THE PHARMACEUTICAL EVALUATION OF *I. GABONENSIS*

BY

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DECLARATION

I hereby certify that the work reported in this thesis was carried out by me in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. The works of other investigators are acknowledged and referred to accordingly.

I solemnly declare that no part of this thesis has been accepted in substance or concurrently submitted in candidature for any other degree.

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CERTIFICATION

This thesis entitled THE PHARMACEUTICAL EVALUATION OF IRVINGIA GABONENSIS by EZZELDIN MUKHTAR ABDURAHMAN meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY (Pharmacognosy) of AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To all those who in their lives have endured the bitterness of great pain, and who, from that pain, have been able to go forward, strong in hope, to yet greater achievements.

And

To my father Alhaji Mukhtar Abdurahman and my late mother Hajia ASMA.

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ABSTRACT

This study deals with the pharmacognostic aspects of the leaves, fruits and seeds of Irvingia gabonensls (O'Rorke) Baill, with special reference to its seed fat content.

I. gabonensls is a forest tree up to 25 meters high and 2 meters in girth. The plant is widely-distributed in Nigeria especially in the Eastern and Western regions of the country, where the kernels are an important source of food. They are eaten in various parts of West Africa. The plant has two varieties, namely gabonensls with sweet edible fruits and excels a with bitter inedible fruits. Detailed macroscopical, microscopical and phytochemical studies were carried out on the two varieties of the plant and pharmacognostic parameters were established for the identification of the two varieties. Also, some pharmacopoeial standards have been recommended for I. gabonensls leaves and kernels.

The composition of the fatty acid in the seed fat was determined by different chromatographic techniques including thin layer chromatography (TLC), gas chromatography/mass spectrometry (GC/MS) and high resolution gas chromatography (HRGC). TLC of the hydrolysed seed fat on silica gel plates impregnated with $AgNO_3$ revealed the presence of

lauric, myristic, stearic and oleic fatty acids.

For GC/MS analysis, the seed fat was first hydrolysed and the free fatty acids (FFE) were derivatized to methyl esters (FAME) before injecting onto a carbowax column. Identification was made by comparing the retention time and mass spectra of the FAME with computerized reference data. The fatty acids identified were capric, lauric, myristic, palmitic, stearic, oleic and linolic acids.

The so called "Dika Fat" was indeed found to be a mixture of fat and wax. The "fat" was only partially hydrolysed with aqueous KOH to afford glycerol and the fatty acids. On the other hand, alcoholic KOH hydrolysed the "fat" completely to afford glycerol and an alcohol which was immiscible with water.

The percentage composition of the fatty acids in the seed fat was determined by the HRGC of the FAME on a GC equiped with fused silica column. It was found that myristic acid (52.3190 and lauric acid (36.9250 were the principal saturated acids in I. gabonensis var. excels a seed fat. The unsaturated acids were present In very low concentration. Similar results were ontained for I.gabonensis var. gabonensis.

The seed fat was evaluated as a possible base for ointment and suppository dosage forms. Wool fat and castor oil were mixed separately with the seed fat in different proportions to produce semisolid preparations of such consistency and composition that

can be applied on the skin by inunction. The seed fat mixed with either 20% wool fat or with 30% castor oil produced two suitable ointment bases with the desired properties. The rate of release of drugs from these bases was studied using the water soluble resorcinol and the less water soluble salicylic acid. The results indicate that the rate of release of resorcinol was lower than the rate of release of salicylic acid while the ointment bases formulated with the seed fat and wool fat released more drugs than those formulated with the seed fat and castor oil.

A suppository base consisting of wool fat (10%) and the seed fat (90%) was formulated and chloroquine suppositories prepared by incorporating chloroquine phosphate into these bases was further evaluated.

The physiological studies on adult rats fed with this formulated seed fat for four weeks, showed significant increase in the serum cholesterol, triglyceride and total lipid levels, indicating that pharmaceutical applications of the seed fat should be regulated to avoid its possible side effects.

This work highlights the economic potentials of the plant I. gabonensis and indicates the possibility of exploiting its fat content in addition to the kernel mucilage as a source of raw materials for the pharmaceutical industry.

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

The plant Irvingia gabonensis (O'Rorke) Baill (Keay, 1989), is a forest fruit tree species belonging to the family Irvingiaceae. The plant is widely distributed in Africa from Senegal to the Sudan and south to Angola (Irvine, 1961).

1.1 DESCRIPTION OF THE FAMILY IRVINGIACEAE

This is a small tropical family composed of three genera, namely Klainedoxa, Desbordesia and Irvingia. All the species are trees. Branchlets, leaves and inflorescences are entirely glabrous. The leaves are simple, alternate and entire, usually arranged along the branchlets in two rows. The stipules are distinctive, long, narrow and pointed, enfolding the bud as in many Moraceae and, like the stipules in that family, leave a prominent annular scar around the stem when they fall off (Keay, 1989). The leaves are medium-sized to moderately large, with stalks up to 12mm long and upcurving lateral nerves running out parallel with the margin or rather faintly looped a short way from it. The inflorescences are racemes or panicles about as long as the leaves, bearing regular flowers about 6mm across, usually

with 5 free petals, and 10 prominent free stamens with slender filaments as long as the petals. The anthers are small, the superior ovary terminates in a single style and is surrounded by a fleshy disc (Keay, 1989).

The key to the family genera is

(i) Klainedoxa, fruits large and fleshy, flattened from top to bottom, usually 5-lobed and containing from 1-5 hard woody stones.

(ii) Desbordesia, fruits winged and remarkably leaf-like.

(iii) Irvingia, fruits mango-like and containing one stone.

1.2 DESCRIPTION OF THE GENUS IRVINGIA

The genus was named after Dr. E. G. Irving, who died at Abeokuta, Nigeria in 1855 (Keay, 1989). The flowers in this genus resemble those in the Klainedoxa genus but the fruits are quite different. Three species occur in Nigeria, namely, smithii, grandifolia and gabonensis.

The general description of the species is:

I. smithii Hook F. The plant is a tree up to 20m high, the leaves are ovate and the fruits are about

2-3 cm in diameter. It is found near river banks in savanna regions. The plant's habit extends from Northern Nigeria to the Sudan and Angola. The local name of the plant in Hausa is "goron biri" meaning "Monkey's kola nut" (Keay, 1989).

I. grandifolia (Engl.) Engl. The plant is a forest tree, the leaves are much larger than those of the other two species, about 10 - 25cm long. The leaves turn bright red before falling off. The flowers are stalkless and distinctive. In young trees the leaves and stipules are much like those of Klainedoxa gabonensis but there are no spines. The plant extends from South-Western Nigeria to Angola. The local name of the plant in Yoruba is "apepere" and in Igbo "akpulu" (Keay, 1989).

I. gabonensis (O'Rorke) Baill. The plant is a forest tree up to 25 - 40 meter high or more with grey trunk slightly buttressed (Irvine, 1961). The tree may be readily recognized by its dense dark green evergreen foliage and characteristic stipules which are similar to those of Klainedoxa but smaller. Okafor (1975) has distinguished two varieties as follows:-

Variety gabonensis has sweet edible fruits with scantily fibrous pulp, the bole is fluted or cylindrical, the lateral branches are ascending, making the crown spherical or narrow (Keay, 1989).

Variety excelsa (Mildbr.) Okafor;its fruits have bitter inedible very fibrous pulp, bole buttressed, lateral branches horizontal making a wide umbrella-shaped crown (Keay, 1989).

1.3 DESCRIPTION OF IRVINGIA GABONENSIS (O'RORKE) BAILL. VARIETY EXCELSA

According to Keay (1989) the plant is a forest tree up to 25m high and 2m in girth, occasionally more. Bark greyish, smooth or very slightly scally with yellowish brown to light yellow brittle slash. The wood is pale brown, very hard and fine grained (Keay, 1989).

The stipules are curved, the ones terminating the shoot are up to 2.5cm long, the others are smaller (Keay, 1989).

The leaves are about 5-15cm long and 2.5 - 6cm broad, they are elliptic to slightly obovate with one margin often a little more rounded than the other, the leaves have acute or shortly acuminate apices and slightly rounded bases, they are leathery and



Plate 1: A Full Grown Tree of Irvingia gabonensis var. gabonensis

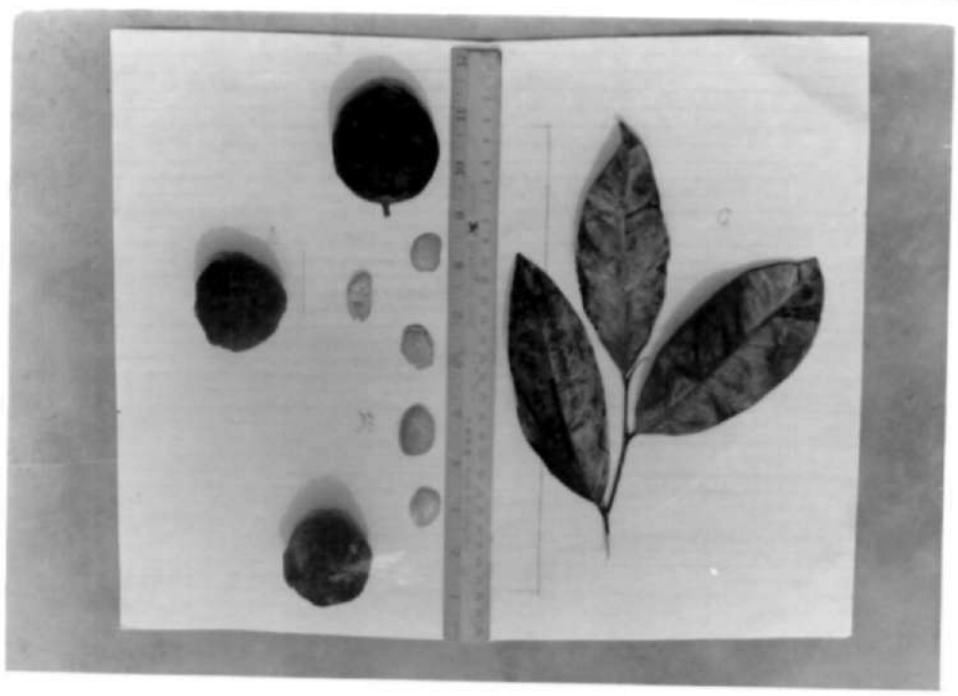


Plate 2: Irvingia gabonensis var. excelsa

A - Fruits
B - Seeds
C - Leaves

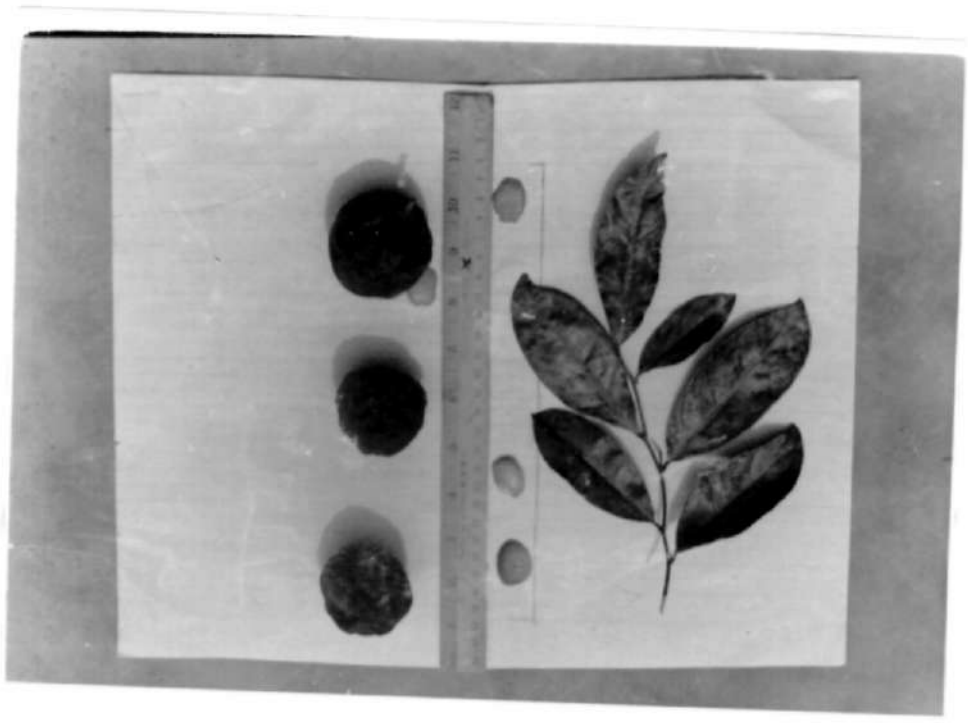


Plate 3: Irvingia gabonensis var. gabonensis

A - Fruits
B - Seeds
C - Leaves

dark green in colour, with 5-10 pairs of irregular lateral nerves, the lower ones running out vaguely to the margin, the upper ones looped; minor nerves are more or less parallel and at right angles to the laterals, vennation is reticulate, the stalk is stout about 6mm long (Keay, 1989).

The flowers (Nov. - Mar.; June) are yellowish to greenish white, found in slender clustered racemes or smaller panicles among the leaves and about as long as the leaves. The flower stalks are slender, about 6mm long, the petals are bent right back and soon fall off. The discs are bright yellow (Keay, 1989).

The fruits (Apr. - July; Sept.) are yellowish in colour, about 5 - 7.5cm in diameter with a yellowish fibrous pulp surrounding a large stone.

The plant's habit extends from Senegal to the Sudan and South to Angola. The plant is widely spread in Nigeria especially the Eastern and Western parts of the country. It has many local names in Nigeria, they are Hausa: "Goron biri", Nupe: "Pekpeara", Yoruba: "Oro" or "Apon", Igbo: "Ogbono" and in Efik: "Oyo". Its kernels are an important source of human food in various parts of Nigeria.

1.4 ETHNOMEDICAL, ECONOMIC AND PHARMACEUTICAL USES OF IRVINGIA GABONENSIS

Ethnomedical uses

In Gabon, the bitter bark is added to palm wine, while in Sierra Leone, the bark is ground and mixed with water and used to rub on the body for pains (Irvine, 1961). In Ghana, the root shavings in roasted banana are used for treating diarrhoea and dysentery (Abbiw, 1990). In Nigeria the powdered kernels are applied to burns and are also used to make astringent remedies (Irvine, 1961).

Economic uses

The kernels are an important source of human food in various parts of West Africa. The fruits are collected and the nuts are split open, the seeds are then dried in the sun or by fire and split into two flat white cotyledons often seen in local markets. These are cooked and eaten in Uganda, and are often used in other West African countries (e.g. Nigeria) in making soups and sauces used in eating food prepared from major staples such as yam and cassava (Irvine, 1961).

In Gabon, a soft paste is also made from the fresh kernels, by soaking the kernels for 2-3 weeks in water and then mould them into a rather bitter paste which is used for cooking with fish and meat. This is called "dika paste", which contains about 70-80% of a fat known as "dika butter". It is comparable to cocoa butter (Irvine, 1961), for which it is used as a substitute. In chocolate making it is used as an adulterent. The "dika butter" is of similar value as palm-kernel oil in soap making (Irvine, 1961).

The tree is highly valued in most parts of Nigeria for the income that is earned from its production of fruits/seeds (kernels). The income yielding potential depends on factors such as age (productivity), variety (excelsa, bitter inedible fruit pulp, variety gabonensis, edible sweet fruits), number of trees owned and form in which the kernels are sold (fresh fruits or processed seeds)(Okafor, 1990).

The nutritional importance and dietary contributions of the seed kernels have been reported (Okafor, 1975; Okigbo, 1977; Abaelu and Akinrimisi, 1980; Eka, 1980). The economic potentials of the sweet variety (var. gabonensis) for the production of table jelly, wine and fruit juice have also

been highlighted (Okafor, 1983; Aina 1986). The wood is suitable for the planking of ship's decks, wooden paving and for house posts (Irvine, 1961).

Pharmaceutical uses

The importance of I. gabonensis kernel for pharmaceutical industry is mainly due to its mucilage and fat contents and their possible application in the production of pharmaceuticals. The plant kernel contains a high percentage of mucilage, about 18.8% w/w (Isimi, 1990) which is responsible for the drawability of the kernels when mixed with water. This mucilage can be used in the pharmaceutical industry as suspending and emulsifying agent to increase the viscosity of mixtures, and as binding agent in the formulation of tablets. Udeala et al (1980) reported the possibility of incorporating the plant fat in tablet formulation as a tablet lubricant. Thus the value of the plant as a source of food stuff and also as a source of pharmaceutical raw material cannot be over emphasized and the high content of the kernel is of special interest.

1.5 CLASSIFICATION OF LIPIDS

The term lipid is used to designate a group of natural substances which are soluble in non-polar solvents (ether, light petroleum, benzene and others).

The term comprises a variety of chemically different compounds e.g. fats (glycerides), waxes (cerides), sterol esters (steroids), and phosphatides. Also included are the fatty acids, free sterols, and higher alcohols soluble in light petroleum (Ramstad, 1959). Bernfeld (1963) classified lipids (Table 1) into simple lipids, compound lipids, derived lipids and complex lipids according to the substances associated with lipids in nature.

1.6 CHEMICAL AND PHYSICAL PROPERTIES OF SIMPLE LIPIDS (FATS, OILS AND WAXES)

Simple lipids are esters of fatty acids with various alcohols, they can be classified according to the nature of the alcohols into, oils and fats (esters of fatty acids with glycerol) and waxes (esters of fatty acids with alcohols higher than glycerol, Finar, 1982).

The physical and chemical properties of a fat are determined by the nature of the fatty acids present in the glycerides. According to Finar (1982)

a. Saturated fatty acids containing from 2 to 10 carbon atoms are liquid at room temperature, more or less soluble in water and volatile i.e. can be distilled with steam (e.g. butyric, caproic and capric acids).

Table 1: Simple Classification of Lipids (Bernfeld, 1963)

A. Simple lipids (mainly esters of fatty acid and alcohol).
   (a) glycerol esters (mono-, di- and triglycerides) (fats and oils).
   (b) cholesterol esters
   (c) higher alcohol esters (waxes)
   (d) glycerol ethers
   (e) hydrocarbons

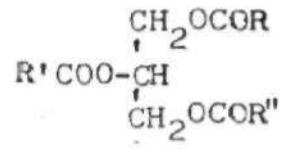
B. Phosphoglycerides (esters of phosphate and the free -hydroxyl of -diglyceride or -(vinyl alkane ether), -monoglyceride).
   (a) phosphate-monoester (phosphatidic acid)
   (b) phosphate-diesters containing:
      (i) choline (lysolecithin, lecithin, choline plasmalogen).
      (ii) ethanolamine (phosphatidyl ethanolamine, ethanolamine plasmalogen).
      (iii) serine (phosphatidyl serine, serine plasmalogen).
      (iv) glycerol (phosphatidyl glycerol, cardiolipin).
      (v) inositol (phosphatidyl inositol, di- and tri-phosphoinositides).
   (c) phosphate-triesters (complexes of phosphatidyl ethanolamine and phosphatidyl serine).

C. Sphingolipids (sphingosine containing N-fatty acyl group).
   (a) sphingomyelin
   (b) cerebrosides
   (c) sulfatides
   (d) mucolipids (gangliosides and strandin)

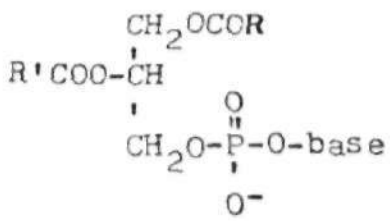
D. Derived lipids
   (a) fatty acids
   (b) cholesterol
   (c) higher alcohols
   (d) sphingosine

E. Complex lipids
   (a) lipoproteins
   (b) proteolipids
   (c) phosphatidopeptides

Triglycerides

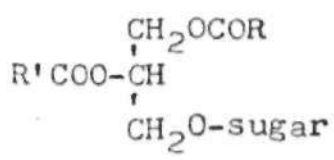


Phospholipids



base = choline, $-CH_2CH_2\overset{+}{N}Me_3$
ethanolamine, $-CH_2CH_2NH_2$
serine, $-CH_2CH(NH_2)CO_2H$

Glycolipids



sugar = galactose
galactosylgalactose
quinovose

(R,R',R" = hydrocarbon chains of different fatty acids, see Fig. 2

Fig. 1: General structures of plant lipids (Bernfeld, 1963).

Carbon chain length	Trivial Name	Formula
	Saturated acids	
C_{14}	Myristic	$CH_3(CH_2)_{12}CO_2H$
C_{16}	Pamitic	$CH_3(CH_2)_{14}CO_2H$
C_{18}	Stearic	$CH_3(CH_2)_{16}CO_2H$
C_{20}	Arachidic	$CH_3(CH_2)_{18}CO_2H$
	Unsaturated acids	
C_{16}	Palmitoleic	$CH_3(CH_2)_5CH = CH(CH_2)_7CO_2H$
	Oleic	$CH_3(CH_2)_7CH = CH(CH_2)_7CO_2H$
C_{18}	Linoleic	$CH_3(CH_2)_4CH = CHCH_2CH = CH(CH_2)_7CO_2H$
	Linolenic	$CH_3CH_2CH = CHCH_2CH = CHCH_2CH = CH(CH_2)_7CO_2H$
	Unusual acids	
C_{16}	Petroselinic	$CH_3(CH_2)_7CH = CH(CH_2)_5CO_2H$
C_{22}	Erucic	$CH_3(CH_2)_7CH = CH(CH_2)_{11}CO_2H$
C_{19}	Sterculic	$CH_3(CH_2)_7C = C(CH_2)_7CO_2H$ CH_2
	Cutin acids	
C_{24}	Lignoceric	$CH_3(CH_2)_{22}CO_2H$
C_{26}	Cerotic	$CH_2(CH_2)_{24}CO_2H$
C_{16}	10,16-dihydroxy-palmitic	$HO(CH_2)_6CHOH(CH_2)_8CO_2H$

Fig. 2: Structures of some representative fatty acids (Bernfeld, 1963).

b. Saturated fatty acids which contain more than 10 carbon atoms are solids at room temperature, insoluble in water, and non-volatile i.e. cannot be distilled with steam (e.g. palmitic and stearic acids).

c. All the unsaturated fatty acids are liquids at room temperature.

d. Fatty acids form salts (soaps) with metal ions because of the presence of a terminal carboxyl groups.

e. Due to the presence of double bond in unsaturated fatty acids, the addition of halogens such as Br_2 or I_2 is done easily.

f. Oxidation of the hydrocarbon chain by various reagents at the double bond is possible forming peroxide derivatives, ketones, aldehydes and acids with smaller number of carbon atoms which may react further to form complex resinous products with unpleasant odour and flavour, the fat then is said to be rancid (Finar, 1982).

Waxes: Are defined as esters of fatty acids with alcohols higher than glycerol. However, most waxes also contain free fatty acids, alcohols and paraffins (Ramstad, 1959).

Trease and Evans (1983) confined the term wax to those natural mixtures containing appreciable quantities of esters derived from higher monohydric alcohols of the methyl alcohol series combined with fatty acids. In this series of alcohols the members change from liquids to solids, become less soluble in water and have higher melting points with increase in molecular weight. According to Trease and Evans (1983) waxes have higher acid value than fats, have lower iodine value and also higher unsaponifiable matter value (Table 3 and Table 5). An important practical difference between fats and waxes is that fats may be saponified by means of either aqueous or alcoholic alkali but waxes are only saponified by alcoholic alkali (Trease and Evans, 1983).

1.7 CHEMICAL AND PHYSICAL ANALYSIS OF FATS

The nature of fatty acids which constitute a fat plays a very important role in determining the physical and chemical characteristics of a fat. The usual physical characters that are determined are, the melting point, solidifying point, density and

Table 2: Important Characteristics of Some Common Waxes (Trease and Evans, 1983).

Wax	Acid value	Saponification value	Iodine value	Important constituents
Spermaceti	Below 1	120-136	Below 5	Cetyl palmitate and cetyl myristate
Beeswax	18-24	70-80 (ester value)	8-11	72% esters mainly myricyl palmitate: free cerotic acid: steryl esters.
Carnauba	4-7	79-95	10-14	
Wool fat	Below 1	90	18-32	Steryl esters, esters of other aliphatic alcohols, fatty acids and hydrocarbons.

refractive index (Finar, 1982). The chemical tests, which gave an indication of the nature of fatty acids present in the fat are, the acid value, the saponification value and the iodine value (Finar, 1982).

The desirable characteristics of vegetable fats, from a pharmaceutical point of view, include their unique sharp melting (or softening) point over a narrow range of temperature. This characteristic melting point profile is due to their fatty acids composition which consists of either a single triglyceride or a few triglycerides of nearly identical melting point. For example, more than 75% of the glyceride content of cocoa butter (Table 4) comprises of a single oleic acid moiety in combination with stearic and palmitic acids or both (Onaga, 1988); also about 90% of the fatty acids of coconut oil are saturated, with lauric acid contributing nearly 50% (Table 4) thus its physical properties are not very much affected by hydrogenation since its unsaturation is low, making the oil resistant to rancidity. Also the preponderance of low molecular weight glycerides gives the coconut oil low melting point of 21-23^{o}C (Table 5) which means that it may be semi-solid or liquid at room temperature (Onaga, 1988).

Table 3: Approximate Range of Oil Content of Selected Seeds/Fruits Indigenous to Nigeria (Onaga, 1988).

Vegetable Oil Sources	Oil Content % (On Dry Weight Basis)
Palm fruit	45 - 50
Groundnut	40 - 50
Palm kernel	45 - 55
Coconut	50 - 70
Shea butter	45 - 55
Cocoa butter	50 - 55

Table 4: Fatty Acid Composition of Some of Nigerian Vegetable Oils (Onaga, 1988).

Fatty acids	Cocoa butter	Shea butter	Palm oil	Coconut oil
Caprylic	-	-	-	8.2 ± 0.7
Capric	-	-	-	6.4 ± 0.7
Lauric	-	-	-	48.7 ± 1.0
Myristic	-	-	1.6 ± 0.5	17.1 ± 1.1
Palmitic	24.4	5.7	42.3 ± 1.8	8.3 ± 0.6
Stearic	35.4	41.0	52.0 ± 0.8	2.4 ± 0.3
Arachidic	-	-	-	-
Total Saturates	59.8	46.7	49.1 ± 1.5	91.1 ± 0.7
Oleic	38.1	49.0	40.6 ± 2.3	5.6 ± 0.5
Linoleic	2.1	4.3	9.9 ± 0.3	1.9 ± 0.4
Total Polyunsaturates	40.2	53.3	50.5 ± 1.3	7.5 ± 0.1

- = Absent or not determined

Table 5: **Important Characteristics of Some Common Vegetable Oils (Onaga, 1988)**

	Palm Oil	Palm Kernel Oil	Coconut Oil	Groundnut Oil	Cocoa Butter	Shea Butter
Iodine Value	40-60	14-22	20-24	84-100	33-40	53-65
Saponification Value	196-209	245-255	250-264	188-194	190-198	178-190
Specific Gravity	-	0.860-0.873	0.869-0.874	0.910-0.915	0.856-0.864	-
Melting Point	27-45°C	24-26°C	21.8.23	-	32-35	32-45
Acid Value	0.2	0.2	1	5	4	-

- = Absent or not determined

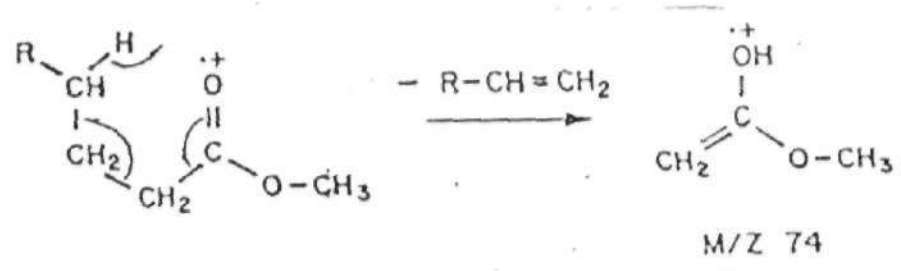
Thus it is clear that the fatty acid composition of an oil or fat affects its physical properties and it is obvious that determination of these physical and chemical properties are useful in the identificatio and quality assessment of fats.

1.8 CHROMATOGRAPHIC TECHNIQUES FOR THE STUDY OF LIPIDS FATTY ACID COMPOSITION

There are different methods for fatty acids analysis e.g. thin layer chromatography (TLC) using silica gel or silica gel impregnated with silver nitrate as adsorbent; gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). GC is better than TLC due to its higher resolution, speed of analysis, automated chart readout and extreme sensitivity for which reasons it is universally adopted for fatty acid analysis.

It is common practice to derivatise fatty acids in order to enhance their volatility and to facilitate chromatographic separation (Weintraub, 1990). Frequently used derivatives for the carboxyl group are methyl, trimethylsilyl (TMS), and t-butyldimethyl-silyl (TBDMS) esters, each with particular advantages and disadvantages (Weintraub, 1990). Methyl esters can be readily prepared through the use of reagents such as diazomethane, boron trifluoride-methanol or

acetyl chloride methanol. The last reagent is of particular value in convenient transesterification of bound fatty acids, thus eliminating the need for a separate hydrolysis step (Weintraub, 1990). Excellent gas chromatographic separation of these compounds, based on the number of carbon atoms in the chain as well as the degree of unsaturation, can be accomplished with either capillary or packed columns (Weintraub, 1990). When a capillary column is used its high resolution makes overlapping peaks less likely and identification by retention time more accurate. Fatty acid methyl esters (FAME) are very stable. Their mass spectra are well documented in the literature, and many homologs are represented in commercially available reference libraries. The mass spectrum of saturated fatty acids produces an extremely characteristic electron impact (EI) fragmentation pattern which makes identification of these compounds very easy. The m/z 74 ion is formed by the McLafferty rearrangement (Weintraub, 1990).



Equation 1: McLafferty rearrangement of fatty acid methyl esters.

This ion is the base peak for straight chain methyl esters with 6-26 carbons (Budzikiewics et al, 1967) and is observed to a decreasing extent as the number of double bonds increases.

The relative intensities of the molecular ion M^+ and the m/z 143 ions are very prominent and consistent for the various saturated members of this series. However, unsaturated fatty acid methyl esters exhibit lower intensity molecular ions (if any) and produce fragmentation patterns which become progressively less distinctive, particularly with three or more double bonds (Weintraub, 1990).

The expansion of GC/MS instrumentation is based on the unique combination of a number of practical features which are key to its tremendous potential in qualitative and quantitative analysis, (1) sensitivity; (2) specificity (i.e. detection for quantitation is based on structural fragments as well as GC retention time); (3) greatly reduced intraassay variability afforded by the use of deuterated internal standards; (4) the simultaneous detection of the compound being measured; (5) reduced interference from impurities in reagents and solvents; (6) speed of analysis (i.e. short run time); and (7) simplified sample preparation.

Thus due to these factors GC/MS technique is more advanced than GC technique alone in the analysis of fatty acids.

1.9 FATTY ACIDS COMPOSITION OF THE SEED FAT IN DIFFERENT VARIETIES OF IRVINGIA SPECIES

Bushell and Hilditch (1939) reported the percentage composition of "Dika fat" in the seed fat of I. gabonensis (Table 6). The chief component of glyceride was 65% of dilauromyristin. Also they reported that the seed fats of I. barteri (Nigeria) and I. el-Livieri (Cochin China) are similar but differ in their component acids which include 40% of lauric, 55% of myristic acids and the oleic acid content varying from 2 to 10% of the total fatty acids.

A sample of Irvingia seed "Dika fat" (species not known) obtained from West Africa and analysed by Meara and Patel (1950) showed the chief component glycerides of the fat to be trilaurine 7.1, myristodilaurin 32.5, caprolauromyristin 5.2, lauromyristopalmitin 13.1, laurodimyristin 28.7, trimyristin 5.1, and oleolauromyristin 5.1 (% mol).

I. gabonensis Baill seeds were found to contain 54-66.8% fat and analysis of the fat indicated that a small amount of oleic acid was present in the fat and

the solid fatty acids were chiefly stearic and palmitic (Pieraerts, 1921).

I. barteri seed were found to contain 69% fat, the seed fat fatty acids percentage composition was determined and reported by Collin and Hilditch, 1930 (Table 6).

The physical constants (melting point, acid value, iodine number, etc) of the seed fat were determined for I. smithii (Pieraerts, 1922), I. gabonensis Baillon (Pieraerts, 1921) and I. barteri (Collin and Hilditch, 1930) (Table 6). Recently, Omogbai (1990) analysed the lipid and fatty acid composition of I, gabonensis (Aubry-lecombe exO'Rorke) Bail (Table 6) using a GC technique with a Hitachi 063 unit equipped with a hydrogen flame ionisation detector. To show that there are different species and varieties of Irvingia and that the seed fat in each variety has different physical and chemical value.

1.10 THE EFFECTS OF DIETARY FAT ON SERUM CHOLESTEROL, TRIGLYCERIDES AND TOTAL LIPID LEVELS

Levy et al (1971) found that three hours after a meal containing 60gm of fat, the total fatty acids in sera of nine subjects increased by an average of 21% and with a higher intake larger rises were found. Keys et al (1950) found that human plasma cholesterol

Table 6: Percentage Composition of Fatty Acids in the Seed Fat of Different Species of Irvingia

Fatty acid	I. gabonensis Bushell and Hilditch (1939)	Dika fat Irvingia spp Meara and Patel (1950)	Irvingia barteri Colin and Hilditch (1930)	Irvingia gabonensis var. excesla, Omogbai (1990)
Capric	3.1	2.0	-	-
Lauric	58.6	46.6	38.8	-
Myristic	33.4	44.11	50.6	1.0
Palmitic	2.0	5.2	-	14.6
Stearic	1.1	-	-	8.6
Oleic	1.8	1.9	10.6	20.5
Linoleic	-	0.2	-	21.5
Linolenic	-	-	-	21.3
Arachidonic	-	-	-	1.3
Others	-	-	-	10.2

- = Absent or not determined

Table 7: Physical and Chemical Characters of the Seed Fat of Different Species of Irvingia.

Physical characters	I. gabonensis Baillon (Pieraerts, 1921)	I. smithii (Pieraerts, 1922)	I. barteri Collin and Hilditch, 1930)
Melting point	37.5°C	37.8 - 39°C	41 - 42°C
Solidifying point	36.25°C	36.8-36.4°C	-
Saponification number	244.9	237.3	233.9
Acid number	-	7.1	-
Iodine number	2.9	2.96	9.1

- = Absent or not determined

can be decreased by eating a diet low in total fat, while Corner (1970) showed that marine oil lowers and coconut oil raises cholesterol levels. What affects the cholesterol is not whether a fat is of animal or plant origin but the degree of saturation of the fatty acids in the dietary triglycerides. Also it was found that serum triglycerides fasting concentration increases when fat and starch are added to an experimental diet.

Thus it is important to study the fatty acids content of food or pharmaceutical preparations to avoid or minimise the side effects of dietary fats.

1.11 THE PROSPECTS FOR COMMERCIAL PROPAGATION OF IRGINGIA GABONENSIS TREES

The planting of I. gabonensis in Nigeria has increased rapidly in recent years following its successful vegetative propagation (Okafor, 1978) which has drastically reduced fruiting age from 10 to about 3 years. Okafor (1990) used parameters of desirable characters such as fruit size and quantity, flavour and good taste (for var. gabonensis), lack of fibrousness (for var. gabonensis), and short gestation time before flowering as basis for the selection of a superior strain of I. gabonensis.

The propagation methods developed for the selected strains of I. gabonensis were vegetative methods (bud grafting and stem cuttings) (Okafor, 1971 and 1978) and propagation by seed (suitable nursery practices) (Okafor, 1981).

According to Okafor (1990), the improvement work on I. gabonensis trees has been limited to vegetative propagation techniques and to the forest zone in Southern Nigeria. But due to the promising results obtained there is a need to expand both the scope and intensity of the work to cover sexual propagation techniques and to other parts of Nigeria.

1.12 FATS AND OINTMENT FORMULATIONS

Ointments are semisolid preparations for external application and which are of such consistency that they may be readily applied to the skin (Seymour, 1970). They always carry medicaments for percutaneous absorption and action on deeper layers of tissues. Some ointments release medicaments to the blood stream as well (Mithal, 1980). All ointments consist of a base which chiefly acts as a carrier for the medicaments, and the nature of the base controls the ointment's performance. Hence, selection of the ointment base is a very important aspect of formulation. Ointment bases can

be classified into four groups as follows:- hydrophilic bases (water soluble), emulsion bases (act as emollients since their water content hydrates the skin), absorption bases (capable of taking up appreciable amounts of aqueous fluids) and oleaginous bases (composed of lipophilic materials) (Mithal, 1980).

Oleaginous bases may consist of a single lipophilic substance or a combination of two or more of such substances. Groupwise, the commonly used materials are hydrocarbons (e.g. ceresin, paraffin wax and white petrolatum), vegetable oils and animal fats (e.g. olive oil, coconut oil and bees wax), hydrogenated and sulfated oils (e.g. hydrogenated cotton seed, corn and castor oils), acids, alcohols and esters (e.g. stearic acid, lauryl alcohols and ethyl oleate) and silicones (e.g. dimethylpolysiloxanes) (Mithal, 1980). Each of these bases has its characteristics and suitable application.

Generally, ointments are evaluated for the following qualities (Mithal, 1980; Seymour, 1970; Gemmel and Morrison, 1975):-

(a) Penetration: Weighed quantities of the ointments are rubbed over definite areas of the skin for a given length of time. Thereafter the unabsorbed ointment is collected

from the skin and weighed. The difference between the two weights roughly represents the amount absorbed.

(b) Rate of release of medicaments.

(c) Absorption of medicaments into blood stream - for ointments meant to penetrate deep tissues and release medicaments to body fluids. This test can be run in vivo only, and

(d) Irritant effect - In general no ointment should possess irritant effects on the skin or mucous membranes. The tests for irritancy can be carried out on the skin and eyes of rabbits or by injecting the ointment into thigh muscles and under the abdominal skin in rats, reactions are noted at time intervals. Lesions on cornea, iris and conjuctiva are used for judging the irritancy to the eyes. Presence of patches on the skin within 2 weeks indicate irritancy to pressing skin.

1.13 FATS AND SUPPOSITORY FORMULATION

Suppositories are conveniently shaped medicated solids of various shapes and sizes suitable for introduction into one of the body orifices other than oral cavity and designed to soften and melt or to

dissolve or disintegrate at the body temperature (Mithal, 1980 and Paterson, 1970). Suppositories generally carry medicaments such as emollients, astringents, antiseptics and local anaesthetics designed to exercise local action at the site of placement. They are often used as a means of projecting medicaments such as hypnotics, tranquillisers, anti-spasmodics, etc. for systemic action. The advantage of administration of such medicaments in the form of suppositories is that portal circulation is bypassed thus preventing or retarding biotransformation of drugs in liver. Similarly pH conditions and activities of gastrointestinal enzymes are bypassed. Sometimes drugs may not be tolerated orally as in condition of nausea, etc. and under such circumstances also suppositories come in handy. The absorption of drugs from rectal mucosa has been studied by Mithal (1980).

The most important aspect of suppository formulation is the selection of the proper base since efficacy of the medicaments in suppositories depends on the base used. Currently, there are two categories of bases in use. These are water soluble/miscible bases (which dissolve slowly in the body fluids liberating contained medicaments) and oleaginous bases (which melt at body temperature slowly and

release their content of medicaments in the body fluids (Mithal, 1980). The best among oleaginous bases and the most popular is cocoa butter, the solid fat obtained from the crushed and roasted seeds of Theobroma cacao L (F. Sterculiaceae). It consists chiefly of glycerides of stearic, palmitic, oleic, arachidic and linoleic acids (B.P., 1973). The valuable characteristics of cocoa butter include melting point range, thus it is solid at normal room temperatures but melts at body temperature, liquefaction is easy on warming and setting is rapid on cooling, miscibility with many ingredients and blandness, which is a valuable feature when a suppository is intended to allay irritation (Peterson, 1970). However, it suffers the drawbacks of rancidity, melting in warm weather, leakage from body cavities, etc. The most serious drawback is its strong tendency of isomerization to forms which melt at lower temperature (Peterson, 1970). Because of these drawbacks of cocoa butter search has been on for possible substitutes. Wool fat, paraffins, olive oil, poppy oil, stearyl alcohol, cetyl alcohol and several waxes have all been investigated (Mithal, 1980). However, the hydrophobic base should necessarily possess the following characteristics: (a) It must melt at 37^{o}C

or in the alternate disintegrate to fine particles in body cavities. (b) It must be completely non-toxic, non-irritant and non-sensitising. (c) It should be compatible with a broad spectrum of drugs and should not exist in metastable forms. (d) It should have a high water number, and wetting and emulsifying properties. (e) It should be stable on storage and must shrink sufficiently on cooling so as to be easily removable from the moulds. (f) If it is vegetable or animal fat, its acid value should be less than 0.2, saponification value less than 250 and the iodine value between 7 and 15 and (g) The difference between the melting and solidification points should be small (Mithal, 1980).

Since all of the above qualities can rarely be found in a single fatty material, judicious combinations may be the answer. Generally, suppositories are evaluated for general appearance and suppositories carrying medicaments in suspension should be sliced longitudinally to determine uniform disposition of the medicament (Peterson, 1970). Assay of the therapeutically active ingredients is also a must to establish whether each unit carries labelled amounts of the drug or not. Other tests include determination of the melting range, determination of the disintegration/dissolution times and

drug uptake rates (Mithal, 1980). The melting range determination is necessary only for oleaginous bases while the disintegration and dissolution times are applicable only to water soluble bases. Fragility is determined to assess the tensile strength of the suppository and its ability to withstand the rigours of normal handling. In the case of suppositories which carry medicaments for local action, there is no need for drug uptake tests. However, those carrying medicaments for systemic action must be subjected to *in vitro* and *in vivo* tests for drug uptake. In the *in vitro* tests, the conditions of the test must simulate the situations obtaining in the body cavity. The *in vitro* methods for estimation of drug uptake are akin to the determination of the rate of release of medicament from ointments. After bringing the suppositories into contact with the simulated fluids, aliquot portions are withdrawn at different intervals of time and the amounts of drug released is noted. In the *in vivo* methods, suppositories are administered onto experimental animals and drug concentration in their plasma or urine is determined after specified intervals of time. Physiological responses wherever measurable can also be used for estimation of the drug concentrations.

1.14 AIM AND SCOPE OF THIS PROJECT

Vegetable oils and fats although abundant in Nigeria have not been fully investigated for their potential uses as pharmaceutical excipients. Studies by Odusote and Ifudu (1987) and Onaga and Odusote (1988) indicated the possibility of using indigenous fats and oils as raw materials for the pharmaceutical industry.

Along this line I. gabonensis seed fat (62-67% w/w of the seed) (Irvine, 1961) offers an example of a Nigerian vegetable fat which can be exploited for the preparation of pharmaceuticals. The specific objectives of this study are:-

(i) To prepare a general pharmacognostic profile of I. gabonensis leaves, fruits and seeds.

(ii) To extract the fat from the kernel and determine its physical (melting point, specific gravity, etc) and chemical (acid value, saponification value, etc) parameters.

(iii) To establish some pharmacopoeal standards that may be useful in preparation of a monograph of this plant.

(iv) To analyse the fat and determine its fatty acid composition.

(v) To carry out pharmaceutical evaluations of the fat in semisolid dosage formulations, particularly in ointment and suppository preparations.

CHAPTER TWO

MATERIALS AND METHODS

2.0 MATERIALS AND METHODS

2.1 PLANT COLLECTION AND IDENTIFICATION

Samples of Irvingia gabonensis (O'Rorke) Baill var. excelsa and var. gabonensis leaves, fruits and seeds were collected from Ibadan (Oyo State), Enugu (Anambra State) and Ajide (Benue State) in Nigeria.

The plant was identified at the herbarium of the Forestry Research Institute, Ibadan, Nigeria. Voucher specimens were deposited at the Forestry Research Institute, Ibadan.

2.2 EQUIPMENT, SOLVENTS AND REAGENTS

All solvents and reagents used in this work were of analytical grade B.P. All references to water indicate distilled water. A compound microscope (Wild MII) fitted with camera lucida was used.

2.3 PHARMACOGNOSTIC STUDIES ON I. GABONENSIS LEAVES, FRUITS AND SEEDS

2.3.1 Pharmacognostic Studies on I. gabonensis Leaves

General methods

Macroscopical, microscopical, chemomicroscopical, preliminary phytochemical screening and quantitative microscopy were carried out on the leaves using

standard procedures (Trease and Evans, 1983). The procedures described in B.P. (1973) were used to determine the ash and extractive values.

2.3.2 Pharmacognostic Studies on I. gabonensis Fruit

General methods

Macroscopical, microscopical, chemomicroscopical, preliminary phytochemical screening (Trease and Evans, 1983); ash and extractive values (B.P. 1973) were carried out on the fruit.

Specific Methods

(i) Estimation of the tannin content in the fruits of the two varieties of I. gabonensis

2gm of the powdered plant fruit was extracted by boiling for one hour with 100ml of water and then filtered. The filtrate was boiled and 30ml copper acetate solution (5%) was added to it with stirring. The precipitate formed was then collected on an ash-less filter paper. The precipitate was washed several times with water until it became free from copper acetate. The precipitate with the filter paper was ignited in a tared metal crucible. The residue of copper oxide was completely oxidised by the addition of a few drops of concentrated nitric acid. The residue was ignited several times till a constant weight was obtained.

The weight of the residue was then calculated by difference. The percentage of tannins in the fruits was calculated using the following equation:-

1gm of copper oxide = 1.305 gm tannin (Balbaa et al., 1976).

(ii) Estimation of reducing and non-reducing sugars in the fruits of the two varieties of I. gabonensis

(a) Estimation of reducing sugars

5ml of each of standard Fehling's solution A and B were taken in a 200ml boiling flask by means of a pipette, then 40ml of water were added and the mixture was boiled very gently over a small flame.

The sugar solution was placed in a burette, and used for titrating the boiling Fehling's solution (without removing the flame). About 2ml of the sugar solution was added at a time and the solution was brought to boiling again after each addition of the sugar solution, and after each addition, the precipitate was allowed to settle before the next addition was made.

The sugar solution was added, until the blue colour of the mixture disappeared, then 4 drops of methylene blue indicator were added to the mixture, and the sugar solution was added

in drops. The end point was reached when the colour of the indicator disappeared. The number of mls of the sugar solution used in the titration was read and recorded. This preliminary result was an approximate one.

A second titration was carried out by adding the sugar solution used in the first titration less 1ml, in order to effect complete reduction to the boiling liquid. The titration was continued without removing the flame and adding the sugar solution in drops, until the end point was reached (the colour of the methylene blue indicator disappeared). This exact titration was carried out in a total boiling time of three minutes (to avoid re-oxidation of the solution by the air).

The process was repeated until two readings, which did not differ one from the other by more than 0.2ml were obtained. The mean of these two readings was taken and 0.2ml was substracted to get the correct result. The percentage of reducing sugar in the solution was calculated as follows:-

The sugar solution taken (in ml) which reduces 10ml, Fehling's solution contains 0.05gm of any of the reducing hexoses (e.g. glucose, fructose, etc).

In case of reducing disaccharides (e.g. maltose or lactose) each 10ml of Fehling's solution is equivalent to 0.08gm of maltose or lactose.

$$\text{Percentage of glucose} = \frac{0.05 \times 100}{\text{ml. of sugar solution taken}}$$

$$\text{Percentage of maltose} = \frac{0.08 \times 100}{\text{ml. of sugar solution taken}}$$

(b) Estimation of non-reducing sugars

20ml of the sugar solution was pipetted into a conical flask, then 5ml of normal hydrochloric acid was added and the mixture was heated on a water bath for 15 minutes at 70°C. Sodium carbonate powder was added to the mixture gradually in small amounts to neutralize the solution.

The mixture was then transferred to a 100ml measuring flask, and the conical flask was washed several times with distilled water, the washings were added to the measuring flask and made up with distilled water up to the mark. The burette was filled with this solution and titrated against Fehling's solution following the copper reduction method for reducing sugars. The percentage of sucrose in the same was calculated as follows:-

- 10ml of Fehling's solution is equivalent to 0.05gm of invert sugar (glucose + fructose).

- Each 1gm of invert sugar originates from 0.95gm of sucrose.

The percentage of sucrose = $\frac{100}{15}$ x 0.05 x 0.95.

To calculate the percentage of sucrose in the original sample (sugar solution) we have to multiply the figure obtained by

$$\frac{100}{\text{Volume of sample taken (20ml)}}$$

2.3.3 Pharmacognostic Studies on I. gabonensis Seeds

General methods

Macroscopical, microscopical and chemomicroscopical studies were carried out on the seeds using standard procedures (Trease and Evans, 1983).

Specific methods

Estimation of I. gabonensis seed fat

The sun-dried seeds were cleaned from impurities such as dust and sand by sifting and blowing, then the seeds were decorticized by hand. The decorticized seeds were milled to smaller pieces and then blended to a coarse powder form. The fat from the ground seeds were extracted with boiled water or light petroleum (60 - 80°) as described below:-

(i) Separation of the fat with hot water

50g of the ground seed powder was mixed with 200mls of distilled water and heated over water bath for 30 minutes. The contents were allowed to cool at room temperature. The fat could not be separated with this method due to the formation of a sticky viscous mixture.

(ii) Extraction with light petroleum (60 - 80°C)

50g of the ground seed powder was extracted with light petroleum (60 - 80°C) in a Soxhlet apparatus for three hours. The light petroleum was later evaporated under vacuum. The fat was filtered whilst hot through Whatman No.1 filter paper and collected in a colourless bottle and kept in a cool dry place. This procedure was repeated three times.

Both varieties of I. gabonensis seeds were subjected to these extraction procedures.

2.3.4 Determination of the Physical and Chemical Characters of I. gabonensis Seed Fat

(a) Melting point

The melting point was determined using a Gallenkamp capillary melting point instrument.

(b) Specific gravity

It was determined by using the density bottle. The specific gravity of the seed fat at 42°C was calculated using the following equation:

$$\text{Specific gravity} = \frac{\text{weight in air of a volume of fat}}{\text{weight in air of the same volume of water.}}$$

(c) Determination of the saponification value

This was determined using the procedure illustrated in the B.P. (1973), the procedure was as follows:-

(i) Saponification of the fat

One gramme of the fat was placed in a 25ml pear shaped Quickfit flask. About 3 pieces of anti-bumping granules were added in the flask. The neck of the flask was securely clamped in a burette clamp and 20ml of alcoholic KOH (approx. 5% w/v)were carefully pipetted into the flask. A condenser was attached to the flask and then the flask was placed in a water bath and heated gently for one hour under reflux. During the heating process, the flask was removed two times and shaken gently to break up the lipid into globules to ensure a steady and rapid saponification.

(ii) Standardization of KOH used for the saponification

20mls of the KOH used (approximately 0.5N in 90% ethanol were pipetted into a conical

flask and 1ml of phenolphthalein (0.5% in ethanol) was added as indicator. The KOH was titrated with standard HCl (0.5N). The contents of the flask were constantly shaken during the titration.

(iii) Titration of the KOH remaining after saponification

The pear shaped flask was removed from the water bath and allowed to cool. The contents were transferred to a clean conical flask and 1ml of phenolphthalein (0.5% in ethanol) was added to it as indicator. The KOH remaining in the flask after the saponification of the fat was titrated with standard HCl (0.5N). The volume of standard HCl equivalent to the KOH used to saponify 1gm of the fat was calculated. The number of mgs of KOH required to saponify 1gm of the fat was calculated from the equation:

$$\text{Saponification value} = \frac{\text{Conc. of KOH x Vol. of KOHx1000}}{1000}$$

(d) Determination of the iodine number

This was determined using the procedure illustrated in the British Pharmacopoeia (1973). The procedure was as follows:-

Although the basis of the determination of iodine number is the addition of iodine across double bonds of unsaturated fatty acids, other halogens can be used to measure the degree of unsaturation. In this method Dam's reagent (pyridine bromide solution; 8gm of pyridine and 10gm of sulphuric acid in 20ml of glacial acetic acid mixed with 8gm of bromine dissolved in 20ml of glacial acetic acid and the mixture was diluted to 1000ml with glacial acetic acid, B.P. 1973) was used, in which bromine is used in preference to iodine because bromine reacts more readily with the unsaturated bonds than does iodine. The reagent is allowed to react with a known amount of fat in chloroform solution and the excess bromine is then determined. The total bromine in the original reagent is also determined. Thus the iodine value of the lipid can be calculated from the bromine absorbed by the lipid.

The bromine content of Dam's reagent in terms of iodine is found by adding potassium iodide and estimating the liberated iodine by titration with sodium thiosulphate.

Procedure:

(i) <u>Determination of the iodine equivalent of bromine in Dam's reagent</u>

5mls of chloroform was placed into a clean dry conical flask and then 5mls of Dam's reagent were added into the flask. The flask was kept

in a dry dark place for 10 minutes. 5mls of KI (10%) and 10ml of water were added to the flask with shaking. The contents were titrated with standard sodium thiosulfate with vigorous shaking to ensure the mixing of the two layers. When the mixture turned pale yellow, the titration continued drop-wise until both phases just turned colourless.

(ii) Determination of the iodine equivalent of bromine not absorbed by the fat

One gramme of I. gabonensis seed fat was added to 20mls of chloroform in a conical flask, then 5mls of Dam's reagent were added into the flask, which was kept in a dark place for 10 minutes. 5ml of KI (10%) and 10ml of water were added and the contents were titrated with standard sodium thiosulfate with vigorous shaking to ensure the mixing of the two layers. When the mixture turned pale yellow, the titration continued drop-wise until both phases just turned colourless. The volume of standard sodium thiosulphate equivalent to the bromine absorbed by the fat was calculated. The number of mg of iodine equivalent to the bromine absorbed by the fat was calculated. 1ml of 0.025N sodium thiosulfate is equivalent to 3.17 mg of iodine.

The number of gms of iodine equivalent absorbed by 100 parts of fat was then calculated using the following equation:

$$\text{Iodine Number} = (\text{Blank-Test})\ \text{Titration} \times 0.003175 \times 0.4 \times 100$$

(e) Determination of the acid value

10gm of the seed fat were placed in a 250mls conical flask. 50mls mixture of equal volumes of ethyl alcohol (95%) and ether which had been neutralized after the addition of 1ml of phenolphthalein were mixed with the seed fat then the flask was shaken gently until the fat was completely dissolved. The flask contents were then titrated with 0.1N aqueous potassium hydroxide, shaking constantly until a pink colour which persisted for fifteen seconds was obtained. The procedure was repeated several times, and the average reading was taken. The acid value of the fat was calculated from the equation (B.P. 1973).

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{\text{weight (in gm) of fat}}$$

a = Number of ml required in the titration.

2.4 STUDY OF THE HYDROLYTIC PRODUCTS OF I. GABONENSIS SEED FAT

The nature of I. gabonensis seed lipid was studied by hydrolysing the seed lipid with aqueous and alcoholic KOH separately and the hydrolysate of each process was examined for its alcoholic and fatty acid composition as follows:-

2.4.1 Study of the Composition of the Alcoholic Part of I. gabonensis Seed Lipid Hydrolysate

2.4.1.a Aqueous KOH hydrolytic products

3gm of the seed lipid was mixed with 60mls of 5% KOH solution in a porcelain dish and the mixture was allowed to boil on a gauze over a burner for one hour, distilled water was added to the boiling mixture from time to time to maintain the initial volume of the mixture.

Completeness of the hydrolysis was tested by taking a few drops of the boiling mixture and mixing it with boiling water. If the sample was fully dissolved in hot water without liberating oily drops, this would indicate complete hydrolysis of the lipid (Rosinkin, 1978). This test was repeated three times at 10 minute intervals; and test indicated the presence of unhydrolysable lipid.

After 60 minutes, 60ml of hot saturated solution of NaCl was added to the hydrolysate. This was to effect the separation of the soap and the unhydrolysable lipid from the aqueous solution (they form a layer which floats on top of the aqueous solution). The soap and the unhydrolysable lipid were separated from the aqueous solution by filtration and each fraction was treated as follows:-

(i) The soap and the unhydrolysable lipid were mixed with 20mls of light petroleum (40-60°C) and the mixture was filtered through a filter paper (soap is not soluble in petroleum). The petroleum filtrate was left to evaporate at room temperature and the residue was collected and designated as fraction LW. This fraction was later hydrolysed with alcoholic KOH and its alcoholic and fatty acids fractions were separated using the same procedure as in experiment 2.4.1b. Thus LW fraction was divided into alcoholic fraction designated as LWA and fatty acid fraction designated as LWF. Each fraction was examined using the same procedure as in experiment 2.4.1.b.

(ii) The aqueous layer was made neutral to pH test paper, then the liquid was filtered through a wetted filter paper. The filtrate was evaporated in a porcelain dish until crystallization started,

then the mixture was allowed to cool and 10mls of ethyl alcohol were added to the mixture and the liquid mixture was decanted slowly to another porcelain dish. The alcohol mixture was evaporated over a water bath to a syrupy consistency mixture. This mixture was designated as fraction LG and it was tested for the presence of glycerol by TLC techniques (Scheme 1).

Test sample

Fraction LG (page 56).

Reference sample

Glycerol (BDH Chemicals, England).

Adsorbent

Silica gel GF (Type 60).

Technique

Ascending

Solvent systems

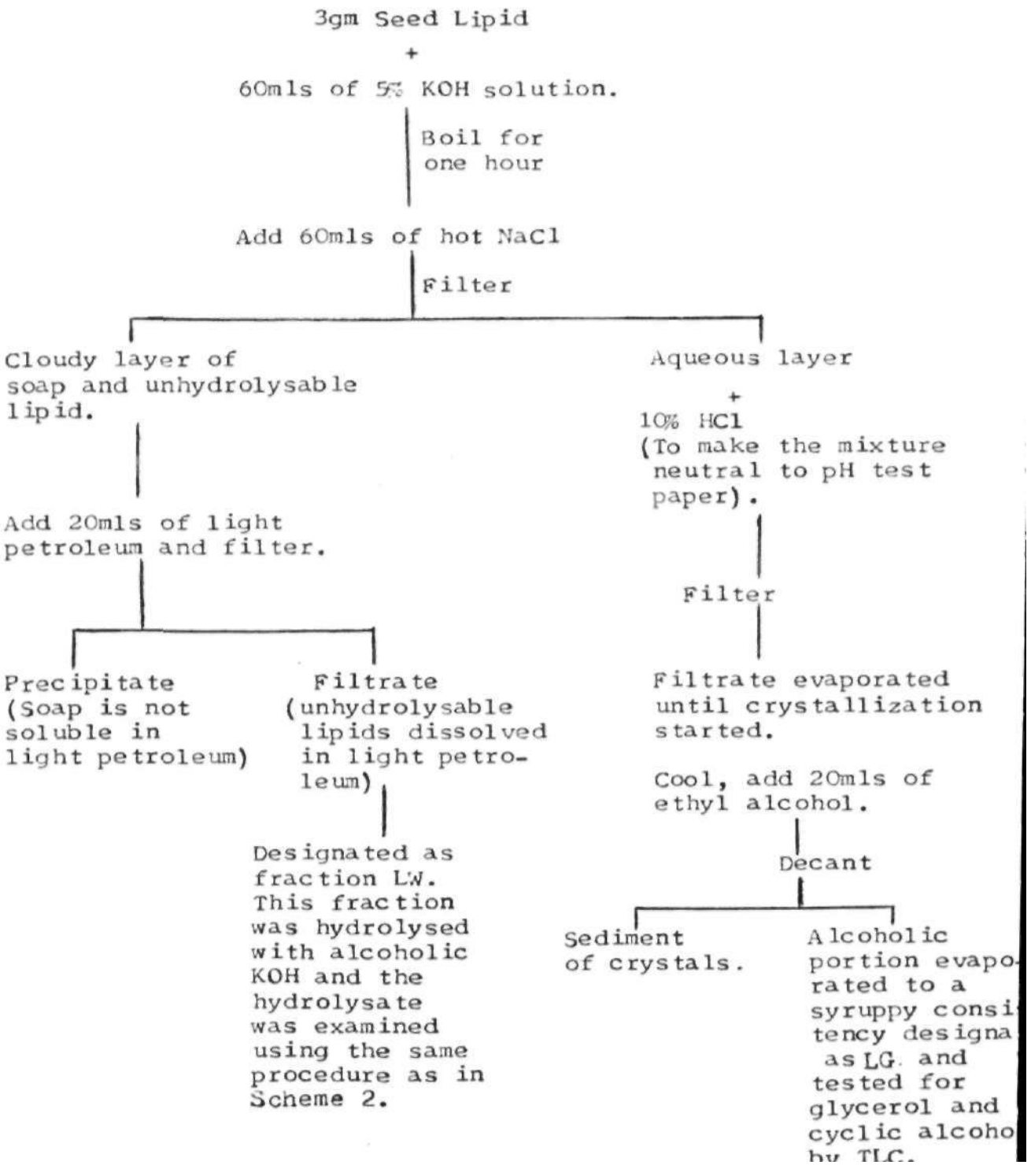
1. Ethyl acetate : Ethyl alcohol : Water : Toluene
   (4 : 2 : 1 : 0.5)
2. Chloroform : Methanol : Water
   (15 : 4 : 1)
3. Toluene : Ethyl alcohol
   (10 : 1)

Visualisation

Exposure to iodine vapour.

Scheme 1: A flow chart for Analysis of the Alcoholic portion of I. gabonensis Seed Lipid Hydrolysate (Aqueous hydrolysis).

Aqueous Hydrolysis of I. gabonensis Seed Lipid



2.4.1.b. Alcoholic KOH hydrolytic products

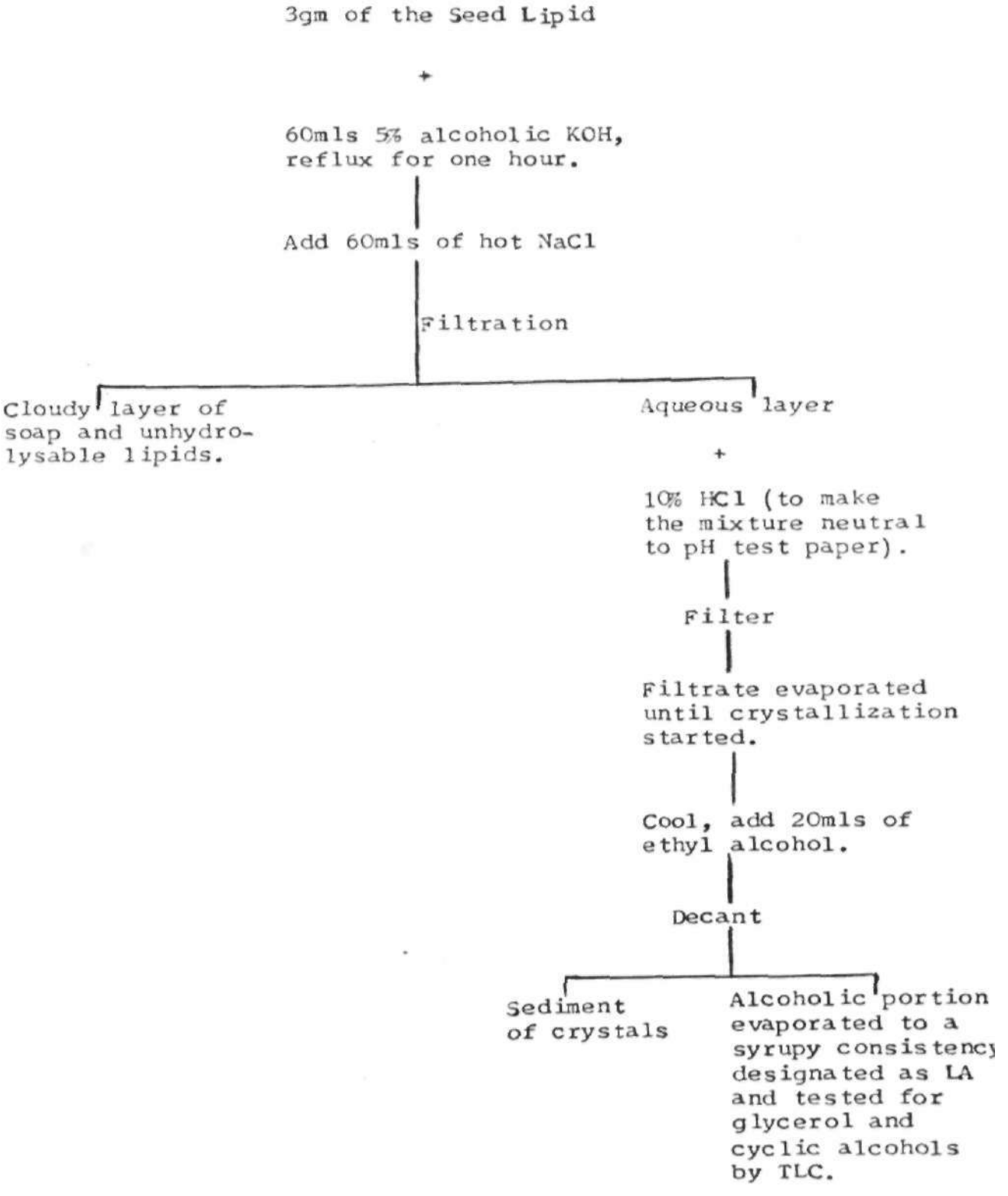
3gm of the seed lipid were mixed with 60mls of 5% alcoholic potassium hydroxide. The mixture was allowed to boil for one hour over a water bath in a round bottom flask fitted with a condenser, then a hot saturated solution of NaCl was added to the mixture until the mixture became cloudy and a layer of soap rose to the surface, then the mixture was allowed to cool and the soap was separated from the aqueous layer by filtration through a wetted filter paper. 10% HCl was added to the filtrate to make it neutral to pH test paper then it was filtered. The filtrate was evaporated in a porcelain dish until crystallization started, then the mixture was allowed to cool and 15ml of alcohol were mixed with it, the alcoholic mixture was decanted and evaporated over a water bath to a syrup consistency mixture. This mixture was designated as fraction LA and was tested for the presence of glycerol, cyclic alcohols (sterols or terpenes) by TLC. The test samples were co-chromatographed with standard glycerol, cetyl alcohol and cholesterol. (Scheme 2).

Test sample

LA (from 2.4.1.b).

Scheme 2: A flow chart for Analysis of the Alcoholic Portion of I. gabonensis Seed Lipid Hydrolysate (Alcoholic Hydrolysis)

Alcoholic KOH Hydrolysis of I. gabonensis Seed Lipid



Reference sample

Glycerol (BDH)

Cholesterol (BDH)

Cetyl alcohol (BDH)

Adsorbent

Silica gel GF (Type 60).

Technique

Ascending

Solvent systems

4. Light petroleum : ethyl acetate (9 : 1)
5. Toluene : ethyl alcohol (2 : 1)
6. Toluene : ethyl acetate : ethyl alcohol (3 : 1 : 1)
7. Chloroform : acetic acid (99.5 : 0.5).

Visualisation

- Iodine vapour (as a general spray reagent).
- Conc. sulfuric acid (for detecting the presence of sterols; Trease and Evans, 1983).
- Antimony trichloride in chloroform (for detecting the presence of sterols; Trease and Evans, 1983).

2.4.2 Study of the Composition of the Fatty Acid Part of I. gabonensis Seed Lipid Hydrolysate

The composition of the fatty acids present in I. gabonensis var. excelsa seed fat was analysed by TLC, GC/MS and high resolution gas chromatography (HRGS).

2.4.2.a Thin Layer Chromatography

Test Samples

The seeds of I. gabonensis were extracted with light petroleum (60 - 80°C). Then light petroleum was evaporated under vacuum. The extracted fat was hydrolysed by refluxing it for 10 minutes with a solution of sodium hydroxide in methanol (1 : 6) (Rosinkin, 1978).

Reference Samples

- Lauric acid
- Myristic acid
- Oleic acid
- Stearic acid

All samples were prepared in chloroform : methanol mixture (2:1).

Adsorbent

1 - Silica gel GF (Type 60).

2 - Silica gel G (Type 60) impregnated with 5% (w/w) silver nitrate.

Technique

- Ascending

Solvent Systems

1 - Chloroform : Benzene (7:3)

2 - Chloroform : Benzene (5:3)

3 - Light petroleum : Diethyl ether : Acetic acid (90:10:1)

4 - Light petroleum : Diethyl ether : Acetic acid (70:30:3)

5 - Chloroform : Acetic acid (99.5 : 0.5).

Visualisation

Exposure to iodine vapour and heating at 100°C for 10 minutes.

2.4.2.b Gas Chromatography/Mass Spectrometry

This system provides complete confirmation of each component eluting from the GC capillary column into the mass spec. detector. Confirmation is achieved by electron bombardment of the molecules eluting, forming ionized fragments that are separated in the mass analyzer to give a mass spectrum for each component. The mass spectrum is then compared to standards through a library search that confirms the identity of the compound. For I. gabonensis var. excelsa seed fat analysis, the triglycerides were converted to fatty acid methyl esters (FAME) suitable for GC analysis according to the method described below (Sheppard and Iverson, 1975).

Reagents

- Sodium hydroxide in methanol (20% w/v)
- Boron trifluoride in methanol (14% w/v)
- Magnesium sulphate (anhydrous)
- Sodium chloride.

Test samples

- The seed fat of I. gabonensis was treated as follows:-

(a) Hydrolysis

200mg of the seed fat sample was placed in an ordinary test tube, then 4ml of sodium hydroxide in methanol (0.5ml : 3ml) were added along with one or two anti-bump granules. The mixture was allowed to reflux gently for 5 minutes in a water bath at 65°C. Methanolic NaOH was used for the hydrolysis instead of aqueous NaOH because it completely hydrolysed the seed fat.

(b) Re-Esterification/Transmethylation

The contents of the test tube were allowed to cool, then 2mls of 14% boron trifluoride in methanol was added along with two anti-bumping granules, then the mixture was refluxed for 2 minutes in a water bath at 65°C.

(c) Extraction

The contents of the test tube were allowed to cool, then poured into 20mls of a saturated salt solution (NaCl) in a small conical flask. The mixture was extracted twice with 10mls of hexane, using a separating funnel, the extracts were then combined and anhydrous magnesium sulphate was added to dry the extract. The magnesium sulphate was then filtered off, and the hexane solution was stored in a refrigerator until it was required.

Reference samples

			MW
-	Methyl laurate	$CH_3(CH_2)_{10}COOCH_3$	214
-	Methyl myristate	$CH_3(CH_2)_{12}COOCH_3$	242.40
-	Methyl palmitate	$CH_3(CH_2)_{14}COOCH_3$	270.20
-	Methyl stearate	$CH_3(CH_2)_{16}COOCH_3$	298.51
-	Methyl oleate	$C_{17}H_{33}COOCH_3$	296.32
-	Methyl linoleate	$C_{17}H_{31}COOCH_3$	294.46

All obtained from BDH Chemicals Ltd, Poole, England.

Equipment

- Gas Chromatography with mass spectra GC/MS, Hewlett Packard, Model No. 5995C (Quadrupole).

Operating conditions

Column : Capillary direct union - Carbowax 20u - 25 meter. Internal diameter 0.31mm. Film thickness 0.3um.

Carrier gas : Helium 1ml/min. at 220°C.

Detector : Flame Ionisation Detector.

Total run time: 10 min.

2.4.2.c High Resolution Gas Chromatography (HRGC)

The method of HRGC analysis was as follows:-

(i) Extraction

The lipids were extracted from the seeds of I. gabonensis Var. excelsa and Var. gabonensis separately by mixing the samples with chloroform - methanol (2:1), then centrifuged at 4000 rpm for 10 minutes. After centrifugation, the lower phase was separated and evaporated to dryness under a nitrogen stream. Light petroleum (0.5ml, 40-60°C) was added immediately and the samples were transmethylated as described below.

(ii) Derivatisation for GC (Transesterification)

Derivatisation was performed by modifying an earlier method developed for rapeseed fatty acid analysis (Hiltunen et al., 1979) as follows:-

(a) Sodium methoxide in dry methanol (0.5ml, 0.5N) and a couple of boiling stones were added to the samples dissolved in light petroleum and the transmethylation was carried out in a water bath at 40°C for 5 minutes (Seppanen-Laakso et al., 1989).

(c) The excess of sodium methoxide was neutralized with a 15% aqueous solution of sodium hydrogen sulphate (1ml).

(d) The fatty acid methyl esters (FAME) and the free fatty acids (FFA) were extracted by adding 0.5ml of light petroleum. A 1ul aliquot from the upper layer was used for GC analysis.

(iii) Gas Chromatography Condition

The gas chromatography analysis was carried out on a DANI 3865 GC using a programmed temperature vapourized (PTV) sampling technique with split mode.

Column : NB - 351 fused silica column (25m, 0.32mm i.d., film thickness 0.20um).

The column oven was programmed from 100°C to 235°C increasing at 10°C/min under the following run conditions:

Carrier gas : H_2 (2.5ml/min.)

Split Ratio : 50 : 1

Detector : Flame ionization detector (FID) 250°C.

PTV - Injector 70°C —— 250°C.

(iv) Identification

Identification was based on the retention times of standard FAMEs and FFAs obtained from NU Chek Prep, Inc. (Elysian, MN, U.S.A., Mixture 68A), Applied Sciences (State College PA, U.S.A., Mixture 3), Supelco (Bellefonte, PA, U.S.A., Rapeseed oil Reference Mixture and Sigma (St. Louis, MO, U.S.A., FFAs) and on those presented in the literature (Bohov et al, 1984, Slower and Lanza, 1979).

The peak areas were measured with a Shimadzu C - R_3A integrator. And from the peak areas, the approximate percentage composition of the seed fat samples were calculated for Var. excelsa and Var. gabonensis.

2.5 EVALUATION OF I. GABONENSIS SEED FAT AS A BASE FOR SEMISOLID AND SOLID DOSAGE FORMS

2.5.1 Evaluation of I. gabonensis Seed Fat as an Ointment

I. gabonensis seed fat was used as an ointment base for different medicaments and the formulated ointments were evaluated for the following qualities:-

(a) Assessment of the rate of release of medicaments from an ointment base consisting of I. gabonensis seed fat.

Materials: Salicylic acid, Resorcinol, Ferric chloride, yellow soft paraffin, castor oil and wool fat. All these were of analytical grade and conformed with B.P. standards.

Preparation of ointment bases: Bases containing varying proportions of I. gabonensis seed fat were prepared by the fusion method (Seymour, 1975) to select a base of optimal consistency, melting and solidification points (Tables 24 & 25). On this ground the base consisting of I. gabonensis seed fat 80% w/w and wool fat 20% w/w was chosen for comparison with simple ointment B.P. which was used as standard. The same procedure as for simple ointment B.P. was used to produce an ointment consisting of I. gabonensis seed fat 70% w/w and castor oil 30% w/w.

For the preparation of medicated ointments, the water soluble drug, resorcinol, and the poorly water soluble, salicylic acid, were incorporated into each base to a final concentration (Seymour, 1975).

Procedure: The release of drugs from the different ointment bases was studied by evaluating the *in vitro* release of resorcinol and salicylic acid, respectively using the colorimetric method (Onaga and Odusote, 1988). 20ml of 1% w/w bactoagar gel, previously melted and cooled to 42°C, was poured into clean petri dishes of 9cm internal diameter, and allowed to set. After 30 minutes, 5ml of 5% w/v ferric chloride solution was poured on the surface of the agar layer. After 30 minutes, four uniform cups having 6mm diameter were removed by means of cork borer No. 3 in such a way that they were uniformly spaced on the solid agar surface, thus allowing triplicate sampling of each ointment base per plate and the fourth cup was used for the plain unmedicated base (control). Two agar plates were used for the study of each base.

The test ointment was introduced into each agar cup by means of a 5ml glass syringe, 0.3ml (about 250 $\pm$ 5mg) of the ointment was gently pushed

down to make uniform contact with the agar gel and to level off with the agar surface. The diameters of the colour zones produced around each cup were measured at 1, 2, 8 and 24 hours, (salicylic acid produced clear blue and distinct colour zones upon contact with the ferric chloride incorporated into the agar gel, while resorcinol produced faint violet zones). The mean diameter of each colour zone was converted into the area of diffusion zone produced which was corrected for the area of the ointment sample in the cup. The experiments were repeated at room temperature of 28 ± 2°C and also at 37 ± 1°C.

(b) Assessment of the irritant effect of I. gabonensis seed fat on rats

Two groups (A and B) of rats were used, Each group had five rats, four rats were used in testing, while the fifth rat served as control. The rats were injected with 0.1ml per kg (group A) and 0.5ml per kg (group B) of I. gabonensis seed fat into the thigh muscles. The control rat of each group was injected with 0.1ml per kg of simple ointment B.P. (1973). The rats were observed at intervals of 24, 48, 72 and 96 hours for any possible reactions (Mithal, 1980).

(c) Assessment of the stability of I. gabonensis seed fat ointment bases

The different ointment bases formulated with I. gabonensis seed fat, were packed in well-closed ointment jars and stored at both room temperature 28 ± 2°C as well as at 37 ± 1°C for 12 weeks during which period visible evidence of instability such as colour change, change in texture, rancid odour development and phase separation were noted.

2.5.2 Evaluation of I. gabonensis seed fat as a suppository base

Suppository bases containing varying proportions of I. gabonensis seed fat and wool fat were prepared by the fusion method, using a metal mould with six cavities. This was done to select a suitable suppository base of optimal melting point, liquefaction point and collapsing weight. To study the rate of release of medicaments from the selected base, chloroquine phosphate was incorporated into the suppository base selected and its release characteristics were investigated. The suppository bases formulated were evaluated by the assessment of the following parameters.

(a) Melting point and melting time:

The melting point of the different suppositories were determined by using the open capillary tube method. The melting time was measured by immersing the suppositories in a constant temperature water bath, then recording the time needed for complete melting or dispersion of the suppositories at 37 ± 2°C.

(b) Hardness and collapsing weight:

The tensile strength of the different suppositories was tested to assess whether it would be able to withstand the rigors of normal handling or it might collapse at room temperature 28 ± 2°C. The method used by Malaneau (1948) was applied. The apparatus consists of a moulded cylinder with a diameter of 9mm, the tested suppository is inserted inside the cylinder and a weight is applied on the top of the suppository. The weight under which the suppository collapses is the collapsing weight.

(c) Assessment of the rate of release of medicaments from a suppository base consisting of I. gabonensis seed fat:

A suppository base consisting of 90% I. gabonensis seed fat and 10% wool fat was selected because of its optimal characters (Table 26) to prepare chloroquine suppositories. The displacement value of chloroquine

phosphate in the suppository was calculated. Chloroquine suppositories containing different percentages of chloroquine (1% and 10% w/w) were prepared. To study the release characteristics of the prepared suppositories, the apparatus described in the B.P. 1980 was used and the procedure applied was as follows: 1000 mls of 0.1M HCl (the dissolution medium) was introduced into the vessel of the apparatus, the dissolution medium was warmed to 36.5 - 37.5°C, one suppository was placed in the dry basket at the beginning, then the basket was lowered into position before rotation at 100 rpm. 5mls samples were taken at 30 minutes intervals, the samples were filtered and the amount of chloroquine phosphate released from the suppository into the dissolution medium was determined spectrophotometrically at 344 nm. The volume of the dissolution medium was kept constant by replacing the volume of the sample with equal volumes of the release medium. The amount of chloroquine released at 30 minutes intervals was calculated from a standard curve. The experiment was repeated five times for each of the 1% and 10% chloroquine suppositories.

2.6 EVALUATION OF THE EFFECTS OF I. GABONENSIS SEED FAT ON SERUM CHOLESTEROL, TRIGLYCERIDES AND TOTAL LIPIDS LEVELS IN RATS

Materials and reagents

All reagents used were of analytical grade and of B.P. standards.

Animals

Adult male rats of the albino Wister strain, weighing 100-200gm, bred in the Animal House Unit of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used. These animals had never been used in any experiment. 45 rats were used, they were divided into three groups of 15 each. Each group was placed in a separately labelled cage. The initial weights of all the rats were noted.

Experimental procedure

The rats were divided into three groups of 15 each, they were labelled as follows:

(i) Group I (Control group)

This group was maintained on growers mash, which was placed into moulds by addition

of water and then moulding. This was dried in the sun and then used to feed the animals in this group. The average weight of each mould was found to be 280gm.

(ii) Group II

The feed for this group of rats was prepared in the form of moulds containing 5% w/w I. gabonensis seed fat. This was prepared by weighing the required quantity of growers mash for a week's feeding calculating 5% of it and substituting it with I. gabonensis seed fat. This was then mixed, a small quantity of water added and mixed again, moulded and dried in the sun.

(iii) Group III

The feed for this group was prepared in the form of moulds of growers mash containing 10% w/w I. gabonensis seed fat.

Serum collection

Before feeding all the rats with their respective diets, 3 rats from each group were sacrificed by decapitation. The blood was collected in clean centrifuge tubes. These blood

specimens were then allowed to clot at room temperature. They were centrifuged after one hour at 3000 rpm and the serum collected. The following estimations were carried out on the serum collected:-

(i) Determination of total lipids.
(ii) Determination of total cholesterol.
(iii) Determination of triglycerides.

These values were also estimated for the first, second, third and fourth week for the rats in each of the three groups. The average values of the three sets of serum parameters were then determined. All the values were statistically analysed using the standard student's T-test or 0.05 degree confidence assumed.

Methods of evaluation

(i) Determination of total lipids

Serum total lipids were determined using the phosphoric acid - vanillin reaction method (Frings et al, 1970). Aliquots of the serum sample (200ml) were heated with 5ml concentrated sulphuric acid at 100°C for 10 minutes. After allowing it to cool to room temperature, 200ul of the contents of each tube was transferred to a clean cuvette (12 x 75mm)

and 3ml of vanillin - phosphoric acid reagent (1 : 3.2 w/w in 100ml of water) was added. The cuvettes were allowed to stand in the dark for 60 minutes. Absorbances of specimens and standards were taken at 520 nm.

Standards of 200, 400, 600, 800 and 1,000mg/100ml concentrations of stock standard (280 mg of tripalmitin and 719 mg of triolein in 50ml of chloroform) were used to prepare a calibration curve (Appendix 13).

Total lipids concentration mg/100ml was determined as follows:-

$$\text{mg total lipids/100ml serum} = \frac{Au \times C}{A}$$

Where:

Au = Absorbance of sample (nm)

A = Absorbance of standard (nm)

C = Concentration of standard (mg/100ml)

(ii) Determination of total cholesterol

Total serum cholesterol was determined by the colorimetric method of Zlatkis (1953). 100 ul of serum samples were transferred into glass stoppered centrifuge tubes and 4.9ml of 10% ferric chloride - acetic acid (1:2 v/v) was added to each tube. The contents of the tubes were mixed well and allowed

to stand for 10-15 minutes for the protein to flocculate. These were then centrifuged for 5 minutes. 1.5ml of concentrated sulphuric acid were added to 2.5ml of the clear supernatant and the mixture was allowed to stand for 20-30 minutes. Absorbances were read at 560 nm.

Standards of 100, 200, 300 and 400 mg/100ml of stock standard (400mg of cholesterol in 100ml of acetic acid) were used to prepare a concentration curve (Appendix 14).

Total serum cholesterol was calculated as follows:-

mg total serum cholesterol/100ml serum

$$= \frac{Au \times Cs}{As}$$

Where:

Au = Absorbance of samples (nm)

As = Absorbance of standard (nm)

Cs = Concentration of standard (mg/100ml)

(iii) Estimation of triglycerides

Serum triglycerides were estimated as described by Kates (1986). To a blank sample, 5.2ml (5ml isopropanol and 0.2ml water) were added, then to a standard, 5.2ml (4.8ml isopropanol,

0.2ml water and 0.2ml triolein) were added and lastly to the test, 5.2ml (5ml isopropanol and 0.2ml serum) were added while the tubes were gently swirled.

The tubes were then shaken with a mechanical mixer for a few seconds until absorbent started to settle. To facilitate separation of fluids from solids each vial was given a single sharp snap with the wrist while holding the capped vial from the top. They were then centrifuged at about 3000 rpm for 5 minutes after which a clear supernatant was transferred to correspondingly labelled tubes, then into each labelled tubes, 0.5ml potassium hydroxide solution was added and mixed by swirling. The tubes were incubated at 60 $\pm$ 4^{o}C for 5 minutes and then removed, cooled to room temperature with tap water then, 0.5ml periodate solution was added to each tube. After 10 minutes, 3ml of the colour reagent (vanillin-phosphoric acid reagent, 1 : 3.2 w/w in 100ml of water) was added to each tube, mixed, covered and then placed in 60 $\pm$ 4^{o}C water bath for 30 minutes, after which, the tubes were removed and cooled to room temperature and then transferred to correspondingly labelled cuvettes. The absorbance of standard (A) and of the test (Vs) were determined using the

reference at the same wavelength. Standards of 100, 200, 300, 400, and 500mg/100ml of stock standard (0.2ml triolein, 4.8ml isopropanol and 0.2ml water) were used to prepare the calibration curve (Appendix 12).

The triglycerides value was calculated as follows:

$$\text{Triglycerides (mg/ml)} = \frac{(V_s)\ \text{Test} \times C_s}{(A)\ \text{Standard}}$$

C_s = Concentration of standard (mg/ml).

CHAPTER THREE

RESULTS AND DISCUSSION

3.0 RESULTS AND DISCUSSION

3.1 PLANT COLLECTION AND IDENTIFICATION

The leaves of Irvingia gabonensis (O'Rorke) Baill Var. excelsa (Keay, 1989) were collected and identified by the herbarium keeper of the Forestry Research Institute, Ibadan, Oyo State, Nigeria. A voucher specimen of this plant was deposited in the Institute Herbarium under voucher Number FHI 103947.

The fruits of Irvingia gabonensis (O'Rorke) Baill Var. excelsa (Keay, 1989) were collected and identified by the herbarium keeper of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

The seeds of Irvingia gabonensis (O'Rorke) Baill Var. excelsa were purchased from the open market at Samaru Village, Zaria, Nigeria.

The leaves, fruits and seeds of Irvingia gabonensis (O'Rorke) Baill Var. gabonensis were collected from Ajide-Edumoga Village, Okpokwu Local Government, Benue State, Nigeria. A voucher specimen (No. 1957, E.M.) of this plant was depoisted in the herbarium of Biological Sciences Department of Ahmadu Bello University, Zaria, Nigeria.

All the plant parts were obtained in large quantities (1 kg to 2 kg) and left to dry at room temperature for two days after which they were transferred to oven and further dried at 35-40°C for thirty six hours. Some of the dried plant parts were kept in their whole form, and the rest were ground into coarse powder in an electric grinder. The powdered plant parts were kept in an air tight glass container and stored in a dry place.

3.2 PHARMACOGNOSTIC STUDIES OF IRVINGIA GABONENSIS LEAVES FRUITS AND SEEDS

3.2.1 Pharmacognostic Studies on I. gabonensis Leaves

(a) Macroscopical examination

Whole leaves

Botanical source : Irvingia gabonensis (O'Rorke) Baill Var. excelsa.

Family : Irvingiaceae

Geographical source: Near Forestry Research Institute, Oyo State, Ibadan,

Habitat: forest edge.

Form	:	Entire
Condition	:	Fresh

Lamina

Shape	:	Obovate elliptic
Size	:	Length 10.2 - 8.2 - 6.2cm
	:	Width 5.5 - 4 - 2.5cm
Venation	:	Reticulate
Margin	:	Entire
Apex	:	Acute
Base	:	Symmetric
Surface	:	Glabrous

Sensory characters

Colour	:	Pale green
Odour	:	Odourless
Taste	:	Acrid
Texture	:	Papery, thin

(b) Microscopical examination

(i) Surface preparations

To study the epidermal characters of the leaf, both upper and lower surfaces were examined.

Description of the leaf upper epidermal characters

Epidermal cells

Shape	:	Polygonal
Anticlinal walls	:	Straight and wavy
Thickening	:	Thick
Papillae	:	Absent
Cuticle	:	Smooth
Stomata	:	Absent
Trichomes	:	Absent

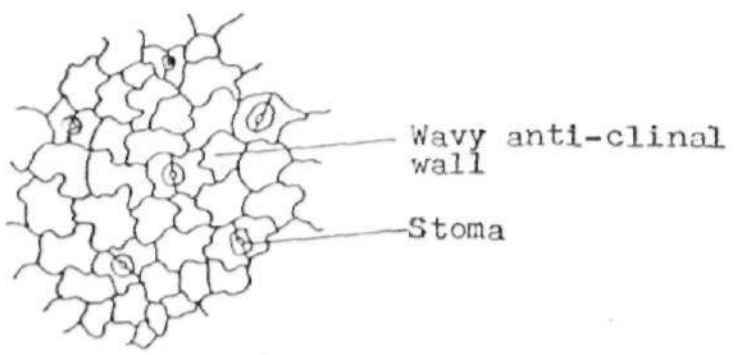
Description of the leaf lower epidermal characters

Epidermal cells

Shape	:	Polygonal
Anticlinal walls	:	Sinuous
Thickening	:	Thin, smooth
Papillae	:	Absent
Cuticle	:	Smooth

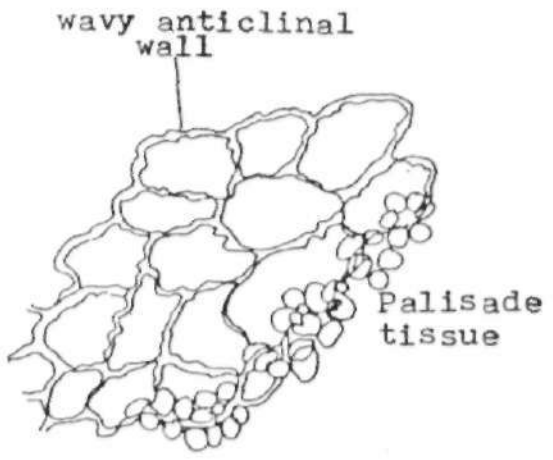
Stomata

Type	:	Paracytic
Frequency	:	Frequent
Trichomes	:	Absent



Lower epidermis in surface view with paracytic stomata

Mag. x 200



Upper epidermis in surface view with part of the underlying palisade.
Mag. x 400

Fig. 3: Epidermal characters of I. gabonensis leaf

Transverse sections through the lamina and midrib tissues:

Transverse sections of the plant leaf through the lamina and the midrib tissues were examined and the results recorded as below:

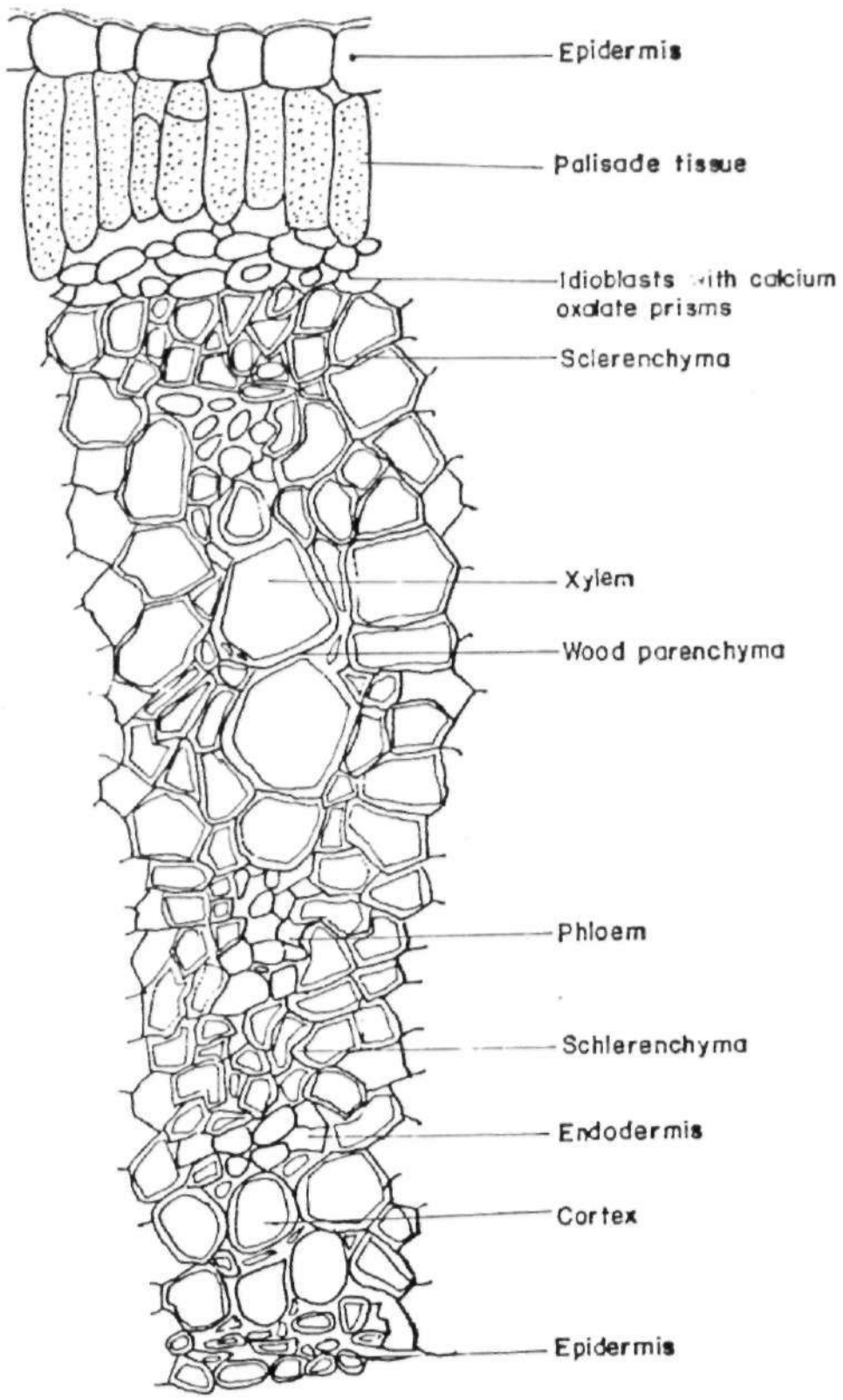
Mesophyll

Epidermal cells: They are mucilaginous, consist of a single row of polygonal tubular cells, with straight anticlinal walls, stomata of the paracytic type present in the lower epidermis. The epidermal cells are covered with a thin layer of cuticle.

Palisade layer: The leaf is dorsiventral, it has only one layer of palisade tissues, made up of cylindrical closely packed cells beneath the upper epidermis. The tissues consist of two layers of columnar chloroplast containing cells. The tissues are interrupted by the cortical tissue in the midrib and via veins.

Spongy layer: It is made of loosely packed cells rich in mucilage and interrupted by sclerenchymatous idioblasts and solitary crystals of calcium oxalate.

Endodermis: It is indistinguishable



Mag.x 400

Fig. 4 Transverse section of Irvingia gabonensis leaf through the midrib.

Pericycle: It is formed of lignified fibres and collenchyma cells forming a continuous ring around the vascular strand.

Vascular system: It appears in transverse section as an arc open above, it has a crescent shape. The tissues are made of pericyclic fibres below and a compact mass of sclerenchyma fibres above, with cells containing crystals of calcium oxalate forming crystal sheath. The phloem tissue appears towards the upper (adaxial) surface of the leaf, while the xylem appears towards the lower (abaxial) surface. The phloem is formed of small thin walled elements, the cambium is not distinguishable. The xylem is mostly primary accompanied by little secondary xylem, it consists of radial rows of vessels with the protoxylem towards the upper surface, and separated by rows of wood parenchyma. Medullary rays traverse the xylem and phloem in the form of radiation tubes.

(c) Examination of the leaf powder

The macroscopical and microscopical features of the powdered leaves were recorded in order to have a profile of its characteristics which would help in the identification of the plant.

Macroscopical characters

Colour : Greyish green

Odour : Characteristic odour

Taste : Acrid

Microscopical characters

The diagnostic characters were as follows:

Fragments of the lamina are seen in surface view, the upper epidermal cells are mucilaginous composed of subrectangular to polygonal cells with straight, moderately thickened walls which show slight beading. The underlying thin-walled palisade cells are small, round, irregularly arranged but distinct. The cells of the lower epidermis are composed of large cells with thin, slightly sinuous walls, fairly numerous paracytic stomata are present. Fragments of the epidermal tissues from over the veins also appear, they are made of elongated cells containing calcium oxalate crystals. Trichomes are absent. Abundant calcium oxalate crystals occur in the vein cells. They occur as prisms scattered in the powder as well as in the vascular tissues. Numerous vessels from the veins occur in small groups of thinly lignified and spirally thickened xylem vessels and thin walled lignified fibers.

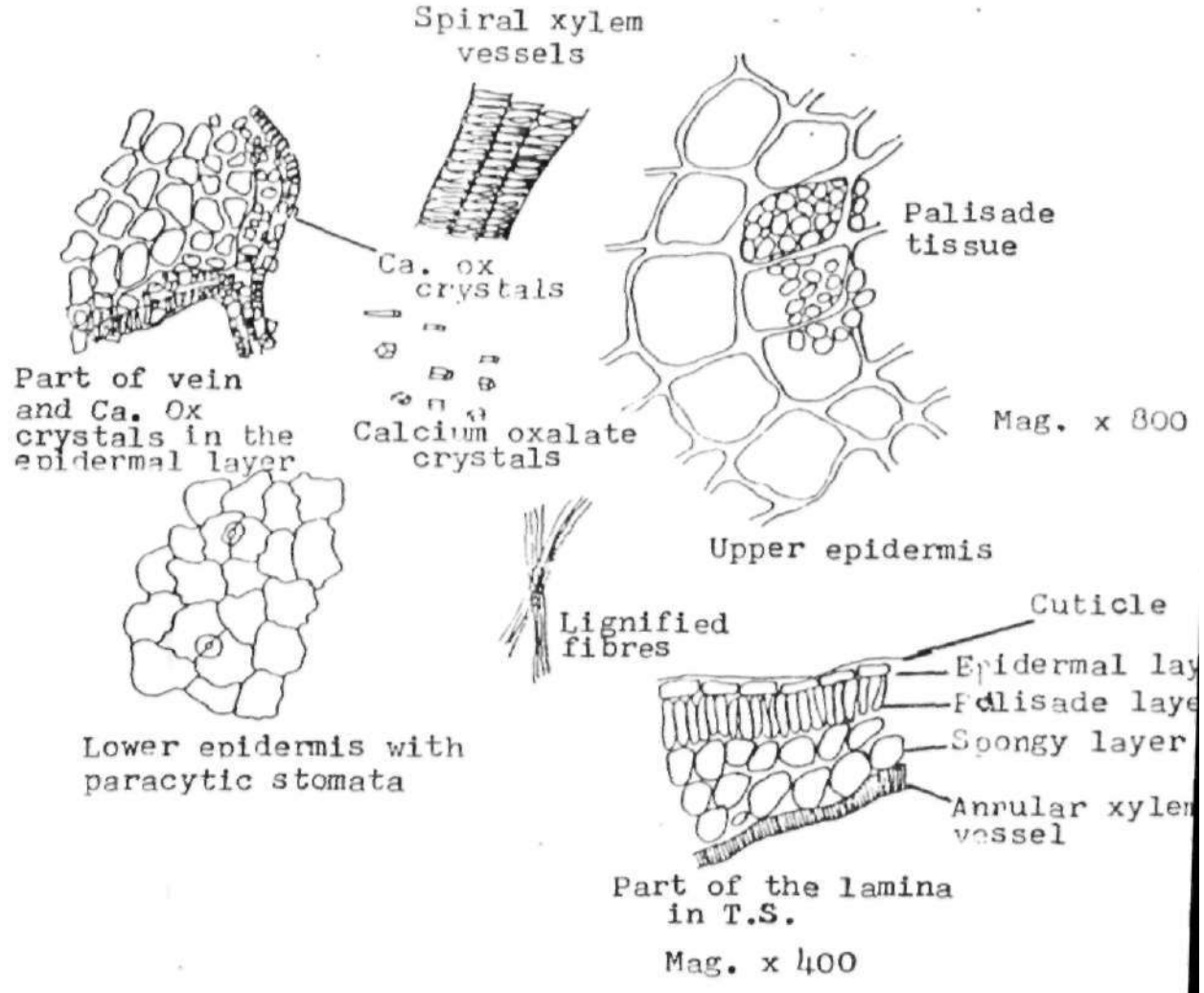


Fig. 5: Characteristic features of I. gabonensis leaf powder.

(d) Chemo-microscopical examination

Test for Lignin

The fibres present in the powdered leaves and in the transverse sections gave positive result (red colour with phloroglucinol and conc. HCl) for lignin. The fibres were thinly lignified.

Test for Cellulose

A blue colour was observed in a transverse section of the leaf (epidermal layer and around the vascular system) after the addition of N/50 iodine followed by 80% w/w sulphuric acid indicating the presence of cellulose.

Test for Tannins

A bluish-black colour was observed after the addition of 5% $FeCl_3$ solution indicating the presence of tannins. A high concentration of tannins was observed in the transverse section around the vascular system.

Test for Mucilage

A dark pink colour was observed in some cells of the spongy parenchyma after the addition of Ruthenium red. This confirmed the presence of mucilage in the leaf, mesophyll tissue.

Test for Starch

A blue colouration was observed after the addition of N/50 iodine to the leaf powder. This indicated the presence of starch in the mesophyll tissue and within the vascular system. The starch granules were found to be simple, oval in shape, about 4-6 um in diameter, hilum at a central point and no striations.

Test for Calcium Oxalate Crystals

The presence of calcium oxalate crystals was confirmed when reflecting structures were observed under the microscope with polarised light and disappeared after the addition of sulphuric acid. They occur as prims in the mesophyll tissue, they were about 2-4 um in diameter.

Test for Oils

No change of colour was observed when Sudan IV reagent was added to the leaf powder and to the transverse sections. This indicated the absence of oils in leaves.

Table 8 Summary of the Results of the Chemo-Microscopical Examination of *Irvingia gabonensis* (O'Rorke) Baill Var. *excelsa* leaves.

Constituents	Results
Lignin	+
Cellulose	+
Tannins	+
Mucillages	+
Starch	+
Calcium Oxalate crystals	+
Oils and Fats	-
Proteins	-

Key: + = Present, - = Absent

(e) Preliminary phytochemical screening

The results of the preliminary phytochemical screening indicate the absence of alkaloids, glycosides, saponins and steroids in the powdered leaf.

(f) Quantitative Microscopy

The leaves of the plant's two varieties were examined and numerical criteria for the authentication of the plant were determined and recorded (Table 7).

Table 9: Quantitative Microscopical Values* of The I. gabonensis Leaves

Type of Determination	Value for Each Variety	
	Var. gabonensis	Var. excelsa
Vein islet number	5 - 6 - 7	4 - 5 - 6
Veinlet termination number	19 - 21 - 23	8 - 15 - 22
Palisade ratio	3 - 3.5 - 4	7.2 - 9.4 - 11.6
Stomatal number	80.32-83.2 - 86	96 - 108 - 120
Stomatal index	21 - 21.8 -22.6	11.6 - 13.3 - 15

Key: Average is underlined.
*Each value represents an average of 25 determinations

(g) Extractive Values* of I. gabonensis Leaves

Table 10: Quantitative Evaluation of I. gabonensis leaf powder

Type of Evaluation	Value for variety % w/w	
	Var. gabonensis	Var. excelsa
Total ash value	11.7	11.4
Acid-insoluble ash value	5.6	6.6
Water-insoluble ash value	3	3
Alcohol soluble extractive	4.6	4.7
Water-soluble extractives	27.4	27.7

*Each value represents an average of 3 determinations.

3.2.2 Pharmacognostic Studies of I. gabonensis Fruit

(a) Macroscopical examination

The macromorphological features of the fresh fruit are recorded below:-

Type : Simple - is formed from one carpel indehiscent succulent.

Shape : Globular

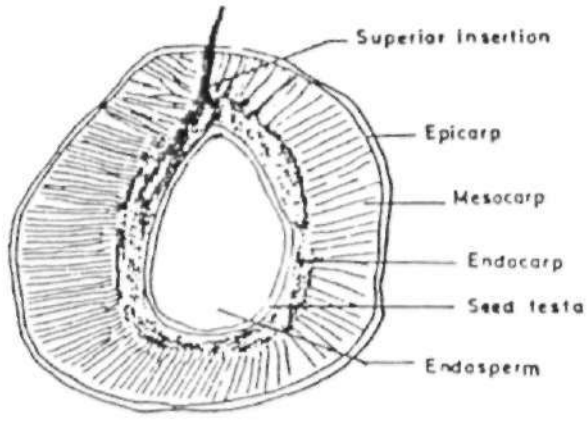
Size : The average size of 30 fruits is 3 - 4cm in diameter.

Insertion : Superior

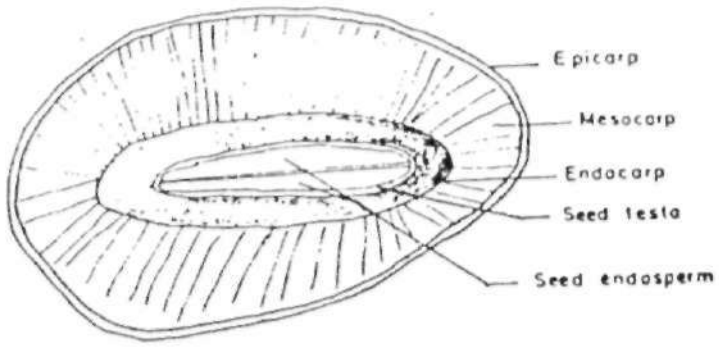
Pericarp : Colour : Yellowish brown
Texture : Leathery
Surface : Smooth with yellowish brown spots
Suture : Nil

- The pericarp differentiated into Epicarp, Leathery, Yellowish brown.
- Mesocarp, yellow in colour, soft, fleshy and fibrous.
- Endocarp, very hard and stony with creamy yellow colour.

Seeds : Single seeded fruit (for description, see under seed).



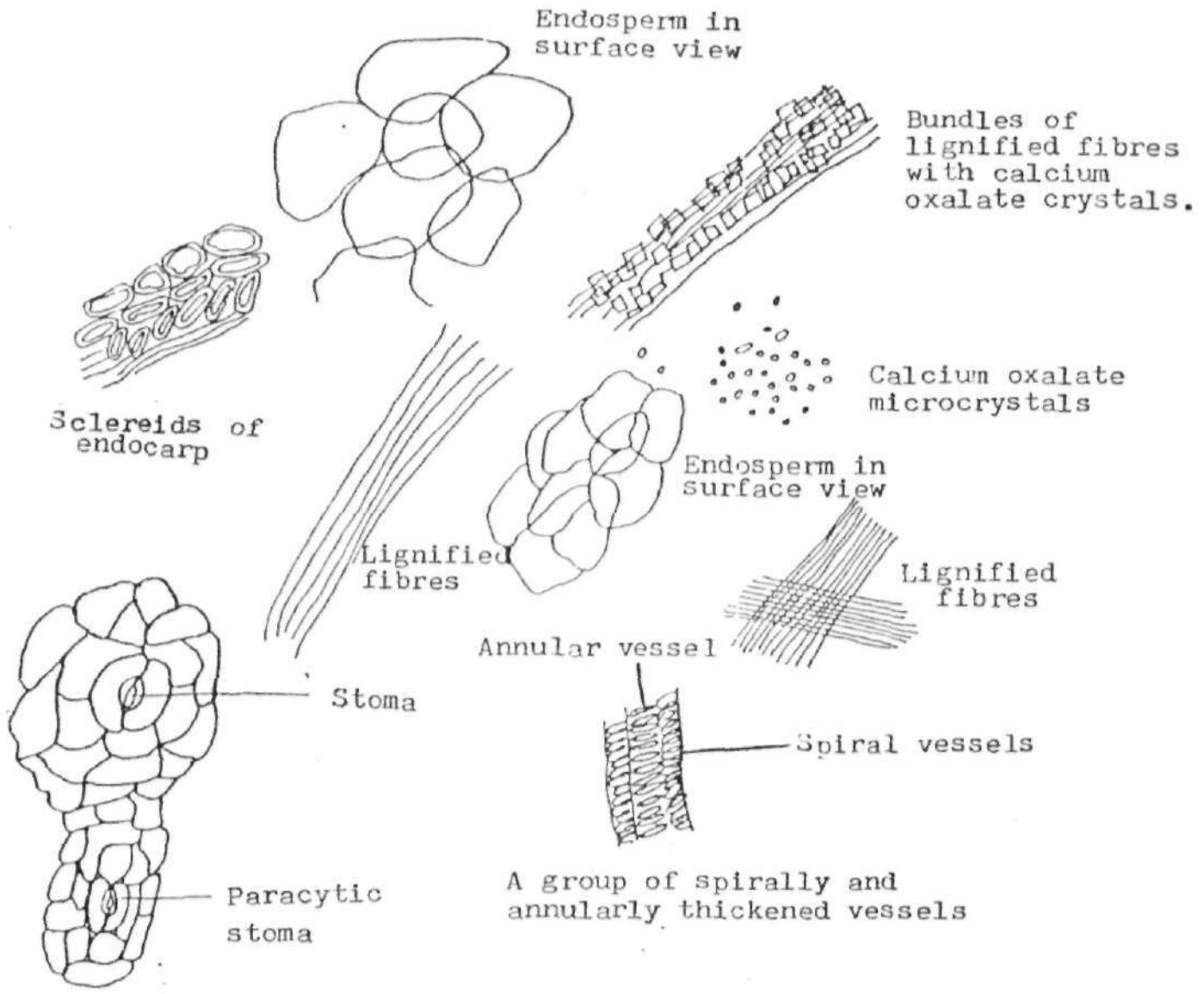
Longitudinal section.



Transverse section.

Mag. x 1

Fig. 6: Sections of I. gabonensis fruit.



Surface view of the epicarp.

Mag. x 400

Fig.7: Characteristic features of I. gabonensis fruit powder.

Odour : Characteristic fragrant smell.

Taste : Sweet bitter taste.

(b) Microscopical examination of the fruit powder

I. gabonensis fruit powder has the following features:

The numerous yellowish brown fragments of the epicarp are observed in the surface view. They are composed of several layers. The upper epidermis is of polygonal, slightly elongated cells sometimes arranged in rows with a paracytic stoma in between.

Abundant lignified fibers from the mesocarp are observed. The fibers are simple, long and lignified with narrow lumen.

The sclereids of the endocarp occur in groups of several layers. The sclereids are polygonal to elongated in surface view, they have sinuous walls which are heavily thickened and have numerous distinct pits.

The fibers of the endocarp are lignified and are surrounded by crystals of calcium oxalate.

The xylem vessels are reticulate and annularly lignified.

(c) Chemo-microscopical examination of the fruit pulp powder:

The results of the examination are listed in the following table:

Table 11 Chemical Constituents of the Fruit Pulp Powder

Constituents	Results
Lignin	+
Cellulose	+
Tannins	+
Mucilage	+
Oils and Fats	-
Starch	+
Proteins	-
Calcium Oxalate	+

\+ Present - Absent

(d) Preliminary phytochemical screening

The results of the preliminary phytochemical screening indicate the absence of alkaloids, glycosides, saponins and steroids in the fruit powder.

(e) Quantitative estimation of tannins and carbohydrates in the fruit pulp.

In order to establish some criteria to differentiate between the two plant varieties, a quantitative estimation of tannins and carbohydrates present in the plant fruit pulp was done; the results are shown in tables 10 and 11.

Table 12: Summary of the Results of the Quantitative Estimation of Some Chemical Constituents of Irvingia gabonensis Fruit Pulp

Chemical Constituent %	I. gabonensis Var. excelsa	I. gabonensis Var. gabonensis
Tannins	6.52%	3.26%
Reducing Sugars		
- Glucose	0.98%	1.04%
- Maltose	1.56%	1.66%
Non-Reducing Sugars		
- Sucrose	0.19%	0.39%

(f) Extractive Values of I. gabonensis Kernel

Table 13: Quantitative Evaluation of I. gabonensis Powdered Kernel

Type of Evaluation	Value for Each Variety % w/w	
	Var. gabonensis	Var. excelsa
Total ash value	5.5	8.6
Acid-insoluble ash value	1.66	2.83
Water soluble ash value	3.33	5.66
Alcohol soluble Extractives	5.6	5.9
Water soluble Extractives	32.4	33.2

3.2.3 Pharmacognostic Studies on I. gabonensis Seeds.

The pharmacognostic features of the whole seed are recorded below:

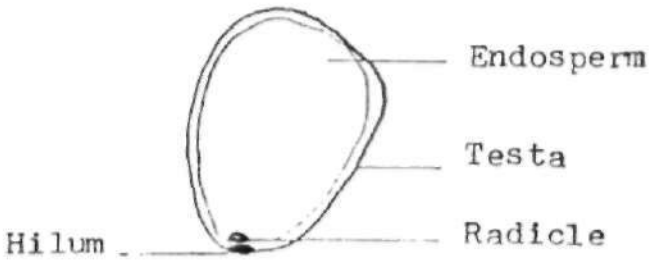
(a) Macroscopical examination:

Size	:	The average size of 30 seeds was found to be 2 - 3cm in length and 1.5 - 2cm. in width.
Shape	:	Flattened, ellipsoidal
Surface	:	Hard, smooth and polished
Colour	:	Brown or light brown
Seed Coat	:	(Testa), single, tough with a smooth surface.
Endosperm	:	Large, smooth, yellowish in colour.
Perisperm	:	Absent
Embryo	:	Dicotyledenous
Odour	:	Characteristic
Taste	:	Mucilagenous oily taste.

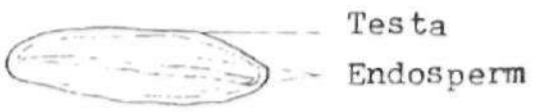
(b) Microscopical examination of the seed powder:

The following pharmacognostic microscopical features were observed:-

The abundant fragments of the pigmented layer of the testa. In surface view of the cells appear square to polygonal. Sclerenchymatous layer of the



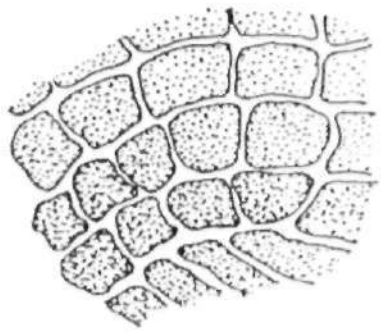
Longitudinal section.



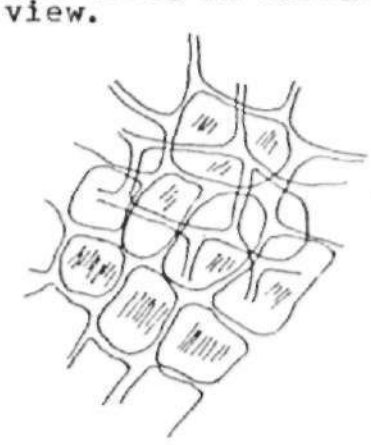
Transverse section.

Mag. x 2

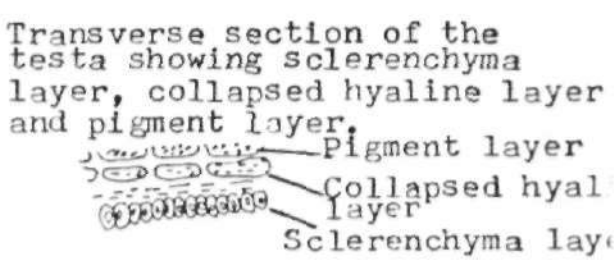
Fig. 8: Sections of I. gabonensis Seed



Pigment layer of the testa in surface view.



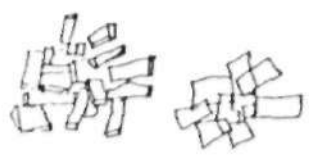
Surface view of the endosperm containing fat.



Transverse section of the testa showing sclerenchyma layer, collapsed hyaline layer and pigment layer.



Fragments of vessels with spiral thickening.



Crystalline masses of fat from a cooled chloral hydrate mount.

Mag. x 400

Fig. 9: Characteristic features of I. gabonensis seed powder.

testa, composed of longitudinally elongated cells, some heavily lignified. Fragments of the epidermis of the testa in surface view, appear as large, thin walled polygonal to rounded cells filled with mucilage. The hyaline layer of the testa is composed of very thin-walled cells which are elongated and lie with their long axes at right angles to those of the sclerenchymatous cells. The abundant parenchyma of the endosperm and cotyledons are composed of irregular polygonal cells, filled with white solid fat and mucilages. There is occasional presence of small, spirally thickened vessels associated with the fragments of the scherenchymatous layer.

(c) Chemo-microscopical examination of the seed powder.

The chemo-microscopical examination of the seed powder indicated the presence of several constituents which are listed in Table 12, starch and calcium oxalate crystals were, however, absent.

Table 14: Chemical Constituents of the Seed Powder

Chemical constituent	Results
Lignin	+
Cellulose	+
Tannins	+
Mucilage	+
Starch	-
Calcium oxalate	-
Oils and fats	+
Proteins	+

3.2.4 Extraction and Quantitative Estimation of I. gabonensis Seed Fat

The seed was found to contain a high content of mucilage, protein, cellulose as well as fat. The mucilage is soluble in water and forms a viscous sticky liquid when dissolved in water. Two methods were tried to extract the seed fat.

(a) Separation with hot water:

Due to the presence of mucilage in the ground seed powder, extraction with hot water produced a white sticky viscous liquid from which the fat could not be recovered. This process proved to be unsatisfactory.

(b) Extraction with light petroleum (60 - 80°C):

The seed powder, after extraction with light petroleum, produced a white solid fat. The fat has a pleasant odour, and melts to a colourless liquid at 40°C. The fat content estimated for the two varieties of I. gabonensis is given in Table 15.

Table 15: The Average Percentage of Fat Content in the Two Varieties of I. gabonensis Seed.

Extraction Number	Var. gabonensis	Var. excelsa
1	61.5	68.0
2	61.2	67.2
3	61.3	68.3
4	60.7	67.1
5	60.3	67.4
Average	61.00 ± 0.48	66.60 ± 0.52

The results indicate that the seeds of variety excelsa contain relatively more fat than variety gabonensis. It has also been shown that variety excelsa contains higher percentage of mucilages than variety gabonensis (Isimi, 1990). This led us to evaluate var. excelsa seed fat for pharmaceutical applications.

3.2.5 Physical And Chemical Characters of I. gabonensis Seed Fat

The physical properties of a fat are determined by the nature of the fatty acids present in the glycerides. There are several numerical values used to express the nature of these fatty acids. These values are used in the identification of individual fats and also to judge the quality of a fat.

The numerical values of the seed fat of I. gabonensis var. excelsa and var. gabonensis were investigated and the results are given in Table 16.

Table 16: The Physical And Chemical Values of I. gabonensis Seed Fat.

Physical and Chemical Character of the Fat	Numerical Value for Each Variety	
	Var. excelsa	Var. gabonensis
Melting point	42 - 44°C	42 - 44°C
Specific gravity	0.927	0.925
Saponification value	203.8 - 204	203 - 203.5
Iodine value	12.7	12.7
Acid value	0.229	0.227

Thus the results of the pharmacognostic studies on the plant show that the leaves are obovate elliptic with reticulate venation like the majority of dicotyledonous leaves. The leaf margin is entire with acute apex and symmetric base attached to a slender grooved, dark green petiole. The leaf powder is pale green with a characteristic odour and an acrid taste.

The microscopical study of the leaf surface preparations revealed that the epidermal cells are polygonal with straight and wavy anticlinal walls and smooth cuticle, the stomata are paracytic type and absent from the upper epidermis. The absence of trichomes was also observed. The quantitative microscopical values (vein-islet number, veinlet termination number, palisade ratio, etc) obtained for variety excelsa and variety gabonensis of the plant (Table 9) could be useful as an aid for the differentiation of the two varieties studied.

From transverse sections of the leaves, the leaf was found to be dorsiventral with a palisade mesophyll below the upper epidermis and a spongy mesophyll above the lower epidermis. The epidermal

cells were found to consist of a single row of polygonal tubular mucilagenous cells and stomata present in the lower epidermis. The palisade layer consists of two layers of column chloroplast containing cells; the spongy layer is made of loosely packed parenchyma cells rich in mucilage which is present in sufficient quantities to fill the cells. The pericycle is made of lignified fibre and collenchyma cells forming a continuous ring around the vascular strand. The vascular system appears in transverse section as an arc open above, it has a crescent shape. They are made of pericyclic fibres below and a compact mass of fibres above. The cells contain prismatic crystals of calcium oxalate forming crystal sheath. The fibres are lignified with blunt ends. The phloem tissue appears towards the upper surface of the leaf, while the xylem appears towards the lower surface. The phloem consists of some narrow sieve-tubes, companion cells and phloem parenchyma. The xylem is mostly primary accompanied by little secondary xylem. These observations are consistent with the general pharmacognostic characters of the family Irvingiaceae.

The fruit is simple, formed from one carpel, indehiscent, succulent, globular in shape and superior insertion. The pericarp is differentiated into leathery yellowish brown epicarp, fleshy, soft, yellow mesocarp and a very hard stony endocarp. The fruit powder is yellowish brown with a characteristic aromatic odour and an acrid taste. The microscopical examination showed fragments of the epicarp in surface view, made of polygonal slightly elongated cells, arranged in rows with a paracytic stomata in between. Also found were abundant lignified fibres from the mesocarp. The fibres are simple, long and lignified with narrow lumen. The scelereids of the endocarp appeared in groups of several layers, they are polygonal or elongated in surface view, they have sinuous walls which are heavily thickened and have numerous distinct pits. Also found are lignified fibres from the endocarp surrounded with prisms of calcium oxalate and also reticulate and annular lignified xylem vessels. The results of the qualitative tests for different chemical constituent groups in the plant (leaves, fruits and seeds) indicate the presence of sugars, tannins, oils and fats and the absence of alkaloids, glycosides, saponins and steroids.

Quantitative estimation of tannin and sugars of the fruit pulp showed that the fruit pulp of I. gabonensis var. excelsa contains a higher percentage of tannins 6.52% w/w and lower percentage of sucrose 0.19% w/w than the fruit pulp of I. gabonensis var. gabonensis which contains 3.26% w/w tannins and 0.39% w/w sucrose (Table 10). This may explain the sour taste of the fruit pulp of I. gabonensis var. excelsa.

The fruits were collected at the same time and were almost at the same stage of ripening. In a study by Aina (1990), mature, hard green I. gabonensis fruits (var. gabonensis and var. excelsa) were ripened 7 days after harvest, by which time the fruits had softened, the peel had turned yellow and the pulp had changed from creamy-white to orange. According to Hulme (1977) the yellow colour development is due to chlorophyll degradation and the subsequent prominence of carotenoids, the major colour pigment in ripe fruits. Aina (1990) found that the yellowness of the fruit was accompanied by a progressive sweetness of the fruit pulp due to formation of sugars resulting from starch hydrolysis. Hulme (1971) found that sourness in fruits decreased with progressive yellowing due to increase in

sugars and a decline in total acidity. Changes in fruit colour were also accompanied by a progressive softening of the unpeeled fruit, which was due to degradation of pectin and other hydrocolloidal substances. This occurred along with changes in fruit colour and texture and development of a characteristic pleasant flavour. This was due to the reduction in total acidity as well as an increase in extractable flavolans resulting from polymerization of tannins and other polyphenolic compounds, all of which contribute to fruit flavour (Hulme, 1971). Production of I. gabonensis fruit in Nigeria has increased rapidly in recent years following its successful vegetative propagation by the Forestry Commission (Okafor, 1978) which has drastically reduced fruiting age from 10 to about 3 years.

The seeds of I. gabonensis were found to be ellipsoidal in shape with hard, smooth polished surface and brown in colour. The microscopical examination of powdered seeds showed abundant fragments of the pigmented layer of the testa. In surface view, the epidermal cells of the testa appear as large, thin-walled polygonal to rounded cells filled with mucilage. The abundant parenchyma of the

endosperm and cotyledons composed of irregular polygonal cells filled with white solid fat. Also observed was the occasional presence of small, spirally thickened vessels associated with the fragments of the sclerenchymatous layer. Extraction and quantitative determination of the seed fat indicated that the decorticized seed of I. gabonensis var. excelsa contains slightly higher fat 67.6% w/w fat while the decorticized seed of I. gabonensis var. gabonensis contains 61% w/w (Table 15).

The physical and chemical analysis of the seed fat (Table 16) indicated that the fat has a low melting point (42 - 44°C). It is slightly lighter than water (sp. gr. 927) and has a low iodine number (12.7) indicating that it has a small percentage of unsaturated fatty acids and that it is mainly composed of saturated fatty acid; also its acid number is low (0.229) which indicates that it has a low percentage of free fatty acids. These results compared with the published data of other vegetable oils (Table 5) show that I. gabonensis seed fat has ideal physical and chemical characters like cocoa butter and shea butter for wide pharmaceutical applications and uses.

3.3 STUDY OF THE HYDROLYTIC PRODUCTS OF I. GABONENSIS SEED FAT

3.3.1 Study of the Composition of the Alcoholic Part of I. gabonensis Seed Lipid Hydrolysate

3.3.1.a Aqueous KOH hydrolytic products

The seed lipid was not completely hydrolysed by aqueous KOH solution, the unhydrolysable fraction was extracted with light petroleum (40-60°C), light petroleum was then evaporated off to produce a waxy material (m.p. 35°C) (Fraction LW, page 55).

This fraction was later hydrolysed with alcoholic KOH and its fatty acid portion (fraction LWF) and alcoholic part (fraction LWA) were analysed. The result of the analysis has shown that fraction LW is a wax, its fatty acids include stearic, myristic, lauric and oleic acids (page 120) and the analysis of fraction LW (alcoholic portion) indicated that its alcohol most probably is aliphatic alcohol or a mixture of alcohols of short chain of carbon atoms since it was not glycerol. It was immiscible with water. It gave negative result for cyclic (terpenoid or steroidal) alcohol (page 118). Fraction LWA is liquid at room temperature and aliphatic alcohol or a mixture of alcohols of short chain lengths, starting from $C_4H_{10}OH$ to $CH_3(CH_2)_9OH$ are liquids at room temperature. The next alcohol

Table 17: TLC Separation of I. gabonensis Seed Fat Hydrolysate (Aqueous Hydrolysis).
Alcoholic portion, Fraction LG (page 56)

Spot identification	Rf value (System I)	Rf value (System II)	Rf value (System III)	Visualization (Iodine vapou
Fraction LG (page 56)	.55	.64	0.32	Dark brown
Reference sample				
Glycerol (BDH Chemicals England).	.54	.64	0.32	Dark brown

Key: Adsorbent : Silica gel GF (Type 60)

Solvent system :

I - Ethyl acetate : Ethyl alcohol : Water : Toluene (4 : 2 : 1: 0.5)

II - Chloroform : Methanol : Water (15 : 4 : 1)

III - Toluene : Ethyl alcohol (10 : 1)

in this series which is dodecyl alcohol is a solid. Fraction LWA was not volatile at room temperature and had a boiling point range of 95°C - 105°C. This wide boiling range suggests strongly that the alcohol is a mixture. This tallies with the fact that waxes contain free alcohols and other substances. The alcohol, if pure, should have even number of carbon atoms since it is natural (Rosinkin, 1978) in other words C_4, C_6, C_8 or C_{10}.

Fraction LG which is the alcoholic part of the seed lipid (page 56) was analysed by TLC (page 56) and it was found to be glycerol (Table 17).

From the above results, the seed lipid was shown to compose of fat and wax as had been proved by the detection of glycerol which is the alcohol part of fats and also by the detection of aliphatic alcohols which are some of the alcohols of wax (Rosinkin, 1978 and Trease and Evans, 1983).

.3.1.b Alcoholic KOH hydrolytic products

The seed fat was completely hydrolysed by alcoholic KOH. Fraction LA, which is the alcoholic part of the seed lipid (page 58) was analysed by TLC (Table 18) and found to be composed of glycerol and some other compounds of non-steroidal nature. These compounds may probably be aliphatic alcohols. The results

Table 18: TLC Separation of I. gabonensis Seed Fat Hydrolysate (Alcoholic Hydrolysis). Alcoholic portion, Fraction LA (page 58)

Spot identification	Rf value (System I)	Rf value (System II)	Rf value (System III)	Rf value (System IV)	Visualisation: Iodine vapour	Visualisation: Conc. H_2SO_4	Visualisation: Antimony trichloride in chloroform
Fraction LA (page 57)	0.21	-	0.7	0.19	Dark brown	-	-
	0.28	0.14	0.14	0.31	"	-	-
	0.34	0.31	0.34	0.42	"	Pale yellow	-
	0.51	0.48	0.46	0.52	"	Pale yellow	Light brown
Reference sample							
Glycerol	0.28	0.16	0.14	0.31	"	-	-
Cholesterol	0.48	0.57	0.61	0.74	"	Pink	Violet
Cetyl alcohol	0.82	0.74	0.77	0.62	"	Light brown	Light yellow

Key: Adsorbent : silica gel GF (Type 60)

Solvent systems:

I - Light petroleum : ethyl acetate (9:1)

II - Toluene : ethyl alcohol (2:1)

III - Toluene : ethyl acetate : ethyl alcohol (3:1:1)

IV - Chloroform : acetic acid (99.5 : 0.5)

indicated that fraction LA was composed of all the fractions in LWA and LG.

Thus the alcoholic KOH hydrolysis of the seed lipid substantiated the results obtained from the aqueous KOH hydrolysis of the seed lipid that the seed lipid is composed of fat and wax together.

3.3.2 Study of the Composition of the Fatty Acid Part of I. gabonensis Seed Lipid Hydrolysate

The determination of fatty acid composition is an essential part of fat analysis. In this study three different chromatographic techniques were used; TLC, GC/MS and high resolution gas chromatography (HRGC). Each of these techniques has its specific applications and was employed in qualitative and quantitative analyses of the fat.

3.3.2.a TLC Separation of I. gabonensis Seed Fat Hydrolysate

The seeds of I. gabonensis var. excelsa were extracted with light petroleum (60-80°C). The extracted fat was hydrolysed by refluxing it for 10 minutes with a solution of sodium hydroxide in methanol (20% w/v). The seed fat hydrolysate was spotted on TLC plates and different solvent systems were used for development. Standard free fatty acids were used as reference samples and the results

are shown in Table 19. In this analysis two solvent systems were tried. While chloroform : benzene (7:3) on silica gel plates separated the seed fat hydrolysate into four different components. Impregnation of the silica gel G plates with silver nitrate and using chloroform:acetic acid (99.5 : 0.5) as solvent system, the seed fat hydrolysate was separated into five different components (Table 19).

Table 19: TLC Separation of I. gabonensis Seed Fat Hydrolysate (Fatty Acid portion)

Spot Identification	Rf value (System I)	Rf value (System II)	Visualization (Iodine vapour)
Hydrolysate of seed fat			
1	0.00	0.24	Dark brown
2	0.56	0.08	" "
3	0.60	0.18	" "
4	0.96	0.35	" "
5		0.68	" "
Reference fatty acids			
Lauric acid	0.00	0.24	" "
Myristic acid	0.55	0.08	" "
Stearic acid	0.60	0.18	" "
Oleic acid	0.95	0.36	" "

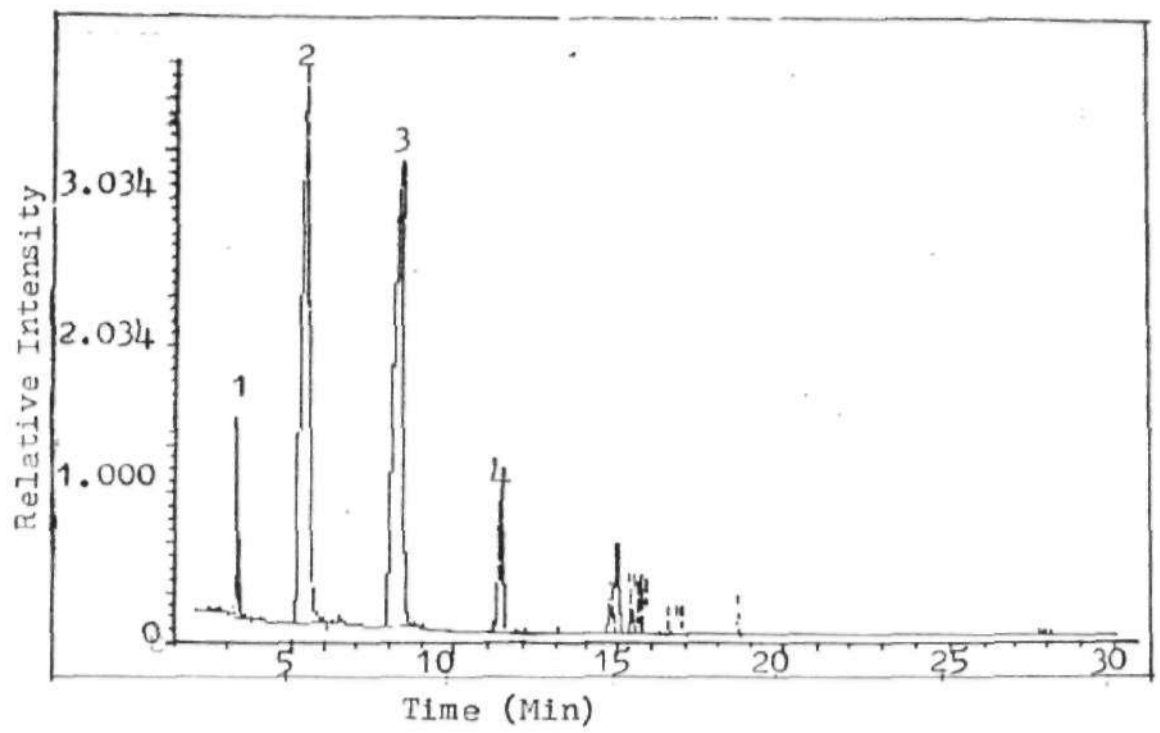
Key: System I
Adsorbent : Chloroform : Benzene (7:3)
: Silica gel G
System II : Chloroform : Acetic acid (99.5:0.5)
: Silica gel G impregnanted with 5% silver nitrate.

The fatty acids identified are lauric, myristic, stearic and oleic.

3.3.2.b Gas Chromatography/Mass Spectrometry (GC/MS)

Gas chromatographic separation of the methylated I. gabonensis seed fat was accomplished using carbowax column (25m x 0.31mm I.D., 0.3 um film thickness) with a linear velocity of helium of 1.0ml/min and a column temperature of 220°C. The GC column was connected directly to the mass spectrum. The gas chromatogram (Fig.10) gave 4 well separated peaks (1,2,3,4) and a number of closely packed peaks, which were expanded (Fig.11) and gave another 4 peaks (5,6,7,8). Each peak (representing a compound) was given a number. The retention time as well as the mass spectra of each compound was determined and compared with the aid of a computer (connected with data based centre) with data previously stored for free fatty acids (FFA) and methylated fatty acids (MFA). Thus the fatty acid derivatives identified are listed in Table 22, peak (7) was identified as free lauric acid. It was probably due to the fact that the lauric acid of the seed fat was not completely methylated. Repeated runs gave exactly the same results.

Fig.10: Gas Chromatogram of (I. gabonensis Seed Fat) Fatty Acid Methyl Esters (FAME).

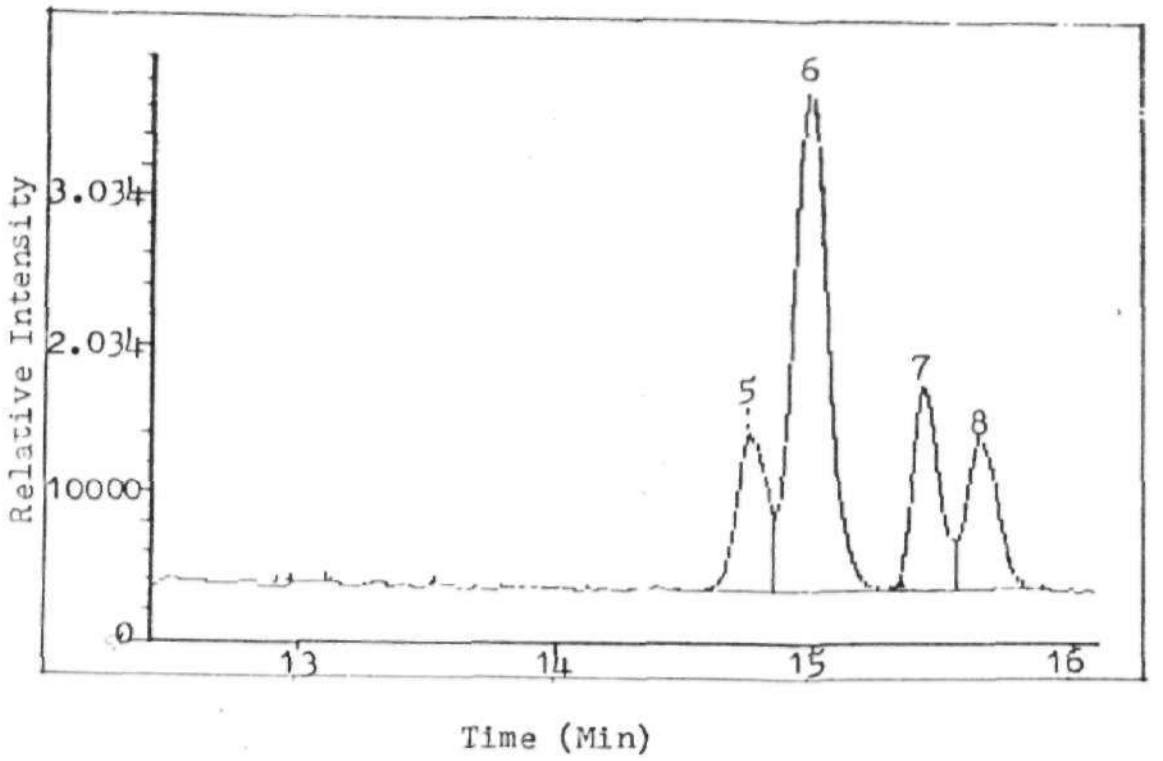


Peak identification was done by comparing the retention time of each peak with the retention time of standard FAME.

Table 20: Retention times of compounds in peaks number 1, 2, 3 and 4 of fig. 8 obtained on Gas Chromatogram of the I.gabonensis seed fat.

Peak No.	Rt (Minute)	FAME
1	3.317	Methyl caprate
2	5.506	Methyl laurate
3	8.395	Methyl myristate
4	11.420	Methyl palmitate

Fig.11: Gas Chromatogram of (I. gabonensis Seed Fat) Fatty Acid Methyl Esters (FAME). (continued)



Peak identification was done by comparing the retention time of each peak with the retention time of standard FAME.

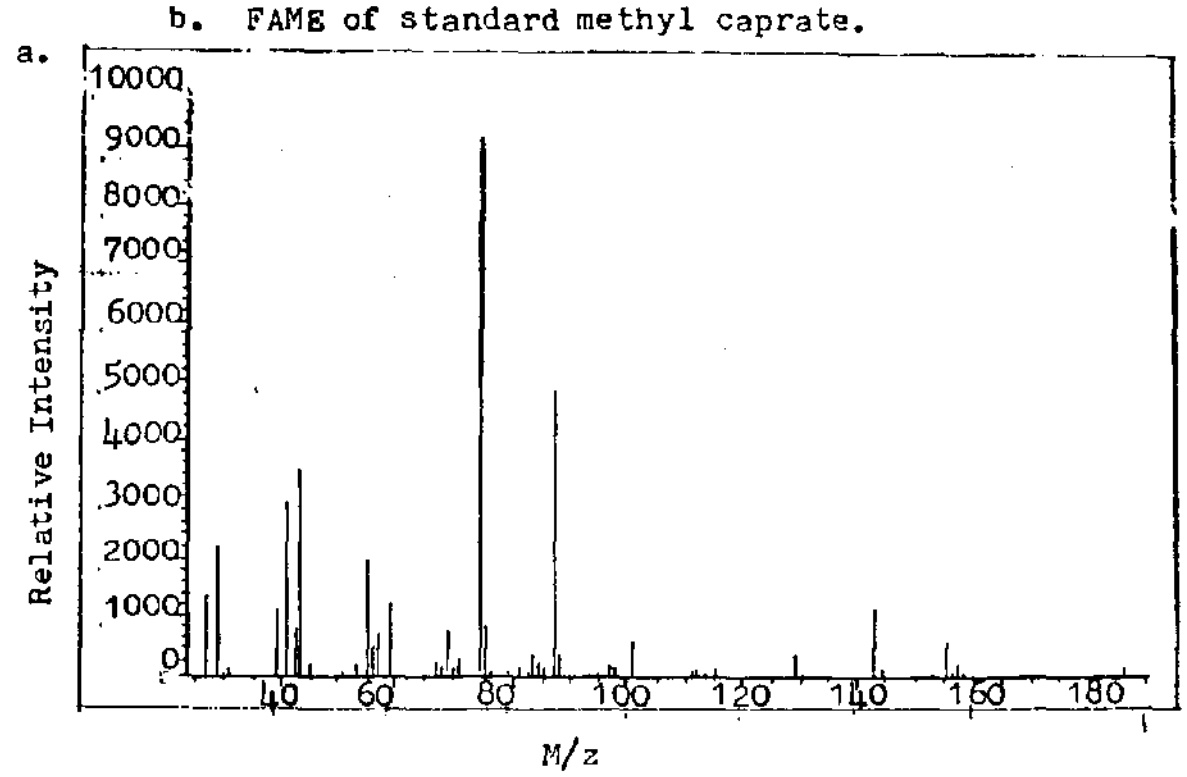
Table 21: Retention times of compounds in peaks number 5, 6, 7 and 8 of fig. 9 obtained on Gas Chromatogram of the I.gabonensis seed fat

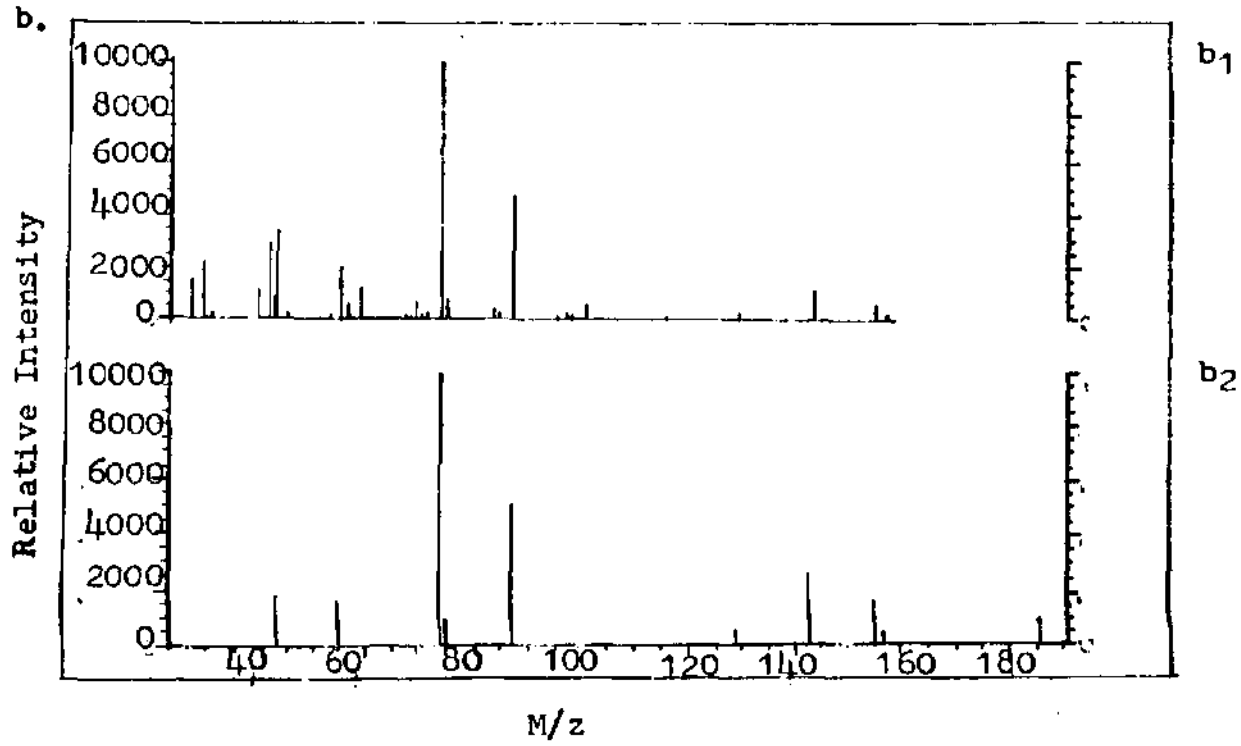
Peak No.	Rt. (Minute)	FAME
5	14.743	Methyl stearate
6	14.990	Methyl oleate
7	15.418	Lauric acid
8	15.636	Methyl linolate

Fig. 12: Mass spectrum of the FAME compound in peak 1 and corresponding reference compound.

a. FAME of test compound.

b. FAME of standard methyl caprate.



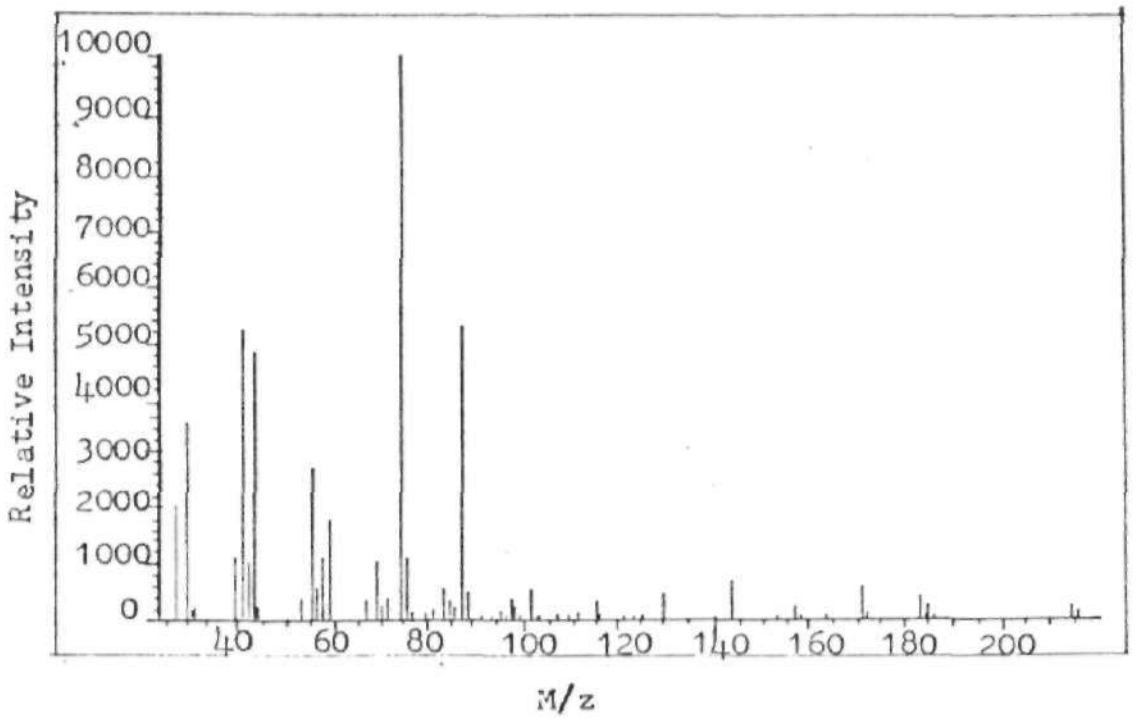


EIMS: M/z (rel. int.): $[M]^+$ $C_{11}H_{22}O_2$, found: 186.17 (1.25) (Calc. 186); 143.1 (11.46); 87.10 (48.93); 74.10 (100). 41.05 (29.55).

b_1 Mass spectrum of FAME test compound

b_2 Mass spectrum of FAME standard methyl caprate

Fig.13: Mass spectrum of the FAME compound in peak 2.

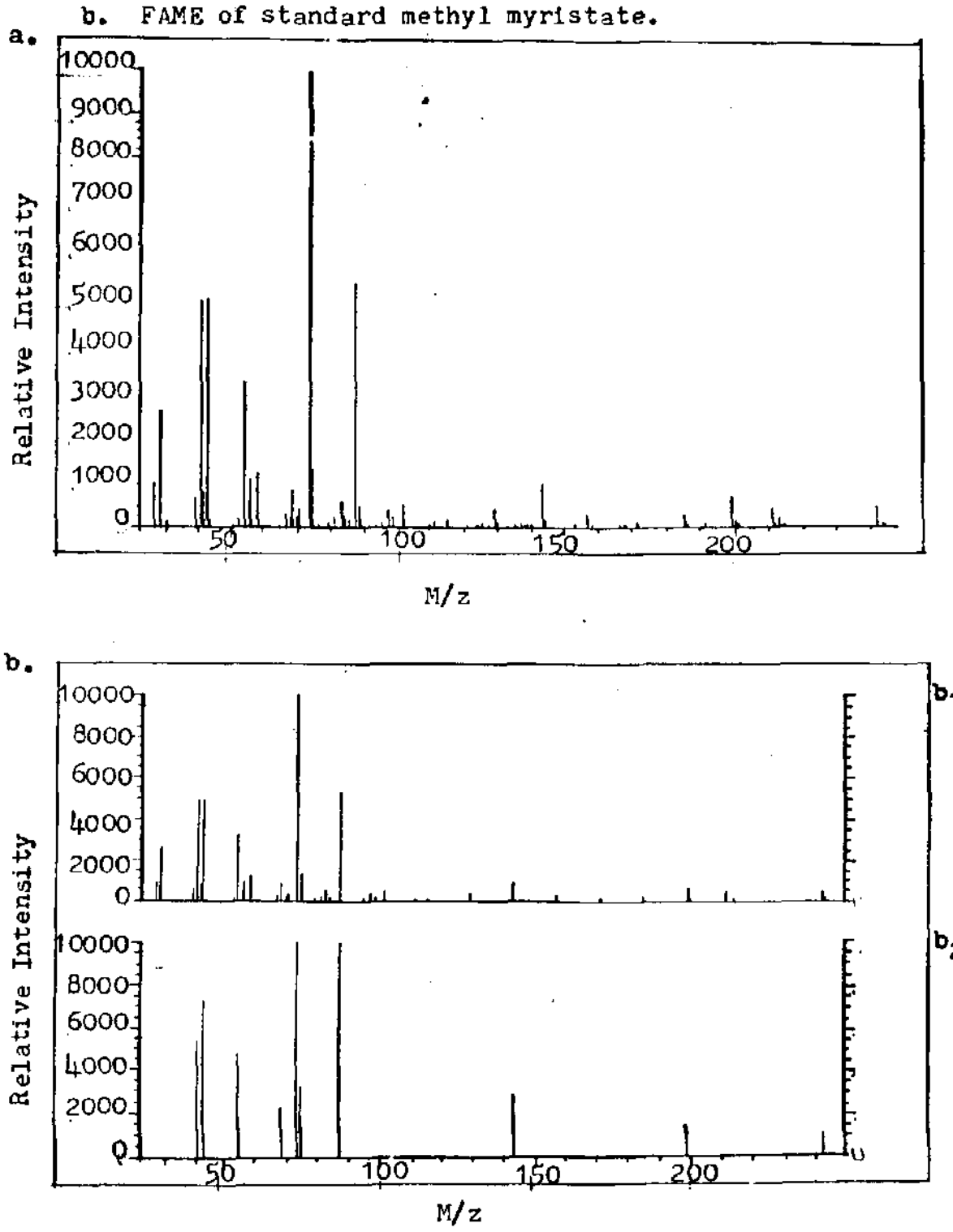


EIMS: M/z (rel. int.) $[M]^+$ $C_{13}H_{26}O_2$, found 214.15 (2.20) (Cal. 214.4); 87.10 (52.20); 74 (100), 59 (17.29); 43 (47.77).

From the mass spectrum and with the aid of Library Search, the compound was identified as methyl lurate.

Fig. 14: Mass spectrum of the FAME compound in peak 3 and corresponding reference compound.

a. FAME of test compound.
b. FAME of standard methyl myristate.



EIMS: M/z (rel. int.): /_M_/$^+$ $C_{15}H_{30}O_2$, found: 242.15 (3.88); (calc. 242.40); 87.10 (53.34), 74 (100); 59 (12.50); 43.00 (50).

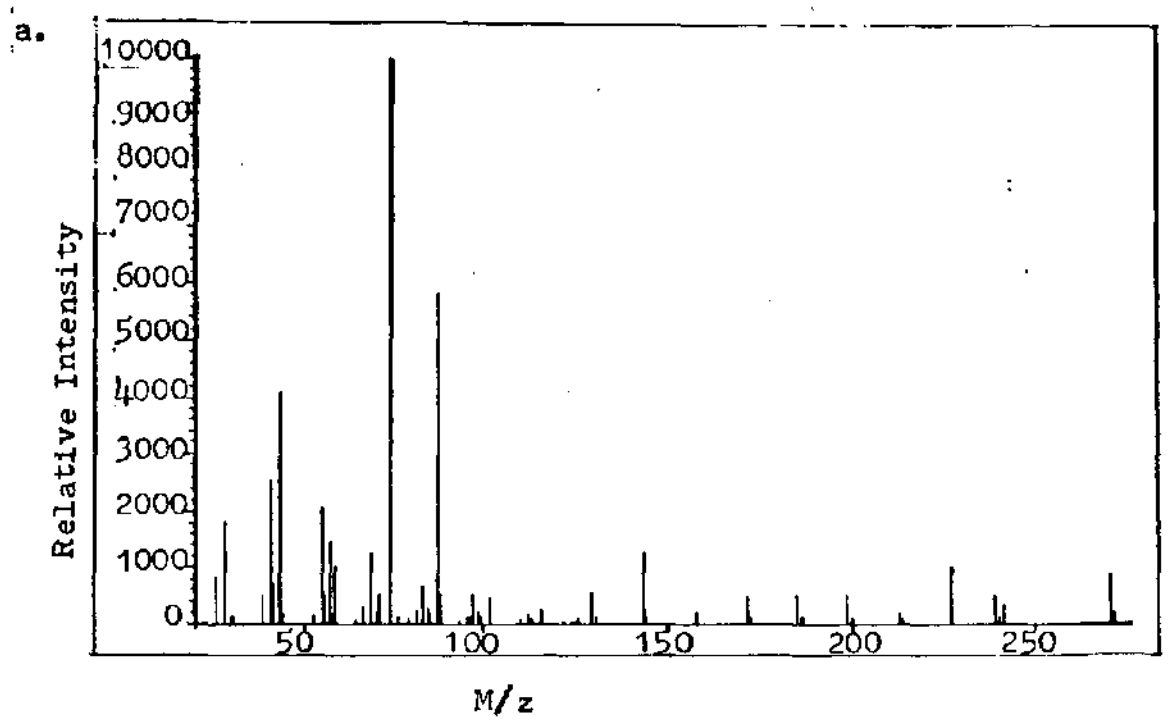
b_1 Mass spectrum of test compound.

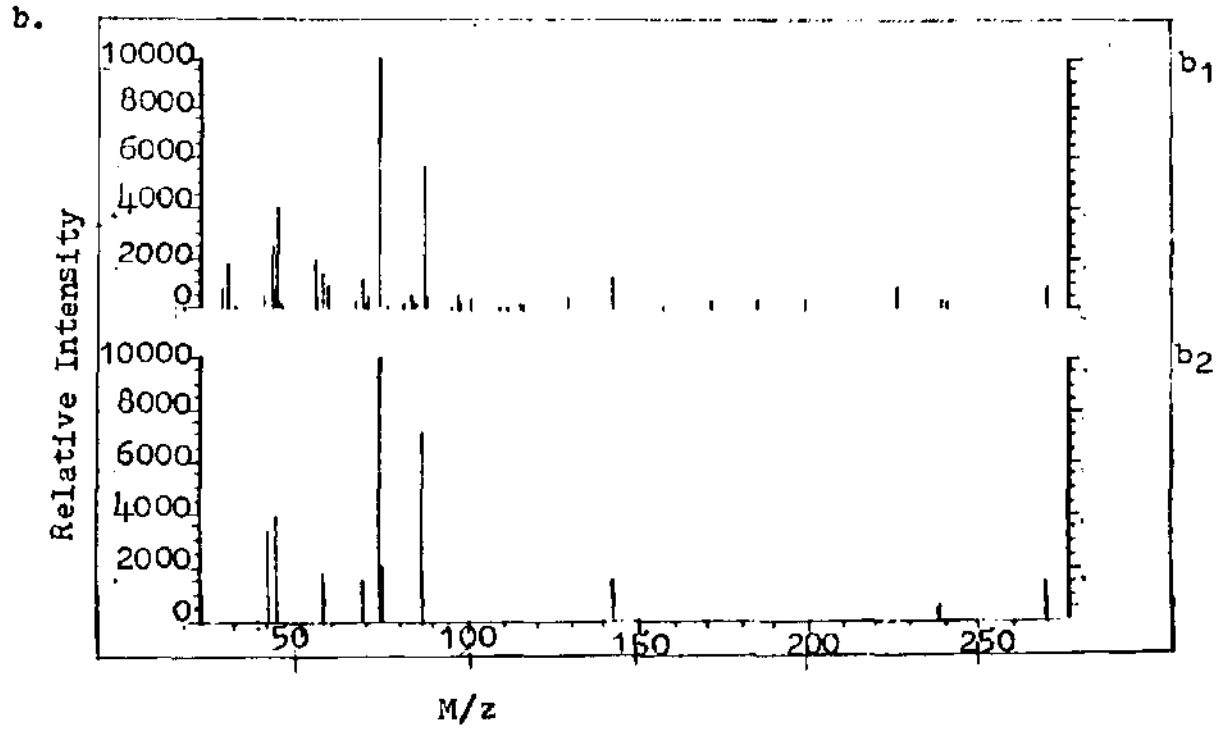
b_2 Mass spectrum of standard methyl myristate.

Fig.15: Mass spectrum of the FAME compound in peak 4 and corresponding reference compound.

a. FAME of test compound.

b. FAME of standard methyl palmitate.



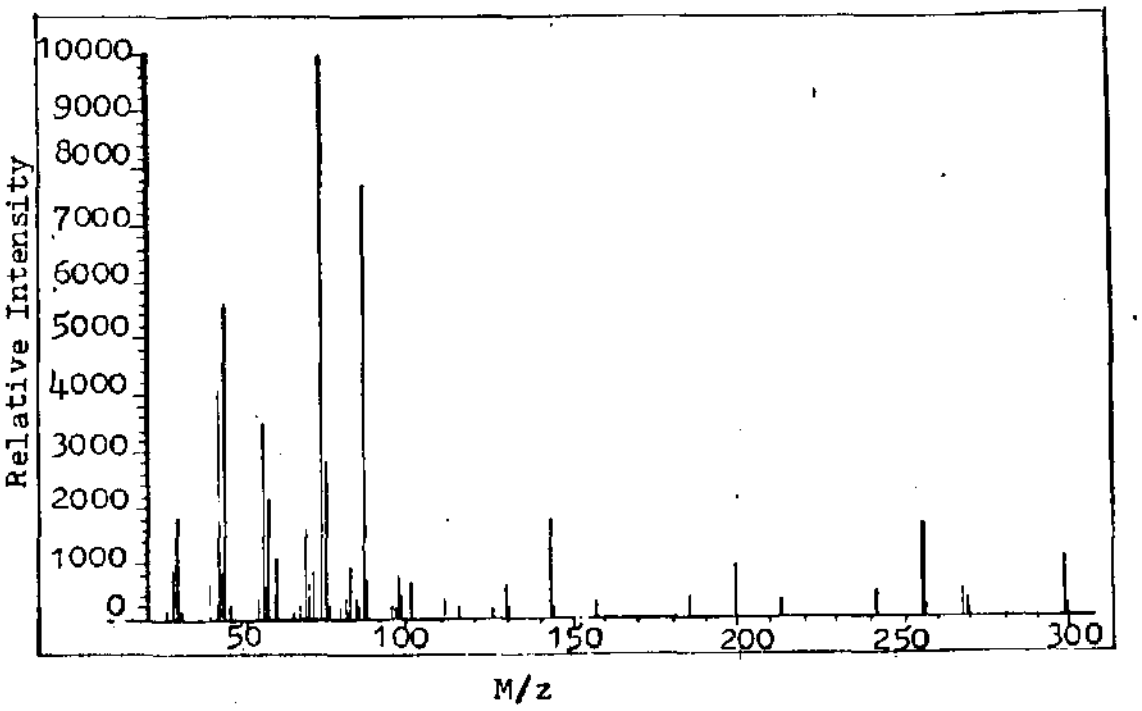


EIMS: M/z (rel. int.): $[M]^+$ $C_{17}H_{34}O_2$, found: 270:15 (8.49), (Cal. 270.20); 113.10 (12.54), 87.10 (58.40); 74 (100), 57.20 (14.60).

b_1 Mass spectrum of test compound.

b_2 Mass spectrum of standard methyl palmitate

Fig. 16: Mass spectrum of the FAME compound in peak 5.



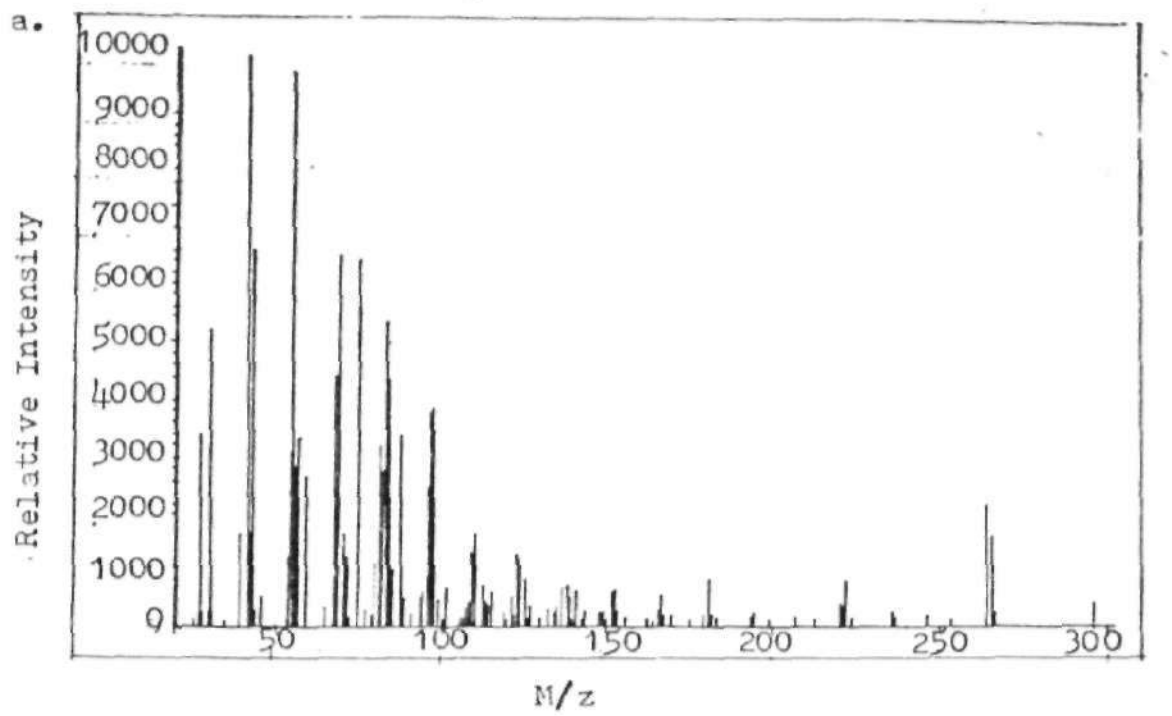
EIMS: M/z (rel.int.) $[M]^+$ $C_{19}H_{38}O_2$ found: 298.30 (10.96); (Calc. 298.51); 143.10 (17.67), 75.10 (28.46), 74.10 (100), 55.05 (35.34), 43.05 (56.36).

From the mass spectrum and with the aid of library search, the compound was identified as methyl stearate.

Fig.17: Mass spectrum of the FAME compound in peak 6 and corresponding reference compound.

a. FAME of test compound.

b. FAME of standard methyl oleate.



EIMS: M/z (rel. int.) $[M]^+$ $C_{19}H_{36}O_2$ found: 296.30 (4.16); (cal. 296.32); 97.10 (38.96); 74.10 (64.58): 69.10 (65.53); 55.15 (97.31); 41.15 (100).

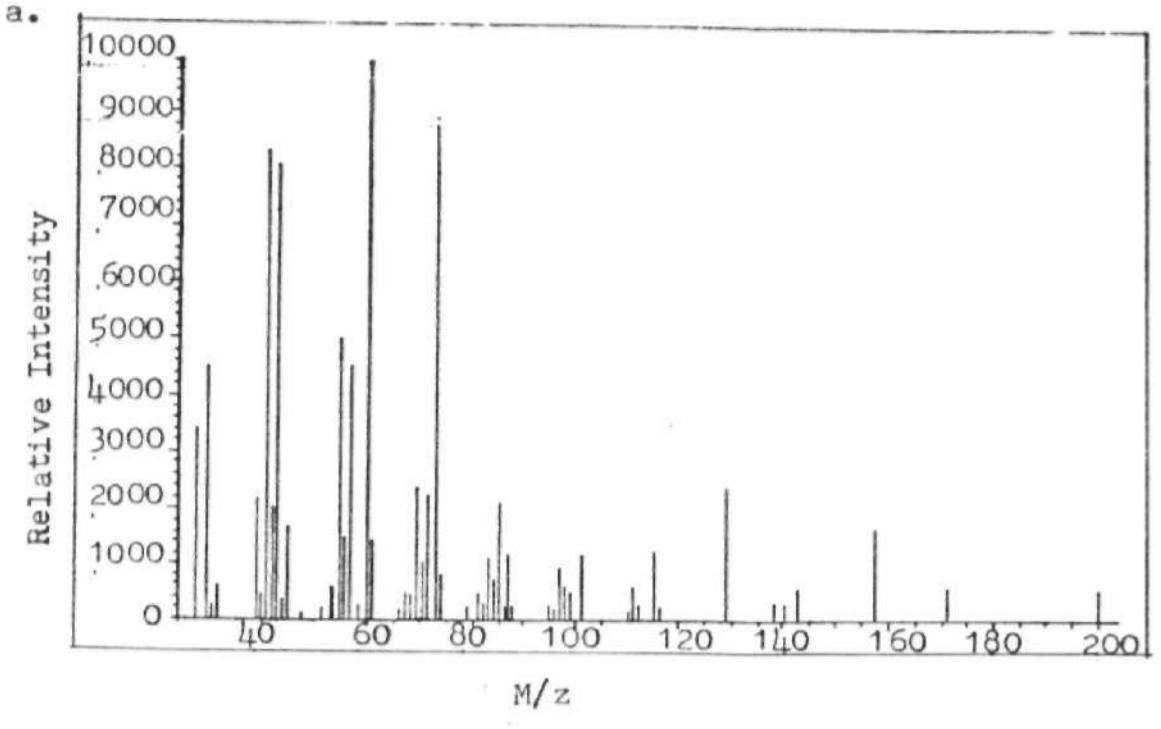
b_1 Mass spectrum of test compound.

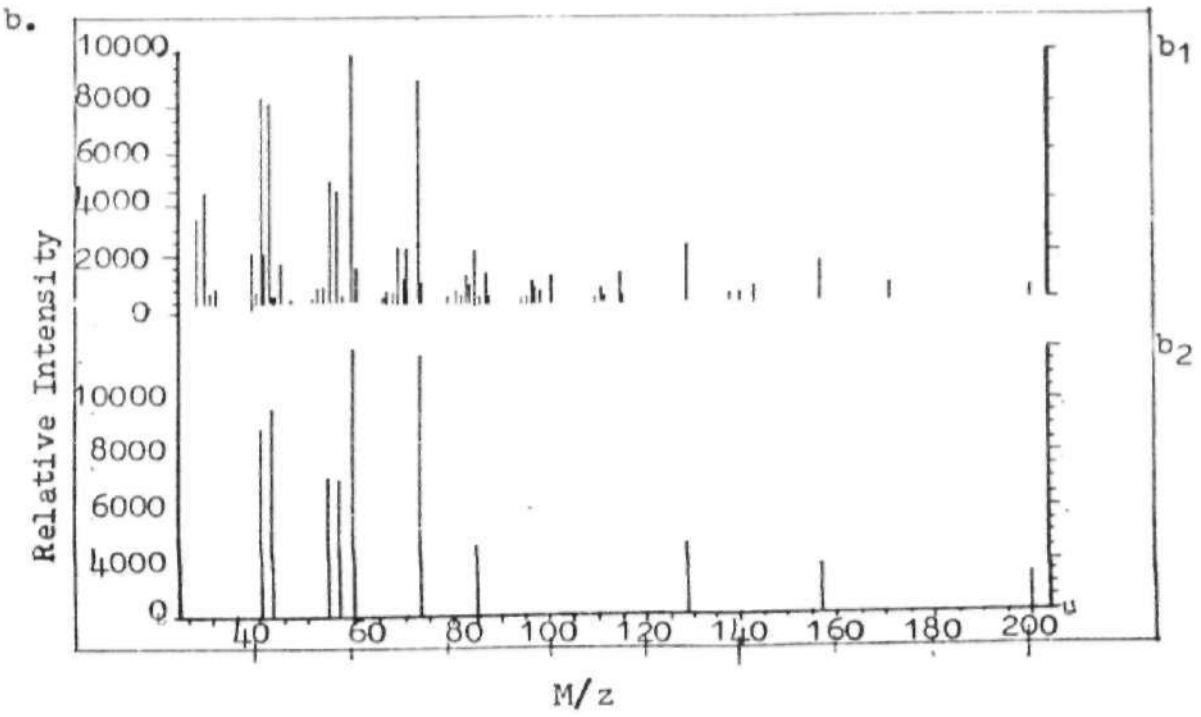
b_2 Mass spectrum of standard methyl oleate.

Fig.18.: Mass spectrum of the compound in peak 7 and corresponding reference compound.

a. FAME of test compound.

b. FFA of standard lauric acid.





EIMS: M/z (rel. int.): $[M]^+$ $C_{12}H_{24}O_2$, found 200.15 (5.88); (Calc. 200.40); 115.10 (12.89); 85.20 (21.49); 73.10 (89.74); 60 (100); 55 (50); 45.05 (16.93).

b_1 Mass spectrum of test compound

b_2 Mass spectrum of standard lauric acid.

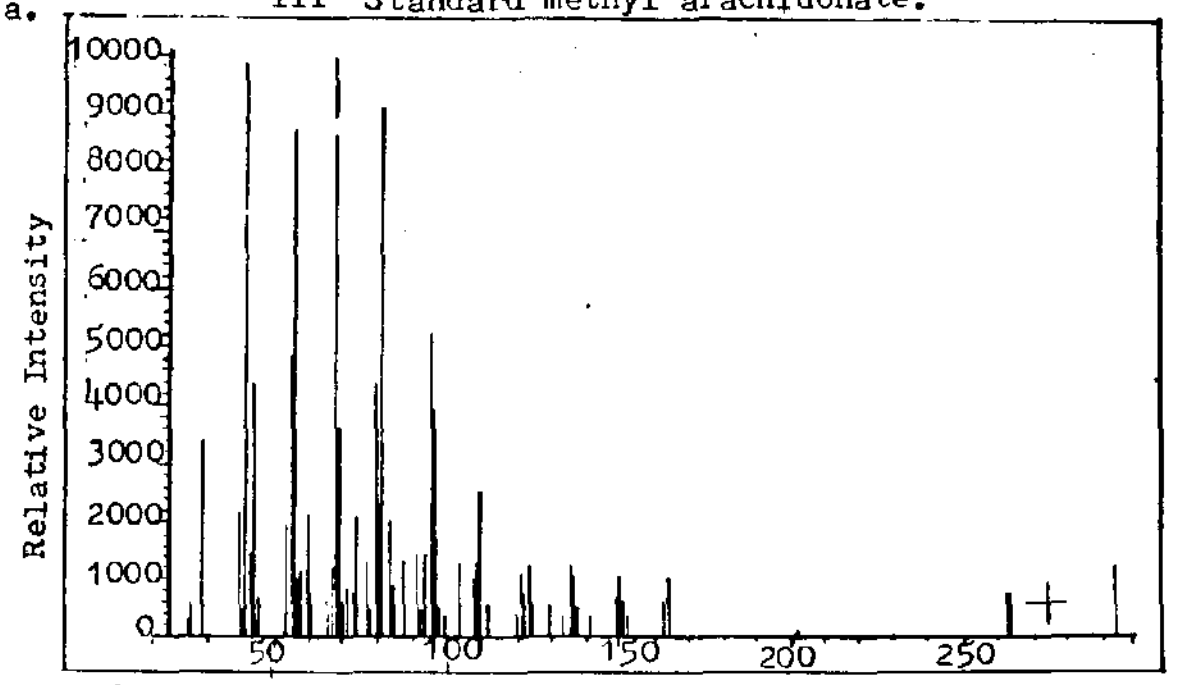
Fig.19: Mass spectrum of the FAME compound in peak 8 and corresponding reference compound.

a. FAME of test compound.

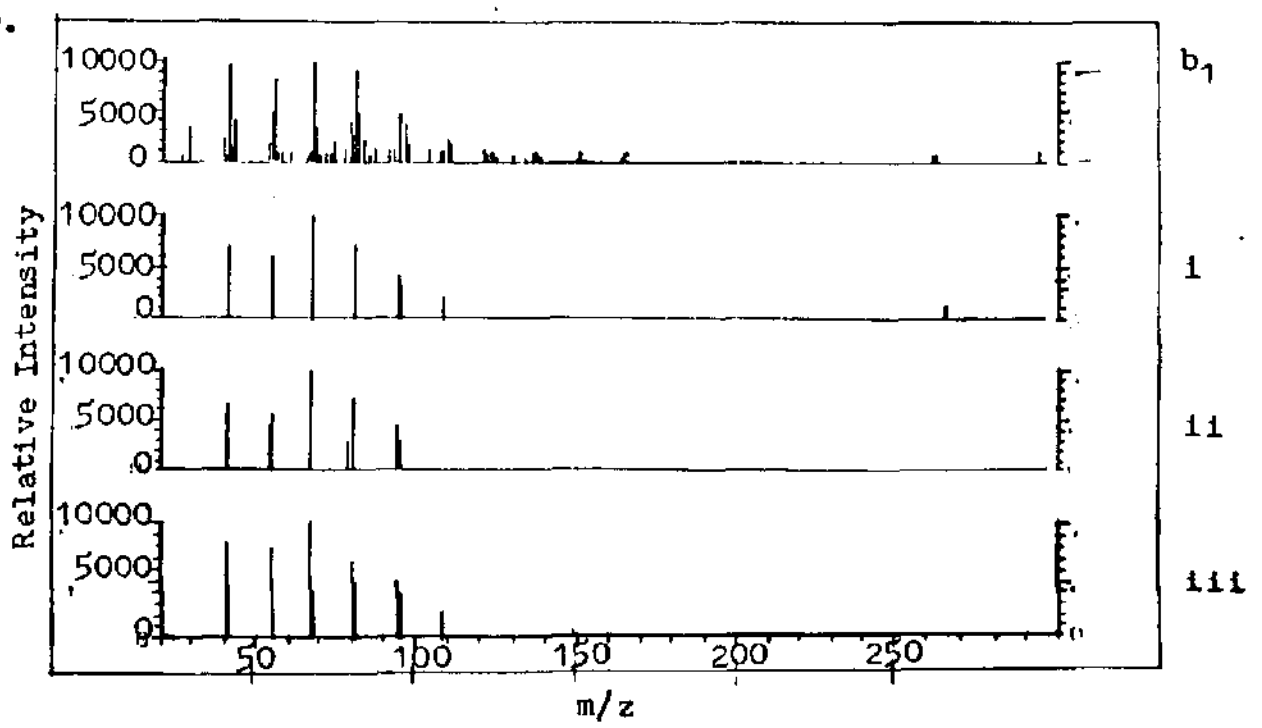
b. FAME of:

i Standard hexadecadienoic acid methylester.
ii Standard methyl linoleate
iii Standard methyl arachidonate.

a.



b.



EIMS: M/z (rel. int.): $[M]^+$ $C_{19}H_{34}O_2$, found: 294.20 (11.90), (Cal. 294.40); 95.10 (49.93), 81.10 (90.68); 74 (20.18); 67.10 (100); 54.05 (49.93).

b_1 Mass spectrum of test compound.

Table 22: Analysis of the Fatty Acid Composition of *I. gabonensis* Seed Fat by GC/MS

Peak No.	Mass Spectra Fig. No.	Methylated Fatty Acid	Fatty Acid
1	Fig. 10	Methyl caprate	
2	Fig. 11	Methyl laurate	
3	Fig. 12	Methyl myristate	
4	Fig. 13	Methyl palmitate	
5	Fig. 14	Methyl stearate	
6	Fig. 15	Methyl oleate	
7	Fig. 16	-	Lauric acid
8	Fig. 17	Methyl linoleate	

3.3.2.c. High Resolution Gas Chromatography (HRGC)

HRGC analysis of the FAME was carried out on a GC equipped with fused silica column (25m x 0.32mm i.d., film thickness 0.20 u.m), carrier gas was H_2 (2.5ml/min). The column oven was programmed from 100°C to 235°C at 10°C/min. Identification was based on the retention times of standard FAME. Peak areas were measured and the percentage composition of the fatty acids in the seed fat was determined. The results of the seed fat analysis obtained by HRGC for the two varieties of I. gabonensis (excelsa and gabonensis) are presented in Figs. 22 and 23. The percentage composition of the fatty acids in the seed fat of the two varieties are listed in Table 25 and also reflected in Figs. 20 and 21.

Thus, the fatty acid composition of I. gabonensis seed fat determined (Table 25) indicate that the seed fat is composed mainly of saturated fatty acids which is reflected in the physical and chemical characters of the seed fat (solid at room temperature and a low Iodine number) (Table 16). The results obtained correlate very well with the results obtained by Bushell and Hilditch (1939), Meara and Patel (1950) and Colin and Hilditch (1930)

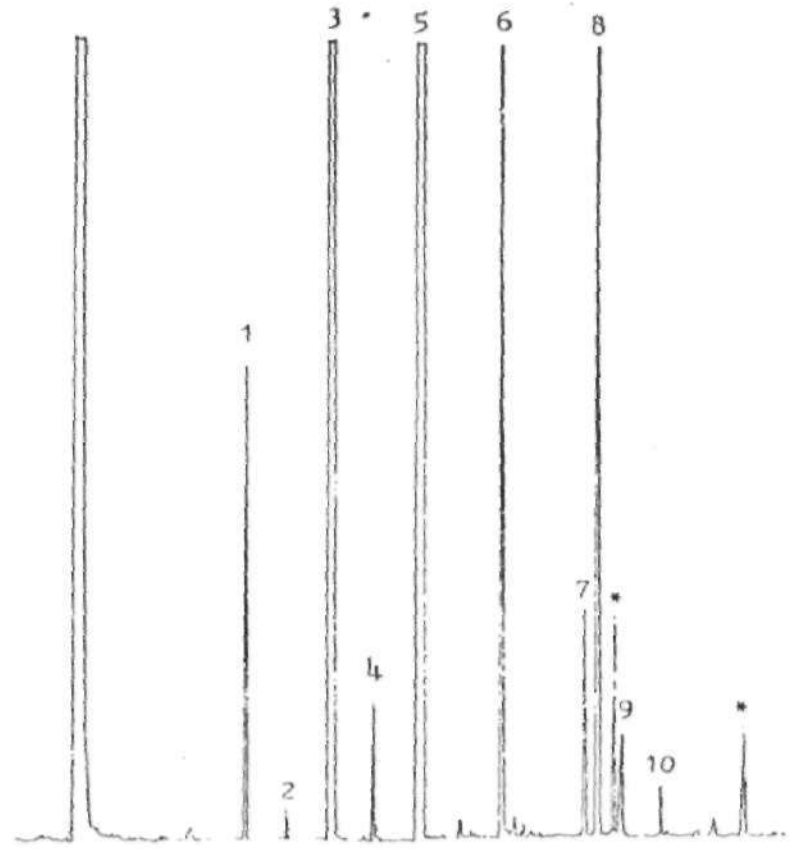


Fig. 20: Gas chromatogram of the FAME of I. gabonensis var. excelsa seed fat, separated by HRGC.

Peak No.	Retention time (min)	Fatty acid Identified
1	4.643	Capric acid
2	5.513	Undecanoic acid
3	6.502	Lauric acid
4	7.302	Tridecanoic acid
5	8.303	Myristic acid
6	9.853	Palmitic acid
7	11.517	Stearic acid
8	11.782	Oleic acid
*	12.122	Unidentified
9	12.257	Linoleic acid
10	13.153	Linolenic acid
*	14.638	Unidentified

Table 23: Retention times of the fatty acid identified in I. gabonensis var. excelsa seed fat by HRGC.

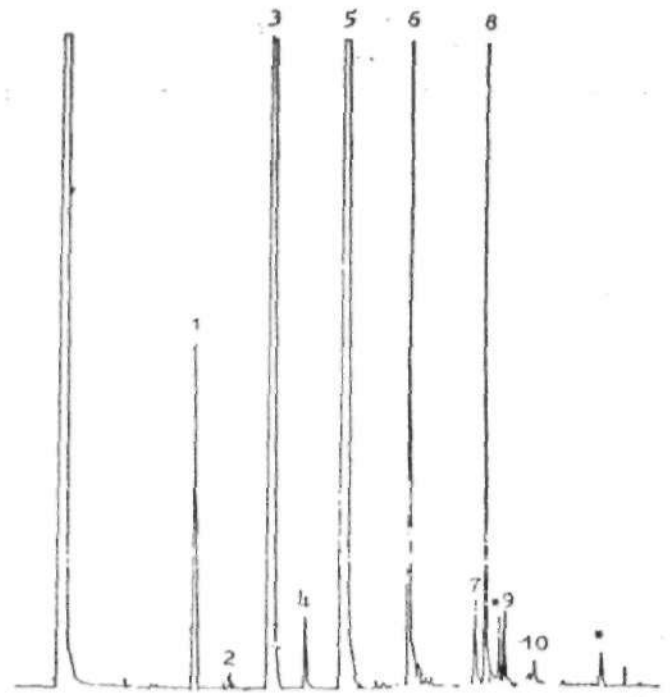


Fig.21: Gas chromatogram of the FAME of I. gabonensis var. gabonensis seed fat, separated by HRGC.

Peak No.	Retention time (min)	Fatty acid identified
1	4.645	Capric acid
2	5.514	Undecanoic acid
3	6.513	Lauric acid
4	7.310	Tridecanoic acid
5	8.308	Myristic acid
6	9.861	Palmitic acid
7	11.518	Stearic acid
8	11.791	Oleic acid
*	12.097	Unidentified
9	12.251	Linoleic acid
10	13.161	Linolenic acid
*	14.712	Unidentified

Table 24: Retention times of the fatty acid identified in I. gabonensis var. gabonensis seed fat by HRGC.

Table 25: The Percentage Composition* of the Seed Fat of Each Variety of I. gabonensis Determined by High Resolution Gas Chromatography.

Fatty Acids of Irvingia gabonensis var.	% of Total Fatty Acids	
	excelsa	gobenensis
Capric acid	1.01	1.50
Undecanoic acid	0.07	0.07
Lauric acid	36.92	37.69
Tridecanoic acid	0.31	0.29
Myristic acid	52.31	51.45
Palmitic acid	5.27	4.83
Stearic acid	0.48	0.40
Oleic acid	2.49	2.94
Linoleic acid	0.22	0.33
Linolenic acid	0.13	0.12
Unidentified	0.79	0.38
	100.00	100.00

*Based on the relative peak areas computed by an electronic integrator.

but these results do not agree with the results published by Omoghai (1990) in which the seed fat composed of 64.6% unsaturated fatty acids (Table 6) and about 35.4% saturated fatty acid, which produce a fat liquid at room temperature and with a high iodine number. Our results were obtained by applying three different chromatographic techniques and using different samples of I. gabonensis seed fat collected from various geographical locations in Nigeria and appear more accurate and related to the physical and chemical characters of the seed fat.

Of the three techniques (TLC, GC/MS and HRGC) employed in this work for the analysis of the fatty acids, it was observed that HRGC was the most versatile and sensitive technique. It afforded the separation of 12 components of which 10 could be identified, whereas TLC and GC afforded the separation of only 5 and 8 components respectively. It was concluded that HRGC is the most favoured technique for the qualitative and quantitative estimation of the fatty acids in I. gabonensis.

It is worth noting here also that the HRGC shows an unidentified acid component. This may be associated with the wax portion of "Dika fat" (Refer page 18).

3.4 EVALUATION OF I. GABONENSIS SEED FAT AS A BASE FOR SEMISOLID AND SOLID PHARMACEUTICAL DOSAGE FORMS

I. gabonensis seed fat was mixed with different proportions of wool fat and castor oil separately to produce bases suitable for ointment and suppository dosage forms. The formulated bases were evaluated and the results were as follows:-

3.4.1 Evaluation of I. gabonensis Seed Fat as an Ointment Base

I. gabonensis var. excelsa seed produced 67% w/w solid vegetable oil. The fat extracted with petroleum ether (60 - 80°C) was white in colour with a pleasant odour. It melts into a colourless liquid at 42 - 44°C. The fat did not spread easily when applied to the skin, due to its high melting point. Wool fat (m.p. 36°C) and castor oil (liquid at room temperature) were mixed separately with I. gabonensis seed fat in different proportions to produce semisolid preparations of such consistency and composition that they soften but not necessarily melt when applied to the skin by inunction (Tables 26 & 27). The seed fat mixed with 20% wool fat produced a semisolid preparation with such consistency that

Table 26: Comparison of Melting Characteristics of Ointment Bases Formulated from I. gabonensis Seed Fat and Different Percentages of Wool Fat

S/No.	Base	Melting Point °C	Liquefication Point °C	Liquefica-Time °C	Softening Point °C	Collapsing Weight at 28 ± 2°C kg
1	I. gabonensis fat	39 - 40	39 - 40	5	39	1, 000
2	I. gabonensis fat + 5% w/w wool fat	38 - 39	39 - 40	5	39	00.750
3	I. gabonensis fat + 10% w/w wool fat	37 - 38	37 - 38	4	35	00.600
4	I. gabonensis + 15% w/w wool fat	36 - 37	36 - 37	3	35	00.400
5	I. gabonensis + 20% w/w wool fat	33 - 35	33 - 35	2	33	00.300
6	I. gabonensis + 25% w/w wool fat	30 - 32	30 - 32	1	32	00.100

Table 27: Comparison of Melting Characteristics of Ointment Bases Formulated of *I. gabonensis* Seed Fat and Different Percentages of Castor Oil

S/No.	Base	Melting Point °C	Liquefication Point °C	Liquefication Time, Min	Softening Point °C	Collapsing Weight at 28 ± 2°C kg
1	*I. gabonensis* fat	39 - 40	39 - 40	5	39	1,000
2	*I. gabonensis* fat + 10% w/w castor oil	38 - 39	38 - 39	4	38	00.950
3	*I. gabonensis* fat + 15% w/w castor oil	37 - 38	37 - 38	4	37	00.800
4	*I. gabonensis* fat + 20% w/w castor oil	36 - 37	36 - 37	3	36	00.600
5	*I. gabonensis* fat + 25% w/w castor oil	35 - 36	35 - 36	3	35	00.500
6	*I. gabonensis* fat + 30% w/w castor oil	32 - 34	32 - 34	1.5	33	00.400
7	*I. gabonensis* fat + 35% w/w castor oil	30 - 32	30 - 32	1	32	00.200

Table 28: Rate of Salicylic Acid Release from I. gabonensis Ointment Bases

Temperature °C	Time Hours	Average diameter of diffusion colour zone of salicylic acid formulations (mm) A	B	C	D
Room Temperature 28 ± 2°C	1	8	5	4	000
	2	9.5	8.5	5.2	000
	6	16.4	15.2	9.6	000
	12	21.5	19.2	26.4	000
	24	29.2	28.4	21.2	000
37 ± 1°C	1	9.5	8	5	000
	2	11.6	11	9.2	000
	6	19.7	19.2	17.4	000
	12	26.4	26	24.2	000
	24	35.4	34	30.6	000

A - 5% Salicylic acid in simple ointment base B.P.

B - 5% Salicylic acid in 20% wool fat and 80% I. gabonensis fat.

C - 5% Salicylic acid in 30% castor oil and 70% I. gabonensis fat.

D - Plain ointment base (control).

(mm) - Mean values of six determinations in millimeter.

it can be applied on the skin by inunction and its melting point (34 - 35°C) is lower than the body temperature. Based on our results obtained in Tables 26&27 a similar preparation was made of I. gabonensis seed fat and 30% castor oil. These bases were evaluated and the results are discussed as follows:-

(a) Assessment of the rate of release of medicaments from an ointment base consisting of I. gabonensis seed fat.

The ability of these preparations to release medicaments, was studied by comparing the rate of I. gabonensis ointment base preparations and their rate of release from simple ointment base B.P. (1973). The results (Tables 28 and 29) indicate that the rate of release of salicylic acid and resorcinol from I. gabonensis ointment base preparations are comparable with their rate of release from simple ointment base B.P. (1973). The rate of release of the water soluble resorcinol was lower than that of the less water soluble salicylic acid. The results (Tables 28 and 29) also indicate that I. gabonensis ointment bases mixed with wool fat release more drugs than those bases made with castor oil. The flow behaviour of the I. gabonensis

Table 29: Rate of Resorcinol Release from I. gabonensis Ointment Bases.

Temperature °C	Time Hours	Average diameter of diffusion colour zone of resorcinol formulations (mm) E	F	G	H
Room Temperature (28 ± 2°C)	1	4	3	5	000
	2	6.5	6	6	000
	6	11.2	10.2	11.5	000
	12	16.4	12.5	12	000
	24	21.2	20.5	18.5	000
	1	6	5	5	000
	2	8.2	8	7.5	000
	6	14.2	13.2	12.6	000
	12	19.4	18.7	17.3	000
	24	28.1	27.3	26.4	000

E - 5% Resorcinol in simple ointment base B.P.

F - 5% Resorcinol in 20% wool fat and 80% I. gabonensis fat.

G - 5% Resorcinol in 30% castor oil and 70% I. gabonensis fat.

H - Plain ointment base (control).

mm - Mean values of six determinations in millimeter.

ointment bases was found to be an important factor controlling the rate of release of salicylic acid and resorcinol. The rate of release of drugs from I. gabonensis ointment base preparations was found to be higher at 37°C than at room temperature (28 ± 2°C). At 37°C the ointment base melted and the drugs released became more available to the absorbent surface.

(b) Assessment of the irritant effect of I. gabonensis seed fat on rats.

No patches were observed on the rats skin throughout the period of study (2 weeks). This indicated the absence of any irritant action of I. gabonensis seed fat.

(c) Assessment of the stability of I. gabonensis ointment bases.

I. gabonensis seed fat ointment base formulations studied did not show any evidence of instability after storage for 12 weeks at both room temperature 28 ± 2°C and 37 ± 1°C or incompatibility during storage.

These results indicate that the physical characters of the vegetable oil of I. gabonensis seed qualify it to be used as a natural ointment base (Table 16).

2.4.2 Evaluation of I. gabonensis Seed Fat as a Suppository Base

Suppositories, if well formulated, should melt, soften, or dissolve when introduced into the body cavities and yet retain their firmness and shape in ordinary storage conditions. I. gabonensis seed fat has a melting point of 42°C and is hard at room temperature 28 ± 2°C, thus it should be mixed with another ingredient to lower its melting point and at the same time maintain to a certain degree its hardness and firmness at room temperature. Bases containing I. gabonensis seed fat and varying proportions of wool fat 10%, 20% and 30% were prepared by the fusion method using a metal mould with six cavities. This produced suppositories with different melting points and varying degrees of hardness (Table 26). These suppositories were evaluated and the results are indicated below:

(a) Melting point and melting time

The melting point of the different suppository bases formulated with wool fat were recorded (Table 26). The results indicate that with the increase in wool fat concentration, the melting point of I. gabonensis seed fat drops. Thus the melting point of the seed fat can be controlled by changing the concentration of wool fat. The base consisting of 10% wool fat was found to be more suitable because of its melting point characteristics of 37 - 38°C.

(b) Hardness and collapsing weight

Setnikar and Fantelli (1963), stated that a suppository, at a temperature of 22°C, should not be deformed by a weight of 500gm. With the increase of the temperature, the softening temperature of the suppository is reduced, its hardness is reduced and it collapses easily. The determination of the collapsing weight and softening temperature are particularly important for suppositories which must be handled and stored in warm or hot climates. The results (Table 26) indicate that the suppository made of 10 and 20% wool fat are suitable but the 20% wool fat

suppositories have a very low melting point, far lower than the body temperature 37°C. Thus suppositories made with I. gabonensis seed fat (90%) and wool fat (10%) were found not only hard enough for handling (collapsing weight 600 gm) but also found to have a suitable softening temperature (35°C).

(c) Assessment of the rate of release of medicaments from a suppositories base consisting of I. gabonensis seed fat.

Chloroquine is widely used for the prophylaxis and treatment of malaria. The drug is available as the phosphate as well as the sulphate salt for oral administration in tablets and syrups, and as the hydrochloride salt for intramuscular administration. Taking chloroquine orally by some patients may cause gastro-intestinal disturbances including nausea, vomitting, abdominal pain and anorexia; pruritis can be very troublesome and seems to occur more frequently in dark-skinned individuals (Catchpool, 1972).

The drug chloroquine is very bitter and thus unpalatable to most patients. Efforts at masking the bitter taste of chloroquine include the addition of a high concentration of sugar

to paediatric liquid preparations and more recently film coating of the tablets. The suitability of formulating chloroquine phosphate into a capsule dosage form was recently investigated (Esezobo and Ibiezugbe, 1982).

Schanker (1957) reported that once a drug is released into the aqueous solution in the rectum, the mechanism of passage through the mucosa and tissues into the blood stream is the same as in the higher regions of the gastro-intestinal tract. Thus Ifudu and Odiamgbe (1987a and 1987b) reported the possible formulation of chloroquine phosphate into a suppository dosage form; they used different suppository bases mainly cocoa butter and polyethylene glycol.

In this project, the suitability of Irvingia gabonensis seed fat as a suppository base for formulating chloroquine phosphate suppositories was investigated (rate of drug release and bioavailability), and the results were compared with those results obtained by Ifudu and Odiamgbe (1987a).

The preparation of chloroquine phosphate suppositories using I. gabonensis seed fat was as follows:-

(a) Preparation of the suppository base

The base consisting of 10% wool fat and 90% I. gabonensis seed fat was selected as the suitable base, due to its melting point ($37^{o}C \pm 0.5$) and hardness (Table 26). Thus 30gm of the base were prepared by the fusion method using 3gm of wool fat and 27gm of Irvingia gabonensis seed fat.

(b) Determination of the displacement value of chloroquine into the base

The displacement value of chloroquine phosphate in the prepared suppository base was calculated as follows:-

1. Six suppositories without chloroquine were prepared and their weight was recorded (agm).

2. Six suppositories with 40% chloroquine phosphate were prepared and their weight was recorded (bgm).

3. The amount of the base used to prepare the 40% chloroquine phosphate suppositories was calculated and recorded (cgm).

4. The amount of chloroquine phosphate used to prepare the 40% chloroquine phosphate suppositories was calculated and recorded (dgm).

5. Thus the amount of base displaced by dgm of chloroquine phosphate is equal to agm - cgm.

6. The displacement value $= \frac{dgm}{agm - cgm}$

7. a = 6 gm
   b = 6.96gm
   c = 4.176gm .
   d = 2.784gm

8. The displacement value of chloroquine phosphate in the base $= \frac{d}{a-c}$
   $= \underline{1.526}$

(c) Preparation of 1% chloroquine phosphate suppositories

The prepared base was used, the amounts of chloroquine phosphate and base to be used was calculated as follows:-

- To prepare six suppositories each weighing 1 gm and containing 1% chloroquine phosphate 10mg chloroquine phosphate is required i.e. the total chloroquine phosphate for the six suppositories will be 60mg. The 60mg of chloroquine phosphate will displace 39.32mg

of the base (d.v = 1.526). Thus the amount of base used is 5.60gm.

(d) Preparation of 10% chloroquine phosphate suppositories

- To prepare six suppositories each weighing 1gm and containing 10% chloroquine phosphate 100mg chloroquine phosphate is required i.e. for the six suppositories 600mg chloroquine phosphate was required. 600mg chloroquine phosphate will displace 393.18mg of the base (d.v. = 1.526). The amount of base used was 5.606gm.

To examine the pattern of drug release, the per cent of drug released was plotted against time for varying drug concentrations.

Figs. 20 and 21 show the pattern of *in vitro* release of chloroquine as a function of time for 1% chloroquine phosphate suppositories made of *I. gabonensis* seed fat and 10% wool fat. A similar plot was also made for 10% chloroquine suppositories. Also the rate of chloroquine phosphate release was indicated for each concentration (Table 30 and Table 31). These results showed that for chloroquine phosphate concentration of 1% of the release of chloroquine was higher than that of chloroquine

Table 30 : The Amount of Chloroquine Phosphate Released per Time and its Percentage for 1% Chloroquine Phosphate Suppositories.

Sample	Time (min)	Concentration(mg)	Percentage of drug released
(1)	30	2.75	27.5
	60	8.57	85.7
	90	8.57	85.7
	120	8.33	83.3
(2)	30	5.95	59.5
	60	9.05	90.5
	90	8.81	88.1
	120	8.81	88.1
(3)	30	6.19	61.9
	60	9.52	95.2
	90	9.05	90.5
	120	9.05	90.5
(4)	30	3.81	38.1
	60	9.05	90.5
	90	9.05	90.5
	120	8.81	88.1
(5)	30	3.05	30.5
	60	8.57	85.7
	90	8.57	85.7
	120	8.33	83.3
Average	30	4.35 ± 1.61	43.5
	60	8.95 ± .39	89.5
	90	8.81 ± .24	88.1
	120	8.66 ± .32	86.6

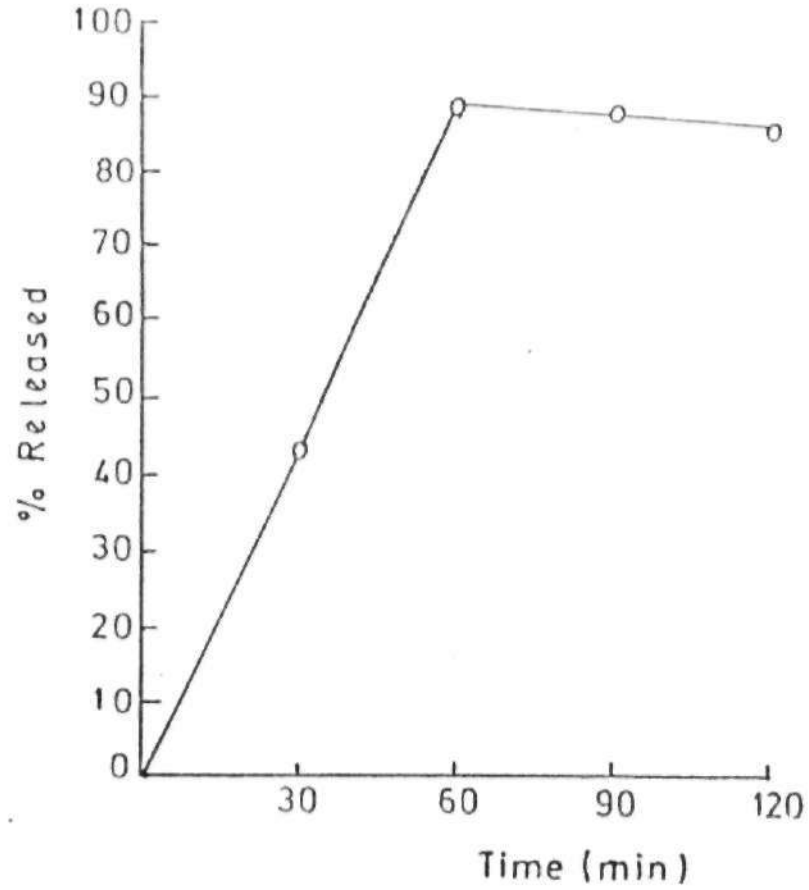


Fig.22 : Rate of release of 1% Chloroquine Phosphate from I. gabonensis seed fat suppositories.

Table 31: The Amount of Chloroquine Phosphate Released per Time and its Percentage for 10% Chloroquine Phosphate Suppositories.

Sample	Time (min)	Concentration(mg)	Percentage of drug released
(1)	30	8.64	8.64
	60	86.45	86.45
	90	86.45	86.45
	120	85.20	85.20
(2)	30	7.26	7.26
	60	76.43	76.43
	90	76.43	76.43
	120	76.05	76.05
(3)	30	7.26	7.26
	60	76.93	76.93
	90	76.93	76.93
	120	76.43	76.43
(4)	30	7.89	7.89
	60	85.95	85.97
	90	85.95	85.95
	120	85.07	85.07
(5)	30	8.64	8.64
	60	86.45	86.45
	90	86.45	86.45
	120	85.20	85.20
Average	30	7.93 ± .69	7.93
	60	82.44 ± 5.26	82.44
	90	82.44 ± 5.26	82.44
	120	81.59 ± 4.88	81.59

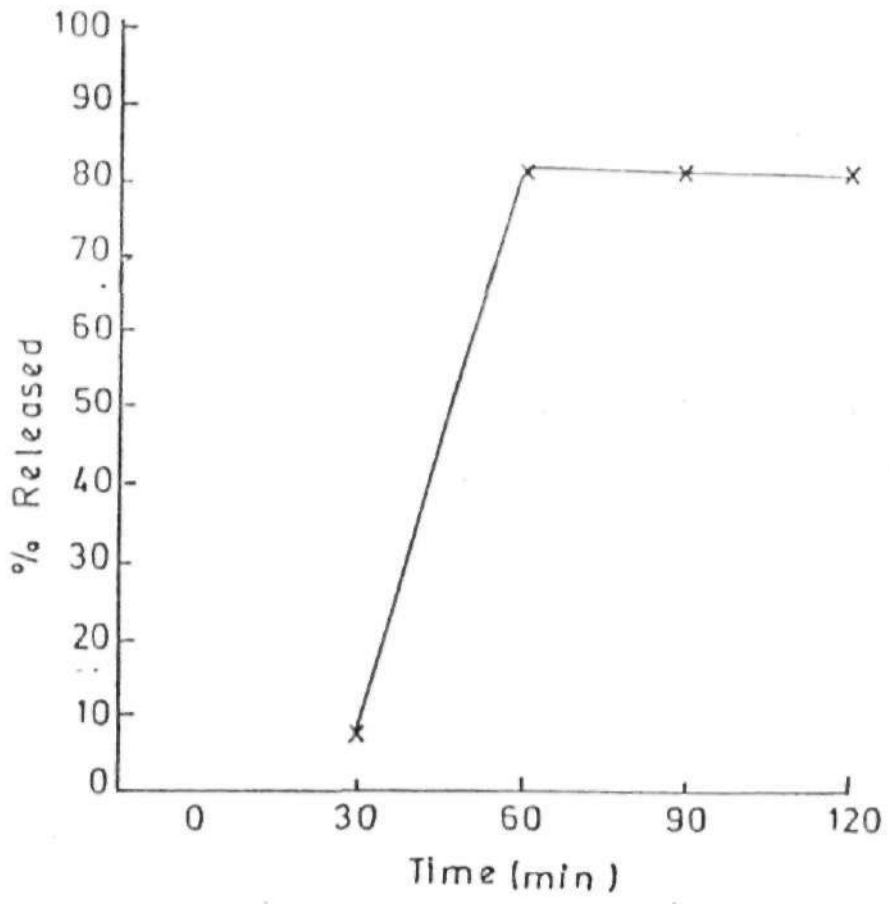


Fig.23 Rate of release of 10% Chloroquine Phosphate from I. gabonensis seed fat suppositories.

phosphate concentration of 10% at the initial 30 minutes. This may be due to the mechanism of release of chloroquine phosphate from the base, at low drug concentrations, drug release from the fatty base is mainly diffusion - controlled as evidenced by the linearity of the plot of the 1% of chloroquine released against time (Fig. 22).

At higher chloroquine concentration (10% w/w), the release pattern of chloroquine from the suppository base changes and becomes surface controlled (Fig. 23). The results show that when the fatty base melts at body temperature its surface area increases and this will lead to an increase of the rate of leaching of chloroquine from the base.

The results of the in vitro release of I. gabonensis seed fat chloroquine suppositories are quite consistent with the results obtained by Ifudu and Odiamgbe (1987a), who formulated chloroquine suppositories using cocoa butter as the suppository base. Their study indicated the possibility of using the rectum as a route for chloroquine administration.

In a follow up study, the in vivo release of chloroquine phosphate suppositories formulated with cocoa butter as a suppository base, was studied by Ifudu and Odiamgbe (1987b). They administered

chloroquine phosphate to male subjects orally and through the rectum. In their study the oral and rectal profiles of absorption were compared using urinary excretion data. Their results indicated that rectal absorption of 300mg chloroquine base from cocoa butter compared favourably with oral administration in terms of the peak time and the total amount of drug absorbed. Thus the in-vivo results indicate that chloroquine is well absorbed from the rectum and from a cocoa butter base.

I. gabonensis seed fat chloroquine suppositories released more than 75% of their chloroquine phosphate content in the first 45 minutes in the dissolution studies. Correlating these results with the in-vivo results obtained by Ifudu and Odiamgbe (1987b), indicates the possibility of using I. gabonensis seed fat as a suppository base.

3.5 EVALUATION OF THE EFFECTS OF I. GABONENSIS SEED FAT ON SERUM CHOLESTEROL, TRIGLYCERIDES AND TOTAL LIPIDS LEVELS IN RATS

The effects of feeding rats with I. gabonensis seed were studied and the following parameters were used.

(i) Serum total lipids levels:

The effect of feeding rats with I. gabonensis seed fat on the levels of serum total lipids is given in Table 33. The control rats recorded serum total lipids levels of between 209.3 ± 9.5mg/100ml and 220.60 ± 14.33mg/100ml values which fall within the range recorded by earlier workers (Friedman, 1962). No significant increase ($P > 0.05$) in serum total lipids was recorded throughout the duration of the investigation in the control group A. The effect of feeding rats with 10% I. gabonensis seed fat on serum total lipids manifested itself only in the fourth week of the experiment ($P < 0.05$) with an increase of 17.9% compared with the normal value.

(ii) Serum total cholesterol levels:

Serum total cholesterol determined as mg cholesterol per 100ml for group C, A and D are given in Table 34. The control rats recorded

Table 32: The Serum Level of Total Lipids, Cholesterol And Triglycerides on Rat Groups The Week Before Feeding Them With I. gabonensis Seed Fat.

Rat Grous	Total Lipids (mg/100ml)	Cholesterol (mg/100ml)	Triglycerides (mg/100ml)
C_1	217.0	53.0	123.0
C_2	219.9	48.9	108.0
C_3	220.0	52.0	113.0
Average C	218.6	51.3	114.6
A_1	219.1	51.1	118.0
A_2	227.8	56.8	120.0
A_3	201.5	45.5	107.0
Average C	216.13	51.13	115.0
D_1	217.1	50.1	119.0
D_2	212.0	48.0	113.0
D_3	220.4	53.2	121.0
Average D	216.5	50.43	117.6

Table 33.: Effect of Feeding 5% (Group A) and 10% (Group D) I. gabonensis Seed Fat on Serum Total Lipids of Rats.

(Values in mg Palmitin and Triolein/100ml)

Experiment Period	Week I			Week II			Week III			Week IV		
Rat Groups	C	A	D	C	A	D	C	A	D	C	A	D
1	228.40	219.20	250.70	218.90	212.00	243.00	221.00	215.00	240.00	209.00	216.00	250.00
2	209.10	237.90	231.10	209.00	242.00	234.00	207.00	240.00	242.00	220.00	243.00	250.90
3	224.20	218.80	246.00	200.00	220.00	249.00	211.00	218.00	251.00	213.00	231.00	254.00
Mean	220.60	225.30	242.60	209.30	224.70	242.00	213.00	224.30	244.30	214.00	230.00	252.20
± SD	14.35	10.91	10.23	9.45	15.53	7.55	7.21	13.65	5.86	7.87	13.50	1.57

Comparison :	Week I	Week II	Week III	Week IV
C & A	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
C & D	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
A & D	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$

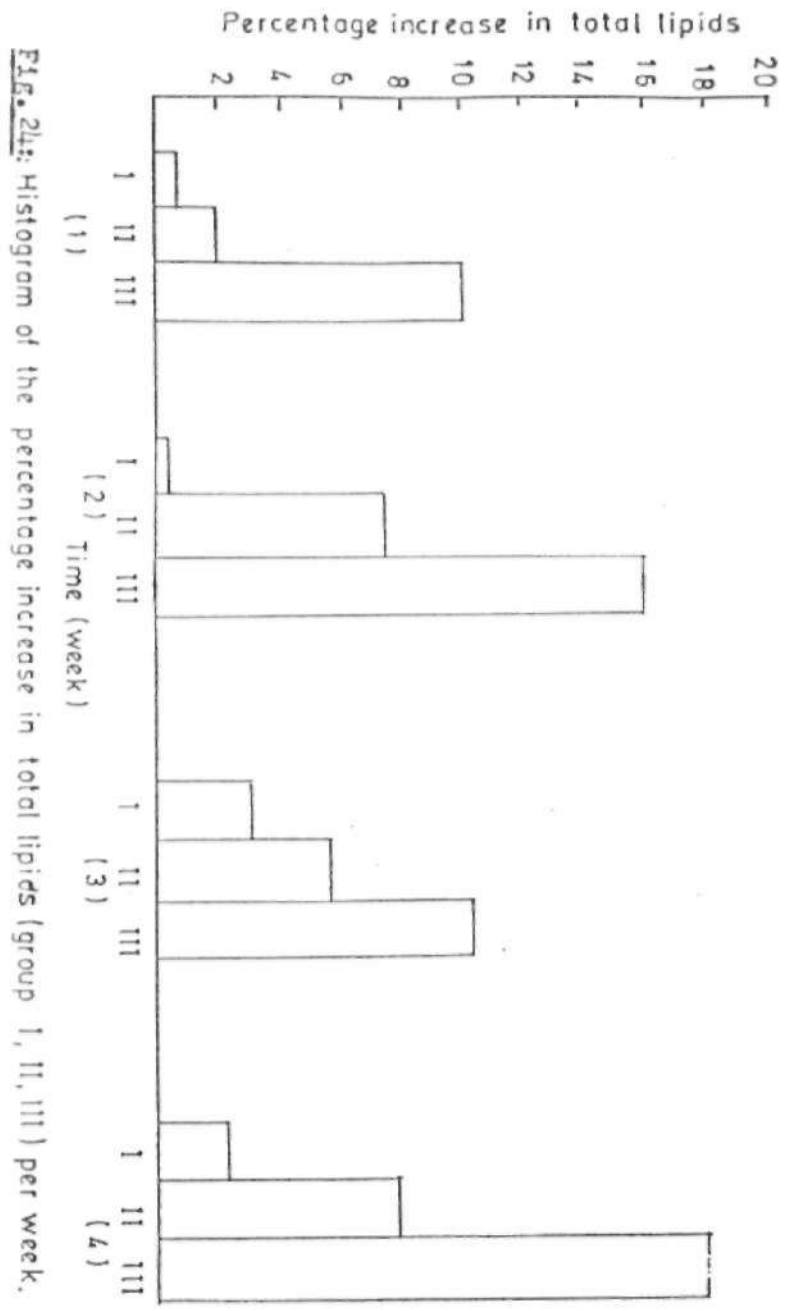


Fig. 24: Histogram of the percentage increase in total lipids (group I, II, III) per week.

levels between 49.5 and 50.7mg cholesterol per 100ml, which agrees with earlier findings (Kates, 1986). A significant increase ($P<0.05$) was recorded in the second week of the experiment in group A, and by the fourth week the value increased by 20.6%. For group D the increase in serum total cholesterol was quite significant ($P<0.05$) even in the first week of the experiment when a value of 62.00 $\pm$ 2.65mg cholesterol per 100ml was recorded, which was a 24% increase from the normal value.

The rats were given measured amounts of fats in their feeds and observations showed that the addition of the fat in feeds of Group A and D, did not in any way affect the feeding pattern of the rats, since rats in all the groups appeared to take equal amounts of feeds.

(iii) Serum triglycerides levels:

Serum triglycerides were determined as mg triolein per 100ml. The results of the serum triglycerides levels in rats in Group C, A and D are given in Table 35. The control rats recorded a mean serum triglycerides level of 112.7mg/100ml $\pm$ 5.7mg/100ml and this agrees with earlier findings (Kates, 1986).

Table 35: Effect of Feeding 5% (Group A) and 10% (Group D) I. gabonensis Seed Fat on Serum Cholesterol of Rats.

(Values are in mg Cholesterol/100ml)

Experiment Period	Week I			Week II			Week III			Week IV		
Rat Groups	C	A	D	C	A	D	C	A	D	C	A	D
1	50.40	54.20	55.70	49.60	56.00	57.00	49.00	58.00	58.00	49.00	61.00	59.00
2	47.80	57.90	54.90	49.00	59.00	55.70	52.00	60.00	57.00	49.10	62.00	63.00
3	53.20	47.80	57.00	50.00	52.50	57.90	51.00	54.00	60.00	52.00	58.00	64.00
Mean	50.46	53.30	55.86	49.53	55.83	56.86	50.66	57.33	58.33	50.03	60.33	62.00
± SD	2.7	5.11	1.06	0.50	3.25	1.10	1.52	3.05	1.52	1.70	2.08	2.64

Comparison :		Week I	Week II	Week III	Week IV
P Values :	C & A	$P > 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.05$
	C & D	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
	A & D	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

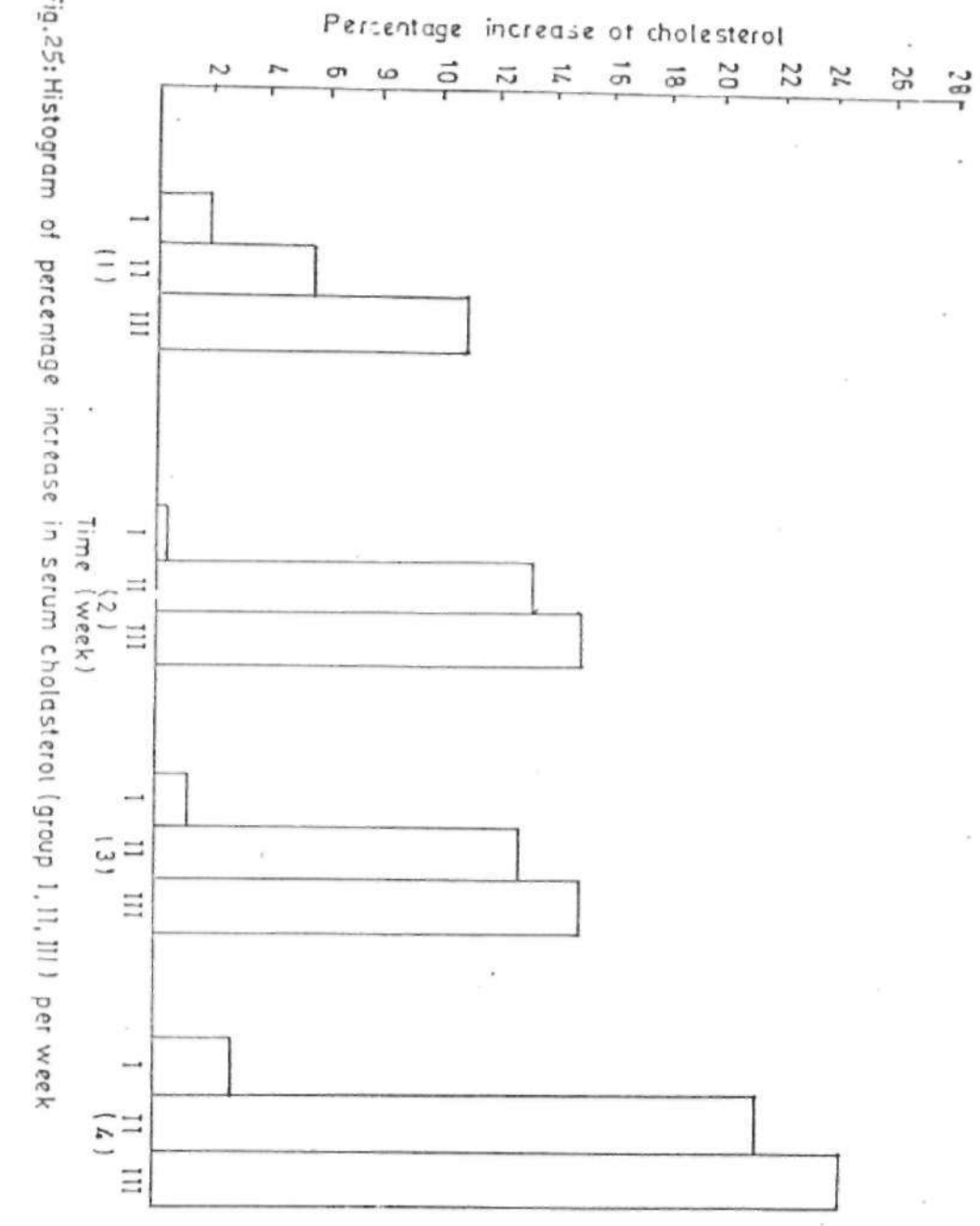


Fig.25: Histogram of percentage increase in serum cholasterol (group I, II, III) per week

For rats in Group A a significant increase ($P<0.05$) of serum triglycerides level was noticed only after the second week of feeding, while the increase in the first week was not significant ($P>0.05$) (Table 35). By the fourth week of feeding a 15% increase in serum triglycerides level was recorded (Table 35).

For rats in Group D a significant increase ($P<0.05$) was noticed in the first week of feeding and this value had increased by 30% in the fourth week.

In this study, the feeding of I. gabonensis seed fat has therefore been shown to significantly increase the fasting level of serum triglycerides in rats.

I. gabonensis seed fat contains predominantly saturated fatty acids of the medium chain length (Table 25). It is known that fats containing saturated fatty acids are very good hyperlipidaemic agents especially those of medium chain length e.g. palmitic, lauric and myristic acids (Grande et al, 1972).

The difference in levels of the fat in the diet of Group A and D animals manifested itself in an increase in serum triglycerides only in the

Table 35: Effect of Feeding 5% (Group A) and 10% (Group D) I. gabonensis Seed Fat on Serum Triglycerides of Rats.

(Values are in mg Triolein/100ml)

Experiment Period	Week I			Week II			Week III			Week IV		
Rat Groups	C	A	D	C	A	D	C	A	D	C	A	D
1	113.00	132.00	127.00	114.00	135.00	129.00	116.00	136.00	140.00	113.00	138.00	148.00
2	120.00	122.00	134.00	119.20	126.00	136.00	121.00	128.00	136.00	123.00	130.00	150.00
3	105.00	121.00	139.00	106.00	125.00	141.00	105.00	128.00	144.00	108.00	128.00	150.00
Mean	112.66	125.00	133.33	113.06	128.66	135.33	114.00	130.66	140.00	114.66	132.00	149.33
± SD	7.50	6.08	6.02	6.64	5.50	6.02	8.18	4.61	4.00	7.63	5.29	1.15

Comparison :	Week I	Week II	Week III	Week IV
C & A	$P > 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
C & D	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
A & D	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$

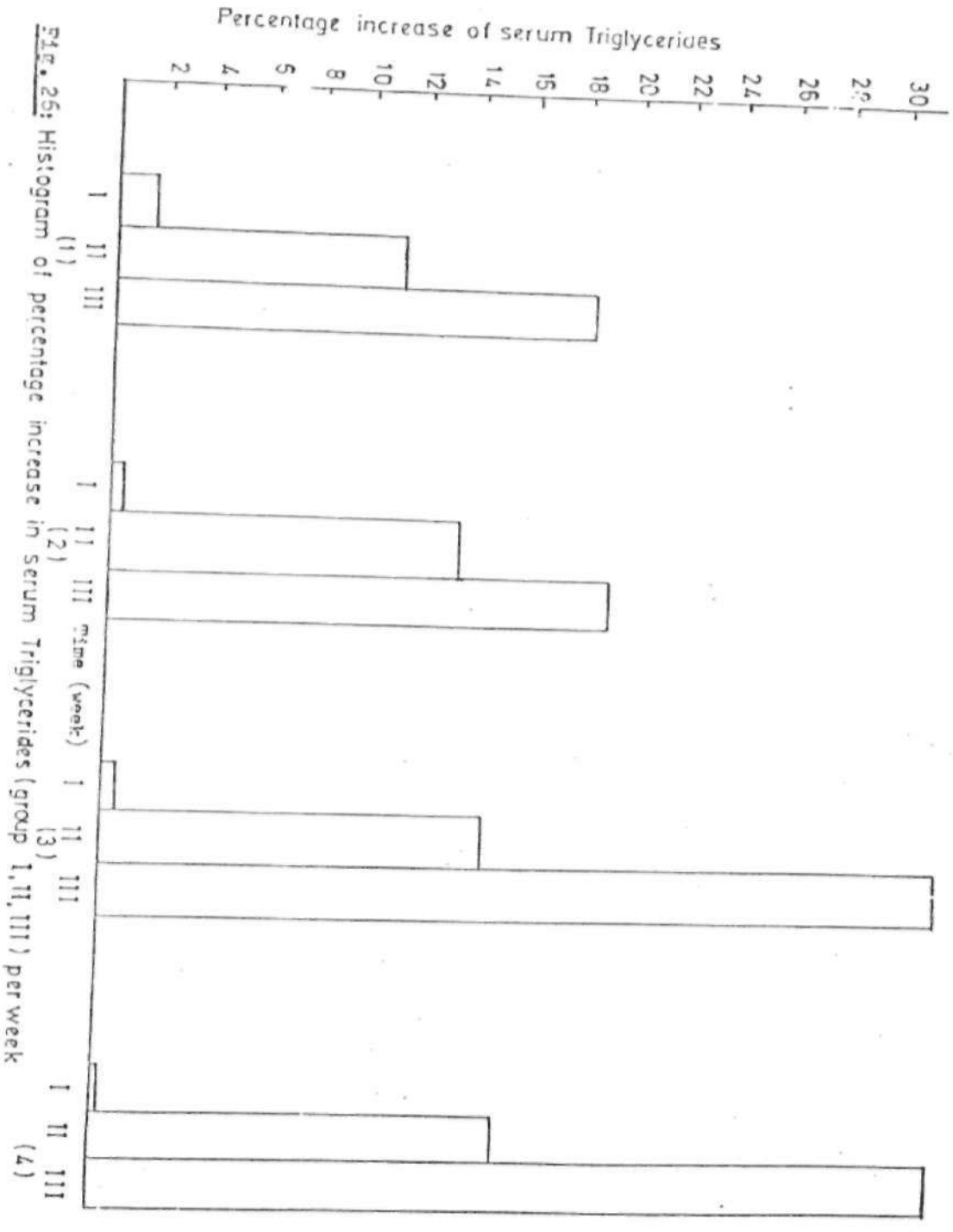


Fig. 26: Histogram of percentage increase in serum Triglycerides (group I, II, III) per week

Table 36: **Percentage Increase in Rats Serum Total Lipids, Cholesterol And Triglycerides After Feeding Them With** *I. gabonensis* **Seed Fat.**

Experiment Period	Week I			Week II			Week III			Week IV		
	TL	CHO	TRI	TL	CHO	TRI	TL	CHO	TRI	TL	CHO	TRI
C Mean	220.60	50.46	112.66	209.30	49.53	113.06	213.00	50.66	114.00	214.00	50.03	114.66
A Difference	4.70	2.84	12.34	15.40	6.30	15.6	11.3	6.67	16.66	16.00	10.3	17.34
A % Increase	2.1	5.62	10.95	7.35	12.71	13.79	5.3	13.16	14.61	7.4	20.58	15.12
D Difference	22.00	5.4	20.67	32.7	7.33	22.27	31.3	7.67	26.00	38.2	11.97	34.67
D % Increase	9.97	10.7	18.34	15.6	14.79	19.69	14.69	15.14	22.80	17.85	23.92	30.23
C Difference	2.00	0.84	2.00	-0.30	-1.77	-1.54	3.4	-0.64	-0.6	4.4	-1.27	0.06
C % Increase of Decrease	0.9	1.63	1.77	-0.14	-3.4	-1.34	1.62	-1.24	-0.52	2.09	-2.47	0.05

TL = Total Lipids, CHO = Cholesterol, TRI = Triglycerides

fourth week of the experiment, probably due to the time lag that is necessary for the fat depots to be saturated with lipids, hence the delay in the increase of fasting serum triglycerides levels in rats fed with the diet of higher concentration of the fat. By the fourth week of the experiment the serum total cholesterol levels of rats in Group A and D had increased by 20.6% and 24% respectively. Although vegetable oils contain negligible amount of cholesterol, it is known that some of them increase serum cholesterol levels when fed to animals (Bronte-Steward et al, 1956).

The fatty acids chain length of triglycerides have been correlated with the level of cholesterol in the serum and body tissues, the longer the chain length the higher the cholesterol level (Keys et al, 1958). Palmitic and myristic acids have shown a greater ability to increase cholesterol level in the serum than fatty acids of shorter chain length such as capric and lauric acids (Young et al, 1972).

The degree of unsaturation of a fat has also been related to cholesterol levels in the body (Keys et al, 1958). Polyunsaturated fatty acids lower body levels of cholesterol while saturated

fatty acids increase the level of cholesterol in the body. Fatty acids in the body are catabolised to acetyl CoA, which is the starting material for cholesterol synthesis. Although increases in serum triglycerides and cholesterol levels were observed in rats in Group A, there was no concomittant increase in the total serum lipids levels; even Group D rats showed a significant increase only in the fourth week. While stress may increase plasma cholesterol and triglycerides the total lipids content was unaffected (Holloszy et al, 1964). The rats could have been stressed by their new environment that the total lipids were not affected from the first to the third week. The increase in the fourth week could be because the rats have adopted to the environment which did not exert physical and mental stress anymore.

I. gabonensis seed fat is consumed as a food additive, as a whole seed and not in the form of pure fat extract as the one used to conduct this study. However, if the fat is taken in the pure form, or the seed consumed with other saturated fats like animal fat, there could be significant increase in serum lipids, which on chronic

consumption could lead to the deposition of fat in the intima of blood vessels, which may lead to atherosclerosis.

Thus the results of this study indicate that the pharmaceutical application of I. gabonensis seed fat should be restricted to external applications only.

CONCLUSION

A detailed pharmacognostic study of *Irvingia gabonensis* leaves, fruits and seeds has been carried out. The two varieties of the plant available in Nigeria were collected and various pharmacognostic parameters were established in order to distinguish and identify each variety.

The seed fat was extracted and its fatty acid composition was determined using three different chromatographic techniques. The data obtained indicate that the seed fat is composed mainly of saturated fatty acids. It was also shown that the fat is in fact a mixture of fat and wax. The seed fat was evaluated as an ointment base and as a suppository base. The results indicate that seed fat can be used as a base for solid and semi-solid pharmaceutical dosage forms.

The physiological studies on adult rats fed with the seed fat for four weeks, showed significant increase in the serum cholesterol, triglyceride and total lipid levels, indicating that pharmaceutical applications of the seed fat should be regulated to avoid its possible side effects.

It is suggested that further work be directed towards a detailed phytochemical investigation of the leaves and fruits of the plant.

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APPENDIX 1

Determination of Some Physical Constants of the Powdered Leaf of I. gabonensis

(a) Determination of the Ash Value

	I. gabonensis var. gabonensis			I. gabonensis var. excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.22	27.24	27.23	27.20	27.22	27.22
2. Weight of crucible and content (g)	29.22	29.24	29.23	29.20	29.22	29.22
3. Weight of crucible and ash (g)	27.45	27.48	27.46	27.43	27.45	27.44
4. Weight of ash (g)	0.23	0.24	0.23	0.23	0.23	0.22
5. Ash value (%)	11.5	12.0	11.5	11.5	11.5	11.0
Average mean		11.66			11.33	

Calculation procedure

Weight of ash = Step 3 - Step 1

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

APPENDIX 2

(b) Determination of Acid Insoluble Ash of the powdered leaf of I. gabonensis (continuation of (a))

	I. gabonensis var. gabonensis			I. gabonensis var. excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.22	27.24	27.23	27.20	27.22	27.22
2. Weight of crucible and acid insoluble ash (g)	27.31	27.36	27.36	27.32	27.36	27.36
3. Weight of acid insoluble ash (g)	0.09	0.12	0.12	0.12	0.14	0.14
4. Acid insoluble ash value (%)	4.5	6.0	6.5	6.0	7.0	7.0
Average mean (%)		5.6			6.6	

APPENDIX 3

(c) Determination of Water-Soluble Ash of the Powdered Leaf of I. gabonensis

	I. gabonensis var. gabonensis			I. gabonensis var. excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.23	27.20	27.24	27.22	27.20	27.21
2. Weight of crucible and ash (g)	27.43	27.43	27.46	27.46	27.43	27.44
3. Weight of ash (g)	0.22	0.23	0.22	0.24	0.23	0.23
4. Weight of water insoluble ash (g)	0.17	0.16	0.16	0.17	0.17	0.18
5. Weight of water soluble ash (g)	0.05	0.07	0.06	0.07	0.06	0.05
6. Water soluble ash value (%)	2.5	3.5	3.0	3.5	3.0	2.5
Average mean (%)	-	3.0			3.0	

APPENDIX 4

Determination of Extractive Values of the Powdered Leaf of I. gabonensis

(a) Alcohol-Soluble Extractive Value.
5gm of the powdered leaves was used in 100ml of 90% ethanol.

	I. gabonensis var. gabonensis			I. gabonensis var. excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.60	27.70	27.70	27.30	27.40	27.40
2. Weight of crucible and content before heating (g)	28.80	28.80	28.80	28.50	28.60	28.60
3. Weight of crucible and content after heating (g)	27.83	27.93	27.93	27.54	27.63	27.63
4. Alcohol extractive content (g)	00.23	00.23	00.23	00.24	00.23	00.23
5. Alcohol extractive value (%)	4.6	4.6	4.6	4.8	4.6	4.6
6. Average (Mean)	4.6			4.7		

Example of calculation

$$\text{Alcohol extractive value (\%)} = \frac{\text{Step 3} - \text{Step 1}}{\text{Initial weight of drug}} \times 100$$

APPENDIX 5

(b) Water-Soluble Extractive Value of the Powdered Leaf of I. gabonensis
5gm of powdered leaves were used in 100 mls of 0.25% chloroform water.

	I. gabonensis var. gabonensis			I. gabonensis var. excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.40	27.50	27.50	27.30	27.40	27.40
2. Weight of crucible and content before heating (g)	28.60	28.50	28.70	28.50	28.60	28.60
3. Weight of crucible and content after heating (g)	27.68	27.77	27.77	27.59	27.67	27.67
4. Water extractive content (g)	00.28	00.27	00.27	00.29	00.27	00.27
5. Water extractive value (%)	28.00	27.00	27.00	29.00	27.00	27.00
6. Average (Mean)	27.4			27.7		

Example of calculations

$$\text{Water extractive value} = \frac{\text{Step 3 - Step 1}}{\text{Initial water of drug}} \times 5 \times 100$$

APPENDIX 6

Determination of Some Physical Constants of the Powdered Fruit of *I. gabonensis*

(a) Determination of the Ash Value

	I. gabonensis var. gabonensis			*I. gabonensis* var excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.24	27.32	28.74	27.26	27.23	28.76
2. Weight of crucible and content (g)	29.24	29.32	30.74	29.26	29.23	30.76
3. Weight of crucible and ash (g)	27.35	27.44	28.84	27.43	27.39	28.95
4. Weight of ash (g)	0.11	0.12	0.10	0.17	0.16	0.19
5. Ash value (%)	5.5	6.0	5.0	8.5	8.0	9.5
Average mean		5.5			8.6	

Example of calculations procedure

Weight of ash = Step 3 - Step 1

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

APPENDIX 7

(b) Determination of Acid Insoluble Ash of the Powdered Fruit of I. gabonensis - (continuation of (a))

	I. gabonensis var. gabonensis			I. gabonensis var. excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.24	27.32	28.74	27.26	27.23	28.76
2. Weight of crucible and acid insoluble ash (g)	27.27	27.36	28.77	27.31	27.30	28.81
3. Weight of acid insoluble ash (g)	0.03	0.04	0.03	0.05	0.07	0.05
4. Acid insoluble ash value (%)	1.5	2.0	1.5	2.5	3.5	2.5
Average mean (%)		1.66			2.83	

APPENDIX 8

(c) Determination of Water-Soluble Ash of the Powdered Fruit of I. gabonensis

	I. gabonensis var. gabonensis			I. gabonensis var excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.20	27.22	28.69	27.21	27.23	28.73
2. Weight of crucible and ash (g)	27.32	27.33	28.80	27.37	27.41	28.88
3. Weight of ash (g)	0.12	0.11	0.11	0.16	0.18	0.16
4. Weight of water-soluble ash (g)	0.04	0.05	0.05	0.05	0.06	0.05
5. Weight of water soluble ash (g)	0.08	0.06	0.06	0.11	0.12	0.11
6. Water soluble ash value (%)	4	3	3	5.5	6	5.5
Average mean (%)		3.33			5.66	

APPENDIX 9

Determination of Extractive Values of the Powdered Fruit of I. gabonensis

(a) Alcohol-Soluble Extractive Value

5gm of the powdered fruit was used in 100 mls of 90% ethanol.

	I. gabonensis var. gabonensis			I. gabonensis var. excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.80	27.40	27.40	27.30	27.20	27.20
2. Weight of crucible and content before heating (g)	28.60	28.60	28.60	28.50	28.60	28.60
3. Weight of crucible and content after heating (g)	28.08	27.68	27.68	27.60	27.49	27.40
4. Alcohol extractive content (g)	00.28	00.28	00.28	00.30	00.29	00.29
5. Alcohol extractive value (%)	5.6	5.6	5.6	6.0	5.8	5.8
6. Average (Mean)	5.6			5.9		

Example of calculation

$$\text{Alcohol extractive value (\%)} = \frac{\text{Step 3 - Step 1}}{\text{Initial weight of drug}} \times 100$$

APPENDIX 10

(b) Water-Soluble Extractive Value of the Powdered Fruit of I. gabonensis
5gm of powdered fruits were used in 100ml of 0.25% chloroform water.

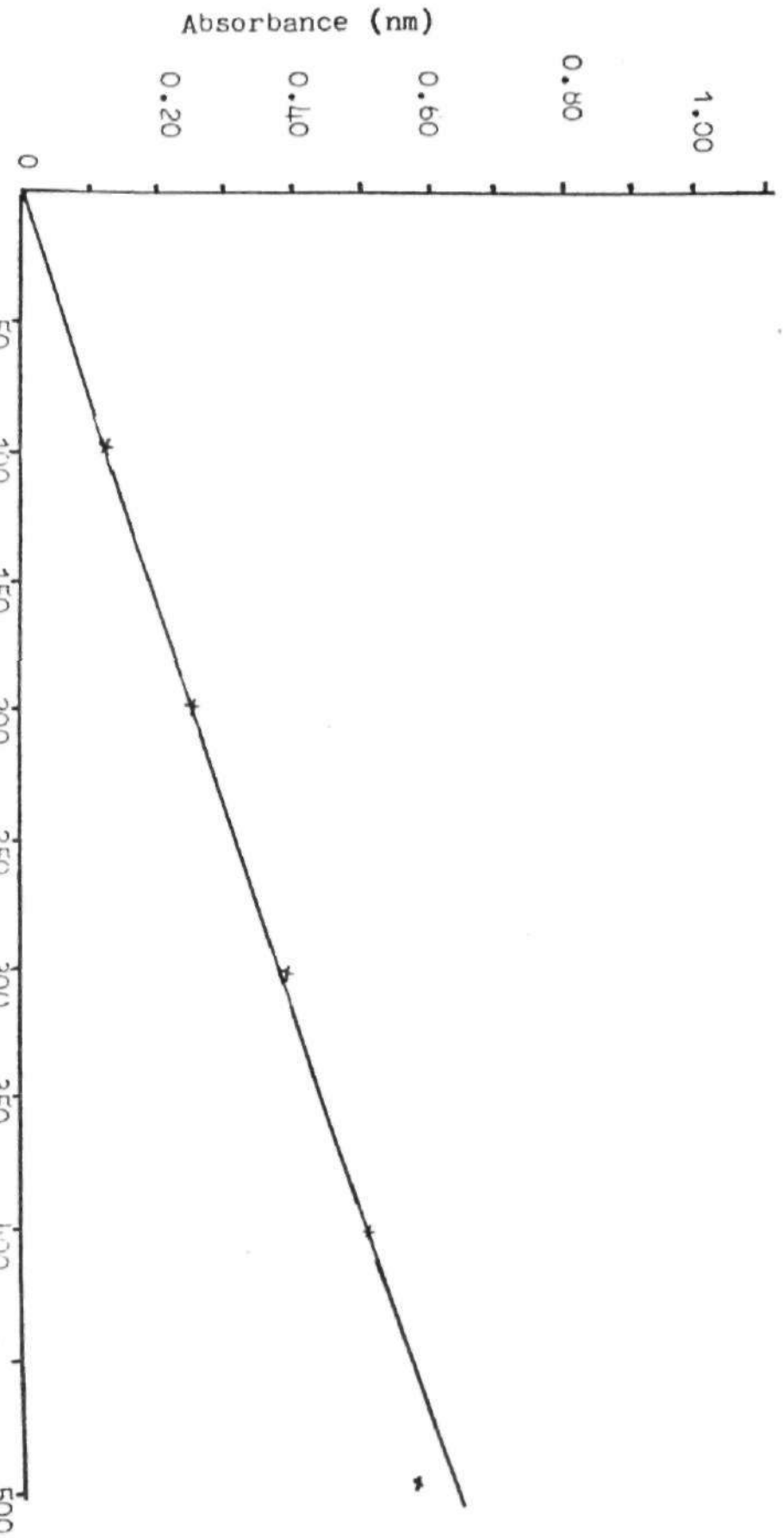
	I. gabonensis var. gabonensis			I. gabonensis var. excelsa		
	1	2	3	1	2	3
1. Constant weight of crucible (g)	27.30	27.20	27.20	27.30	27.30	27.30
2. Weight of crucible and content before heating (g)	28.50	28.62	28.45	28.73	28.74	28.74
3. Weight of crucible and content after heating (g)	27.63	27.52	27.52	27.64	27.63	27.63
4. Water extractive content (g)	00.33	00.32	00.32	00.34	00.33	00.33
5. Water extractive value (%)						
6. Average (Mean)	32.4			33.2		

Examples of calculations

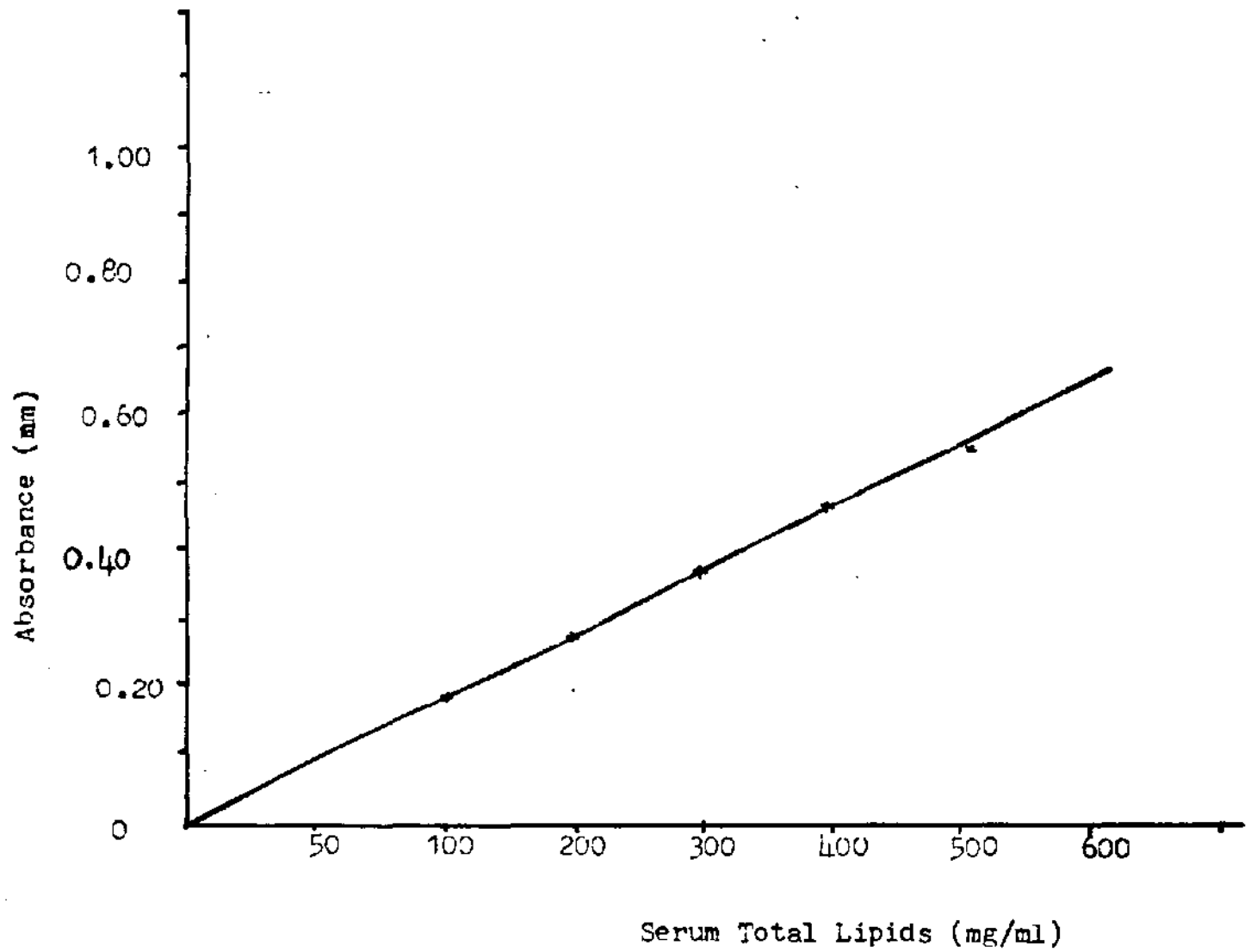
$$\text{Water extractive value} = \frac{\text{Step 3} - \text{Step 1}}{\text{Initial weight of drug}} \times 5 \times 100$$

Appendix 11 Calibration Curve for Total Lipids Cholesterol and Triglyceride.

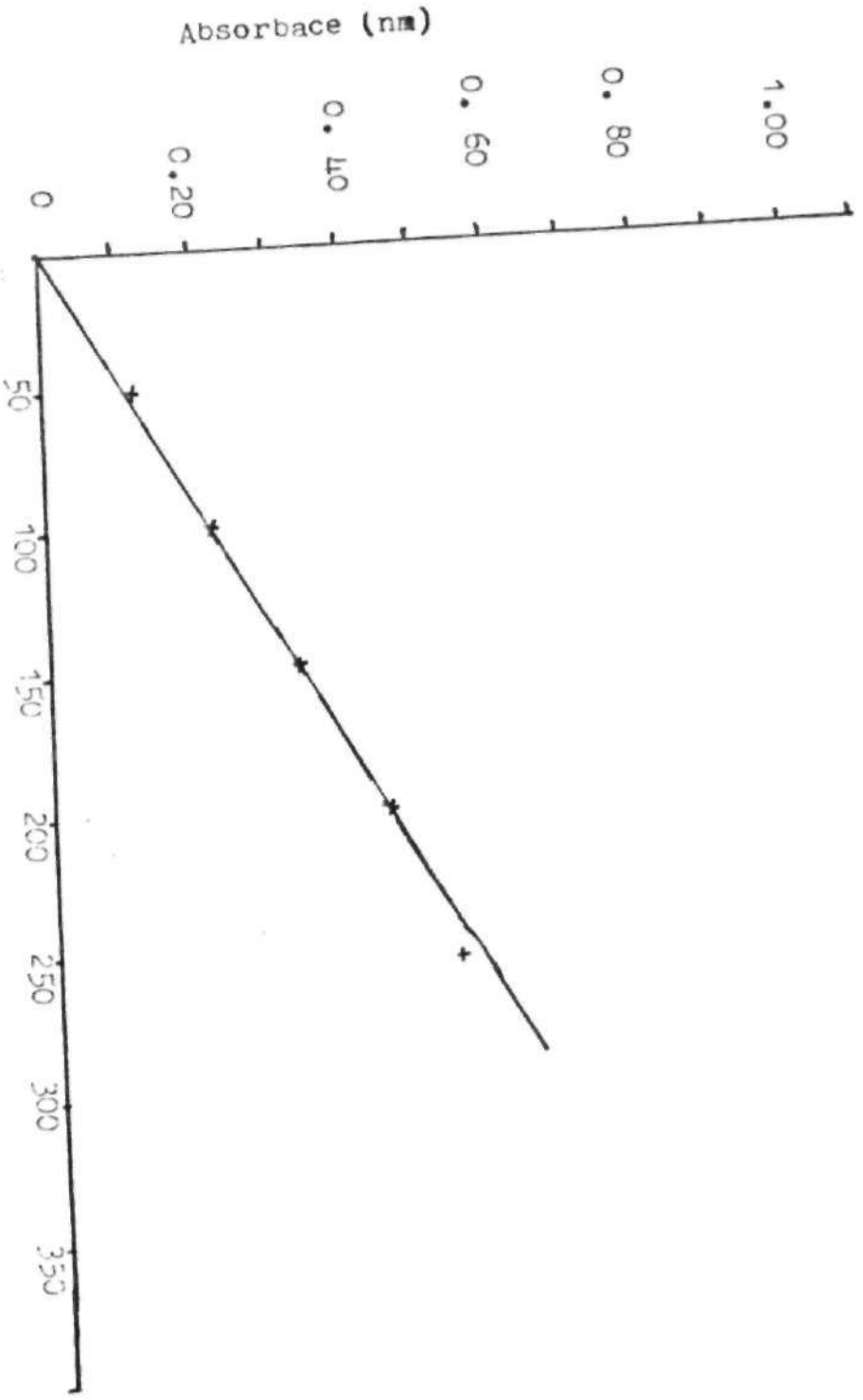
Total Lipids		Cholesterol		Triglyceride	
Standard mg/100ml	Absorbance (nm)	Standard mg/100ml	Absorbance (nm)	Standard mg/100ml	Absorbanc (nm)
100	0.140	50	0.120	100	0.130
200	0.270	100	0.230	200	0.250
300	0.380	150	0.340	300	0390
400	0.470	200	0.460	400	0.510
500	0.560	250	0.550	500	0.580



Appendix 12: Calibration Curve for Serum Triglyceride



Appendix 13: Calibration Curve for Serum Total Lipids.



Appendix 14: Calibration Curve for Serum Cholesterol