Phytochemical and Some Pharmacological Studies on the Stem Bark of *Anogeissus lieocarpus* (Combretacea)

By

El-Tayeb Einas Abdel Farrag

Being:

A Thesis submitted to the Post Graduate School, Ahmadu Bello University, in partial fulfilment of the requirements for the Award of the Master's Degree of Science in (Pharmaceutical Chemistry).

Department of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

March, 2004

Declaration

I hereby declare that this research work was carried out and written by me under the supervision of Dr. A.K. Haruna and it has not been presented in any previous publication for a higher degree elsewhere. The work of other investigators is acknowledged and referred to accordingly.

El-Tayeb, Einas Abdel farrag, B.Sc. (Chem.)
Department of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

Certification

This thesis entitled Phytochemical and Some Pharmacological Studies on the Stem Bark of *Anogeissus lieocarpus* (combretacea) Meirs by El-Tayeb Einas Abdel Farrag meets the regulations governing the award of the degree of Master of Science in Pharmaceutical Chemistry and is approved for its contribution to knowledge and literary presentation.

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Dr. A.K. Haruna B.Sc. (Pharm), M.Sc., PhD., MPSN **Date**
Department of Pharm. & Medicinal Chemistry,
Ahmadu Bello University, Zaria.
Chairman Supervisory Committee & Head of Department

---------- ----------

Prof. M. Ilyas B.Sc. (Chem), M.Sc., Ph.D., Chem. **Date**
Department of Pharm. & Medicinal Chemistry,
Ahmadu Bello University, Zaria.
Member of Supervisory Committee.

---------- ----------

Prof. H.A.B. Coker B.Sc. (Pharm), ABU., Ph.D. (Strathclyde, Glasgow) **Date**
Faculty of Pharmacy,
University of Lagos,
External Examiner.

---------- ----------

Prof. S. B. Ojo B.Sc., M.Sc. (ABU), Ph.D. (W. Ontario), FNIP **Date**
Dean of Post Graduate School.
Ahmadu Bello University, Zaria.

Dedication

To Allah (S.W.T.) for his guidance.

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Abstract

The plant *Anogeissus lieocarpus* (Combretacea), is a plant widely used in Africa as a remedy for abdominal pains, cough and chest pain. The plant is also beneficial in leprosy, malaria, anthelmentic, fungal and bacterial infections. The bark of *Anogeissus lieocarpus* was screened phytochemically and it was found to be rich in alkaloids, flavonoids, other poly phenols, sterols, saponins, carbohydrates, tannins and anthraquinones.

Ethyl acetate extract of the bark was fractionated into fractions **C** and **P**, and these fractions were subjected to Gas chromatography-Mass spectroscopy (GC-MS). Fraction **C** gave 17 - (1,5 – dimethyl – 3 - phenyl sulfanyl hex – 4 - enyl) - 4,4,10,13,14 – pentamethyl - 2,3,4,5,6,7,10,11,12,13,14,15,16,17 - tetradeca hydro - 1H - cyclopent phenanthrene; 3`H - cyclo prop (1,2) - 5-cholest - 1- en – 3 - one, 1`-carboethoxy - 1`- cyano - 1,2 - dihydro and Ursa, 9(11), 12 – dien – 28 - oic acid, 3 - (acetyloxy)-methyl ester, (3beta), while fraction **P** gave n-hexadecanoic acid; 1- nonadecene; Oleic acid and Oleic acid, 3 - (octadecyloxy) propyl ester; and steroids such as 3, 9, beta, 14, 15 – Diepoxy preg – 16 – en - 20-one, 3,11, beta, 18 - triacetoxy; (22S) – 21 - Acetoxy – 6 - alpha, 11- beta – dihydroxy - 16 alpha, 17 - propylmethylene, dioxy-pregna -1,4 – diene - 3,20 – dione,; Glycine, N-[(3. alpha, 5.beta, 12.alpha) – 3,12 –dihydroxy - 24 - oxocholan-24-yl]; 25 – Norisopropyl - 9,19 - cyclo lanostan – 22 – en – 24 – one; 3 –acetoxy – 24 - phenyl; Beta - sitosterol acetate; and cholesterol.

The ethanolic extract of the bark was observed to antagonize histamine-induced contractions on isolated guinea pig ileum in a dose dependant manner. Similarly the extract reverses acetylcholine induced contractions on the isolated guinea pig ileum. The extract also relaxes the isolated tissue.

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CHAPTER ONE

1.0 INTRODUCTION

Through gradual process of various trials mankind was able to detect plants that are edible, those that are nourishing, and those that could even poison or modify his health. It is through this process that he was able to observe those plants that heal due to some medicinal properties. Such knowledge of medicinal plants has been handed over successively from one generation to another. It is these plants that are now being used as traditional medicine and are recognised as folkloric remedies.

Traditional medicine is an important part of human health care, it is the sum total of the knowledge, skills, and practise based on the theory, beliefs and experiences used in maintaining good health as well as in curing diseases using medicinal plants. Through various means of communications, for example, the use of plants in medicine has been recorded in the pharmacopoeia of the Emperor Shen Nung of China around 3000 B.C. when chaulmoogra oil from species of Hydnocarpus Gaerth was used in the treatment of leprosy. Also it was confirmed by Papyri as the Beers papyrus, that castor oil seeds were used by ancient Egyptians as far back as 1500 B.C. Around 460 B.C, Hippocrates compiled *materia _medical* which consisted essentially of herbal recipes of over 400 remedies which include opium, mint, sage, rosemary and verbena. Theophrastus (370 B.C), a Greek botanist, also produced a number of manuscripts including the famous *historia plantarium* which was used as a standard medicinal text book in his time and few years after his death.

Various civilisations recorded the Arts and sciences of healing. These have been transferred to other centuries for example, Arab physicians practised medicine with healing arts as they travel through Europe for preaching's. In the South American countries, the use of medicinal herbs and plants is being practised. Gardens with medicinal

plants such as Cocoa, Tobacco and Potato were found by Spanish settlers between 1531 and 1536 in Mexico and Peru. Similarly, European settlers in Australia found the aborigines using medicinal herbs together with traditional rites (Sofowora, 1982).

The use of medicinal plants in Europe is on the increased. This could possibly be due to the marked increase in the number of Asian settlers in Europe. The Asian immigrants have retained their traditions, religions, dietary and their folkloric medicines. Recently, allopathic system of medicine, coexist with the traditional system which could be divided into 2 types: The first type being Unani which was originally from ancient Greece and has been influenced by Persian and Egyptian/African medicine while the other being Ayurvedic, which is purely of Indian origin and has not been influenced by other countries (Trease and Evans,2002)

The World Health Organisation (WHO) recently recognised many herbal remedies and it encourages developing countries to incorporate the use of folkloric plants for obvious reasons of availability and affordability apart from being effective alternative to allopathy practice. In Nigeria, ethenopharmaco-treatment using herbal remedies succeeded in the treatment of several diseases including breast cancer, skin disorders, sickle cells anaemia, some viral diseases, diabetics and some forms of mental disorders (Trease and Evans, 2002).

About 80% of the world's population are believed to be using herbal medicines and governments of developing countries are being encouraged to develop their national traditional treatments (Trease and Evans, 2002). Despite their importance, only a small fraction of the earth's plants have been screened for medicinally useful ingredients. Thousands of plants have become practically extinct before their usefulness could be determined (Gbile, 1985). Scientists are currently working on an estimated 25,000 – 5000,000 plant species in the world, of which 85% of them are in environment that are

traditional homes of indigenous users (Buger, 1990). Due to this large number of known medicinal plants we have nowadays on earth, the plant kingdom has been described as 'sleeping giant of drug derivation' (Verpoorte, *et al*, 1987), while the forest has been referred to as 'God's own Pharmacy' (Treben, 1986). From medicinal plants, compounds which revolutionalised medicine were isolated such as: quinine, artemisin, reserpine, vincristine and vinblastine alkaloids, digoxin, digitoxin, and many others (Hardman, 1991).

The usefulness of plants and their protection was emphasised in ancient literature. More than 100 medicinal plants are used in modern medicine where they occupy a very significant place as raw materials for important drugs, and also plants used in traditional systems of medicine of pharmaceutical houses are collected from wild sources (Singh and Pandey, 2003). Medicinal plants are cultivated commercially nowadays for extraction of some important active constituents for use in modern medicine.

In Nigeria, the uncontrolled human activities and ignorance of the role of the forests play to man's continued existence in this chaotic world, threatens the existence of this great masterpiece of nature's handiwork. Therefore planting and cultivation of medicinal plants should be encouraged (Gbile, 1985).

The world is dynamic and so are diseases and therefore their remedy. Because of this reason the search for new agents with more efficacies and less toxicity is more than justified from natural sources that could be presented as future drugs or their precursors.

Consequent upon this, we decided to screen an important medicinal plant; *Anogeissus leiocarpus* that is claimed to have beneficial effects in the traditional treatments of some viral diseases and more popularly used as an antitussive in different parts of sub-saharan Africa.

1.1 Literature Review

1.1.1 *Morpholigical features of Anogeissus lieocarpus*

Anogeissus leiocarpus belongs to the family *combretaceae* and genera *Anogeissus.* It is a woody tree of 90ft (28m) high, which is greyish outside and dark brown at the heart. Its bark is hard with grey or pale brown in colour, gets darker with age. The bark peals off in very thin patches, which often curl at the edge; slash yellow with darker streaks, excluding a born soluble gum. The branches are usually ascends from head down and often dropping at the ends. The young foliage are densely and often silky hairy.

The tree can be easily recognised by it's dedicate leaves of the length 1-3 inches (2.5 – 7.5cm) and 0.5-1.5 inches (12-30 mm) broad, elliptic, acute, and bluntly pointed. The midrib is projecting in a short spine rounded at the base or broadly connate, alternate, entire, finely hairy on both surfaces. The leaf has 8-10 pains of thin, up curving lateral nerves, which fades out at the margin and not looped, its hairy stalk is of 0.1 – 0.2 inches (2-5mm).

Anogeissus leiocarpus flowers around July to November, the greenish to yellow in compact globase flowers, heads on a stalk up to 0.5 inch (12mm) long. Each flower has a calyx – tube. This is fused to the ovary and resembles a stalk with 5 calyxes – teeth forming a short simple style. It has 10 free stamens. The scaly-like fruits of *Anogeissus leiocarpus* starts coming out on August to January. They are about 0.25 inch (6mm) across, with 2 wings and sharp beak, collected in a rather prickly dark brown head.

Anogeissus leiocarpus trees growing in drier areas tend to have smaller leaves and more hairy flowers than those growing under water conditions. *Anogeissus leiocarpus* is known locally in Northern part of Nigeria as "*marke*" by Hausa tribe "*Kojoli*" by Fulani

tribe and "*annum*" by Kanuri tribe. It is also known locally in the southern part of Nigeria as "*avin ainy*" or "*orin-odan*" by Yoruba tribe and in the Eastern part of Nigeria is known locally as "*atara"* by Igbo tribe (Keay, 1960).

The seeds soon loose their vitality and their germinating capacity is rather low (Irvine, 1961).

1.1.2 *Distribution*

Anogeissus leiocarpus occurs in most of the savannah areas from the driest regions to the borders of the forest zone (Keay, 1989). It extends from Senegal to Cameroon, the Sudan, upper Nile region like Abyssinia and Eritrea.(Irvine, 1961). It is locally abundant in Northern, Western and Eastern Nigeria (Keay, 1989).

1.1.3 *Ethnomedicinal Uses*

Bark infusion of *Anogeissus leiocarpus* in Ivory Coast is applied to sores on the feet and its powder together with that of *terminalia* are rubbed on gums to relieve toothache. The decoction of the young bark is used to disinfect syphilitic sores and ulcer.

The bark in warm lotions and infusions is used in Guinea as a febrifuge. A bark decoction is given to the new born child to drink and the bark being chewed or its infusion or decoction taken orally is used as medicine for cough.

The bark decoction is used for treatment of body and chest pains, orally, together with capsicum *annuum* (red pepper) are being applied externally together with *Aframomum.*

Nankani women in Guinea use the plant bark against diarrhoea and the leaf decoction after parturition. It is also used for treatment of skin disease especially itch by washing and fumigation. A decoction of the leaves together with *Rhaphiostylis reninesis*

and *Diospyros kekemi* is drunk for leprosy while the decoction with *terma* and *Nauctea* is drunk for yellow fever or jaundice (Irvine, 1961).

Adults take a decoction of the leaf in Nigeria, made by soaking tight-packed leaves in an earthenware pot orally as anti malaria. Roots in Senegal are used as Emmenagogue. In Ivory Coast pulped roots are applied to wounds. Seeds and bark of the plant mixed with Guinea corn or soaked in water as a remedy or preventative as vermifuge, especially for *Tinea spp* (tape worm) in equines (horse) in the markets .While the stem is used in Sudan and Nigeria as chewing stick (Irvine,1961).

1.1.4 *Biological Activities*

The leaf of *Anogeissus lieocarpus* has been reported to possess antibacterial activity (Sanogo, *et al*.,1998). It was also reported to posses' antifungal activity (Bhatt and Saxena, 1979) and weak antiparasital activity (Elsheikh, *et al.,* 1990). While dried aerial parts of the plants were reported to show anthelmintic activity (Ibrahim , 1992). The stem bark has also been reported to show a CNS depressant activity (Vasileva , 1969).

1.2 Phytochemistry

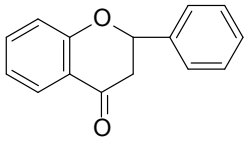
In spite of the numerous uses of this medicinal plant, the only reported phytochemical works conducted on it was by Adigum, *et al.,* (2000), where they reported the isolation of coumarin from the bark of the plant.

Inorganic elements were also isolated, such as fluoride from the stem of the plant (Odusanya, et, al., 1979), and other inorganic elements such as Al, K, Fe, V, Na, Sc, Ca, Br, Zn, Mg, La and Cs from the root of the plant (Asubiojo, *et al.,* 1982) has also been reported.

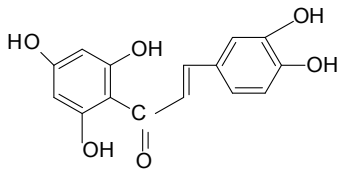
Earlier reports indicated the absence of alkaloids while the phytochemcial screening carried out on the bark of the plant indicated the presence of alkaloids in the bark of *Anogeissus lieocarpus* beside the presence of flavonoids, terpenoids, steroidal saponins and tannins, therefore, it pertinent, here-in to discuss concisely their chemistry.

1.2.1 *Flavonoids*

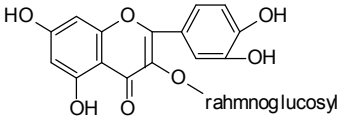
Flavonoids are structurally derived from the parent substance flavone (I) and they share common properties. They usually occur in all plants parts, both in free state and as glycosides and are the largest group of naturally occurring phenolic compounds. Flavonoids are coloured substances, which impart colouration to most plant's tissues. They are often yellow and the intensity of their colour increases with increase of number in the hydroxyl groups and with the increase in pH. They all have γ - pyrone moiety and phenolic ring with the exception of chalcones (II), which are biosynthetically related to flavonoids. Flavonoids exist as aglycones such as quercetin (VI), flavonone e.g. hesperetin (VII), flavonanone eg naringanin (VIII), flavonanol eg taxifolin (IX) and isoflavonal eg daidzein (X). The dimeric compounds known as biflavonyls and a typical example of this are amentoflavone (XI) (Trease and Evans, 2002) or conjugated with sugar units as glycosides, mostly O, glycosides such as quercetin 3-rutinoside (rutin) (III) and some known C-glycosides, e.g. 8-C-glucoside of luteolin (orientin) (IV) are soluble in aqueous and alcoholic solvents and insoluble in organic solvents. While free flavone (glycones), dissolve in non-polar solvents like ether and only sparingly in aqueous solvent (Trease and Evans, 2002).



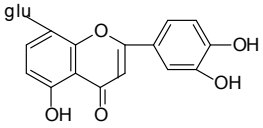
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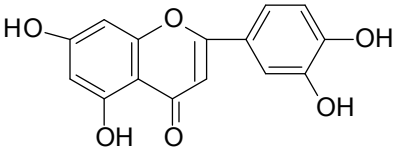
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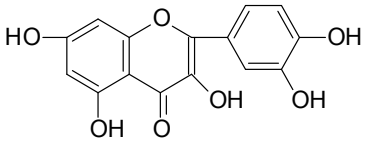
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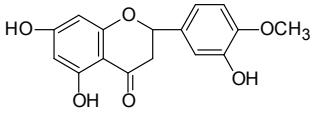
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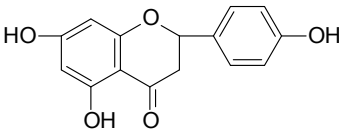
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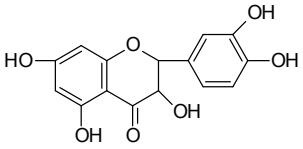
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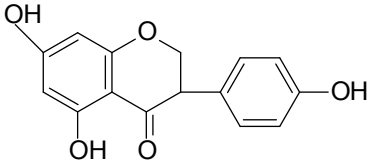
(VII)



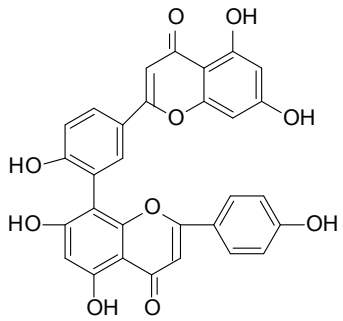
(VIII)



(IX)



(X)



(XI)

1.2.1.1 *Extraction of Flavonoids*

Extraction of flavonoids from plant tissues depends on their nature. Flavonoid glycosides and polar aglycones are extracted with acetones, alcohol, aqueous or a combination of these (Bhutani, *et al.,* 1969a). Aqueous methanol is used frequently as solvent for extraction of flavonoid while boiling aqueous solvent is used for the extraction of polyglycosides (Okigawa, *et al*., 1971a). Although there is possibility of hydrolysis of glycosidic flavonoids, the very rare use of traces of acid with the solvent for the extraction of flavonoids has been reported (Wallace, *et al*., 1969). Cold maceration of flavonoid may also lead some times to hydrolysis of glycosides due to occurrence of enzyme action (Trim, 1955). This problem could be avoided by extraction with boiling solvents or by rapid drying prior to extraction (Seshadri, 1962).

1.2.1.2 *Purification / Isolation of Flavonoids*

Preliminary purification of flavonoids particularly glycosides can be achieved by treatment of crude plant extract with charcoal powder (Mabry, *et al.,* 1970). Dialysis is also another preliminary method for isolation of high molecular weight flavonoids (and

tannins), which are water-soluble and are mixed with sugar or soluble inorganic material in the crude extract (Markham, 1972).

Column chromatography remains the single most useful technique for the isolation of large quantities of flavonoids from crude plant extracts, but it is more difficult to separate individual members of one class of flavonoids by this technique, using silica gel, cellulose and polyamide as the best adsorbent or kieselgulr, magnesol, alumina, sephadex or ion exchange resins (Harborne, 1959b).

Paper chromatography is used as a method of separation and identification using n-butanol – acetic acid – water as one of the best developing solvents. Precipitation with lead acetate is frequently employed (Sayed and Ashkan, 1976).

1.2.1.3 *Methods of Identification of Flavonoids*

(i) **Ultraviolet spectra (UV)**

UV spectra are useful both in determining the structure of flavonoids and in estimating the number and position of hydroxyl groups and of sugar attachments. Flavanones and related compounds have spectra very similar to the hydroxyl acetophenones from which they are derived. Flavans absorb strongly around 280nm and other have different wave - length absorption bands.

Diagnostic reagents such as sodium ethylate, sodium acetate, aluminium chloride and a boric acid – solid sodium acetate mixture, when added to solutions of flavonoids cause shifts in the position of the maxima. These shift reagents are usually used to determine the structure of flavonoids (Sayed and Ashkan, 1976).

(ii) **Infra red Spectra (IR):**

This has not been proven to be useful in flavonoids work, so only few reports have been published (Sayed, and Ashkan, 1976).

(iii) **Gas Chromatography (GC)**

Gas chromatography of flavonoids compounds is still not well developed, although in earlier reports was indicating that methyl ether of thirty-six flavons were separated with a silicone liquid polymer (SE-30) as the stationary phase (Sayed, and Ashkan, 1976).

1.2.1.4 *Medicinal and Biological Properties of Flavonoids*

Flavonoids possesanti- inflammatory, anti- allergic, anti- thrombiticand vasoprotective properties. They are beneficial in inhibition of tumour and as protective of gastric mucosa. Many flavonoid-containing plants are diuretic (e.g. buchu, and broom) or antispasmodic (e.g. liquorice and parsley). Some flavonoids have anti-tumour, antibacterial or anti-fungal property and others like tanning (fustic and sumac). They decrease capillary fragility and are therefore employed in case of hypertension and radiation injuries. Some are used in domestic veterinary medicine, particularly in the form of ointment as anti-hepatoxic properties (Trease and Evans, 1996).

1.2.2 *Terpenoids*

Terpenoids are group of compounds occurring in plant kingdom (while few obtained from other sources). They have the molecular formula $(C_5H_8)_n$ where the value of n is the basis for their classification. Terponoids are classified into monoterpene, sesequiterpene, diterpene, sesterpene, triterpene, tetraterpene and polyterpene.

1.2.2.1 *Extraction*

Extraction of terpenoids is usually employed with non-polar solvents like ether or benzene, followed by saponification in alcoholic alkali for removal of lipids, waxes and esters (Robinson, 1969). While terpenoidal glycosides are extracted from plants with 70 – 95% ethanol since they

are moderately polar. Removal of lipids is done by partition with non-polar solvent such as benzene.

1.2.2.2 *Purification / Isolation of Terpenoids*

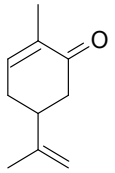
Terpenoids are usually isolated by express, adsorption in purified fats or steam distillation. They are popularly isolated by extraction with volatile solvents. Recently chromatography has been used both for isolation and separation of terpenoids. Gas chromatography has been particularly useful for isolating pure form (Finar, 1984).

1.2.2.3 *Methods of Identification of Terponoids*

(i) **Ultraviolet spectroscopy (UV)**

The main application of this technique is its detection of conjugated system. In simple acyclic diene, λmax is 217 – 228nm, while in hetero annular diene λmax is 230 – 240nm, and for homoannular diene λmax are 256 – 265nm. For α, β - unsaturated, carbonyl system λmax is 220 – 250nm. Appearance of a weak band at λmax 315 – 330 nm is also found.

Substitution affect the absorption maximum of a diene system and hence wood ward develop an empirical rule for calculating λmax from the molecular structure of the compound which was later modified by the Scientist Fieser. For example, the observed λmax value for Carvone (XIII) is 235nm while the calculated value based on wood ward rule is 237nm (Finar, 1984). UV spectra may also be used for detecting the presence of an isolated double bond at λmax 175 – 200nm and - unsaturated acids, esters and lactones at 220nm



(XII)

(ii) **Infra red Spectroscopy (IR)**

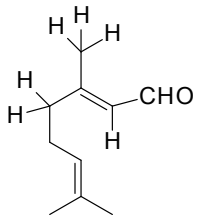
IR is used in structure elucidation in terpenes. It detects the presence of OH hydroxyl group (~ 3400cm$^{-1)}$ or an oxo saturated group (1750 – 1700cm^{-1}) and oxo, α, β - unsaturated group, (1700 – 1660 cm^{-1}). It is also used for detecting the presence of isopropenyl group (895-885 cm-1) and to distinguish between cis and trans isomers and between hetroannular diene and α, β, unsaturated ketones, where UV failed to distinguish between them (Finar, 1984).

(iii) Nuclear Magnetic Resonance Spectroscopy (H^{1}NMR)

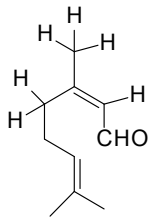
The main application of NMR in structure elucidation in terpene is to detect and identify double bonds and to determine the nature of end group, number of rings present and to ascertain the orientation of methyl groups present in the molecule. For example:

Value of CH_2 in trans- citral (XIII) is δ 7.76 while in cis – citral ((XIV) is δ 7.42.

The value of CH_3 in trans-citral is δ 7.84 while in cis-citral is δ 8.02.



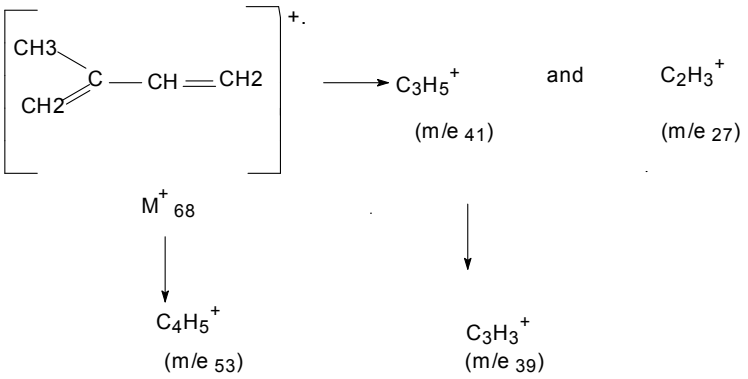
(XIII)



(XIV)

(iv) **Mass Spectroscopy (MS)**

With the means of mass spectroscopy it is possible to detect molecular weight and molecular formula, the nature of various functional groups and relative position of double bonds, then elucidating the complete structure of terpenoid is possible especially using mass spectroscopy spectrum comparing with reference spectrum of an authentic specimen. The following peaks can give its mass spectroscopy isoprene as a building unit of the terpenoid, 68 ($C_5 H_8^+$, M^+), 67 ($C_5 H_7^+$)(100%), 53 ($C_4 H_5^+$), 51($C_4 H_3^+$), 41 ($C_3 H_5^+$), 39($C_3 H_3^+$), 29 ($C_2 H_5^+$), 27($C_2 H_3^+$).



1.2.1.4 *Medicinal and Biological Properties of Terpenes*

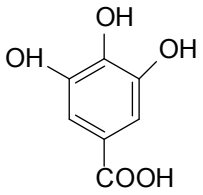
Monoterpenes and sesequiterpene are widely used in perfumery industries to their sharp scent; sesequiterpenes are used in pharmaceutical purpose. For example, the sesequiterpene lactones, Artemisin is Chinese antimalaria used in the treatment of cerebral malaria and plasmodium falciparum resistant malaria. Various diterpenes are being used in treatment of diseases for example forskohlin (coleanol) has been used since by Hindus in Ayruvedic traditional medicine for treatment of heart disease, abdominal colic which

showed also anti-hypotensive effect, cardiotonic effect and spasmolytic effect (Amman and Muller, 1985).

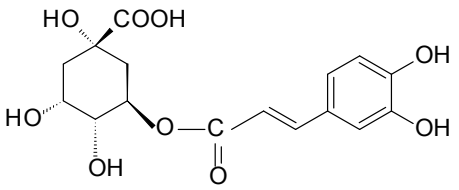
1.2.3 *Tannins*

Tannins are colourless, non-crystalline substances, which form colloidal isolations in water; these solutions have an astringent taste. They precipitate protein from solutions and they form a bluish – black colour with ferric salts, they also precipitate many alkaloids from their solutions (Finar, 1975).

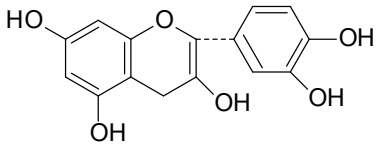
True tannins are of a very high molecular weight of 1000 – 5000, while pseudo – tannins are relatively of a low molecular weight and often present with Gallic acid (XV), catechin (XVI) and cholrogenic acid (XVII). Complex tannins are newly discovered group of compounds, they are biosynthesised from both hydrolysable and condensed tannins through carbon-carbon bond between the two (Trease and Evans, 2002)



(XV)



(XVII)



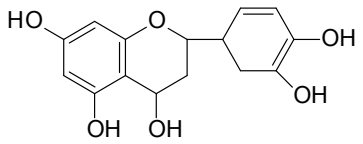
(XVI)

Tannins occur widely in vascular plants, their occurrence in angiosperms being particularly associated with woody tissues, nutgall is one of their best sources. They are divided chemically and structurally into two types:

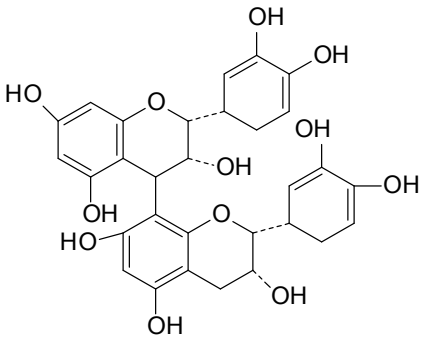
(i) ***Proanthocyanadins (condensed tannins):***

(ii) They occur in ferns and gymnosperms, widely distributed among the angiosperms especially in woody species. They are related to flavonoid pigments on having polymeric flavan – 3 – ol structure (XVIII). Biosynthetically, they are formed by the condensation of single catechins (or gallocatechins) to form dimmers (XIX), then higher oligomers with carbon – carbon bonds linking one-flavan units by 4 – 8 or 4 – 6 link.

On treatment with hot acid or enzymes (Topoisomerase 1 & 2, T – 1 and T – 2) Some carbon-carbon linking bonds are broken and anthocyandin monomers are released forming a red insoluble compounds known as phlobaphens e.g. red cinchona bark. While prodelphinidins and propelargonidins on acid degradation they yield cyanadin and delphinidin e.g. of condense tannins are bark of cinnamon, wild cherry, roots of krameria, leaves of hamamelic, tea, etc (Harborne 1984)



(XVIII)



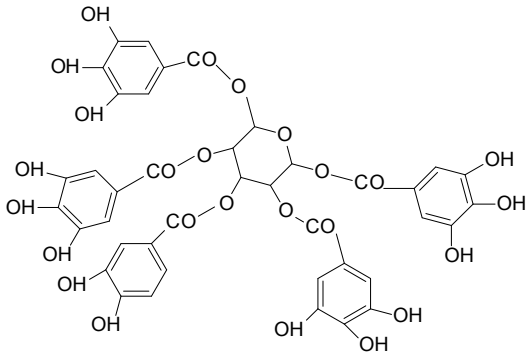
(XIX)

(ii) *Polyesters (hydrolysable tannins):*

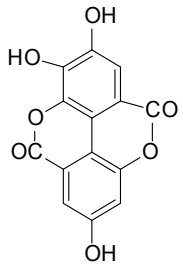
Polyesters occur in dicotyledonous plants and hydrolysed by acid and enzymes such as tannase. They are of two classes:

(a) Galloyl glucose depside, here the glucose is surrounded by 5 or more galloyl ester group, e.g. Gallo tannins (XX).

(b) Core molecular is a dimmer of gallic acid (XXI), hexahydroxy dipheric acid, again with glucose attachment or ester linkage e.g. ellagitannins which yield ellagic acid in hydrolysis. (Haddock, *et al*, 1982). Hydrolysable tannins are present in cloves, red rose petals, bearberry leaves, eucalyptus leaves, and others.



(XX)



(XXI)

1.2.3.1 *Extraction of Tannins*

Tannins being highly polar phenolic compounds are extracted with polar solvents like alcohol, aqueous alcohol, mainly methanol. They can also be extracted with chloroform then followed by washing with 1% aqueous sodium chloride, with the upper phase discard and the chloroform portion then dried with anhydrous Na_2S0_4 for few hours. Washing with 1% sodium hydroxide solution NaOH can be effective (Richard, 1998).

1.2.3.2 *Purification / Isolation of Tannins*

Isolation is tedious but is done by means of usual column chromatography. Aqueous or non polar extracts are passed through polyamid collagen and then sephadex LH – 20, or silica gel. Isolation of tannins from methanolic extract using DE 52 (diethyl amino ethyl cellulose-based ion exchanger) is also effective (Richard 1998).

Separation can be done by its precipitation with excess lead salt and the latter can be Precipitated as sulphide by passing in sulphuratted hydrogen (Denston, 1951). Purification and separation of proanthocyanins can be achieved on sephadex G-50, and

sephadex LH – 20 in aqueous methanol 1:1. While separation of hydrolysable tannins using HPLC has also been successful (Harborne, 1984).

1.2.3.3 *Medicinal and Biological Properties of Tannin*

Tannins – containing drugs will precipitate protein which used traditionally as astringents and used internally for the protection of inflamed surface of mouth and throat. They act as anti-diarrhoeal and have been employed as antidotes in poisoning by heavy metals, alkaloids and glycosides. Their study of anti-tumour effect and anti HIV activity has been of interest. Epigallocatechin –3 – gallate as a component of tea has shown to prevent anagogic in mice. Canberry juice has long been used for reducing bacterial infection of the bladder (Avorn *et, al*., 1994).

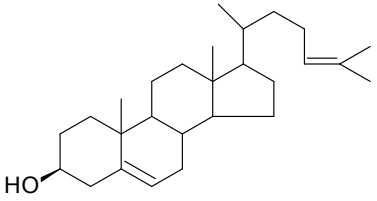
1.2.4 *Saponins*

Saponins are highly polar glycosidic compounds, which are used widely all over the world as soap. They often occur as complex mixtures and differ slightly from each other in the nature of the sugar present or the aglycone structure. They are characterised by their property of producing frothing aqueous solution and their haemolytic property. Also being highly toxic when injected into the blood stream but harmless when taking orally. There are two types of saponins:

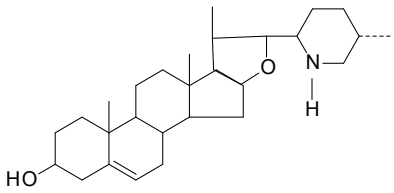
(i) The steroidal saponins (tetracyclic triterpenoids):

Steroidal saponins are less distributed in nature than the penta cyclic triterpenoid type (XXIV). They have a glycosidal linkage at C – 3 and have a biogenetic origin via mevalonic acid and isoprenoid units. Exception is the steroidal alkaloid which poses a

hetrocyclic nitrogen – containing ring to give basic properties e.g. solasodine (XXV).



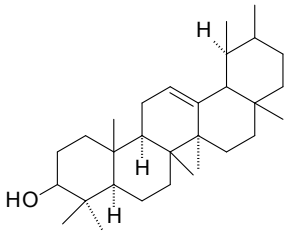
(XXIV)



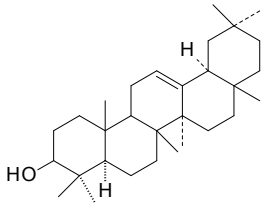
(XXV)

(ii) Pentacyclic Triterpenoid Saponins:

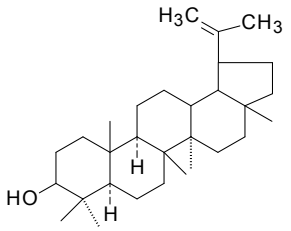
Unlike the steroidal saponin, pentacyclic triterpenoid saponins are rare in monocotyledous but abundant in many dicotyledonous families. The sapogenins triterpenic Saponins consists of two units and a sugar. The triterpenic pentacyclic Saponins are attached to a chain of sugar or uronic acid units, or both, often in the 3-position. They are classified into α - amyrin (XXVI), β -amyrin (XXVII) and lupeol (XXVIII)



(XXVI)



(XXVII)



(XXVI)

1.2.4.1 Extraction of Saponins

Saponin extraction is usually done with polar solvents such as methanol, 95% ethanol or aqueous solvent. Precipitation of the crude saponins from n-butanol portion by diethyl ether or acetone from methanolic extract, has been very effective (Richard, 1998).

1.2.4.2 *Purification / Isolation of Saponins*

Saponins are highly polar compounds with high molecular weight so their isolation is some how difficult usually by column chromatography using sephadex LH – 20, silica gel or counter current. Saponins are hydrolyzed using mineral acids to give an aglycone (sapogenin), various sugars and related uronic acids (Trease and Evans, 1997).

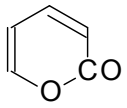
1.2.4.3 *Medicinal and Biological Properties of Saponins*

Steroidal saponins are of great pharmaceutical importance because of their relationship to compound such as the sex hormones, cortisone, diuretic steroids, vitamin D and the cardiac glycosides. Some saponins are used as the starting material for synthesis of steroids. Oleanene Saponins,which occur in the root of umbellifera is used in Chinese

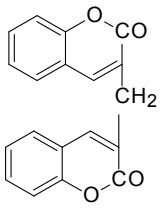
medicine for the treatment of hepato – billiary disorders which also to posse's anti-inflammatory property (Trease and Evans, 2002).

1.2.5 *Coumarins and Glycosides*

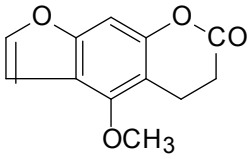
Coumarins are found in plants both in the free state and as glycosides and in about 150 species belonging to over 30 different families. They give blue, blue – green or violent fluorescence in ammonical solution or under UV. There derivates of benzo - α - Pyrone (XXIX). Bi-coumarins are formed from two coumarin moieties eg, dicoumarol (XXX). Furano coumarins eg, bergapten (XXXI) are closely related to coumarins (XXXII). Umbelliferae, scopoletin, fraxin, apterin (XXXIII) are examples of plants containing free and glycosidic coumarin.



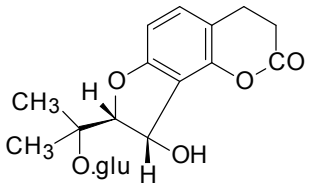
(XXIX)



(XXX)



(XXXI)_



(XXXIII)

1.2.5.1 *Medicinal and Biological Properties of Coumarin*

Coumarins are known to have powerful anti-coagulant and haemorrhagic properties. Roots of angelica that are known to contain coumarins are used in the treatment of rheumatic disease and treatment of bronchitis associated with vascular deficiency. Coumarins are also used as starting material for synthetic hormones. Bergatpten, archangelicin, and apterin are reported to have potent coronary vasodilator effect and are also calcium antagonists. More complex coumarins such as calanolides and inophyllums have received recent attention as potent HIV – 1 - RT inhibitors (Trease and Evans,2002).

1.3 Aims and objectives of the studies

The aims of these studies are to screen the plant and isolate biologically active compounds and validate the traditional claims of the uses of the plant.

The objectives are to search for plant's active constituents in order to understand the rationale of their usage in traditional medicine. This includes the isolation, characterisation of the constituents and studying their biological responses with the aim of obtaining agents with better efficacy and low toxicity.

CHAPTER TWO

2.0 Experimental

2.1 Materials

2.1.1 Plant Material

The plant *Anogeissus lieocarpus*, was collected around Zaria, Nigeria. The plant was identified and authenticated at the Herbarium of Biological Sciences Department, Ahmadu Bello University, Zaria and specimen voucher was deposited

2.1.2 *Chemicals/Reagents*

All solvents were distilled before use.

Thin layer plates were prepared by the usual method using silica gel G. coated evenly (0.25mm thick) on glass plates.

2.1.3 *Instruments*

(1) Melting Point

All the melting points values were uncorrected and were determined using capillary tubes on Gallen Kemp and Melting Point Apparatus.

(2) Gas Chromatography / Mass Spectroscopy (GC/MS):

GC-Varian 3800 with MS –Saturn 2000

Column:	cpsil 8CB low bleed MS; 0.25mm x 0.25 µm x 30m
Oven:	80^0C / min to 275^0C for 20min.
Condition:	Ramprate 10^0C / min
Traptemprature:	150^0C
Manifold:	40^0C
Transfer line:	250^0C
Injection temperature:	270^0C

2.2 Analytical Methods

2.2.1 *Thin layer chromatography (TLC)*

Technique: Ascending

Stationary Phase: Silica Gel G. Slurry made by dissolving 40g in 80ml of distilled water and spread (0.25mm) unto the plates (2cm x 5cm) using a spreader (Stahl, 1975) and activated at 100^{o}C for 1 hour.

Mobile Phase: As in the details in text.

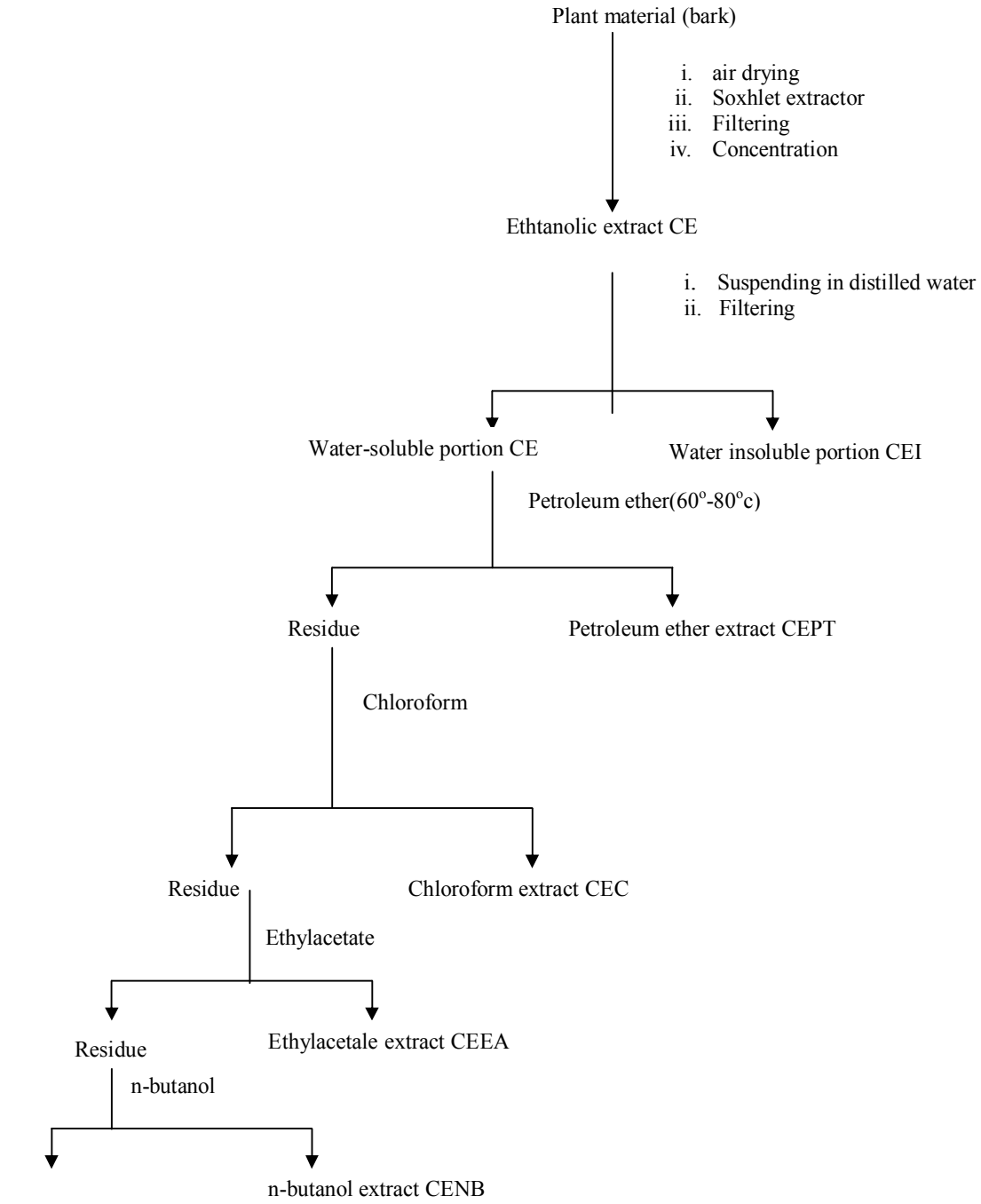
Spotting and development: The spots were applied manually using glass capillary tubes and the plates developed at room temperature using a Shandon chromatotank.

Visualization: The developed chromatogram were dried in a fume cupboard and viewed under: UV light for fluorescent spots; (ii) Iodine vapour chamber; (iii) Sprayed with suitable chromogenic reagents.

2.3 Extraction Procedures

About 2.0 Kg of the air- dried bark of the plant material was crushed and sieved into moderately coarse powder and about 500g of the powered bark was extracted with ethanol 95% using soxhlet extractor. The ethanolic extract was collected and the solvent evaporated at reduced pressure completely to give a gummy dark brown extract, which was coded as CE and weighed 87.23g.

About 10g of CE was suspended in distilled water and filtered. The filtrate was successively partitioned with petroleum ether, chloroform, ethylacetate, and n – butanol to give petroleum ether extract (CEPT), chloroform extract (CEC), ethyl acetate extract (CEEA), n-butanol extract (CENB), and residual aqueous portion (CEAq) respectively. (See fig. 2.1).



Residual aqueous por

CEAQ

Fig 2.1 Scheme of Extraction of Crude Ethanolic Extract (CE)

2.4 Preliminary Phytochemical Screening

About 5g of the ethanolic extracts CE was used for preliminary Phytochemical screening according to procedures summarized blow:

2.4.1 *Alkaloids*

(a) **Mayer's test:** About 2 drops of Mayer's reagent were added to 2ml of the acidified solution of ethanolic extract (HCl or diluted H_2S0_4 solution). Yellow precipitate occurred indicating the presence of alkaloid.

(b) **Dragendroff's test:** About 2 drops of dragendroff's reagent were added to 2ml of the acidified ethanolic extraction. Appearance of an orange – brown precipitate indicated the presence of alkaloid. This was also performed on a filter paper or TLC plate by adding a drop of the reagent into a spot of the sample.

(c) **Wagner's test:** addition of 3 drops to 2ml of wagner's acidified ethanolic extract did not give a brown precipitate.

(d) **Ammonium reineckate:** Addition of 3 drops of the reagent to 2ml of ethanolic extract gave a pink precipitate due to the presence of alkaloid (Richard, 1998).

2.4.2 *Flavonoids*:

(a) **Shinoda test:** About 2ml of the ethanolic extract was replaced on a test tube and by addition of metallic magnesium and 2 drops of concentrated HCl, a red colour was observed indicating the presence of flavonoids.

(b) **Sulfuric acid:** About 2 drops of concentrated H_2S0_4 were added to a test tube containing 2ml of ethanolic extract and the occurrence of a red colour indicated the presence of flavonoids (Richard, 1998).

2.4.3 *Other Poly Phenols:*

(a) **Ferric chloride**: About 5% of ferric chloride solution dissolved in water or ethanol, the occurrence of a brown precipitate indicating the presence of polyphenols when added to 1ml of ethanolic extract.

2.4.4 *Sterols*:

(a) **Libermann-Buckard test:** About 1g of ethanolic extract was dissolved in 1ml chloroform and 1ml of acetic anhydrous all cooled in ice. The solution was transferred into test tube and a drop of concentrated H_2S0_4 was added down the test tube with the aid of pipette. A reddish brown ring colour indicated a positive test.

(b) **Salkowski test:** About 1 g of ethanolic extract was dissolved in 1ml of chloroform, addition of 1ml concentrated H_2S0_4 formed two phases with a red colour at the interphase indicating the presence of steroids (Richard, 1998)

2.4.5 *Saponins*

About 1ml of the aqueous ethanolic extract of the sample was vigourously shaken in a test tube, formation of froth, which persists for approximately 15 minutes (Richard, 1998).

2.4.6. *Carbohydrates*

(a) **Molish test:** About 3 drops of Molish reagent were added to 2 ml of aqueous ethanolic extract in 1 ml of concentrated H_2S0_4 without mixing; occurrence of a purple ring in the interface indicated a positive test.

(b) **Aniline acetate reaction:** About 1g of ethanolic extraction was heated over a flame, and the vapours were allowed to react with aniline acetate impregnated on a filter paper placed on the vapours. Presence of a red colour due to the presence of hetrocyclic aldehydes produced from carbohydrate dehydration indicated a positive test (Richard, 1998).

(c) Reducing Sugars (Fehliling I & II) test: About 1ml of aqueous ethanolic extract was heated with 2ml equal volumes of fehling's solution I and II, formation of a red-prick precipitate indicated the presence of reducing sugars (Trease and Evans, 2002).

2.4.7 *Tannins*: A drop of diluted solution of ferric chloride was added to 0.5ml of aqueous ethanolic extraction. Occurrence of blue-black and brown-green precipitate indicated the presence of mixtures of gallic and ellegitannins and catechol tannins respectively (Trease and Evans, 2002).

2.4.8 *Free Anthraquinones*: About 0.5g of ethanolic extract was shaken with 10ml organic solution (benzene), after filtration, 5ml of 10% ammonia solution (NH_40H) was added, on shaken a pink colour observed in the aqueous layer (lower layer) indicating a positive test (Trease and Evans, 2002).

2.5 Fraction CEEA

TLC was carried out on CEEA in various solvent systems, which includes:

1. Ethyl acetate: methanol: water (100:16.5:135)
2. Chloroform: methanol: water (3:3:1)
3. Ethylacetate: acetic acid: formic acid: water (30:0.8:1.2:8)
4. Ethylacetate: formic acid: water (10:1:2)
5. n-butanol: acetic acid: water (4:1:2)

The chromatograms showed this fraction to be a mixture (fig 3.1).

This fraction on standing after 24 hours at room temperature gave a brownish suspension, which was filtered. The filtrate was kept, while the residue was dissolved in chloroform to yellow solution. To this solution methanol was added and yellow flakes were formed which were filtered out, washed with more methanol and dried to a yellow amorphous

powder (15.7mg) coded fraction (C). TLC of fraction C was conducted in several solvent systems, which includes: chloroform: methanol (19:1) and ethyl acetate: methanol (19:1), showed fraction C to be a mixture as showed in fig. 3.2. Fraction C was soluble in chloroform and insoluble in methanol. The filtrate kept above was concentrated and dissolved in chloroform to form a dark yellow solution. Addition of methanol to this solution give yellowish-grey flakes, which were filtered and washed with methanol to afford yellowish grey amorphous powder coded fraction (P), (6.0mg). Fraction P was soluble in chloroform and insoluble in methanol. The TLC profile of this fraction is shown in fig. 3.3. Fraction C and P were subjected to Gas chromatography / Mass spectroscopy (GC-MS).

CHAPTER THREE

3.0 Results

3.1 Extraction

The air – dried bark after ethanolic extraction gave a Crude ethanolic extract (CE), a dark brown-gummy material (87.23g) which after successive solvent extractions yielded various fractions as summarised in Table 3.1.

3.2 Preliminary Phytochemical Screening of the Bark of *Anogeissus lieocarpus*

The results of the tests conducted on the ethanolic extract (CE) are as summarised in Table 3.2.

Solvents	Extracts	Description
Petroleum ether (60^0-80^0)C	Petroleum ether extract (CEPT)	Brown oily material (0.89g)
Chloroform	Chloroform extract (CEC)	Orange brown material (2.01g)
Ethylacelate	Ethylacelate extract (CEEA)	Dark brown material (2.15g)
n-butanol	n-butanol extract (CENB)	Reddish brown material (3.42g)
Residue	Residual aqueous portion (CEAq)	Brownish- black, slightly gummy material (0.61g)

Table 3.1 Solvent extractions of ethanolic extract of the bark of *Anogeissus lieocarpus*

Table 3.2 Preliminary phytochemical screening of the bark of *Anogeissus Lieocatpus*

Constituents	Test	Observation	CE
1. Alkaloids			
	i. Mayer's test	Yellow precipitate	++
	ii. Dragendroff's test	Orange-brown Precipitate	+
	iii. Wagner's test	No brown precipitate	-
	iv. Ammanium	Pink Precipitate	+
2. Flavonoids	i. Shinoda test	Red colour	+++
	ii. Sulfuric acid	Red colour	+++
3. Other polyphenols		Brown Pricipitate	+++
			+++
4. Sterols	i.Libermann-buckard test	Reddish brown ring colour at the bottom of the test tube.	
	ii. Salkowski test	Red colour with formation of two phases	++
	Froth test	Froth formation	+++
5. Saponins	i. Molish test	Purple ring in the interface	++
6. Carbohydrate	ii. Aniline acetate reaction	Red colour	+
	iii. Reducing sugars (felling I & II test)	Red-prick precipitate	+++
	i. Ferric chloride test	A mixture of blue-black and brown green Precipitate	++
7. Tannins			+
8. Free anthraquinones		Pink colour in the aqueous layer	

Key: + = faintly present; ++ = moderately present; +++ = highly present; - = absent

3.3 Thin layer chromatography of crude ethylacetate extract CEEA, fraction C and fraction P.

3.3.1 TLC of CEEA

Solvent systems:

i. Ethylacetate: methanol: water (100:1.67: 13.5)

ii. Ethylacetate: acetic acids: formic acids: water (30:0.8: 1.2:8)

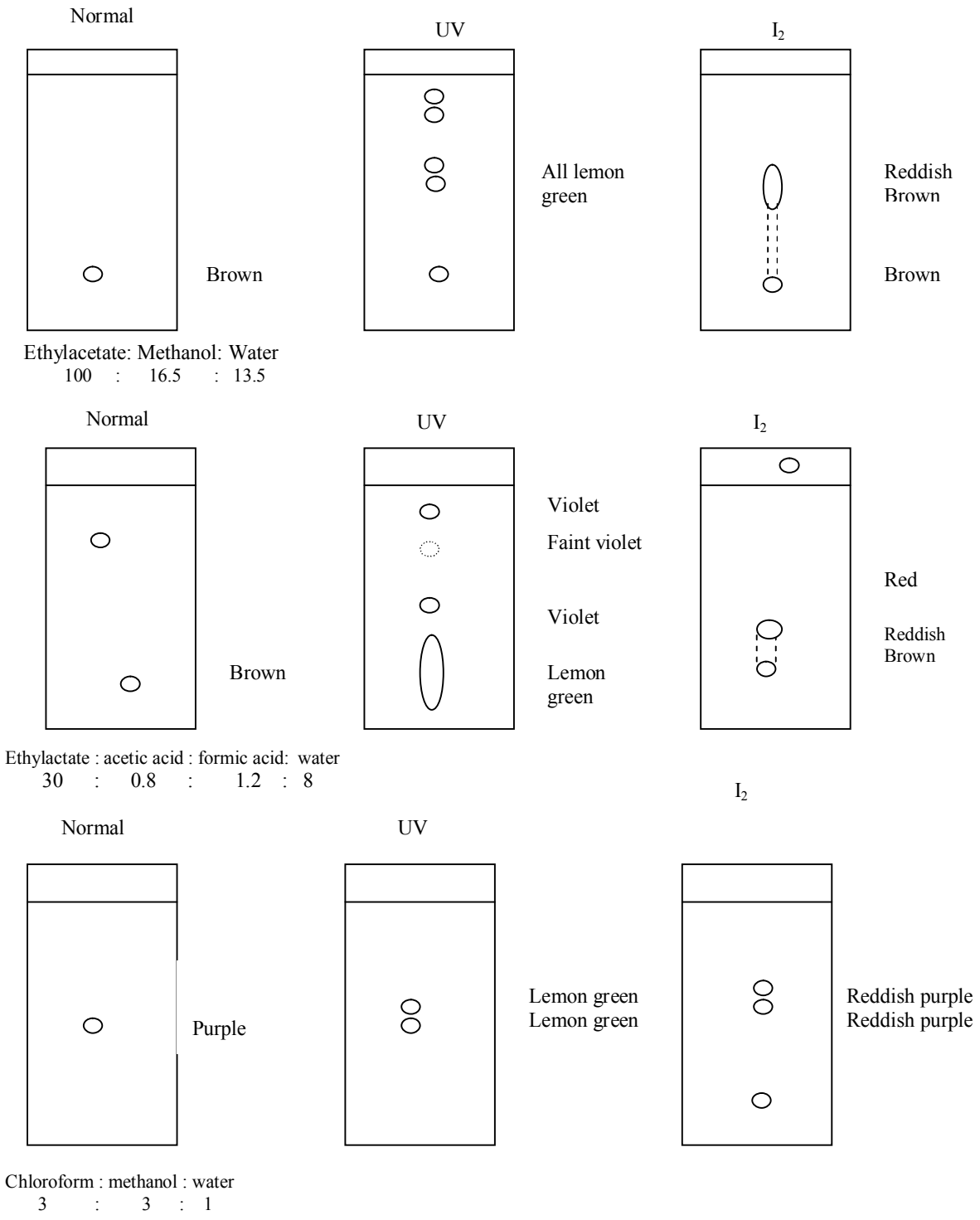
iii. Chloroform: methanol: water (3:3:1)

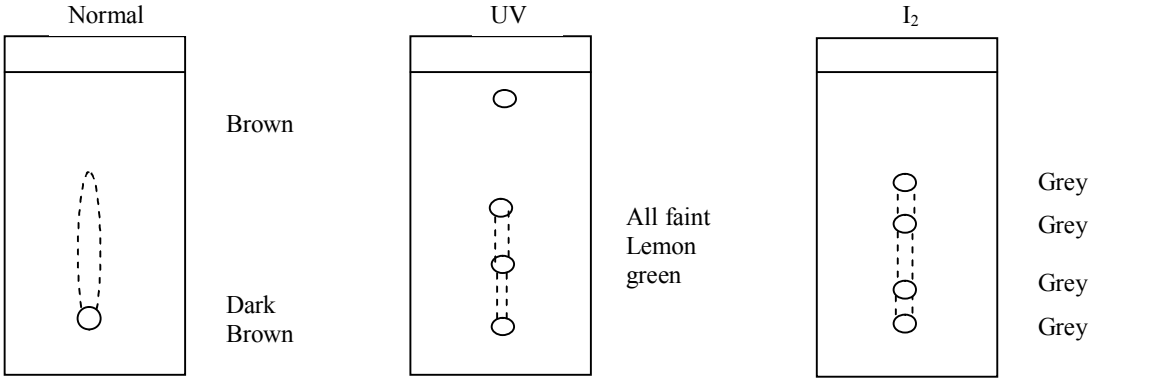
iv. Ethylacetate: formic acid: water (10:1:2)

v. n-butanol : acetic acid: water (4:1:2)

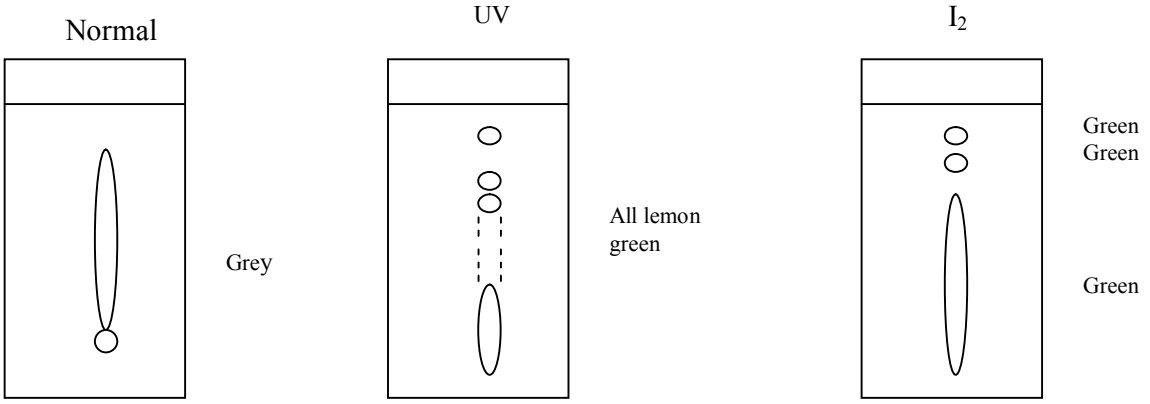
See fig. 3.1

Fig. 3.1 Thin layer chromatography of crude ethylacetate fraction CEEA





Ethylacetate: formic acid: water
10 : 1 : 2



n-butanol: acetic acid: water:
4 : 1 : 2

3.3.2 TLC of Fraction C

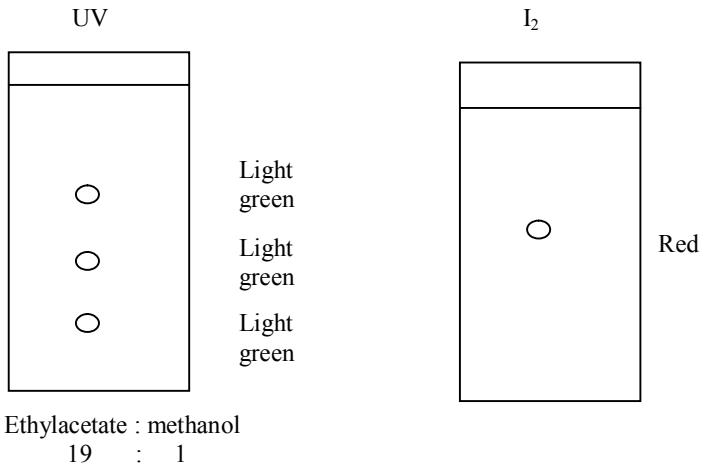
Solvent systems:

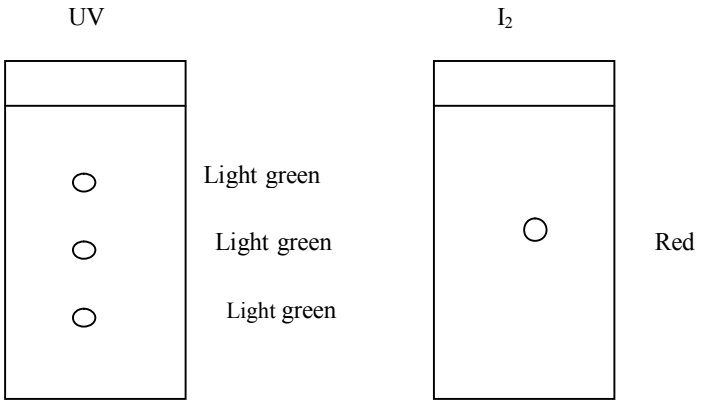
i. Ethylacetate: methanol (19:1)

ii. Chloroform: methanol (19:1)

See fig. 3.2

Fig. 3.2 Thin layer chromatography of fraction C





Chloroform : methanol
19 : 1

3.3.3 TLC of Fraction P

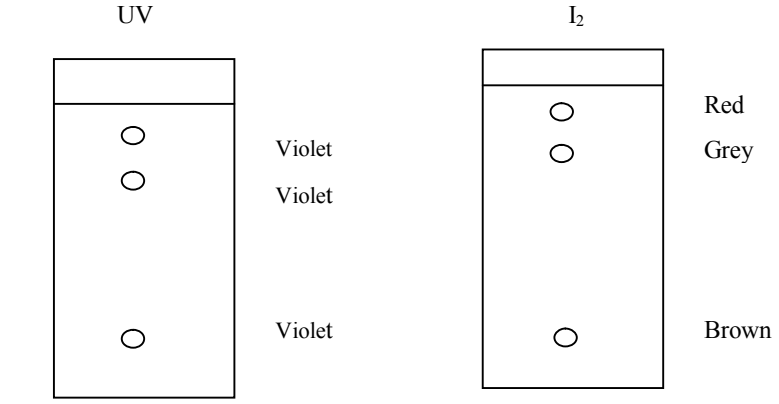
Solvent systems:

i. Ethylacatate: methanol (19:1)

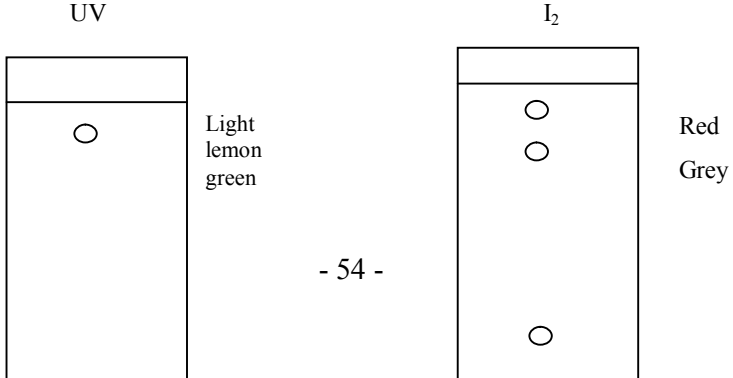
ii. Chloroform : methanol (19:1)

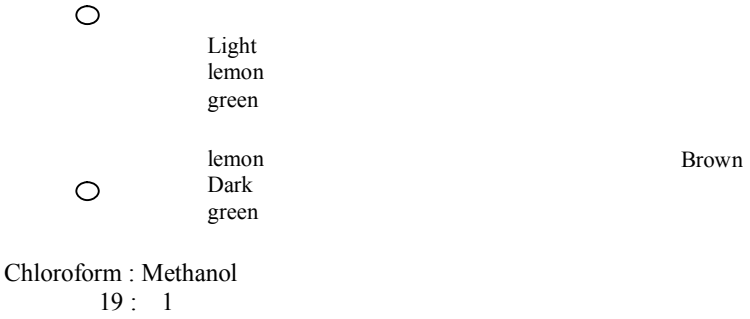
See fig. 3.3

Fig. 3.3 Thin layer chromatography of fraction P



Ethylacetate : Methanol
19 : 1



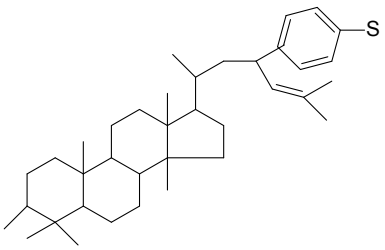


3.4 Gas Chromatography/ Mass Spectroscopy (GC-MS) of fraction C and fraction P

3.4.1 GC-MS of Fraction C

Fraction C, a pale yellow amorphous power, was subjected to GC-MS analysis and a chromatogram of four peaks with the retention time (RT), 20.607min, 22.173 min, 25.779min and 32.254min respectively, was revealed (fig.3.4). The separated compounds were further fragmented by MS and identified by comparing their characteristic fragmented pattern with compound data in the GC-MS computer library.

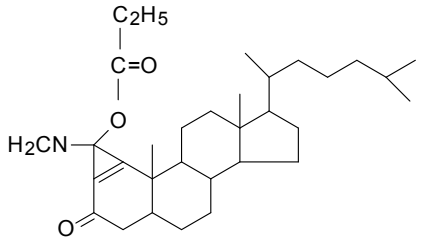
Fig. 3.5 shows the fragmentation peak with ions at m/z 71, 95, 299 (base peak), and 327. this fragmentation pattern was consistent with 17-(1,5-dimethyl-3-phenyl sulfanyl hex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetra deca hydro-1H-cyclopent, ($C_{36}H_{54}OS$) MW 534 (XXXI).



(XXXI)

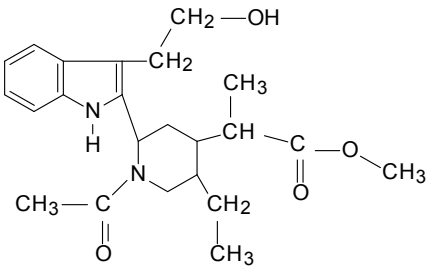
Fig. 3.6 shows a peak with RT 22.173min., which was fragmented to produce peak ions at m/z 71, 377, 355(base peak), and 494 (molecular ion, M^+). This fragmentation

pattern was consistent with 3`H-cyclo prop (1,2)-5-cholest -1-en-3-one, 1`-carboethoxy-1`-cyano-1,2-dihydro, ($C_{32}H_{49}NO_3$), MW 495 (XXXII).



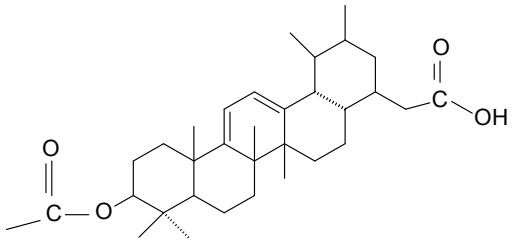
(XXXII)

Fig. 3.7 also shows a peak with RT 25.779min, which was fragmentation to give ions at m/z 81,117, 327 and 355 (base peak), this was consistent with 4-piperidineacetic acid, 1-acetyl -5-ethyl -2-[3-(hydroxyl ethyl)-1H-indole-2-yl], ($C_{23}H_{32}N_2O_4$), MW 400 (XXXIII).



(XXXIII)

Fig. 3.8 shows the last peak in the chromatogram, which in fragmentation gave ions at m/z 81 (base peak), 355, 383, this was consistent with Ursa, 9(11), 12-dien-28-oic acid, 3- (acetyloxy)-methyl ester, (3beta), ($C_{33}H_{50}O_4$), with MW 510 (XXXIV).



(XXXIV)

Fig. 3.4 GC chromatogram of extract C

Fig. 3.5 mass spectrum of compound 1 from fraction c

Fig. 3.6 mass spectrum of compound 2 from fraction c

Fig. 3.7 mass spectrum of compound 3 from fraction c

Fig. 3.8 mass spectrum of compound 4 from fraction c

3.4.2 GC-MS of Fraction P

This fraction, yellowish-grey amorphous powder, was subjected to GC-MS analysis. This fraction gave a chromatogram (fig. 3.9), which revealed ten peaks with retention time (RT), 15.399min, 16.642min, 17.131min, 18.301min., 20.361min, 21.669min, 22.977min, 24.042min, 27.708min and 28.771min respectively. The resolved substances were fragmented by Mass Spectroscopy and identified by comparing the characteristics fragmentation pattern of compounds data in the GC – MS system library.

Fig. 3.10 shows the fragmentation peaks with ions at m/z 83 (base peak), 129, 157, 185, 213 was consistent with the fragmentation patterns of n-Hexadecanoic acid, (C_{16} H_{32} O_2) with MW 256(XXXV).

$$CH_3.(CH_2)_{13}.CH_2.COOH$$

(XXXV)

Fig. 3.11 also shows peak two with RT 16. 642min., which was fragmented to produce ions at m/z 83 (base peak), 97,111, 125, this pattern is consistent with suggested 1- nonadecene, (C_{19} H_{38}), with MW 266 (XXXVI).

$$CH_2=CH.(CH_2)_{16}.CH_3$$

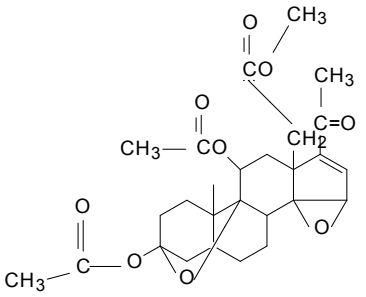
(XXXVI)

Fig. 3.12 shows peak three, RT 17.131min, which was fragmented to produce ions at m/z 69, 83, 97, 111, 129 and this fragmentation was consistent with the suggested Oleic acid, ($C_{18}H_{34}O_2$), with MW 282 (XXXVII).

$$CH_3(CH_2)_7.CH=CH(CH_2)_7.COOH$$

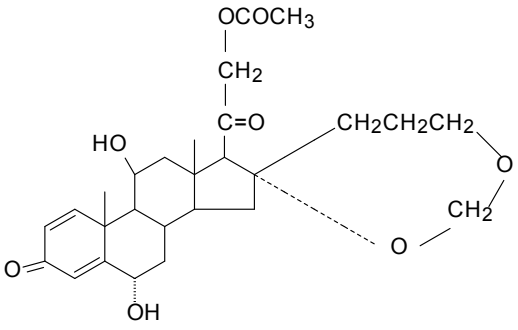
(XXXVII)

Fig. 3.13 shows another peak RT, 18.301min, which was fragmented and the fragmentation pattern showed ions at m/z 83 (base peak), 429. This fragmentation was consistent with that of 3, 9, beta, 14, 15 – Diepoxy preg-16-en-20-one, 3, 11, beta, 18-triacetoxy, ($C_{27}H_{34}O_9$), with MW 502 (XXXVIII).



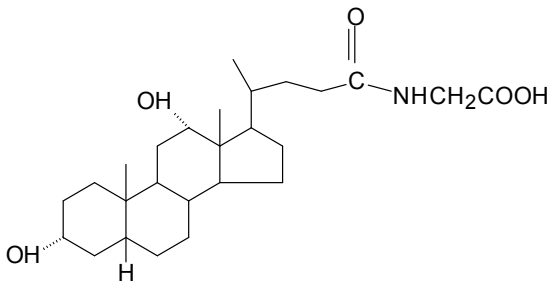
(XXXVIII)

Fig. 3.14 shows peak 5 with RT 20.361min, which was fragmented to produce ions at m/z 83 (base peak), 115, 149, 167 and this fragmentation patterns was consistent with (22S)-21-Acetoxy -6-alpha, 11-beta- dihydroxy-16 alpha, 17 alpha-propylmethylen, ($C_{27}H_{36}O_8$), with MW 488 (XXXIX).



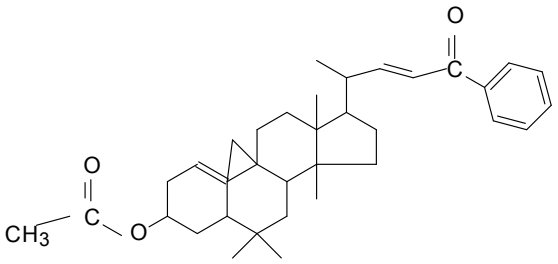
(XXXIX)

Fig 3.15 shows peak 6 with RT 21.669min, which was fragmented to give ions at m/z 83 (base peak), 97, 207, 355, these was consistent with Glycine, N-[(3. alpha, 5.beta, 12.alpha) – 3, 12-dihydroxy-24-oxocholan-24-yl], ($C_{26}H_{43}NO_5$), MW 449 (XL).



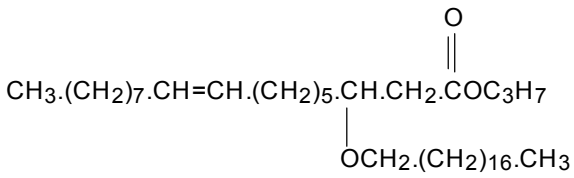
(XL)

Fig. 3.16 shows another peak (RT 22.977min.) which on fragmentation give ions at m/z 81 (base peak), 95, 121, 207. 396, and this fragmentation pattern was consistent with 25- Norisopropyl- 9,19 - cyclo lanostan-22 -en- 24 -one, 3 -acetoxy- 24- phenyl, ($C_{35}H_{48}O_3$), MW 516 (XLI).



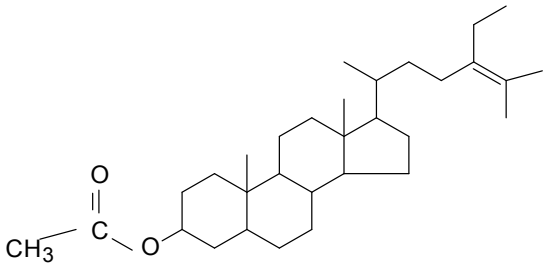
(XLI)

Fig.3.17 shows peak 8 with RT 22.977min. and on fragmentation this peak give ions at m/z 83 (base peak), 129, 207, 396, this fragmentation pattern was consistent with Oleic acid, 3-(octadecyloxy) propyl ester, ($C_{39}H_{76}O_3$), MW 592 (XLII).



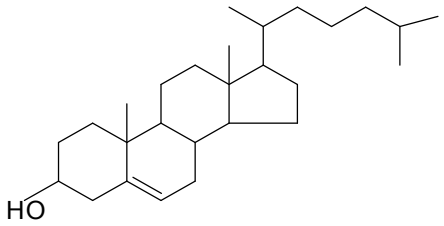
(XLII)

Fig. 3.18 shows peak 9 with RT 27.708min, which was fragmented to produce peak ions at m/z 83, 147, 355, 396 (base peak), this was consistent with beta-Sitosterol acetate, ($C_{31}H_{52}O_2$), MW 456 (XLIII).



(XLIII)

Fig.3.19 shows peak 10 with RT 28.771min, this peak on fragmentation produce peak ions at m/z 83 (base peak), 135, 207, 386 as molecular ions (M^+) and this was consistent with cholesterol, ($C_{27}H_{46}O$), MW 386 (XLIV).



(XLIV)

Fig. 3.9 GC- Chromatogram of fraction P

Fig. 3.10 mass spectrum of compound 1 of fraction P

Fig. 3.11 mass spectrum of compound 2 of fraction P

Fig. 3.12 mass spectrum of compound 3 of fraction P

Fig. 3.13 mass spectrum of compound 4 of fraction P

Fig. 3.14 mass spectrum of compound 5 of fraction P

Fig. 3.15 mass spectrum of compound 6 of fraction P

Fig. 3.16 mass spectrum of compound 7 of fraction P

Fig. 3.17 mass spectrum of compound 8 of fraction P

Fig. 3.18 mass spectrum of compound 9 of fraction P

Fig. 3.19 mass spectrum of compound 10 of fraction P

CHAPTER FOUR

4.0 Discussion

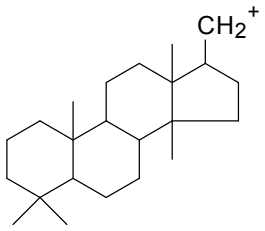
From the preliminary phytochemical tests on the ethanolic extract (CE), the bark of the *Anogeissus lieocarpus* was found to contain among constituents, alkaloids, flavanoids,

polyphenols, sterols, saponins, carbohydrates reducing Sugar, taninns and free anthraquinones. Fraction CE after being "work-up" afforded two fractions, C and P.

GC-MS of fraction C gave four peaks corresponding to four compounds, 1, 2, 3, and 4 in its chromatogram (see fig. 3.4), this revealed the portion to contain four compounds: 17- (1-5-Dimethyl- 3 –phenyl sulfanyl hex -4- enyl) -4,4,10,13,14-pentamethyl -2,3,4,6,7,10,11,12,13,14 ,15,16,17- tetra decahydro-1H – cyclo pent phenanthrene (C_{36} $H_{54}OS$), MW 543; 3`H- cycloprop (1,2)-5-cholest-1-en-3-one, 1`-carboethoxy-1`- cyano-1,2-dihydro, (C_{32} H_{49} NO_3), MW 495 ; 4- Piperidine acetic acid, 1-acetyl-5-ethyl –2- (3- (2-hydroxy ethyl) –1H- Indol –2-yl) – alpha –methyl-methyl ester, (C_{23} H_{32} N_2O_4), MW 400 and Ursa-9 (11), 12-die-28-oic acid, 3-acety 10xy) –methyl ester, (3 beta), (C_3 H_{50} O_4), MW 510. These were identified from their characteristic fragmentation patterns.

Fig. 3.5 showed the fragmentation pattern of peak one (compound 1). With retention time 20.607 min. This mass spectrum shows peaks due to ions at m/z. 466, 327, 299 (100%), 155, 95 and 71, which corresponds to 17-(1,5) Dimethyl–3-phenyl sulfanyl hex –4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17- tetra decahydro-1H-cyclo penta phenanathrene.

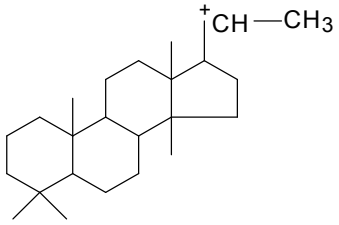
The most abundant peak (base peak) was obtained due to the cleavage of the hexenyl chain at C_1 position with the branched methyl group leaving also, the hydroxyl group-(OH) leave the molecule as water, molecule which leaves the cyclo penta phenanthrene group of m/z 299 as the base peak (XLV)



(XLV)

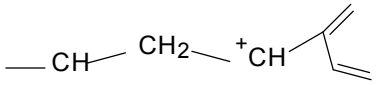
(See appendix 1.1)

From the mass spectrum of compound 1, a signal at m/z 327 is a result of the removal of hydroxyl group as water molecule and the hexenyl chain cleavage at the C_2 position (XLVI) leaving the 1-methyl group attached to the phenenthrene structure as:



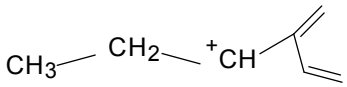
(XLVI)

Another peak at m/z 95 is a result of fission of the pheyl sulfanyl branch in the hexenyl and subsequent cleavage at C_1 in the hexenyl chain with the methyl branch bond also being broken, in addition of cleavage in C_4 of the hexenyl chain to give (XLVII)



(XLVII)

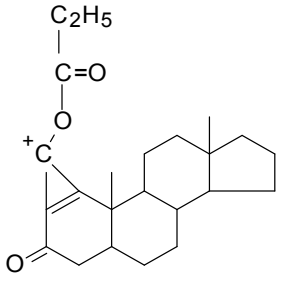
which is in addition of two hydrogen atoms, $H^+/2$ gives a signal (XLVIII)



(XLVIII)

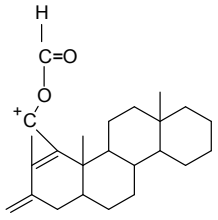
at m/z 95 (see appendix 1.1).

Compound 2, which appear as peak two after 22.173 min. gives m/z 495 as a molecular ion peak, M^+, 496 as M^++1, 383, 355 (100%), 327, 211, 95, which is consistent with 3`H-cycloprop (1,2)-5 cholest –1-en-3-one, 1`-carboethoxy-1`-cyano-1,2-dihydro ( See fig 3.6). The compound gave a signal at m/z 355 is a result of cleavage of 1`-cyano group leaving the carbon atom with a positive charge (+ve), i.e. deficiency in electron, and cleavage of the hydrocarbon alkane chain C_{19} of the cyclo pent of the cholestenone giving (XLIX):



(XLIX)

A prominent peak at m/z 327 results from also cleavage of the cyano group leaving the carbon atom with a deficiency of electron, and cleavage of C_{19} aliphatic chain in addition in cleavage of ethyl group giving (L):

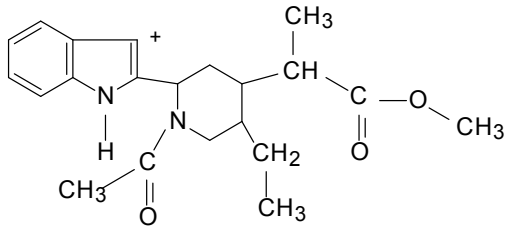


(L)

(See appendix 1.2)

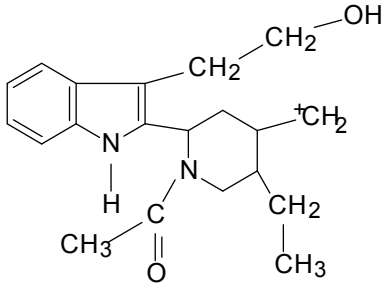
Fragmentation patterns of peaks three (compound 3) with retention time 25.779 min. and m/z 523, 383, 355 (100%), 327, 262, 81 is consists with fragmentation pattern of 4-piperidine acetic acid, 1-acetyl-5-ethyl-2- [3-(2-hydroxy ethyl)- 1H-indole-2-yl] –alpha-

methyl-, methyl ester, (C_{23} H_{32} N_2 O_4), MW 400 (see fig 3.7). This fragmentation pattern gave base peak at m/z 355 which consist with M–45 (cleavage of the hydroxy ethyl chain attached to the indole group leaving the cation) (LI):



(LI)

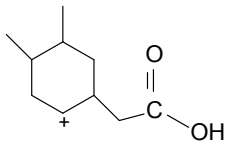
A signal at m/z is plausibly as a result of 327 is a result of M-73, which was due to cleavage of the methyl ester and the alpha methyl branch in the piperidine (and in addition of hydrogen atom, H^+/1 gives) (LII):



(LII)

(See Appendix 1.3)

Compound 4 was identified as Ursa, 9 (11), 12-dien-28-oic acid, 3 (acetyloxy)-, methyl ester, (3,beta)., (C_{33} H_{50} O_4), MW 510, RT 32.254min. (See fig 3.8). The spectrum of this compound showed base peak at m/z 355 as a result of M-155 i.e fission of the bond between C_{16} – C_{17} and C_{13}-C_{18} of the cyclohexane ring to give the cation (LIII) as shown in appendix 1.4



(LIII)

GC-MS of fraction P gave ten peaks in its chromatogram, as shown in fig 3.9. This revealed ten compounds, which were identified from their characteristics fragmentation patterns.

Peak 1, which appeared after 15.399 min., was fragmented and the mass spectrum produced a base peak at m/z 43 (100%), which is due to the cation (LIV)

$$CH_3CH_2CH_2^+$$

(LIV)

Other peaks such as m/z 256 (M^+), 213, 185, 171, 157, 129 and 83 were produced due to ions as shown in fig 3.10.

Signal at m/z 256 is the molecular ion, M^+, while m/z 213 is a result of loss of propyl group from the parent molecule. The signals at m/z 185, 157, 129 are a result of loss of ethyl group from previous fragments, respectively starting from peak 213 as shown in appendix 1.5.

Signal at m/z 83 was obtained as a result of Mclefferty rearrangement of the long aliphatic chain to give (LV)

$$CH_3\text{-}CH_2\text{-}\ CH_2\text{-}\ CH_2\text{-}\ CH_2\text{-}\ C^+H_2 \quad \text{,m/z 85.}$$

(LV)

This in reaching its allylic resonance stabilization losses two hydrogen radicals to give (LVI)

$$CH_3\text{-}\ CH_2\text{-}\ CH_2\text{-}\ CH_2\text{-}\ CH = C^+H, \text{ m/z 83}$$

(LVI)

(See appendix 1.5).

Peak 2 with retention time 16.64 min, have ion peaks of 83 (100%), 97, 111 and 125 which corresponds to 1-Nonadecene as shown in fig 3.11. m/z 125 is obtained by the cleavage of the alkene chain and loss of $-(CH_2)_9 CH_3$ to give (LVII)

$$CH_2 = CH- CH_2- CH_2- CH_2-CH_2-CH_2-CH_2-C^+H_2.$$

(LVII)

Loss of methylene radicals successively yield (LVIII), (LIX) and (LX) respectively

$$CH_2 = CH- CH_2-(CH_2)_4 - C^+H_2 \quad ,m/z\ 111$$

(LVIII)

$$CH_2 = CH- CH_2-(CH_2)_3- C^+H_2 \qquad ,m/z\ 97$$

(LIX)

And $$CH_2 = CH- CH_2-CH_2-CH_2-C^+H_2 \quad ,m/z\ 83(100)$$

(LX)

as shown in appendix 1.6

Peak 3, retention time 17.131 min., produce a mass spectrum with ion peaks at m/z 282 (M^+), 256, 129, 111, 97,83, 69, 55 and 41, which corresponds to Oleic acid ($C_{18}H_{34}O_2$), MW 282 (see fig 3.12).

The resultant signal at m/z 111 was obtained by cleavage at the double bond, MC lafferty rearrangement and hydride shift with the methyl group leaving as a radical to give the catrion (LXI)

$$CH_3-(CH_2)_5-CH =^+CH$$

(LXI)

The signal at m/z 97, 83, 69, 55 and 41 (base peak) were obtained by loss of methyl group as a radical and a hydride shift to give the cations (LXII), (LXIII), (LXIV), (LXV), (LXVI) ,respectively as shown below

CH_3-$(CH_2)_4$-CH=$^+$CH ,m/z 97

(LXII)

CH_3-$(CH_2)_3$-CH=$^+$CH ,m/z 83

(LXIII)

CH_3-$(CH_2)_2$-CH=$^+$CH ,m/z 69

(LXIV)

CH_3-CH_2-CH=$^+$CH ,m/z 55

(LXV)

CH_3-CH=$^+$CH ,m/z 41 (100)

(LXVI)

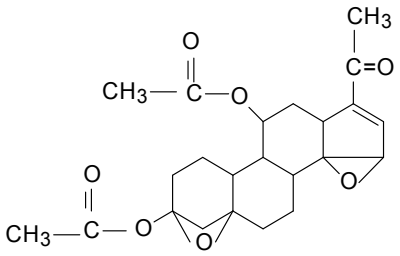
A peak at m/z 129 was obtained from M-153 or a cleavage of the aliphatic chain to give (LXVII)

$^+CH_2$-$(CH_2)_5$-COOH

(LXVII)

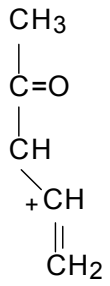
See Appendix 1.7

Fig 3.13 shows the mass spectrum of peak 4 with retention time 18.301 min. produced signals at 503, 429, 221, 147 and 83 (100%), which consistent with the fragmentation pattern of 3,9, β-14,15-triepoxy pregn-16-en-20-one, 3, 11, B-18-tri acetoxy, ($C_4H_{34}O_9$), MW 502. This showed a signal at m/z 429 as a result of M-73 (M-CH_2OCOCH_3) (acetate chain) cleavage (LXVIII).



(LXIX)

Signal at m/z 83 as the base peak is due to the cleavage of the epoxy cyclo pentene and in addition two hydrogen atoms, $H^+/2$ gives (LXIX):



(LXIX)

(See Appendix 1.8)

Peak 5, retention time 20.361 min, produce a signal due to ion at m/z 355, 207, 167, 149 and 83 (100%), consistent with (22S)-21-acetoxy-6-alpha, 11. beta- dihydroxy-16 alpha.,

17. alpha-propyl methylene dioxy pregna-1,4- diene-3, 20-dione ($C_{27}H_{36}O_8$), MW 488 (see fig 3.14). The base peak was obtained due to cleavage of the propyl methlene dioxy chain, fission of acetoxyl group and cleavage of the cyclo pentane ring at C_{17} and C_{15} forming a double bond between C_{16} and C_{15} to give (LXX)

$^{+}CH_2$-CO-CH = CH-CH_3 the stable m/z 83

(LXX)

(See appendix 1.9)

Peak six, retention time 21.669 min., produced mass spectrum with signals at m/z 550, 385, 281, 207 and 83 (100%). This compound was identified as Glycine, N-[(3.alpha., 5. beta., 12.alpha)-3,12-dihydroxy-24- oxocholan-24-yl], (C_{26} $H_{43}O_5$), MW 449 from its fragmentation pattern as shown in fig 3.15. The base peak, m/z 83 was obtained as a result of the carboxyl group leaving the oxocholan and a formation of a double bond being formed between the nitrogen and carbon atoms in addition to a cleavage of the bond between C_{20}-C_{22} and formation of a double bond between C_{22}-C_{23} with two hydrogen radicals, $H^{\cdot}/2$ leaving to give (LXXI)

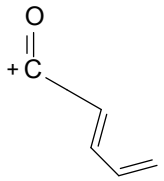
CH_2 = CH-CO-$N^{\cdot +}$=CH_2

(LXXI)

(See appendix 1.10).

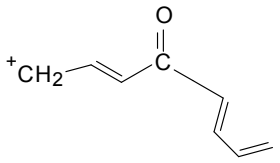
Fig 3.16 shows the mass spectrum of peak 7 (RT, 22.977) with fragment ion peaks at m/z. 429, 281, 207, 121, 95 and 81 (100%), this is consistent with of fragmentation pattern of 25- Norisopropyl- 9, 19- cyclolanostan –22-en-24-one, 3-acetoxy-24-phenyl ($C_{35}H_{48}O_3$), MW 516.

Signal at m/z 81 was observed as the most abundant peak (base peak) and was due to fission on the phenyl ring and cleavage after the carbonyl group in addition to three hydrogen atoms, $H^+/3$ to yield (LXXII):



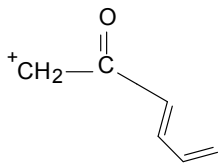
(LXXII)

Signal at m/z 121 corresponds also to ion form fission on the phenyl ring and cleavage of the aliphatic chain with the methyl group branch being also broken in addition to three hydrogen atoms, $H^+/3$ to give (LXXIII):



(LXXIII)

Signal at m/z 95 is from an ion formed as a result of fission on the phenyl ring and cleavage at the double bond of the aliphalic chain in addition to three hydrogen atoms, $H^+/3$, to yield (LXXIV):

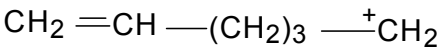


(LXXIV)

(See appendix 1.11)

Peak 8 (RT, 24.042 min.), gave mass spectrum mass with ion peaks at 396, 281, 207, 129 and 83 (100%) with retention time 24.042min., this fragmenantation pattern was consistent with Oleic acid, 3-(octadecyloxy) propylester, ($C_{39}H_{76}O_3$), MW 592 as shown in fig 3.17.

The resultant base peak at m/z 83 was obtained by Mc lefferty rearrangement, from cleavage of the double bond between C_9-C_{10}, formation of a double bond between C_9-C_8 and cleavage between C_3-C_4, in addition of hydrogen atom,H^+/1 to give (LXXV):

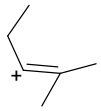


(LXXV)

(See Appendix 1.12)

Fig 3.18 shows the chromatogram where peak of compound 9 with retention time 27.708 min. This compound gave a mass spectrum with signals of ions at m/z 396 (100%), 355, 255, 185, 147 and 83. this consists with beta Sitosterol acetate.

The signal at m/z 83 was due to the cleavage at the quaternary carbon (4^o) of the aliphatic chain from the parent molecule to give the most stable carbonium ion which is the most abundant ion and present itself as the base peak (LXXVI):



(LXXVI)

(See appendix 1.13).

Peak 10 with retention time 28.771 min. gave a mass spectrum with signals at m/z 386 as the molecular ion, M^+, 301, 207, 135, 83(100%). This fragmentation pattern is consistent with cholesterol ($C_{27}H_{46}O$), MW 386 (see fig 3.17).

Signal at m/z 386 appears as the molecular ion peak, M^+ and the m/z 83 is obtained from cleavage of the long alkene chain in the parent structure forming a double bond at the cleaved carbon atom with the two branching methyl group leaving as radicals to give (LXXVII) which is the most abundant ions and represent the base peak (See appendix 1.14).

$$CH_2 = CH\text{-}CH_2\text{-}CH_2\text{-}C^+H\text{-}CH_3$$

(LXXVII)

4.1 Conclusion

From the phytochemical screening it can be concluded that the bark of *Anogeissus lieocarpus* contains alkaloids, flavonoids, other polyphenols, sterols, saponins, carbohydrates, tannins and free anthraquinones.

From ethylacetate fraction, fraction C and fraction P were isolated, which on separation by GC-MS fraction C was found to contain alkaloids such as 4-piperidineacetic acid, 1-acetyl -5-ethyl -2-[3-(hydroxyl ethyl)-1H-indole-2-yl]-alpha-methyl-methyl ester; and steroids such as 17-(1,5-dimethyl-3-phenyl sulfanyl hex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetra deca hydro-1H-cyclopent phenanthrene; 3`H-cyclo prop (1,2)-5-cholest -1-en-3-one, 1`-carboethoxy-1`-cyano-1,2-dihydro and Ursa, 9(11), 12-dien-28-oic acid, 3- (acetyloxy)-methyl ester, (3beta), while fraction P was found to contain fatty acids such as n-hexadecanoic acid; 1-nonadecene; Oleic acid and Oleic acid, 3- (octadecyloxy) propyl ester; and steroids such as 3, 9, beta, 14, 15 – Diepoxy preg-16-en-20-one, 3,11, beta, 18-triacetoxy; (22S)-21-Acetoxy -6-alpha, 11-beta- dihydroxy-16 alpha, 17 -propylmethylene, dioxy-pregna-1,4-diene-3,20-dione Glycine, N-[(3. alpha, 5.beta, 12.alpha) – 3,12-dihydroxy-24-oxocholan-24-yl]; 25-Norisopropyl-9,19-cyclo lanostan-22-en-24-one,3-acetoxy-24-phenyl; Beta-Sisterol acetate and cholesterol.

Further work needed to be carried out to authenticate the basis for the traditional claims of this plant.

Although earlier reports indicated the absence of alkaloids in the plant, alkaloids has been tested present and been isolated from the bark of the plant.

Only coumarins and some inorganic elements have been reported to be isolated from the plant while in this work, alkaloids, fatty acids and steroids have been successively isolated from the bark of the plant.

CHAPTER FIVE

5.0 Pharmacological Screening

5.1 Aim and Objectives of the Studies

The aim of this study is to assay pharmacologically the local use of the plant *Anogeissus lieocarpus* in relieving gastrointestinal complaints by studying the effect of the ethanolic extract (CE) of the bark of the plant *in vitro* on isolated guinea pig ileum.

5.2 Material

5.2.1 Animals

Locally bred guinea pig (456g) was maintained at animal house, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria.

5.2.2 Apparatus

(i) Recording microdynanometer and transducer model No. 7050, (Ugo Basile Italy).

(ii) Thermostatically controlled tissue bath (20ml).

(iii) Dissecting kit.

5.2.3 Standard Drugs and Sample Used

(i) Acetylcholine (2μg/ml)

(ii) Histamine (100μg/ml)

(iii) Adrenaline (20μg/ml)

(iv) Ethanolic extract (25mg/ml)

(v) Tyrode Solution

All drugs and sample solution were freshly prepared.

5.2.4 Isolated Tissue

Guinea pig ileum.

5.3 Experimental Methods

5.3.1 Studies of Gastrointestinal Smooth Muscles on Isolated Guinea Pig Ileum.

After starving for 24 hrs, the animal was sacrificed by a blow on the head, dissected and segments of 2.8 on the ileum were dissected free from adhering mesentery in a petri dish containing tyrode solution aerated by electrical pump. His tissue was then mounted in a 25ml organ bath containing-tyrode solution gassed with air and maintained at 37^0C and allowed to stabilize for 30 min before use. Dose response for acetylcholine (2µg/ml) was investigated. Washing the tissue with the physiological solution three times before the next dose was added follows each addition.

Dose response for histamine (100µg/ml) and adrenaline (20µg/ml) were also investigated after allowing the tissue to rest for 3 minutes before addition, followed by washing the tissue three times with the physiological solution.

The effect of the extract (25mg/ml) was also investigated after allowing the tissue to rest for 15 minutes sub maximal dose of acetylcholine (0.2ml of 2µg/ml) was used to observe the effect of the extract on the response of the dose of histamine (0.4ml of 100µg/ml) was also observed.

Responses were recorded on an Ugo Basile Unirecorder.

5.4 Results

Table 5.1 Effect of Acetylcholine on Isolated Guinea Pig Ileum

Response (cm)	Concentration (µg/ml)	% Response	Log dose
3.3	8×10^{-3}	68.75	- 2.096
5.1	16×10^{-3}	100.00	- 1.795
4.8	32×10^{-3}	94.11	- 1.494

Table 5.2 Effect of Histamine on Isolated Guinea Pig Ileum

Response (cm)	Concentration (µg/ml)	% Response	Log dose
2.6	400×10^{-3}	44.82	- 0.397
5.3	800×10^{-3}	91.37	- 0.096
5.8	1.6×10^{-3}	100.00	0.204
5.6	3200×10^{-3}	96.55	0.505

Table 5.3 Effect of Ethanolic Extract on Isolated Guinea Pig Ileum

Response (cm)	Concentration (mg/ml)	% Response	Log dose
- 0.5	200×10^{-3}	100.00	- 0.698
- 0.4	400×10^{-3}	80.00	- 0.397
- 0.4	800×10^{-3}	80.00	- 0.096

Table 5.4 Effect of the Extract on Induced Acetylcholine Contraction on Isolated Guinea Pig Ileum

Response (cm)	Concentration (µg/ml)	% Response	Log dose
1.4	8×10^{-3}	93.33	- 2.096
1.5	16×10^{-3}	100.00	- 1.795
1.3	32×10^{-3}	86.66	- 1.494
1.2	64×10^{-3}	80.00	- 1.193
1.0	128×10^{-3}	66.66	- 0.892

Table 5.5 Effect of the Extract on Induced Histamine Contraction on Isolated Guinea Pig Ileum

Response (cm)	Concentration (µg/ml)	% Response	Log dose
1.1	100×10^{-3}	100.00	- 1.000

1.0	200×10^{-3}	90.90	- 0.698
0.3	400×10^{-3}	27.27	- 0.397
0.2	800×10^{-3}	18.18	- 0.096
0	1600×10^{-3}	0	- 0.204

Fig. 5.1 Effect of acetylcholine on isolated guinea-pig ileum

Fig. 5.2 Effect of histamine on isolated guinea-pig ileum

Fig. 5.3 Effect of ethanolic extract on isolated guinea-pig ileum

Fig. 5.4 Effect of ethanolic extract on acetylcholine-induced contractions on isolated guinea-pig ileum

Fig. 5.5 Effect of ethanolic extract on histamine-induced contractions on isolated guinea-pig ileum

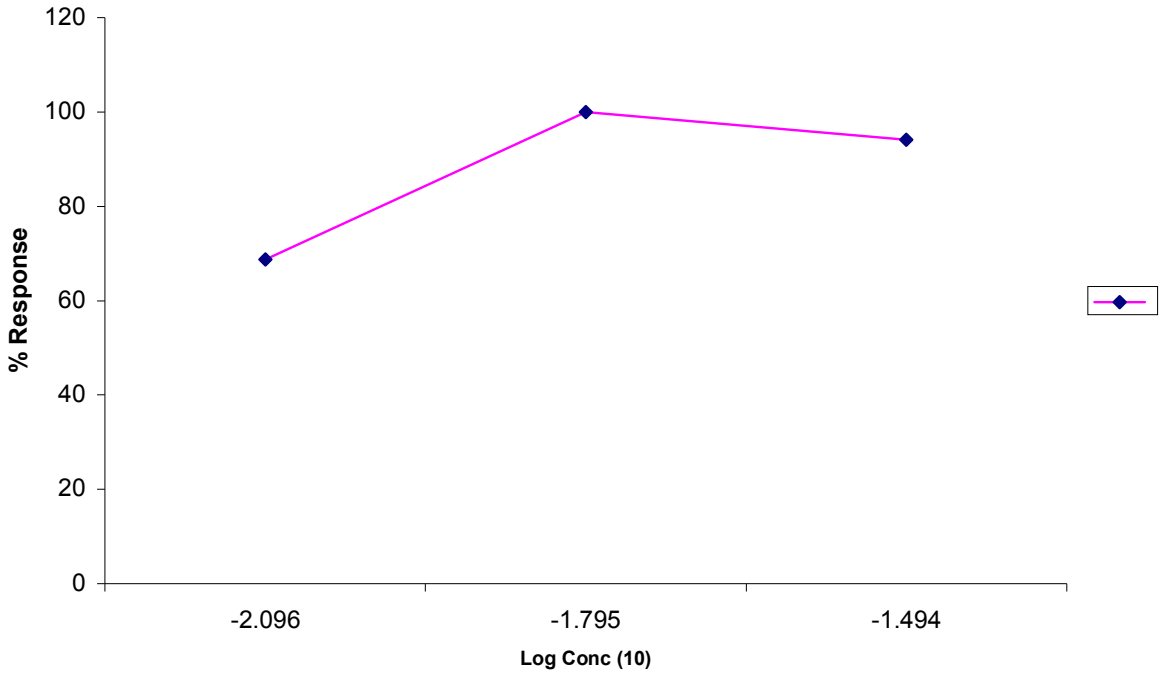


Fig. 5.6 Effects of acetylcholine on isolated guinea-pig ileum

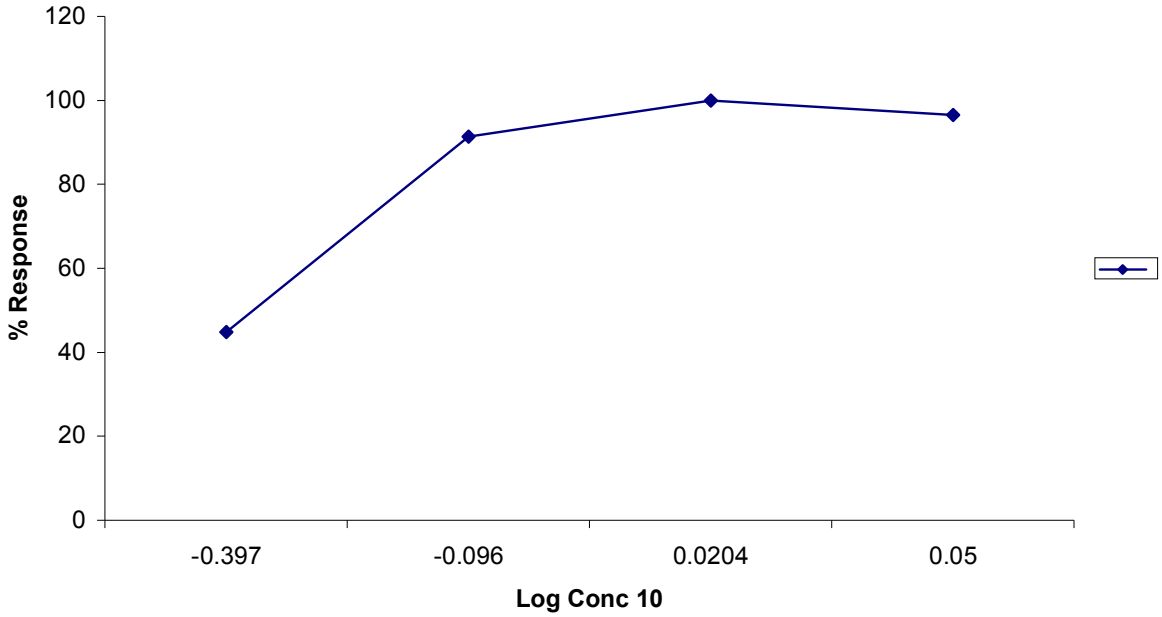


Fig. 5.7 Effects of histamine on isolated guinea-pig ileum

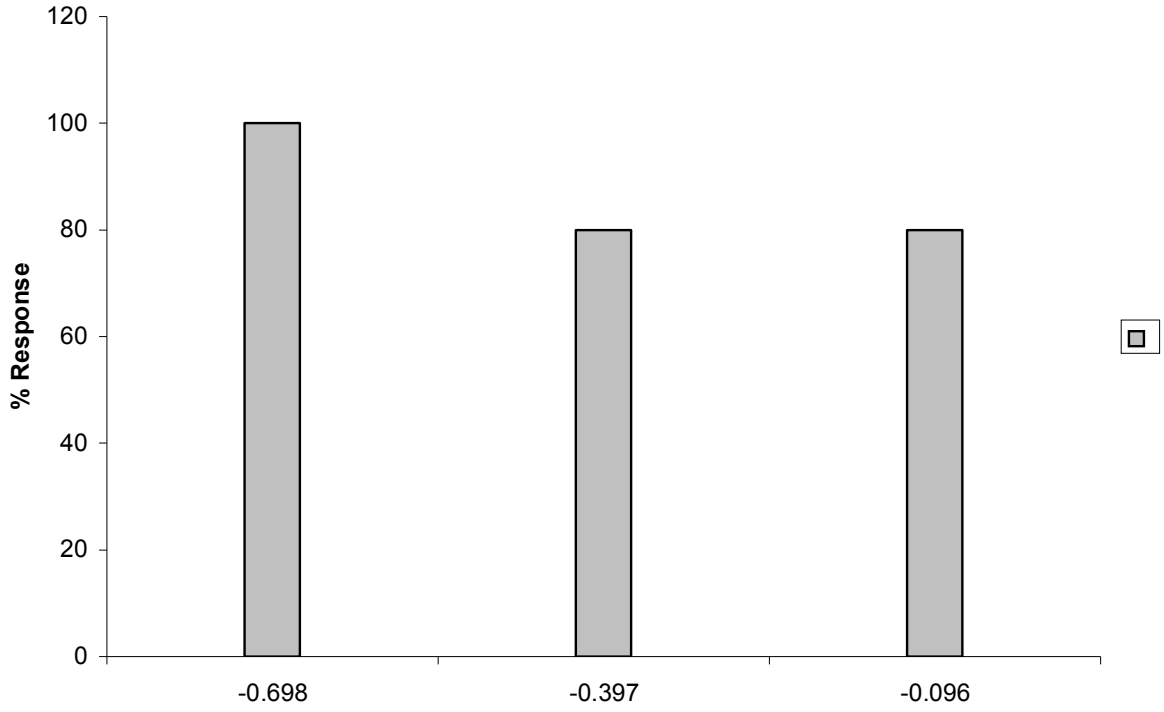


Fig. 5.8 Effects of ethanolic extract on isolated guinea-pig ileum

Fig. 5.4 Effect of the extraction on induced acetylecholine contraction on Guinea Pig Ileum

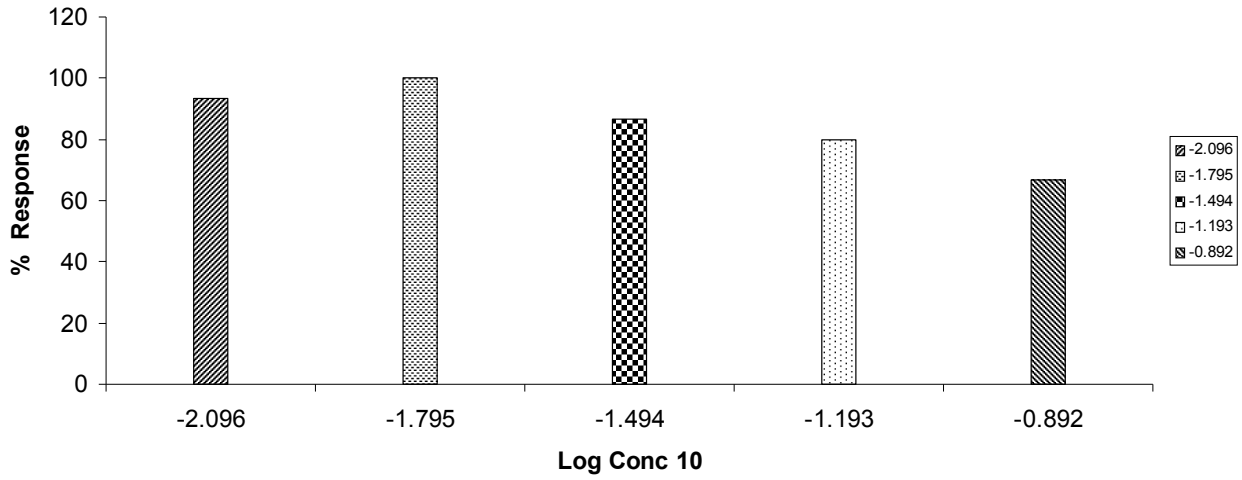


Fig. 5.9 Effects of the ethanolic extract on induced acetylcholine contraction on isolated guinea-pig ileum

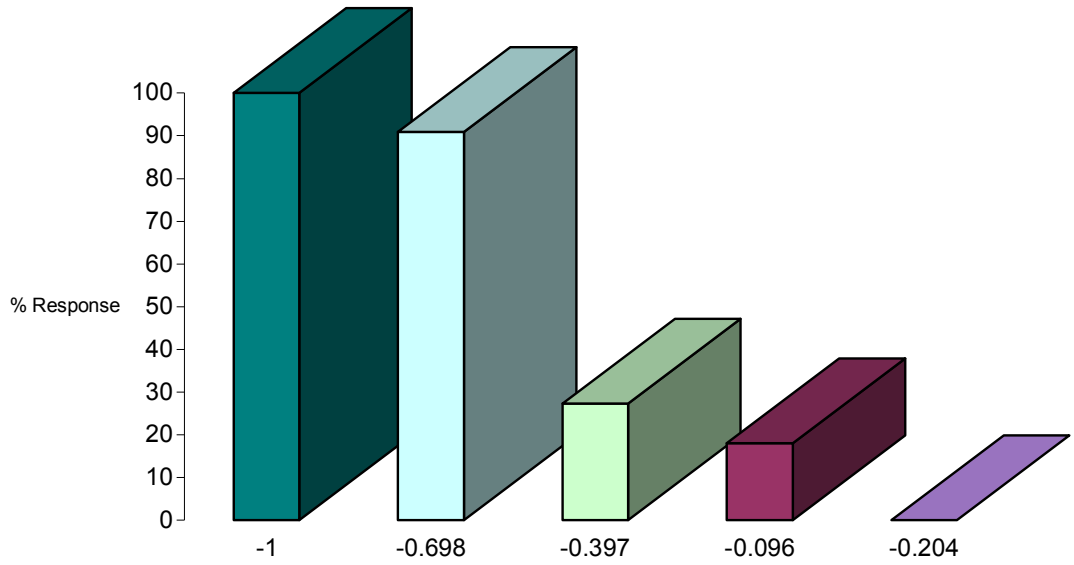


Fig. 5.10 Effects of the ethanolic extract on induced histamine contraction on isolated guinea-pig ileum

5.5 DISCUSSION

5.5.1 Effect of the ethanolic extract (CE) on the cholinergic activity of guinea pig ileum

Acetylcholine causes contraction of smooth muscles of guinea pig ileum through the muscarinic activity. Acetylcholine showed a dose-dependent contraction of guinea pig ileum (fig 5.1), in contrast with the ethanolic extract of the plant, which shows a dose-dependent relaxation of the guinea pug ileum (fig 5.4). The ethanolic extract (CE) showed an antagonis property of Acetylcholine induced contraction of isolated guinea pig ileum (fig 5.5). The maximum contraction of CE that completely blocks the Acetylcholine induced contraction was found to be 128×10^{-3} µg/ml (Table 5.4)

5.5.2 Effect of the ethanolic extract (CE) on the histaminic activity of guinea pig ileum

Histamine causes contraction of smooth muscles through its action on histamine receptors. The guinea pig ileum is very sensitive to histamine due to abundance of histamine receptors, thus it is usually used to screen drugs or extract for antihistaminic activity.

Histamine showed a dose-dependant contraction on guinea pig ileum (fig 5.2), while the ethanolic extract (CE), relaxed the smooth muscles of the guinea pig ileum (fig 5.4). The extract CE antagonized histamine induced contraction of isolated guinea pig ileum fig (5.6) and completely, blocks the histamine induced contraction at 800×10^{-3} µg/ml as a maximum concentration (Table 5.5).

The anti-cholinergic and anti-histaminic activity of the bark of *Anogeisus lieocarpus* can be the basis for it's used by traditional healers as a remedy for abdominal pain, cough, chest pain and its itching rashes.

5.6 Conclusion

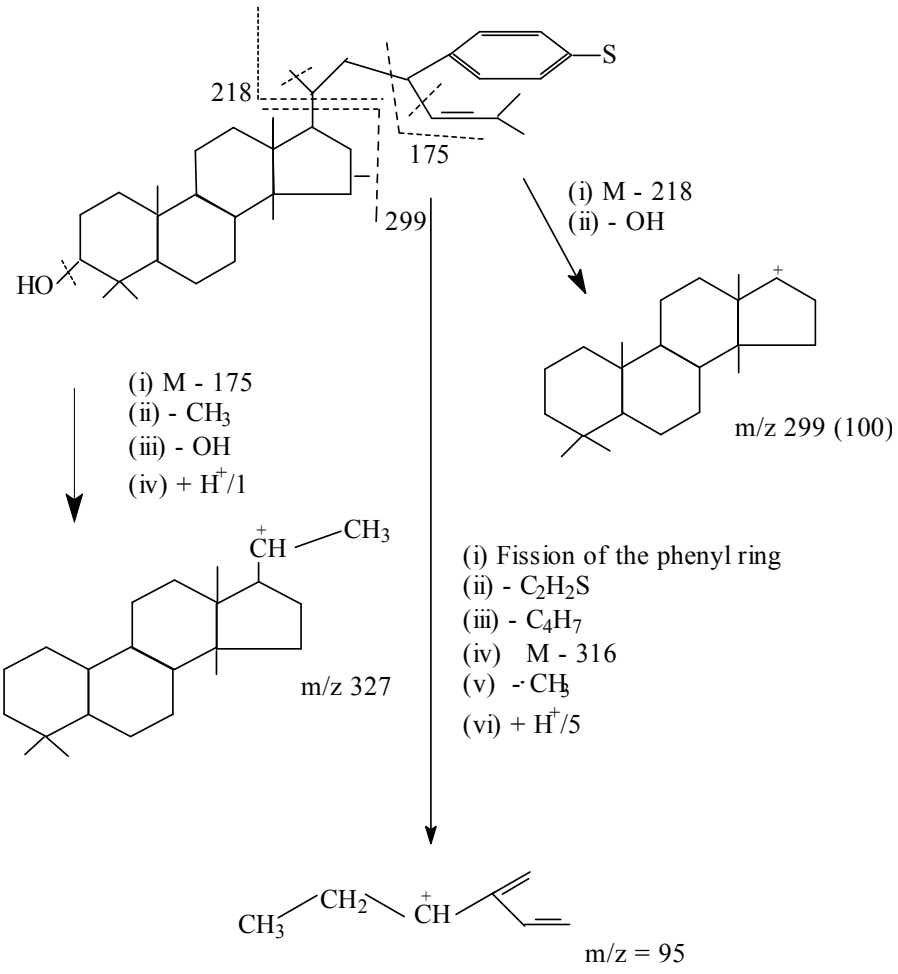
The pharmacological screening has revealed that the ethanolic extract of the bark of Anogeissus lieocarpus (combretacea), has anticholinergic activity. The anti histaminic effect of the extract can explain the use of Anogeissus lieocarpus bark for coughs, chest pain and skim diseases such as rashes.

Appendix 1.1

Fragmentation pattern of peak one from fraction C

17-(1,5-dimethyl-3-phenyl sulfanyl hex-4- enyl) 4,4,10,13,14- pentam - 2,3,4,5,6,7,10,11,12,13,14,15,16,17- tetradeca hydro -1H-cyclo pent phenanthrene.

$(C_{36}H_{54}OS)$, MW = 534



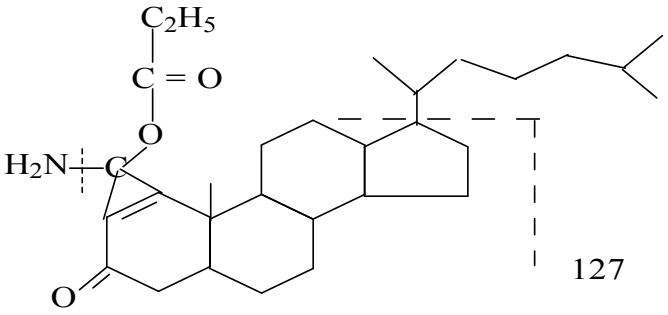
Appendix 1.2

Fragmentation pattern of peak two of fraction C

3' H-cycloprop(1,2)-5-cholest-1-en-3-one, 1'-carboetho

1'-cyano-1,2-dihydro

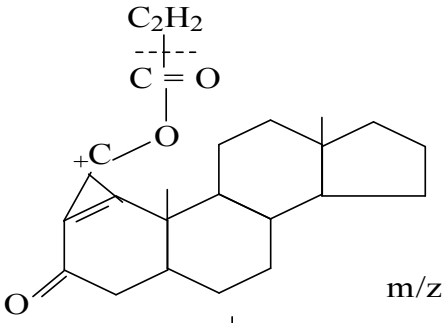
$(CH_{32}H_{49}NO_3)$, MW = 495



(1) [M]+ = m/z 495

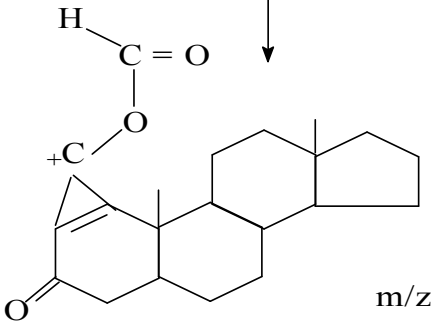
(2) [M+1] = m/z 496

(i) M - 127
(ii) $-NH_2$
(iii) $+ H^+/_1$



m/z = 355(100)

$-CH_2$
$+H^+/_1$



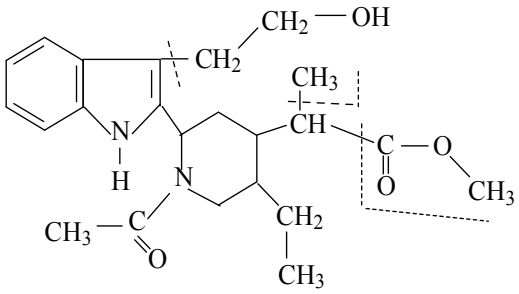
m/z = 327

Appendix 1.3

Fragmentation pattern of peak three of fraction C

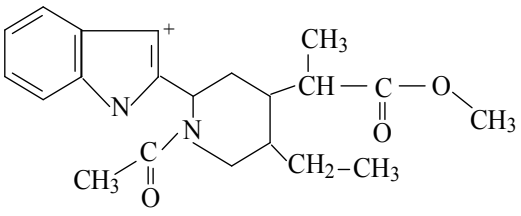
4 - Piperidine acetic acid, 1 - acetyl - 5 - ethyl - 2 - [3 - (2 - hydroxy ethyl) - 1H - indole - 2 - yl] - alpha - methyl - methyl ester

(C_{23} H_{32} N_2 O_4), MW = 400

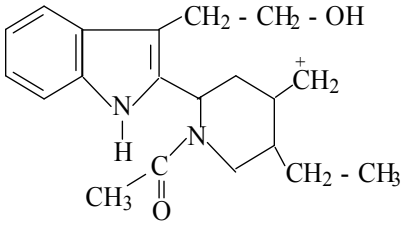


(i) M - C_2 H_4 OH

(i) - $OCOCH_3$
(ii) - $^{\cdot}CH_3$
(iii) + H^+/1



m/z = 355 (100)



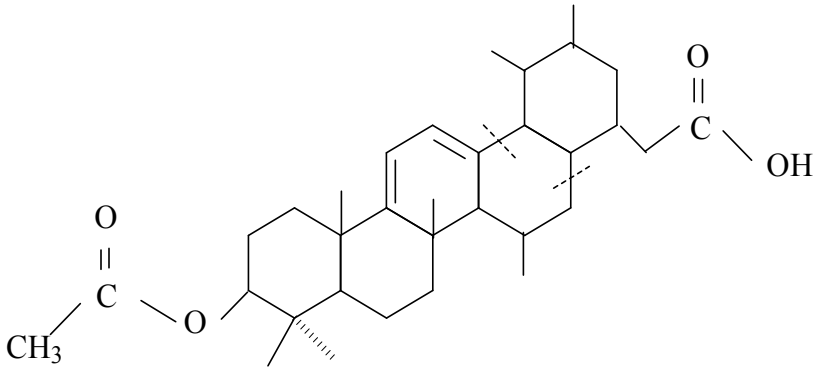
m/z = 327

Appendix 1.4

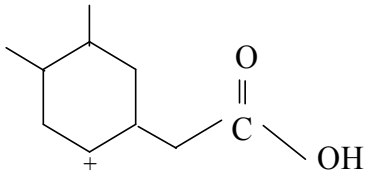
Fragmentation pattern of peak four of fraction C

Ursa, 9(11), 12-dien-28-oic acid, 3-(acetyloxy)-, methyl ester, (3.beta)

($C_{33}H_{50}O_4$), MW = 510



M-155



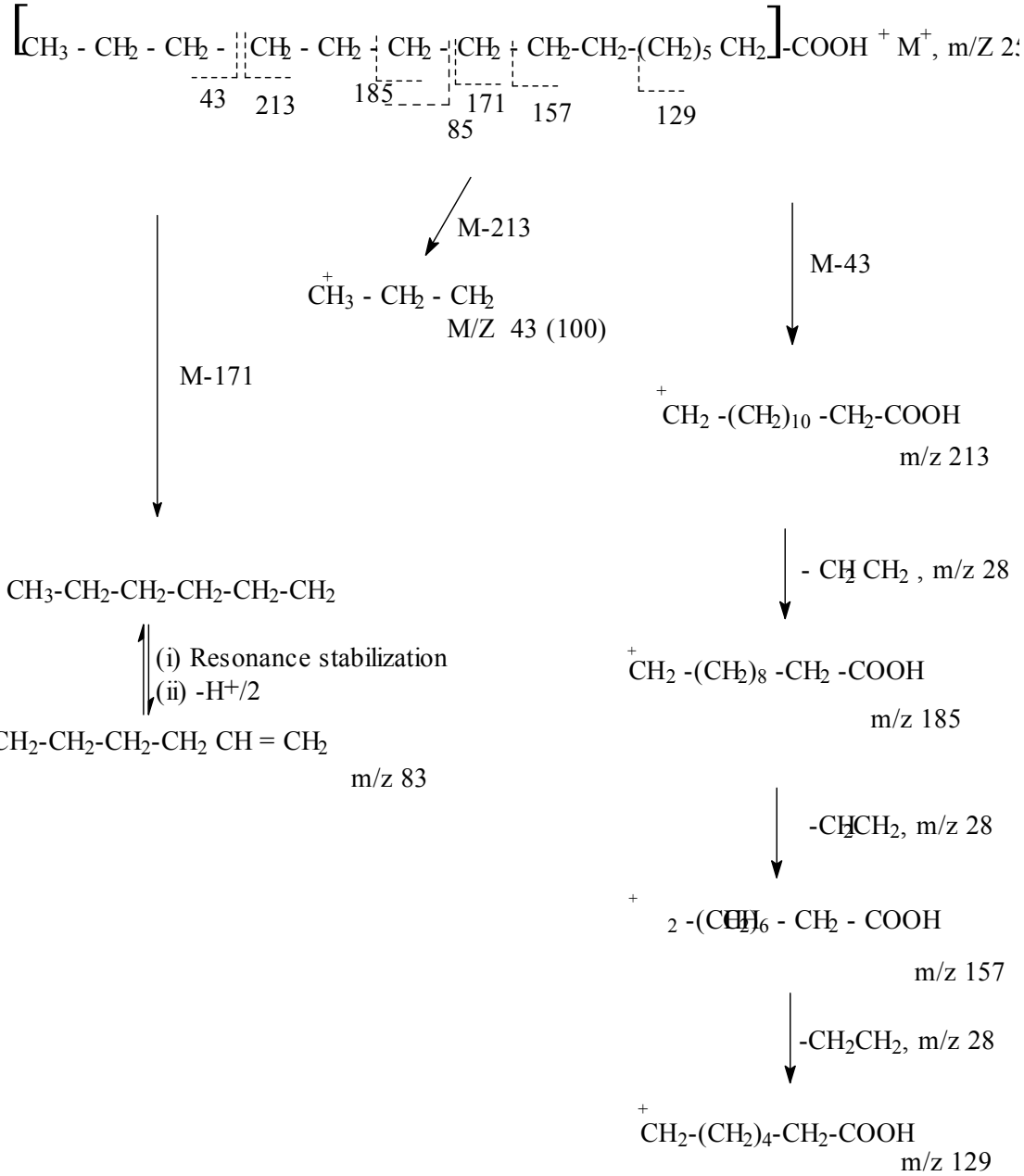
m/z 355(100)

Appendix 1.5

Fragmentation pattern of peak one of fraction P

n - hexadecanoic acid

$C_{16}H_{32}O_2$), mw = 256



Appendix 1.6

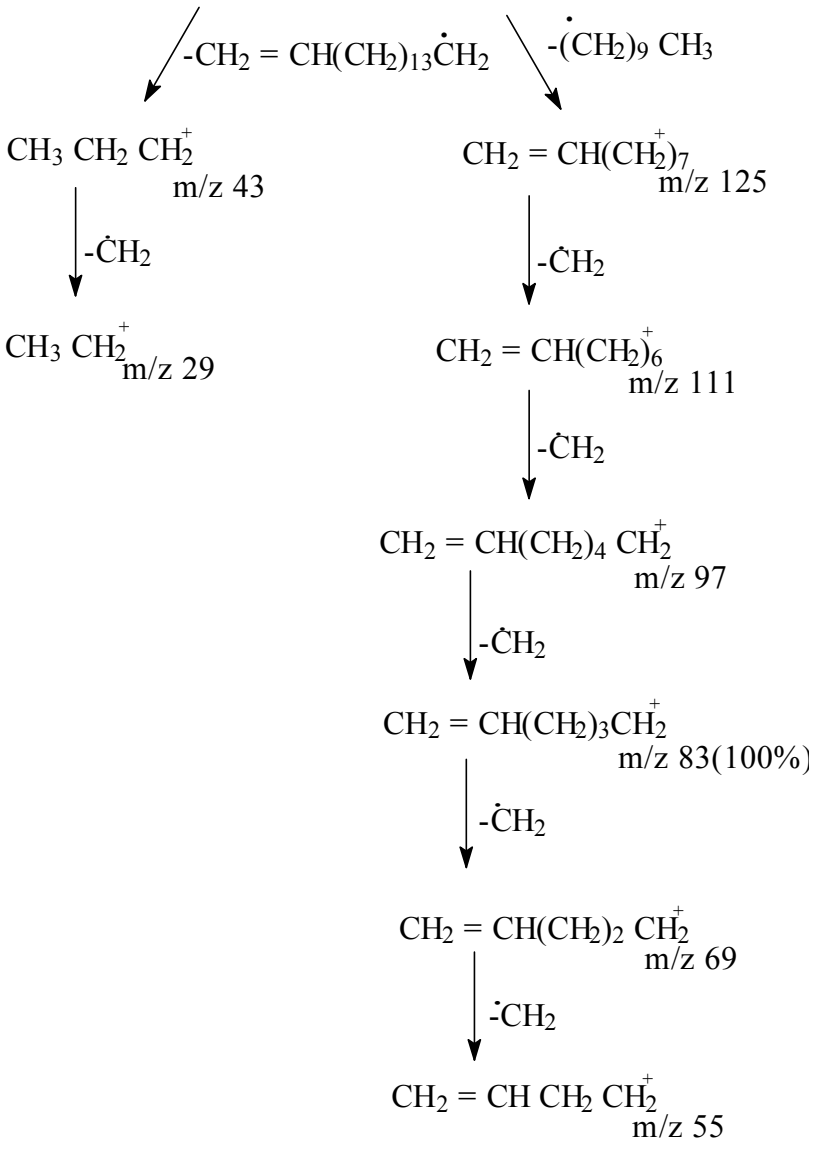
Fragmentation pattern of peak two of fraction P

1- Nonadecene

($C_{19}H_{38}$), MW = 266

$CH_2 = CH-(CH_2)_{16}-CH_3$

$[CH_2 = CH-(CH_2)_{16}-CH_3]^{+\cdot} = M^+ = 266$

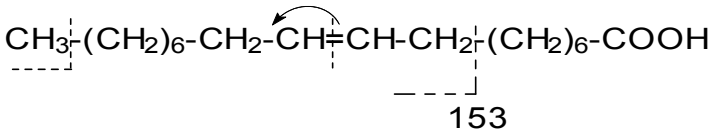


Appendix 1.7

Fragmentation pattern of peak three of fraction P

Oleic acid

$(C_{18}H_{34}O_2)$, MW = 282



(i) $[M]^+$ = m/z 282

(i) Cleavage on the double bond
(ii) MC Lafferty
(iii) $-\dot{C}H_3$
(iv) $-H^+/2$

$CH_3-(CH_2)_5-CH=\overset{+}{C}H$ m/z 111

(i) $-\dot{C}H_2$
(ii) Hydride shift

$CH_3-(CH_2)_4-CH=\overset{+}{C}H$ m/z 97

(i) $-\dot{C}H_2$
(ii) Hydride shift

$CH_3-(CH_2)_3-CH=\overset{+}{C}H$ m/z 83

(i) $-\dot{C}H_2$
(ii) Hydride shift

$CH_3-(CH_2)_2-CH=\overset{+}{C}H$ m/z 69

(i) $-\dot{C}H_2$
(ii) Hydride shift

$CH_3-CH_2-CH=\overset{+}{C}H$ m/z 55

(i) $-\dot{C}H_2$
(ii) Hydride shift

$CH_3-CH=\overset{+}{C}H$ m/z 41(100)

(i) M-153

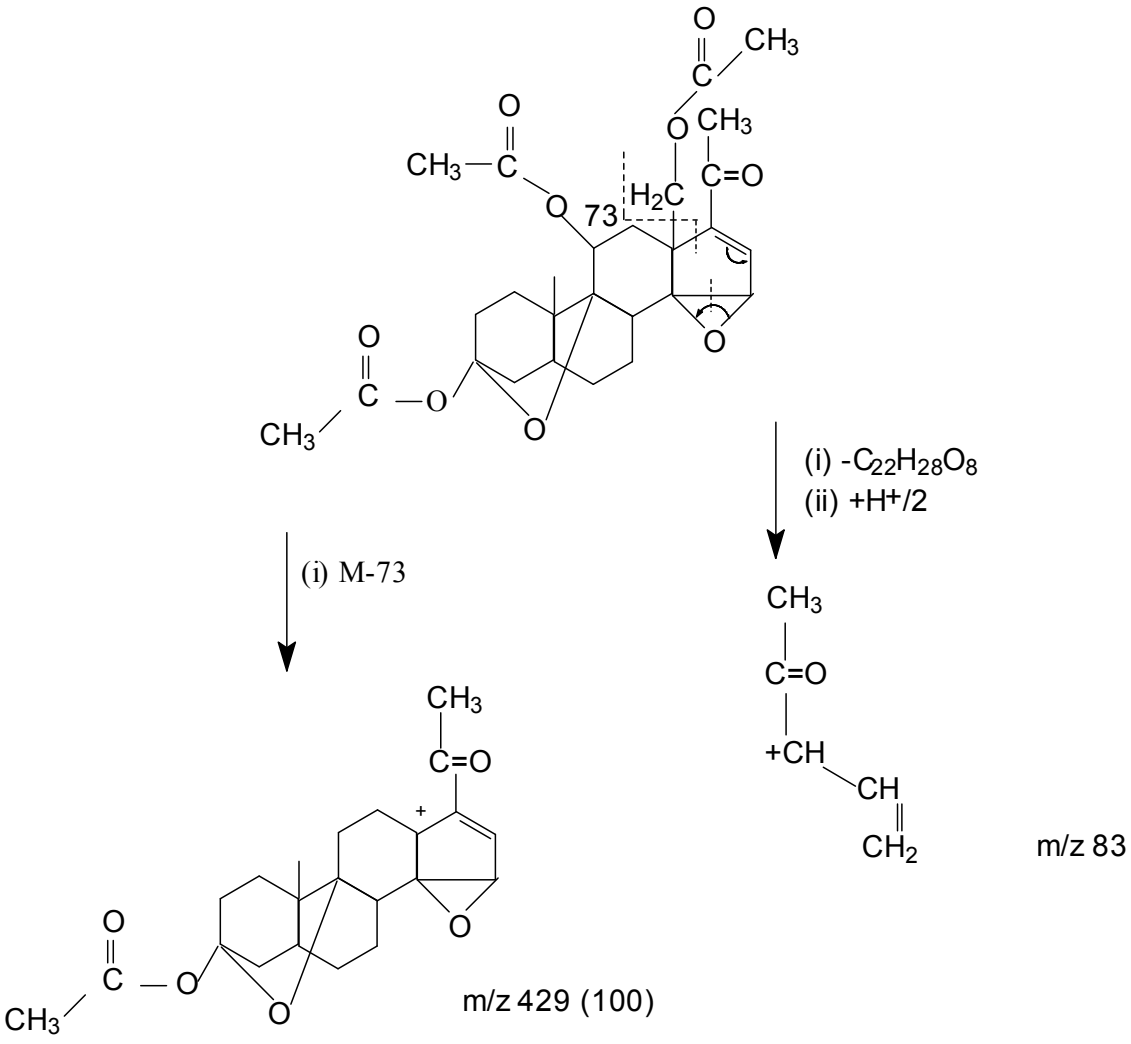
$CH_2-(\overset{+}{C}H_2)_5-COOH$ m/z 129

Appendix 1.8

Fragmentation pattern of peak four of fraction P

3,9,ß-14,15-Diepoxy pregn-16-en-20-one,3,11-ß-18-triacetoxy

($CH_{27}H_{34}O_9$), MW 502

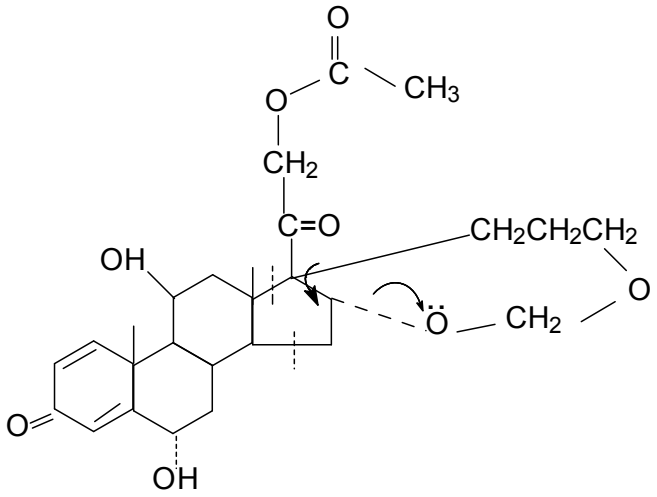


Appendix 1.9

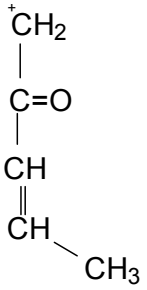
Fragmentation pattern of peak five of fraction P

(225)-21-Acetoxy-6α-11-ß-dihydroxy-16α -17-propyl methylene dioxy-pregna-1, diene-3,20-dione

($C_{27}H_{36}O_8$), MW = 488



(i) $-O-C-CH_3$
(ii) $-CH_2CH_2CH_2-O-CH_2-O$
(iii) $C_{22}H_{30}O_3$
(iv)$+H^+/2$



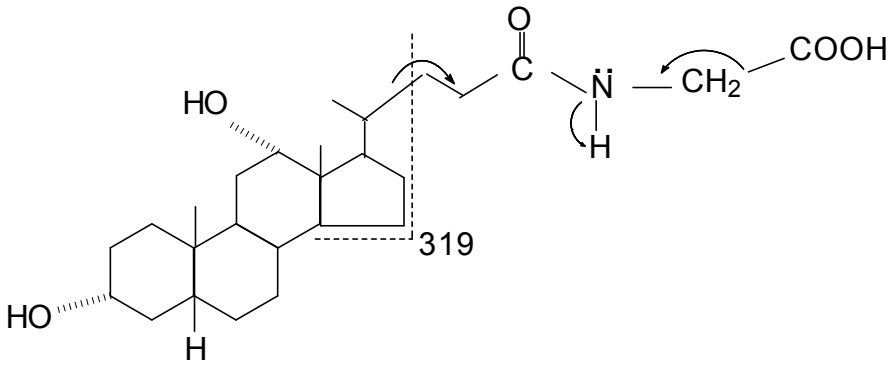
m/z 83(100)

Appendix 1.10

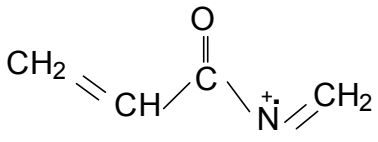
Fragmentation pattern of peak six of fraction P

Glycine,N-[(3-alpha,12,alpha-3,12-dihydroxy-24-oxocholan -24

($C_{26}H_{43}NO_5$), MW = 449



(i) M-319
(ii) MC Lafferty rearangment
(iii) -COOH
(iv) $-H^+/2$



m/z 83(100)

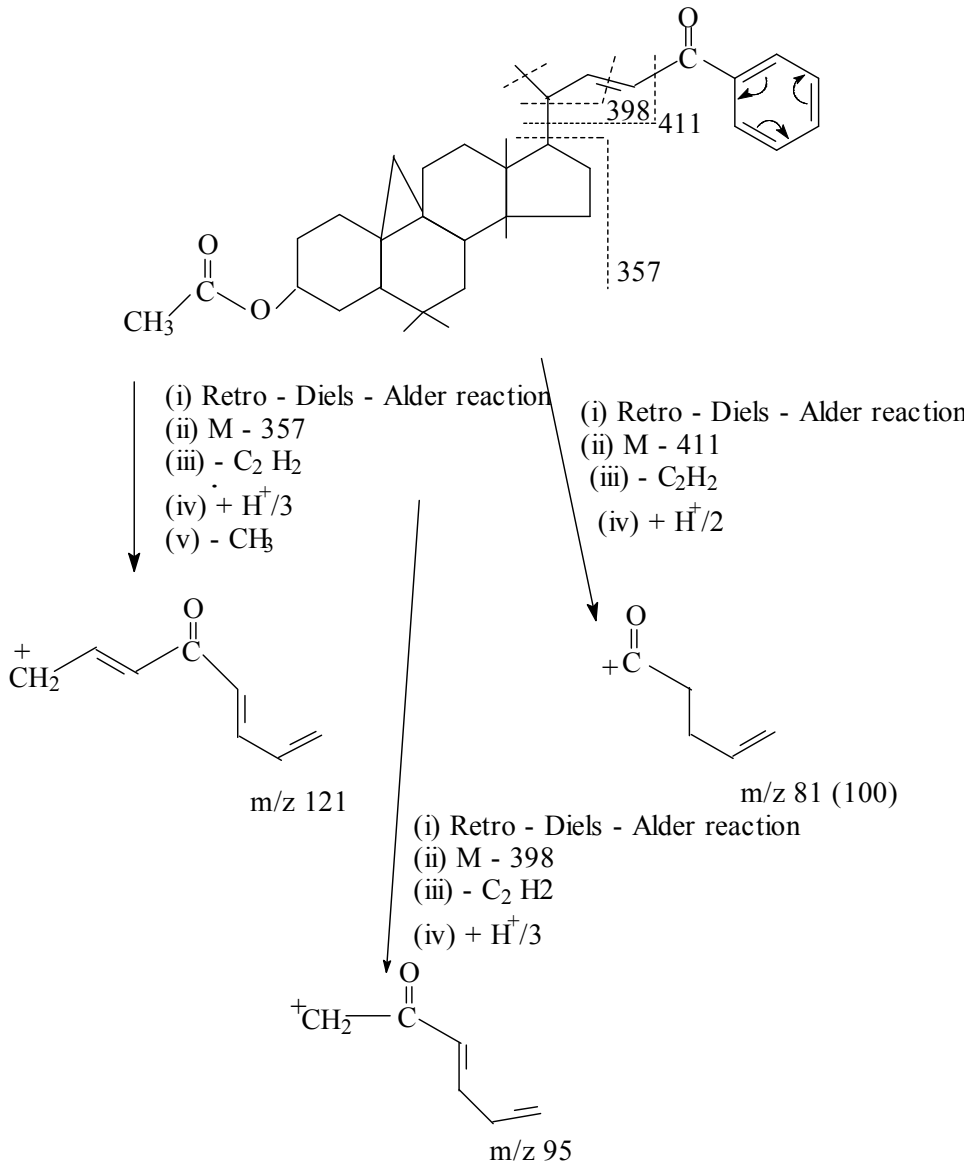
Appendix 1.11

Fragmentation pattern of peak seven of fraction P

25 - Norisopropyl - 9, 19 - cyclolanostan - 22 - en - 24 - one,

3 - acetoxy - 24 - phenyl

(C_{35} H_{48} O_3), MW - 516

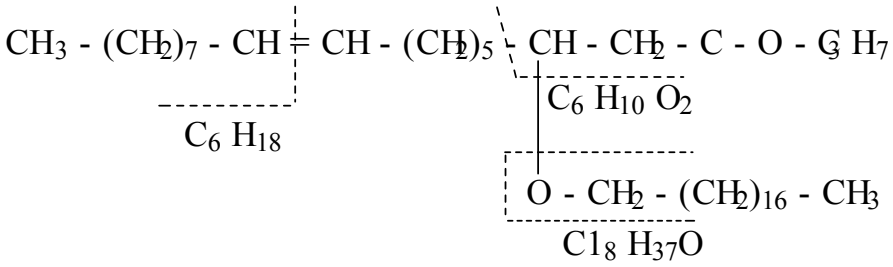


Appendix 1.12

Fragmentation pattern of peak eight of fractio

Oleic acid, 3 - (Octadecyloxy) propyl ester

(C_{39} H_{76} O_3), MW = 592



(i) - C_9 H_{18}
(ii) - C_6 H_{10} O_2
(iii) - C_{18} H_{37} O
(iv) Mc Lafferty rearangmen

$CH_2 = CH - (CH_2)_3 - \overset{+}{C}H_2$

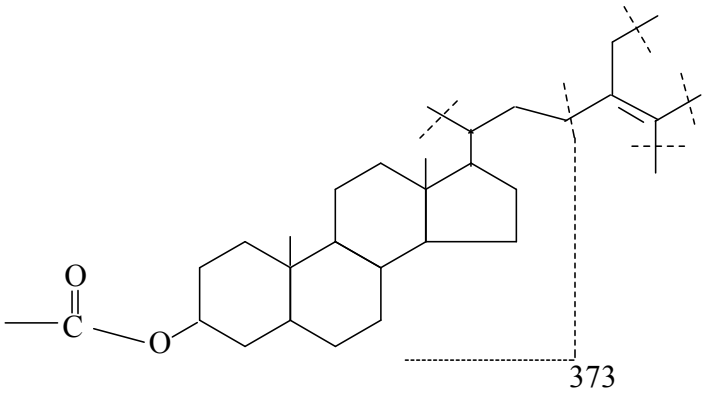
m/z 83 (100)

Appendix 1.13

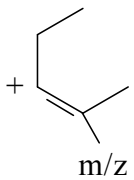
Fragmentation pattern of peak nine of fraction P

Beta - sistosterol acetate

($C_{31}H_{52}O_2$), MW = 456



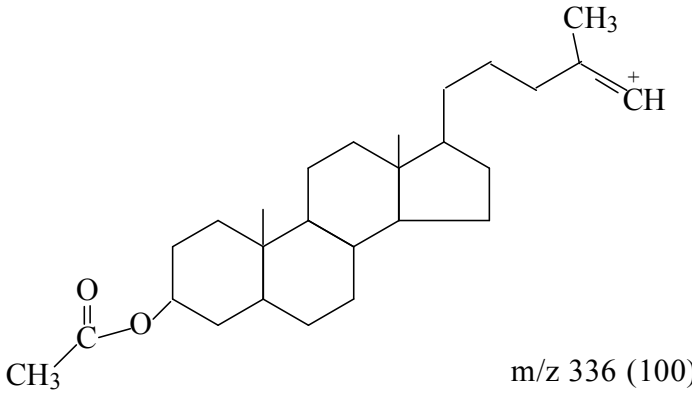
(i) M - 373



m/z 83

(i) M - 4 $\dot{C}H_3$

(ii) $+ H^+/3$



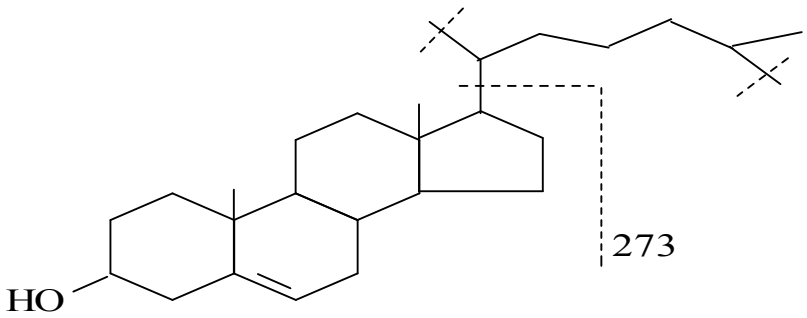
m/z 336 (100)

Appendix 1.14

Fragmentation pattern of peak ten of fractio

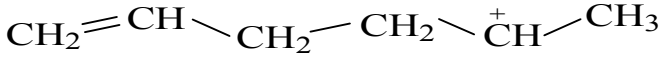
Cholesterol

(C_{27} H_{46} O), MW = 386



$[M^+]$ = m/z = 386

(i) M - 273
(ii) - 2 $^{\cdot}CH_3$
(iii) Mc Lafferty



m/z 83 (100)

Appendix 1.15

Sodium chloride	90g
Potassium chloride	20ml
Sodium dihydrogen phosphate (10%)	5ml
D-glucose	10g
Sodium bicarbonate	10g
Calcium chloride (10%)	20ml
Magnesium chloride ($Mg\ Cl_2\ .6H_2O$)	1ml

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