrheal activity, castor oil, tissue relaxation.

INTRODUCTION

Diarrhea is the frequent passage of watery unformed stools. Its causes are many and include irritable bowel syndrome, infectious disorder, thyrotocosis, malabsorption or maldigestion, and laxative abuse. Medications used to treat other disorders also may induce diarrhea. For example. Xanthenes e.g. theophlline preparations cause diarrhea secondary to alteration of mucosal cyclic adenosine monophasphate (cAMP). Antihypertensive drugs, such as reserpine and guanethidine, may induce diarrhea by changing gut neuronal input and reducing noradrenergic – mediated relaxation (Craig etal, 1997).

The WHO estimation revealed that diarrhea causes 4 – 5 million deaths annually throughout the world. 80% of these deaths are reported in developing countries including Nigeria. In Nigeria, diarrheal infection remains the number one killer disease among children under 5 years, while 7 – 12 month – old babies remains the most susceptible (Audu et al, 2002). In addition, reported cases of diarrhea in many areas, including Kaduna state, still account for more than 30% of admissions to children wards (WHO, 1985). Despite the effective and simple cheap treatment of oral dehydration therapy, majority of the local populace still rely on herbs to treat diarrhea (Ahmadu, 2007). The use of herbal drugs in the treatment of diarrhea is a common practice in many developing countries.

In Hausa ethnomedicine of Northern Nigeria, some medicinal plants are used frequently for treating diarrhea infections and these include; *Stereospermum kunthianum*. The plant has been used traditionally in Northern Nigeria as a remedy for diarrhea, dysentery, venereal diseases and as a cure for gonorrhea. (Hutchinson and Dalziel, 1963). It is also a

remedy for cough (Brandwijk, 1962). *Stereospermum kunthianum* is used in the treatment of ulcer, leprosy, skin eruptions and pneumonia. The roots and the leaves are used for respiratory ailments and gastritis (Dalziel, 1963). As part of our efforts to screen some ethno medicinal plants of Northern Nigeria for anti – diarrheal activity, leaves of *Stereospermum kunthianum* was investigated.

PLANT MATERIALS

Collection and Drying of Plant Materials

Whole fresh plant material bearing fruits and leaves, growing wild was collected at Hanwa village, Sabon-Gari Local Government Area of Kaduna state, Nigeria in the month of December, 2005. The plant was identified by the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria with a specimen Voucher number 1381. The leaves were air – dried for 21 days before powdering.

Extraction of Plant Material

The powdered leaf (165g) of the plant was continuously defatted with Petroleum ether (60 – 80ºC) using Soxhlet extractor until the draining solvent was clear. Solvent used was recovered at reduced pressure to afford a yellowish – green waxy material (6.64g) that was thereafter referred to as the petroleum ether extract coded PE. The marc after defatting was air – dried at room temperature and then extracted continuously with ethanol using Soxhlet extractor. The Solvent was evaporated at reduce pressure to afford ethanolic extract (10.60g) that was coded EE. This extract was evaluated for its anti – diarrheal properties.

Phytochemical analysis

The preliminary phytochemical screening of the ethanolic extract was carried out using the standard procedure (UNIDO, 1970 and Sofowora, 1982).

Animals

New Zealand rabbit weighing 1.5kg and Swiss albino mice 20.0 + 0.5g maintained in the animal house of Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria were used for the experiments. The animals were fed with standard laboratory feeds and water *ad libitum*.

This research was carried out in Ahmadu Bello University, Zaria, Nigeria according to the rules governing the use of laboratory animals as acceptable internationally.

Drugs

Acetylcholine (Sigma chemical, USA), Castor Oil (Bell Sons & Co., England) and Loperamide (Janssen, Germany).

EXPERIMENTAL PROCEDURE

Toxicity Study

The method of Lorke (1983) was adopted. The study was divided into two phases. A total of fifteen mice were used, in phase one, mice were divided into three groups of three mice each with geometrical doses of 10mg/Kg, 100mg/Kg and 1000mg/Kg administered intraperitoneally, the last group received normal saline as a control. No death was recorded after 24 hours. In phase two, 1600mg/Kg, 2900mg/Kg and 5000mg/Kg were administered. The median lethal dose (LD_{50}) was calculated as the geometric mean of the

lowest lethal dose and the highest non lethal dose of which there was 1/1 and 0/1 survival.

Effect on Isolated Rabbit Jejunum

The rabbit was sacrificed by a blow on the head, dislocating the neck, exsanguinations. Segments of the jejunum, about 3cm long, were removed and disserted free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode's solution of the following composition in millimoles (mM): NaCl, 136.8; KCl, 2.7; $CaCl_2$, 1.3; $NaHCO_3$, 12.0; $MgCl_2$, 0.5; $NaPO_4$, 0.14; glucose, 5.5. The tissue was mounted in a 25ml organ bath containing Tyrode's solution maintained at $35 \pm 1.0\ ^0C$ and aerated with air. A lot of 0.5g was applied. A one hour equilibrium period was allowed during which the physiological solution was changed in every 15mins. At the end of the equilibrium period, the effect of acetylcholine (2.0×10^{-8}mg/ml – 3.2×10^{-7}mg/ml) an extract of *Stereospermum kumthianum* were investigated non-cumulatively. The contact time for each concentration was one minute, which was followed by washing three times. The tissue was allowed a period of 15mins before the next addition. Responses were recorded isometrically using Ugo Basile recorder 7050 (Amos *et al*., 1998; Agunu *et al*., 2005 and Ahmadu *et al*., 2007).

Effect on Castor oil-induced diarrhea in mice

The mice were fasted for 12 hours prior to the commencement of the experiment and were randomly divided into 5 groups of five mice each. Mice in the first group received 10ml/Kg (ip) normal saline, the second, third and fourth groups received 2000mg/Kg, 1000mg/Kg and 500mg/Kg of ethanolic extract of *Stereospermum kumthianum* (ip),

while the fifth group received Loperamide 5mg/Kg (ip). After 30mins of administration of extract, Castor Oil 0.2ml/mouse was administered intragastrically. The animals were placed on individual cages over clean filter paper. Three hours after the administration of oil, the cages were inspected for the presence of characteristic diarrhea droppings. Their absence was recorded as the protection from diarrhea, and the percentage protection calculated (Diurno et al., 1996; Akah and Offiah, 1996).

Statistical Analysis

The result on Castor Oil-induced diarrhea were analyzed using the Chi – Square Test and were regarded as significant when $P<0.05$.

RESULTS

The extraction process yielded 6.42% w/w of ethanolic extract of *Stereospermum kumthianum*. Phytochemical test revealed the presence of sterols/triterpenes, saponins, tannins, coumarins and free carboxylic group (Table 1). The median lethal dose (LD_{50}) of the ethanolic extract in mice (i.p.) was found to be 3807.9mg/Kg. The ethanolic extract of *Stereospermum kumthianum* (2.0×10^{-8}mg/ml – 3.2×10^{-7}mg/ml) exhibited a concentration-dependent relaxation of the rabbit jejunum (figure 1). The extract of *Stereospermum kumthianum* (500mg/Kg and 1000mg/Kg) significantly ($p<0.05$) protected the mice against Castor oil-induced diarrhea when compared with the control. This was comparable to that of loperamide (5mg/kg), the standard agents.

DISCUSSION AND CONCLUSION

Phytochemical screen of the ethanolic leaf extract of *Stereospermum kumthianum* reveals the presence of steroids/terpenoids, saponins, tannins, coumarins, and free Carboxylic group. The LD_{50} value of the ethanolic extract of the plant was found to be 3807.9mg/Kg (ip) in mice. Castor Oil is made up of 90% ricinoleate (Mekeon et al., 1999) which when metabolized is responsible for the observed effect of the oil. The active metabolites recinoleic acid is responsible for its diarrhea inducing properties, which diminishes Na^+ and Cl^- permeability in the intestine (Gaginella and Phillips, 1975); it is also associated with endogenous stimulation of prostaglandins release (Zavala et al., 1998). Earlier studies showed that anti-dysenteric and anti-diarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, steroids and/or terpenoids are reducing sugars (Anonymous, 1962; Galvez et al., 1991, 1993; Longanga et al., 2000). The anti-diarrheal activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistant and hence, reduce secretion (Tripathi, 1994).

Loperamide reduces the daily fecal volume, and decreases intestinal fluid and electrolyte loss. Loperamide produces a rapid and sustained inhibition of the peristaltic reflux through depression of longitudinal and circular muscle activity, presumably through an effect on intestinal opiate receptors. Loperamide is effective against a wide range of secretory stimuli and can be utilized in the control and symptomatic relieve of acute diarrhea that is not secondary to bacterial infection.

In conclusion, the plant extract exhibited anti-diarrheal activity. The effect was comparable to loperamide which is presently one of the most widely used anti-diarrheal

drugs and it elicited its activity by antagonizing diarrhea induced by Castor Oil (Niemegeers et al; 1974) and Prostaglandins (Karim and Adaikum, 1997), its therapeutic effect could also be due to its antimotility and its anti-secretory properties (Couper., 1987). The extract similarly inhibited spontaneous agonist induced contractions of rabbit jejunum. The effect may also contribute to the observed anti-diarrheal activity. Tannins have been known to make the intestinal mucosa more resistant and reduce secretion, therefore, inhibit diarrhea induced by Castor Oil (Tripathi, 1994). The presence of tannins in the plant extract could be responsible for the anti-diarrheal activity. The results of this investigation suggest that the leaf extract of *Stereospermum kumthianum* possesses antidiarrheal activity and justify the ethno-medicinal use of the plant in the treatment of diarrhea in Hanwa village, Zaria, Kaduna State Nigeria.

Table 1: Phytochemical Constituents of *Stereospermum kumthianum* leaf extract.

Chemical Constituents	Inference
Steroids/terpenes	+
Saponins	+
Tannins	+
Coumarins	+
Flavonoids	-
Alkaloids	-
Free carboxylic group	+

Key: + Present

_ Absent

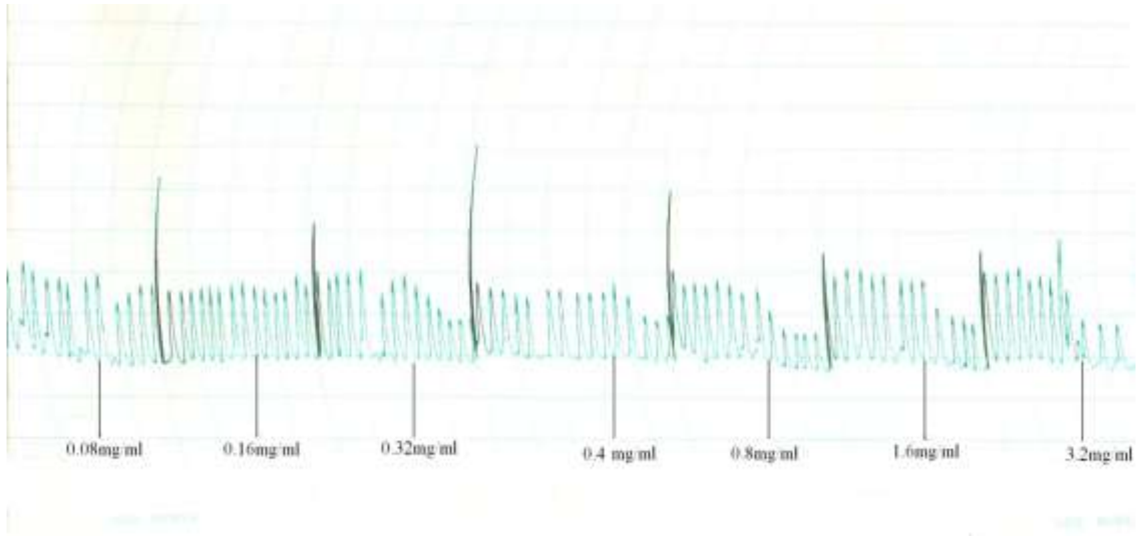

Figure 1: Effect of the ethanolic extract of Stereospermum *kumthianum* on isolated rabbit jejunum

Table 2: The effect of Ethanolic extract of the leaf of *Stereospermum kumthianum* on Castor Oil-induced diarrhea in mice.

Treatment n=5	Dose (*i.p*)	No. of mice with diarrhea	Protection %
Castor Oil	0.2ml/mouse	5/5	0
Stereospermum	500mg/Kg	2/5	60
kumthianum	1000mg/Kg	2/5	60
	2000mg/Kg	5/5	0
Loperamide	5mg/Kg	1/5	80

REFERENCE

Agunu, A; Sadiq, Y; Gabriel, OA; Zezi, AU and Abdurahman, EM (2005). Evaluation of five Nigerian Medicinal Plants used in treatment of diarrhea in Nigeria. Journal of *Ethnopharmacology.* 61 : 209 – 213.

Ahmadu, A. A; Zezi, A.K; and Yaro A. H. (2007). Anti-diarrheal activity of the leaf extracts of Daniellia Oliveri Hutch and Dalz (Fabaceae) and *Ficus sycomorus* Mio (Moraceae). *Afr. J. Trad. CAM* 4(4) : 524 – 528.

Akah, P. A; Offiah, V. N., (1996). Gastrointestinal effects of *Allamanda cathartica* leaf extracts *International Journal of Pharmacognosy*, 30 : 213 – 217.

Amos, S; Okwusaba, F. K; Gamaniel, K; Akah, P. A. and Wambebe, C. (1998). Inhibitory effects of Gastrointestinal and extracts of *Parefta crassipes* leaves on Gastrointestinal and uterine smooth muscles preparations isolated from Rabbit, Guinea Pigs and Rats. *J. Ethnopharmacol*, 61 : 209 – 213.

Anonymous, (1962). *The Wealth of India (Raw Material*). CISR. New Delhi, Vol. 6 : 280 – 281

Audu, R., Umilag, S.A; Renner, J. K; Awodiji (2000). Diarrhea Management. *J. Nigeria Infection Control Association*. 3 : 15

Charles R. Craig and Robert E. Stitzel., (1997). *Modern Pharmacology with Clinical Applications* 5th Edition, Little Brown and Company – Boston: 504 – 506.

Diurno, M. V., Izzo, A. A; Mazzoni, B; Bologgnese, A., Capasso, F; (1996). Anti-diarrheal activity of New thiazolidinones related to Loperamide. *Journal of Pharmacy and Pharmacology* 48; 760 – 762

Galvez, J. Zarzuelo A. Crespo, M. E. (1991). Anti-diarrheal activity of *Silerocarya birreh* bark extract and its active tannin constituent in rats. *Phytother Res*. 5 : 276 – 278.

Galvez, J. Zarzuelo A. Crespo, M. E; Lorente, M.D; Ocets, M. A.; Jimenez J; (1993). Anti-diarrheal activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. *Planta Medica,* 59 : 333 – 336.

J. Hutchinson and J. M. Dalziel, (1963). *Flora of West Tropical Africa*, Vol. II : The Whitefrians Press Limited London. Pp 21-26

Longanga Otshudi, A; Vercruysse, A; Forrier A (2002). Contribution to ethno botanical, Phytochemical and Pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in Lomda area of the Democratic Republic of Congo (DRC). *J. Ethnopharmacol*. 71(3) : 411 – 423.

Lorke, D. (1983). A new approach to acute toxicity testing. Arch Toxicol. 54 : 275 – 287

Mekeon, T. A; Lin, J. J; Stafford, A.E; (1999). Biosynthesis of ricinoleate in Castor Oil. *Adv.Exp.Med. Biol*. 46437 – 46447

Sofowora, A; (1982). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Limited Ibadan.Pp.34-42

Tripathi K. D. (1994). *Essentials of Medical Pharmacology*. Jay Pee Brothers Medicals Publishers (P), New Delhi P. 775

WHO, 1985. *The Management of Diarrhea and the use of Oral Dehydration Therapy*: A joint WHO/UNICEF Statement second edition Geneva

Zavala, M. A; Perez, C., Vargal, R; Perez, R. M. (1998). Anti-diarrheal activity of *Waltheria Americana, Commelins*, *Cuelestris* and *alternanthra* repens; Journal of *Ethnopharmacology*. 61 : 41 - 47