PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON THE EXTRACT OF THE BARK OF *COMMIPHORA AFRICANA* (A. Rich) ENGL. (FAMILY: BURSERACEAE)

BY

EKARIKA CLEMENT JOHNSON

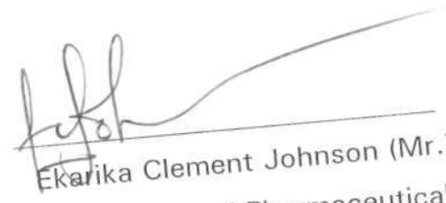
A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA,
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE,
(PHARMACEUTICAL CHEMISTRY)

DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA.

JANUARY 1999.

DECLARATION

I solemnly declare that the work reported in this thesis has been carried out by me, under the supervision of Dr. M.K. Choudhury. It has not been accepted in any previous application for a higher degree elsewhere. The works of other investigators are acknowledged and referred to accordingly.



Ekarika Clement Johnson (Mr.)
Department of Pharmaceutical
and Medicinal Chemistry,
Faculty of pharmaceutical Sciences.
Ahmadu Bello University,
Zaria. Nigeria.

CERTIFICATION

This thesis entitled PHYTOCHEMICAL AND PHAMACOLOGICAL STUDIES OF THE BARK OF *COMMIPHORA AFRICANA* by Ekarika Clement Johnson meets the requirements of the regulations governing the award of the degree of MASTER OF SCIENCE of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

M.K. Choudhury — M.K. Choudhury

Dr. M.K. Choudhury, B.Sc. (Hons.), M.Sc. Ph.D. — Date 16-7-99
Major Supervisor.
Department of Pharmaceutical and Medicinal Chemistry.
Faculty of pharmaceutical Sciences,
Ahmadu Bello University, Zaria.

Prof. A. Mustapha BSc. (Pharm.) Ph.D. (MPSN) — 16-7-99 Date
Co-Supervisor,
Department of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria.

Dr. M. Garba, B.Sc.(Pharm.) M.Sc. Ph.D.(MPSN) — Date
Head,
Department of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria.

Prof. J.A. Owoyale, B.Sc. (ABU) M.P.S. Ph.D (Florida) — 11/5/99 Date
External Examiner,
Department of Chemistry,
University of Ilorin.

Prof. S.B. Ojo, — 04/03/00 Date
Dean, Postgraduate School,
Ahmadu Bello University, Zaria.

DEDICATION

This Project work is dedicated to:

THE GLORY OF THE ALMIGHTY GOD,

My Parents, Etubom CLEMENT and Obonganwan JUSTINA JOHNSON

My Brothers, PHILIPS and MARTINS, My Sisters, GRACE, CHRISTIANA,

HANNAH and their families and My Friend's Daughter MISS

BERNADETTE - AMA UKPE

ACKNOWLEDGEMENT

I wish to express my gratitude to Dr M.K. Choudhury, a renowned scientist and international scholar who designed and supervised this project. He has not only supervised the project thoroughly, but he has also meticulously and painstakingly exposed me to knowledge beyond this level such that the next stage would be an easy affair for me by his expertise. My sincerest thanks also go to my co-supervisor Prof. A. Mustapha for his encouragement and criticism which added up to make the work an authentic reality.

Many thanks to Dr. A.K. Haruna who provided the plant materials and made intelligent suggestions on the work.

I am deeply indebted to Dr. M Garba, Head of Department of Pharmaceutical and Medicinal Chemistry for his contributions both administratively and in offering scholarly advice to make this project a success. I also like to thank Messrs Ilyasu-Mohammed Salisu, M.A Maggaji, Francis Obeta, C. Ochigbo, Ahmadu Zubairu and all other technical and administrative staff of the Department of Pharmaceutical and Medicinal Chemistry for their assistance.

I am indeed very grateful to Messrs J.O. Chidi and Sunday Onwu for helping to type this thesis. I also like to thank Messrs Iyada Kingsley, A.M. Amos, Charles Ebute, John Kono and Jonathan Huji, Dr. Gadaka, all staff of Department of Pharmacology and Clinical Pharmacy, who helped to carry out pharmacological work of this project. I wish to acknowledge and appreciate the contributions of Dr. A.S. Agbaji who provided laboratory space and facilities for most of the phytochemical work of this project at the National Research Institute for Chemical Technology, Basawa, Zaria.

I must admit that I am indebted to a very large number of friends, well-wishers and colleagues who have helped in this endeavour but I hope none will feel slighted by the physical impossibility of listing every name here. They

should be assured that I certainly appreciate their contribution. However, I wish to acknowledge specifically, the special encouragement and interest of Mr & Mrs R.A. Ukpe, Mrs Charity E. Udoh. Mr M.N. Mbokoh, Mrs Ajoke Labake Fadipe (nee Philips), Mrs. M.N. Attoh and Miss Julia Philips.

Above all, thanks be to God for seeing me through the difficult times during the period of this work.

ABSTRACT

Commiphora africana (A. Rich) Engl. (Family: Burseraceae) is a bushy shrub or small tree found mainly in the savannah woodland and driert parts of tropical Africa. The bark and the leaves are used in traditional medicine as a remedy for stomach pains dysentery, snake and scorpion bites as well as a source of gum resins.

The methanolic extract of the bark of the plant gave a homogenous product through chromotographic separation which after crystallization from methanol furnished a needle, mp. 142 - 143°C, characterized as dimethylterepthalate (Benzene - 1, 4- dicarboxylic acid dimethylester), $C_{10}H_{10}0_4$ (M^+, 194), on the basis of spectral analysis (UV, IR, NMR, Mass) and comparison with the synthetic dimethylester prepared from terephthalic acid.

The pharmacological screening of the crude aqueous extract of the plant (bark) showed a dose - dependent relaxation of the isolated rabbit ileum and antagonized the action of acetylcholine on the same tissue in the same manner like atropine. This justified the use of the plant for stomach pains and dysentery in traditional medicine. The pharmacological test on uterus revealed that the aqueous extract lacked agonistic or antagonistic action on the isolated rabbit uterus. However it caused a dose - dependent suppressing action on the rate and the amplitude of the beat of the isolated rabbit heart, a typical B - adrenergic antagonistic effect resembling those potentiated by propranolol. This also confirmed the basis for the use of the plant to treat heart related problems.

TABLE OF CONTENTS

TITLES	PAGES
Title Page	i
Declaration	ii
Certification	iii
Dedication	iv
Acknowledgement	v
Abstract	vii
Table of Contents	viii
List of Tables	xi
List of Tables	xiii
Abbreviations	xv
CHAPTER ONE: GENERAL INTRODUCTION	1
1.1 Brief Introduction on native medicines from plant sources	1
1.2 LITERATURE REVIEW	3
1.2.1 Description of the Plant	3
1.2.2 Medicinal and non Medicinal uses of *Commiphora africana*	5
1.2.3 Aims and Objectives of the Project	6
CHAPTER TWO: MATERIALS AND METHODS	7
2.1 Materials	7
2.1.1 Chemicals and Reagents Used	7
2.1.2 Instruments Used	7
2.2 Methods	8
2.2.1 Extraction Procedures	8
2.3 Preliminary Phytochemical Screening	8
2.3.1 Test for Carbohydrates	9
2.3.2 Test for Alkaloids	9
2.3.3 Test for Tannins	10
2.3.4 Test Saponins	10

2.3.5 Test for Flavonoid....10
2.3.6 Test for Cardiac Glycosides....11
2.3.7 Test for Cardenolides....11
2.3.8 Test for Terpenoids....12
2.4 Analytical Methods....12
2.4.1 Thin Layer Chromatography....12
2.4.2 Thin Layer Chromatographic Examination of the Methanolic Extract....13
2.4.3 Column Chromatography....13
2.4.4 Separation of the Components of Methanolic Extract by Column Chromatography....13
2.5 Characterization of CA....15
2.5.1 Colour Tests for CA....16
2.5.2 Spectral Analysis of CA....17
2.5.3 C,H- Analysis of CA....17
2.6 Preparation of Dimethylterphthalate From Terephthalic Acid....18
CHAPTER THREE: PHYTOCHEMICAL RESULTS AND DISCUSSION....19
3.1 Description of the Methanolic Extract....19
3.2 Result of the Preliminary Phytochemical Screening....21
3.3 Characterization of CA....22
3.3.1 Result of Colour Tests....22
3.4 Result of Spectral Analysis of CA....23
3.4.1 UV Spectrum of CA....23
3.4.2 IR Spectrum of CA....24
3.4.3 NMR Spectrum of CA....24
3.4.4 Mass Spectrum of CA....24
3.4.5 C,H- Analysis....25
3.6 Discussion....34
3.7 Conclusion....37

CHAPTER FOUR: PHARMACOLOGICAL STUDIES OF *COMMIPHORA AFRICANA*.....39
4.1 Objective of the Project.....39
4.2 MATERIALS AND METHODS.....39
4.3 Materials.....39
4.4 METHODS.....42
4.4.1 Preparation of the isolated tissues from the animal.....42
4.4.2 Recording Techniques.....44
4.5 RESULTS.....44
4.5.1 Rabbit ileum.....45
4.5.2 Rabbit uterus.....45
4.5.3 Rabbit heart.....45
4.6 DISCUSSION.....72
4.6.1 Rabbit ileum.....72
4.6.2 Rabbit uterus.....74
4.6.3 Rabbit Heart.....74
4.7 CONCLUSION.....78
REFERENCES.....81

LIST OF TABLES

TABLE	TITLE	PAGES
2a	Separation of Components of Methanolic Extract of *C. africana* by Column Chromatography	14
3a.	T.L.C. properties of Methanolic Extract	19
3b.	Constituents of Methanolic Extract of *C. africana*	21
3c.	R_f values of CA and the synthetic sample	23
3d.	Preparation of physiological salt solution	41
4a.	Effect of contraction produced by acetylcholine on isolated rabbit ileum	46
4b.	Effect of acetylcholine (0.6µg/ml) in the presence of atropine on the isolated rabbit ileum	49
4c.	Effect of adrenaline on isolated rabbit	49
4d.	Effect of aqueous extract of *C. africana* on the isolated rabbit ileum	52
4f	Effect if Carbachol on isolated rabbit uterus	55
4g.	Effect if acetylcholine on isolated rabbit uterus	55
4h.	Effect of acetycholine (0.16µg/ml) on the isolated rabbit uterus in the presence of atropine	58
4i.	Effect of aqueous extract of *C. africana* of the isolated rabbit uterus	58
4j.	Effect if acetylcholine (0.6µg/ml) in the presence of aqueous extract of *C. africana* on isolated rabbit uterus	61
4k.	Effect of acetylcholine on isolated rabbit heart	61
4l.	Effect of isoprenaline on isolated rabbit heart	65
4m.	Effect of propranolol on isolated rabbit heart	65

4n. Effect of aqueous extract *C. africana* on isolated rabbit heart.....66

4o. Effect of isoprenaline (0.16µg/ml) in the presence of propranolol on isolated rabbit heart.....66

4p. Effect of isoprenaline (0.16µg/ml) in the isolated rabbit heart.....72

LIST OF FIGURES

FIGURE		PAGE
Fig. 3a	TLC Picture of Methanolic Extract	20
Fig.3b	UV Spectrum of CA	26
Fig. 3c	IR Spectrum of CA	27
Fig 3d	1H - NMR Spectrum of CA	28
Fig. 3e	^{13}C - NMR Spectrum of CA	29
Fig. 3f	^{13}C - NMR Spectrum (DEPT) of CA	30
Fig. 3g	Mass Spectrum of CA	31
Fig. 3h	Mass Spectrum of Fragmentation Pattern of CA	32
Fig. 3i	NMR Spectral Analysis of CA	33
Fig. 3j	Structure of CA	34
Fig. 4a	Effect of contraction produced by acetylcholine on isolated rabbit ileum	47
Fig. 4b	Effect of acetylcholine (0.16µg/ml) in the presence of .. atropine on the isolated rabbit ileum	48
Fig. 4c	Effect of adrenaline on isolated rabbit ileum	50
Fig. 4d	Effect of aqueous extract of *C. africana* on isolated rabbit ileum	51
Fig. 4e	Effect of cetylcholine (0.16µg/ml) in the presence of aqueous extract of *C. africana* on isolated rabbit ileum	53
Fig. 4f	Effect of carbachol on isolated rabbit uterus	54
Fig. 4g	Effect of acetylcholine on isolated rabbit uterus	56
Fig. 4h	Effect if acetylcholine (0.16µg/ml) in the presence of atropine on isolated rabbit uterus	56
Fig. 4i	Effect of aqueous extract of C. *Africana* on the isolated rabbit uterus	57
Fig. 4j	Effect of acetylcholine (0.16µg/ml) in the presence of aqueous extract of *C. africana* on isolated rabbit uterus	57
Fig 4k	Effect of acetylcholine on isolated rabbit heart	59

Fig. 4l Effect of isoprenaline on isolated rabbit heart.....60

Fig. 4m Effect of propranolol on isolated rabbit heart.....62

Fig. 4n Effect of aqueous extract of *C. africana* on isolated rabbit heart.....63

Fig. 4o Effect of isoprenaline (0.16µg/ml) in the presence of propranolol on isolated rabbit heart.....64

Fig.4p Effect of isoprenaline (0.16µg/ml) in the presence of aqueous extract of *C. africana* on isolated rabbit uterus.....67

Fig. 4q Log Dose - response curves of acetylcholine and atropine Ach + Atr. On isolated Rabbit ileum.....68

Fig. 4r Log Dose - response curves for Aqueous extract of *C. africana* and Ach + Aqueous extract of *C. africana* on isolated rabbit ileum.....69

Fig. 4s Log. Dose - response curves of isopr. And isopr. + propr. On isolated rabbit heart.....70

Fig. 4t Log. Dose - response curves for aqueous of *C. africana* and + aqueous extract of *C. africana* on isolated rabbit heart.....71

Fig. 4u Structures of Standard Drugs used.....80

ABBREVIATIONS

Ach.	=	Acetylcholine
Adr.	=	Adrenaline
Atr.	=	Atropine
C_6H_6	=	Benzene
B.P.	=	British Pharmacopoeia
$CaCl_2$	=	Calcium Chloride
Carb.	=	Carbachol
^{13}C	=	Carbon-13
$CHCl_3$	=	Chloroform
C.C.	=	Column Chromatography
Conc.	=	Concentration
cm^3	=	Cubic Centimeter
^0C	=	Degree Centigrade (Celsius)
$CDCl_3$	=	Deuterated Chloroform
DEPT	=	Distortionless Enhancement by Polarisation Transfer
$FeCl_3$	=	Ferric Chloride
Fig.	=	Figure
e.g	=	For example
g	=	gramme
hr	=	Hour
IR	=	Infrared
I_2	=	Iodine
Isopr.	=	Isoprenaline
LBR	=	Liebermann Buchard Reagent
Log	=	Logarithm
MeOH	=	Methanol
ME	=	Methanolic extract
$MgCl_2.6H_2O$	=	Magnesium Chloride hexahydrate

μg	=	Microgramme
ml	=	Millilitre
mm	=	Millimetre
min	=	Minute
HNO_3	=	Nitric acid
NMR	=	Nuclear Magentic Resonace
%	=	Percentage
cm^{-1}	=	Per centimetre
KCl	=	Potassium chloride
KOH	=	Potassium hydroxide
Propr.	=	Propranolol
^1H	=	Proton
r.t.	=	Room temperature
AcONa	=	Sodium Acetate
NaOH	=	Sodium hydroxide
Soln	=	Solution
H_2SO_4	=	Sulphuric acid
i.e.	=	That is
TLC	=	Thin layer Chromatography
Vol.	=	Volume

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Brief Introduction on Native Medicines from Plant Sources

Mankind's experience for thousands of years with plants has led him to nurse a notion that herbal remedies, being natural products are inherently safer than the potent synthetic drugs which often produce side effects, (Trease and Evans, 1983). This notion conveys a special and popular appeal on the research on medicinal plants by scientists, (Sofowora, 1986).

Most drugs of plant's origin used by medical practitioners are in the form of extracts of the whole plant material or part of it. In view of this, local medicinal plants which show suitable biological effects could be standardized and similarly utilized. Some of the effects elaborated by extracts of plants used in traditional medicine include, antiviral, antitumour, antimicrobial, insecticidal and central nervous system effects (Sofowora, 1986).

Many of these herbal or ancient medicines have given cause for detailed investigation. For instance, the fruit of *Momordica charantia* has outstanding importance to the diabetics as it improves glucose tolerance while *Abrus precatonus* seeds have been used as an oral contraceptive. (Trease and Evans, 1983). These interesting pharmacological and clinical effects of plants used in ancient medicine have attracted further research into their untapped potentials or active principles that may allow these materials to be used in western (modern) medicine.

However, such research into medicinal plants for their bioactive compounds is not that a direct simple task. Investigation of a plant may involve the stepwise extraction procedures including separation and isolation of the constituents; characterization and quantitative evaluation

of the isolated constituents having the particular biological effects. The active principle frequently include the alkaloids, glycosides and those of dietary importance such as carbohydrates, fats, proteins and mineral elements. Saponins, steroid, terpenoids, flavonoids and tannins often reported to occur alongside with the ones mentioned earlier are invaluable to the pharmacy profession. (Trease and Evans, 1983).

The review of these medicinally useful compounds of plants origin is very essential to establish the potency and side effects of the drugs in response to the growing interest in the alternative system. New drugs from plants are not just a relief for a particular ailment, but can serve as new sources of raw materials for the manufacture of known and commonly used drugs which would provide the basis for some local pharmaceutical industries. Drugs from plant origin known to possess high medicinal values promote the cultivation of such plants which eventually saves the scarce foreign exchange and improves the economy of the country.

One of the major set backs often encountered with drugs of plant source is that, most of the active compounds occur in minute quantity which requires tedious extraction procedures. In order to overcome this limitation the synthetic chemist takes the approach of determining the structure - activity relationship of the isolated, biologically active constituents to synthesize simpler analogues, some of which might be more active and less toxic than the natural products. A good example is the antimalarial drug - quinine, obtained from cinchona bark and its synthetic analogue, chloroquine. Structural modification of pure bioactive molecule of plant origin as with Naloxone (narcotic analgesic anatagonist of morphine isolated from *Papavera somniferum* has presented a break through (Trease and Evans, 1983).

It is therefore necessary to review the active chemical constituents of the medicinal plants such as *Commiphora africana* used in this study with the aim of discovering the prospect for the present and future sources of useful drugs for both local utilization as well as providing alternative modern medicines. Besides, inspite of the tremendous advances, certain human ailments have not reached complete remission with these drugs, but evidently some defiant diseases have found complete treatment with bioactive substances of plant sources. For example Vincristine and Vinblastin; two important alkaloids from *Catharanthus roseus* have superseded the synthetic antitumour agents in the palliative treatment of certain carcinomas, while reserpine obtained from *Rauwolfia serpentina* still remains an indispensable drug in the management of malignant hypertension. (Sofowora, 1982)

1.2 LITERATURE REVIEW

1.2.1 Description of the Plant:

Commiphora africana (A.Rich) Engl. is a specie in the family Burseraceae. Burseraceae is a family of about 16 genera and 500 species of tropical shrubs and trees well represented in Africa, Asia and tropical America. The major genera include *Commiphora* (185 species), *Bursera* (80 species) *Canarum* (75 species) and *Boswellia* (24 species).

The best known products of the family are myrrh, frankincense, american elemi and java almond, which are all oleo resins and gums. Myrrh occurs as an irregular or rounded lumps of agglutinated tears with a dry surface covered with fine powder. They are reddish brown or reddish yellow in colour and brittle with an irregular, brown, oily, translucent, fractured surface. It has aromatic acrid and bitter taste. It is made up of volatile oil (2.5 - 8%), gum (57 - 61%) and resin (25-35%). It possesses antiseptic and stimulant properties, hence is used as an

astringent and also as stomachic (Ghani, 1990). It is also used for embalment of human bodies. Frankincense is mainly used in the preparation of perfumes and incense (Anderson, 1956. Herbert, and Ellery, 1948).

The genus of the plant under test is *Commiphora*. The name Commiphora is coined from Greek word meaning "gum bearing". This refers to the resin which exudes from the bark of some certain species. *Commiphora* is a large genus found abundantly in Africa and Asia as shrubs or small trees with short trunk and tangle branches. The species of *Commiphora* yield myrrh, gums and oils resins. Other species under the genus include *C. myrrh, C. pendiculata, C. kerstingii. Commiphora africana* is a shrub, or tree with short lateral branches sharply pointed at the apex, bearing coarsely toothed leaves in small cluster of three below the tip.

It is called "dashi" in Hausa, " badadi" in Fulani, "Kabi" in Kanuri languages in Nigeria. In Nigeria it is mainly found in Kano, Borno, Damaturu and Karasuwa and widespread in the drier parts of tropical Africa. The plant was formerly known as *Balsamodendron africanum*. It is also called *African bdelium* or *African myrrh.*

The tree can grow up to 6 meters in height. The bole is short. The bark is grey or greenish on the bole, reddish on the branches, peeling off in thin papery strips. When slashed a reddish brown fibrous fragment gum-resin exudes slowly.

The leaves have slender finely hairy common stalk (0.625-3.75 cm long), the terminal leaf (largest) is about 1.25 - 3.75 cm long and 0.625 - 2.5cm wide. The shapes of the leaves range from obovate to oblanceolate, rounded at the apex, narrowly cuneate at the base. The pair of leaflets below are relatively broad,

considerably smaller, unsymmetrical and practically stalkless. All the leaves are with coarsely irregular rounded teeth and finely hairy beneath, with 3-4 pairs of irregular nerves. The leaves are fragrant when crushed.

The flowers (October - April) glabrous, stalkless, and reddish in colour usually appear when the tree is leafless in small axillary clusters. The fruits (May) are reddish with purplish bloom, broadly ellipsoid, about 0.75cm long and stalkless. The white stone is surrounded by resinous pulp. (Dalziel, 1937; Keay, 1989).

1.2.2 Medicinal and Non Medicinal Uses of the Plant

Among the local communities in Northern Nigeria, the decoction of the bark of the plant is commonly used in the treatment of diabetic patients and as remedy for stomach pains and dysentery (Jinju, 1990). The root of the plant cooked with millet or sorghum is used to treat heart burns. Jinju, (1990) has also reported that, the water extract of the bark of the plant mixed with some other plants is used as anti-snake venum.

The leaves are pounded with bulrush millet and milk and taken as stomachic. For scorpion bite the bark is applied after chewing it along with natron. The stems are used as chewing stick and the wood for making rosary beads.

The bark and leaves are fragrant. These parts of the plant yield a gum resin which gives an agreeable myrrh like odour when burning and is used to fumigate clothing. The dried and pulverised bark may be burnt for the same purpose or it is put amongst clothing like lavender.

The gum resin forms part of the export commodities of some West African Countries (e.g. Senegal). The gum resin is used in Europe to manufacture medicinal plasters and varnishes while in West Africa it is boiled for the treatment of the inflammation of the eyes by holding the

face over the steaming pot.

There are also some mystical beliefs associated with the plant. Some people say that it has seven lives. Hausa people nickname it `Dashi mai-yawan rai' because "it is cut and not killed" and they therefore plant it to form a hedge in arid districts. It grows well from cuttings and is recommended as a hedge plant in suitable localities. It is also venerated by the Tuaregs (tribe in Morocco and Chad) who regard it as a symbol of immortality and place it on graves. (Dalziel, 1937).

Commiphora africana is chiefly noted for its gum resin products some of which are bdellium, galbanum and african myrrh, which are sources of feed stocks for making polyester resins, fibres, films, plasters and varnishes. (Jessene et al, 1977; Rendle, 1952; Hörhammer et al, 1966).

1.2.3 Aims and Objectives of the Project

Commiphora africana has been reported to be used in traditinal medicine as remedy for some ailments such as stomach pains and dysentery, heart burns and snake-bites. The project was then designed to establish the authenticity of these claims through phytochemical and pharmacological studies of the bark of the plant, with a view to isolating the active principle(s) that could have been eliciting the actions in the gastro-intestinal tract and heart. Besides, it was hoped to establish the effect of the aqueous extract of the bark of the plant on isolated tissues of rabbit namely; ileum and heart which are the sites of the reported action of the drug. The work would be extended to test the effect of the aqueous extract on the uterus for abortificient properties.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals and Reagents Used

All chemicals were obtained from B.D.H. Chemicals Ltd, Poole, England and distilled before use. Silical gel (for TLC and Column) was supplied by E. Merck, Darmstadt, Germany.

2.1.2 Instruments Used

Melting point (mp): Gallenkamp Capillary Melting Point Apparatus.

Ultraviolet Spectroscopy (UV): Pye Unicam 5000 Spectrophotometer SP8 - 100

Ultraviolet Light (254 and 366 nm): Ultraviolet Products. Insurance.

Infrared Spectroscopy: Perkin - Elmer infrared Spectrophotometer (710B)

Mass Spectroscopy: Prospec Electron Ionization (E.I).

Nuclear Magnetic Resonance (NMR): Varian Spectrometer (400 MHz)

2.2 METHODS

2.2.1 Extraction Procedures

(a) Collection and Drying of the Plant Materials.

The bark of the plant, *Commiphora africana* was collected from the matured tree along Zaria - Funtua road in the month of May, 1997. The plant was authenticated by the Herbarium at the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, and a herbarium sample was deposited.

The plant material was dried in air under shade for several weeks, crushed and then pounded into powder form.

(b) Extraction

The air dried powdered bark (250g) was first defatted (Soxhlet, 24 hours) with petroleum ether (60 - 80^{oC}), until the draining solvent was colourless. The extract was concentrated in vacuo to obtain an oil. The oily reddish liquid material was kept for further investigation.

The defatted marc was air-dried to evaporate petroleum ether and extracted with methanol (Soxhlet) until the draining solvent became colourless. The extract was concentrated and a reddish gummy solid mass was obtained. This was weighed.

2.3 PRELIMINARY PHYTOCHEMICAL SCREENING

A small portion of the methanolic extract (**ME**) was used to test for the presence of the following constituents of the plant.

2.3.1 Tests for Carbohydrates

(a) **General Test (Molisch's Test)**

A few drops of the Molisch's reagent was added to a clear solution of the extract dissolved in water followed by the addition of 1ml of conc. sulphuric acid down the side of the test tube, so that the acid formed a layer beneath the aqueous layer. The mixture was allowed to stand for about 2 minutes and then diluted with 5ml of water. Formation of a red colour at the interphase of the two layers confirmed the presence of carbohydrates, (Trease and Evans, 1983).

(b) **Test for Sugars (Barfoed's Test)**

To a clear solution of the extract in water contained in a test tube 1 ml of Barfoed's reagent was added and heated on a water bath for 2 minutes. A red precipitate of Cu_2O was taken as a positive test for the presence of sugars (Brain and Turner, 1975).

(c) **Test for Reducing Sugars (Fehling's Test)**

The clear solution of the extract dissolved in water was heated with 5ml equal volumes of Fehling's solutions A and B. Formation of a red precipitate of Cu_2O indicated the presence of reducing sugars, (Trease and Evans, 1983).

2.3.2 **Test For Alkaloids**

A little of the extract was stirred with 5ml of 1% aqueous hydrochloric acid (HCl) on a water bath and filtered. 3ml of the filtrate was divided into 3 portions of 1ml each. The first 1ml was treated with a few drops of Dragendorff's reagent to give an orange red precipitate.

To the second 1ml, Mayer's reagent was added to give a buff coloured precipitate and the third 1ml portion was treated with Wagner's reagent to give a dark brown precipitate. The colour changes confirmed the presence of alkaloids (Brain and Turner, 1975).

2.3.3 Test for Tannin

A little of the extract was dissolved in water and heated on a water bath. The mixture was filtered and $FeCl_3$ solution added to the filtrate. A dark green precipitate observed was taken as evidence for the presence of annins (Trease and Evans, 1983).

2.3.4 Test for Saponins

A little of the extract was shaken with water in a test tube and frothing which persisted on warming indicated the presence of saponins, (Sofowora, 1982).

2.3.5 Test for Flavonoids

(a) Shinoda's Test

A little of the extract was dissolved in ethanol, warmed and filtered. Three pieces of magnesium turnings were added to the filtrate followed by the addition of a few drops of conc. HCl. A pink colour was an indication of the presence of flavonoids (Ventataraman, 1962).

(b) Ferric Chloride Test

A little of the extract was dissolved in ethanol and boiled with a few drops of 10% $FeCl_3$ solution. A violet colouration was an indication of the presence of a phenolic hydroxyl group, (Trease and Evans, 1983).

(c) **Lead Acetate Test**

A little of the extract was dissolved in 10ml of water and filtered. To the filtrate, 3 drops of $Pb(CH_3COO)_2$ solution were added. A buff coloured precipitate confirmed the presence of flavonoids (Brain and Turner, 1975).

2.3.6 **Test for Cardiac Glycosides**

Test for steroidal Nucleus (Salkawskii Test)

A little of the extract was dissolved in 2ml of $CHCl_3$ followed by the addition of conc. H_2SO_4 to form a lower layer. A reddish brown colouration at the interphase indicated the presence of a steroidal nucleus i.e. aglycone portion of the glycoside (Sofowora, 1982).

2.3.7 **Test for Cardenolides**

(a) **Legal Test for cardenolide (Aglycone)**

A little of the extract was dissolved in pyridine and to it a few drops of sodium nitroprusside together with a few drops of 20% sodium hydroxide solution were added. A deep red colour which faded to brownish yellow was an indication for the presence of cardenolide (Sofowora, 1982).

(b) **Keller - Kiliani Test for Digitalis Glycoside**

A little of the extract was dissolved in 2ml of glacial acetic acid and to it one drop of $FeCl_3$ solution was added followed by the addition of 1ml of conc. H_2SO_4.

A reddish - brown ring obtained at th

presence of a digitoxose sugar component, characteristic of cardenolides (Trease and Evans, 1983).

2.3.8 Test For Terpenoids

(Liebermann - Burchard Test)

A little of the extract was dissolved in $CHCl_3$. To it 1ml of acetic anhydride was added followed by the addition of conc H_2SO_4. A colour change from pink to violet was an indication of the presence of terpenoids.

2.4 ANALYTICAL METHODS

2.4.1 Thin Layer Chromatography (TLC).

Ascending technique was used and the stationary phase was silica gel G. slurry, which was prepared by shaking silica gel (40g) in distilled water (80ml) and spread onto the plate (20 x 10cm) to a thickness of 0.25mm. The prepared plates were activated at 110°C for one hour in the oven.

The spots were applied manually using capillary tubes. The plates were developed at room temperature in an appropriate solvent system (mobile phase - details in the text) using a shandon chromotank. The developed chromatograms were dried in the air and visualized as follows:-

(a) viewed under sunlight

(b) viewed under ultraviolet light (254 and 366 nm)

(c) exposed to iodine vapour in a closed chamber

(d) sprayed with Liebermann - Burchard Reagent and heated in the oven for 5 minutes

2.4.2 Thin Layer Chromatographic Examination of the Methanolic Extract.

Thin Layer Chromatographic examination of the methanolic extract was carried out using $CHCl_3$: MeOH (4:1) solvent system. Visualization of the chromatogram using UV Light and iodine vapour revealed the presence of several spots, most of which were positive towards Liebermann - Burchard Reagent (pink to violet).

2.4.3 Column Chromatography (CC)

The gradient elution technique was employed using sintered glass column (25 x 1.5cm) packed by slurry method. The adsorbent was silica gel (60 - 120 mesh). Details of the eluting solvent systems are given in the text.

2.4.4 Separation of the Components of Methanolic Extract by Column Chromatography

About 1g of the methanolic extract was absorbed on a column packed with silica gel and eluted with the eluants (Table 2a) with increasing polarity. The eluates were collected in 10ml aliquots and subjected to thin layer chromatography to determine the number of components eluted. Similar aliquots were pooled together, concentrated under reduced pressure and left to crystalize at room temperature. Aliquots 224 - 238 yielded long needle like yellow crystals which on recrystallization from methanol became colourless. This was coded `CA'.

Table 2a: Separation of the Components of the Methanolic Extract Eluant systems and the aliquots

Eluants	Ratio	Aliquots	Remarks	TLC Report (UV,LBR and I_2
PE: $CHCl_3$	10:0	1-15	colourless	1-15 combined
PE: $CHCl_3$	9:1	16-25	faint	16-25 combined
	8:2	26-35	yellow	
	7:3	36-45	"	26-75 combined
	6:4	46-55	"	
	5:5	56-65	"	
	4:6	66-75	"	76-85 combined
	3:7	76-85	"	
	2:8	86-130	86-130	86-130 combined
	1:9	131-150	yellow	
	0:10	151-170	"	131-150 combined
$CHCl_3$:MeOH	9:1	171-190	"	151-200 combined
	8:2	191-195	"	
	7:3	196-200	"	
	6:4	201-205	"	201-205 combined
	5:5	206-210	206-240	206-210 combined
	4:6	211-215	deep	211-223 combined
	3:7	216-223	yellow	
	2:8	224-230		224-238 combined
	1:9	231-240		239-240 combined
	0.10	241-270	brown	241-270 combined

2.5 CHARACTERIZATION OF CA

CA was purified by recrystallization from methanol and characterized through melting point, co-chromatography with the authentic sample (synthetic) compound, spectral (UV, IR, NMR & Mass) and C,H analysis.

(a) Purification and Determination of Melting Point of CA

CA was recrystallized from methanol, filtered and dried in oven at 50°C to remove solvent and water. The pure crystal was ground into powder and filled into a small capillary tube sealed at one end. The capillary tube was fitted into the Gallenkamp Capillary Melting Point Apparatus to determine the melting point (mp) of the crystal. Mixed melting point with synthetic sample was also determined.

(b) Solubility Tests For CA

The degree of solubility of CA was determined in various solvents such as Petroleum ether, benzene, chloroform, acetone, ethylacetate, methanol and water.

(c) **Thin Layer Chromatography for CA**

A solution of CA in Chloroform was subjected to thin layer chromatoragraphy in different solvent systems and the R_fs compared with that of the authentic compound in the same solvent systems.

The following solvent systems were used

1. Benzene: 100%
2. Benzene: ethylacetate 5:1
3. Benzene: ethylacetate 4:1
4. Benzene: ethylacetate 2:1

The spots were detected by viewing under UV light (254nm) and long exposure in iodine chamber.

2.5.1 Colour Tests For CA

About 2mg of CA was dissolved in methanol and used for the following test

(a) **Ferric Chloride Test**

A few drops of $FeCl_3$ solution was added to the methanolic solution of CA and the colour change noted.

(b) **Sodium Hydroxide Test.**

A few drops of aq. NaOH solution was added to the methanolic solution of CA and the colour change observed.

(c) **Shinoda's Test**

Two pieces of magnesium turnings and a few drops of conc. HCl were added to the solution of CA and the colour change noted.

(d) **Sulphuric Acid Test.**

A few drops of conc. Sulphuric acid was added to a little of the solid crystal of the CA and the colour change noted.

2.5.2 SPECTRAL ANALYSIS OF CA

The UV spectrum was recorded in methanol, the IR spectrum in nujol; the ^{1}H-NMR and ^{13}C - NMR spectra were run in deuterated chloroform ($CDCl_3$) and the mass spectrum was recorded using E.I. (electron ionization) method.

2.5.3 C, H - ANALYSIS

About 0.02g of CA was completely burnt in a stream of oxygen in the presence of CuO. The resulting water was absorbed into weighed anhydrous magnesium perchlorate and CO_2 into weighed soda lime. Carbon was determined from CO_2 and hydrogen from water.

(Dazeley, 1979)

2.6 Preparation of Dimethylterephthalate from Terephthalic Acid

Dimethylterephthalate was prepared by refluxing a solution containing terepthalic acid (benzene 1,4 - dicarboxylic acid, 1g) dissolved in dry methanol (20ml) and conc. H_2SO_4 (2ml) for 6 hours under dry condition (anhydrous $CaCl_2$ guard tube). The reaction mixture was cooled to room temperature, the precipitated material was filtered, washed with water to remove acid and dried in air (0.9g). The residue was recrystallized from methanol to furnish needless, mp. 142 - 143°C (Vogel, 1974).

CHAPTER THREE

3.0 PHYTOCHEMICAL RESULTS AND DISCUSSION

3.1 Description of the Methanolic Extract

The methanolic extract on concentration gave a reddish, thick gummy mass (30.5g) with some shiny specks on the surface. The extract when shaken in water formed a yellowish emulsion. It was found to be soluble in $CHCl_3$ and methanol; partially soluble in petroleum ether, and benzene.

The TLC ($CHCl_3$:MeOH 4:1) chromatogram of the extract revealed 6 spots in iodine vapour and with Liebermann - Burchard Reagent and 2 other spots when viewed under UV Light (366nm) as shown in Fig 3a.

Table 3a: TLC Properties of ME

Spot	R_f value	Colour under UV (366) nm)	Colour with I_2	Colour with LBR
a	0.00	Dark	Yellow	Yellow to brown
b	0.12	No colour	Yellow	Yellow to violet
c	0.37	No colour	Pink	Pink to violet
d	0.60	Blue flourescent	No spot	No spot
e	0.70	Pink	No spot	No spot
f	0.80	Blue flourescent	Yellow	Pink to Violet
g	0.88	Blue flourescent	Yellow	Pink to Violet
h	1.00	Yellow	Yellow	Pink to Violet

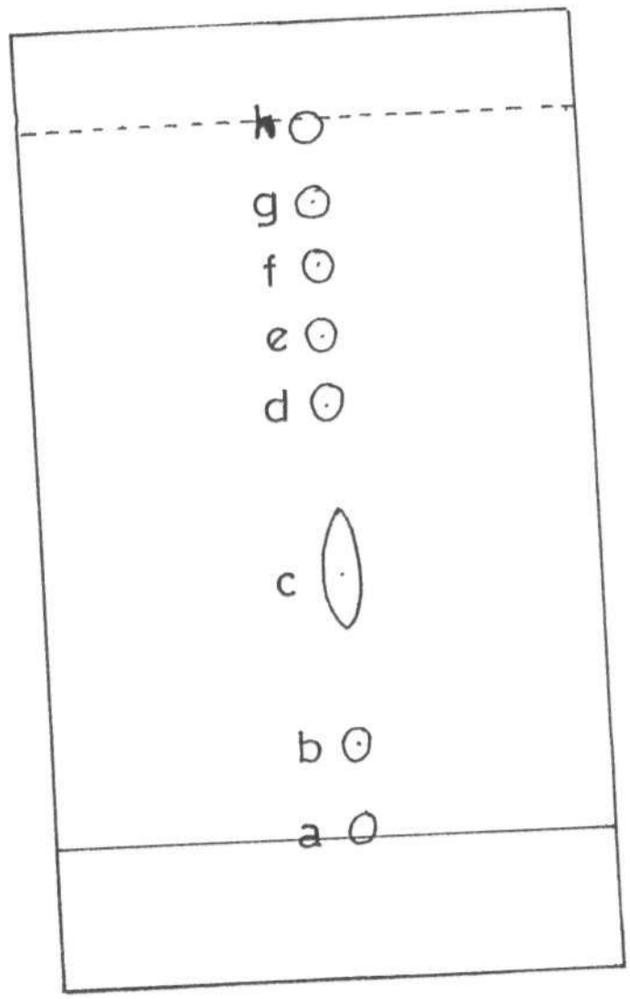


Fig. 3a TLC PICTURE OF METHANOLIC EXTRACT.

3.2 RESULT OF THE PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of the methanolic extract revealed the presence of the plant constituents as shown in Table 3b.

Table 3b: Constituents of ME of *C. africana*

Constituents	Test	Observation	Inference
Carbohydrates i. General Test ii. Sugar iii. Reducing Sugars	 Molisch's Barfoed's Fehling's	 Red Ring Red ppt Red ppt	 + + + + + +
Alkaloids	(a) Dragendorff's (b) Wagner's (c) Mayer's	Orange red ppt Dark brown ppt Buff ppt	+ + +
Tannins	$FeCl_3$	Dark green ppt	+ +
Saponins	Frothing	Frothing	+ + +
Flavonoids	(a) Shinoda's (b) $Fecl_3$ (c) Lead Acetate	Red colour Violet colour Buff colour	+ + + + + +
Cardiac Glycoside Steriodal Nucleus	Salkowski's	Reddish brown at interphase	+
Cardenolide Aglycone	Legal	Deep red colour	+
Digitalis Glycoside	Keler Killiani's	Reddish-brown	+
Terpenoid	Lieberman Buchard	Pink to violet	+ + +

KEY:

- : absent;

\+ : faintly present

+ + : moderately present

+ + + : highly present

3.3 CHARACTERIZATION OF CA

(a) Purification and Melting Point.

CA was recrystallized from methanol to give a colourless needle; melting point 142-143°C (Lit. 141 - 142°C (Harris, 1965). The mixed melting point determination also gave the same range (142-143°C).75 mg.

(b) Solubility of CA in Organic Solvents

CA was found to be sparingly soluble in petroleum ether, soluble in benzene, chloroform, acetone, ethylacetate, methanol and insoluble in water.

(c) Thin Layer Chromatography of CA

CA was seen as a bright pink, non fluoresecent spot under UV light (254nm). Itappeared as a faint yellow spot when exposed for a very long time (overnight) to iodine (I_2) vapour in a closed chamber. It did not give any spot with LBR.

The same observation was made in respect of the synthetic compound. CA also gave the same R_f values when compared with the synthetic compound in different solvent systems as shown in Table 3c.

Table 3c: Rf values of CA and synthetic compound

Solvent System	R_f values of CA	R_f values of Synthetic Compound
Benzene: 100%	0.48	0.48
Benzene: Ethylacetate 5:1	0.67	0.67
Benzene: Ethylacetate 4:1	0.80	0.80
Benzene: Ethylacetate 2:1	0.90	0.90

3.3.1 **Result of Colour Tests**

CA did not give any positive colour test but it appeared as a pink spot on TLC plate when viewed under UV light (254nm)

3.4.0 **RESULT OF SPECTRAL ANALYSIS**

3.4.1 UV spectrum of CA

UV (MeOH): 272 (strong), 292 nm (weak)

UV (MeOH + NaOH: 272 strong, 292 nm (weak)

UV (MeOH + NaOAc): 272 (strong), 292 nm (weak).

The UV absorption spectrum (Fig 3c) was taken in methanol alone, in methanol with NaOH and methanol with NaOAc. It gave a strong absorption at 272 nm and very weak absorption at 292 nm. There was no shift of the band when NaOH or NaOAc was used along with MeOH.

The absorption at 272nm suggested the presence of aromatic nucleus chromophore in the molecule of CA.

3.4.2 IR Spectrum of CA

IR (Nujol): 1730, 1300, 1100, (ester), 1540, 1520, 1475, 1450 cm^{-1}

The diagnostic peak were at 1730, 1540, 1520, 1475 and 1450cm^{-1} (Fig. 3c). The absorption at 1730 1300^{-1} and 1100 cm^{-1} were typical of -C-O-C- of ester(s) while those of 1520, 1540, 1475 and 1450cm^{-1} were absorptions bands of benzene nucleus.

3.4.3 NMR Spectra of CA

^{1}H NMR ($CDCl_3$, 400 MHz, δ): 3.95 (s,6H, 2OCH_3), 8.10 (s,4H, four aromatic protons) (fig 3d).

$^{13}_{C}$ NMR ($CDCl_3$, 100 MHz, δ): 52.38 (2 CH3), 129.52 (4-CH), 138.88 (2QC), 166.24 (2 CO) (Figs 3e) 3f,).

$^{13}_{C}$-NMR ($CDCl_3$,100MH_z DEPT:δ): 52.38 (2CH_3), 138.88 (4CH). (Fig 3f).

3.4.4 Mass Spectrum of CA

The mass spectrum of CA (Fig. 3g) showed the following peaks:

Mass (E.I, m/z, %): $C_{10}H_{10}O_4$, 194(M^+, 36), 179 (M^+-CH_3,2), 164 (M^+-2CH3,10), 163 ($M^{+\cdot}$ OCH_3, 100), 135 (M^+-$COOCH_3$, 15), 120 (M^+-CH_3 -$COOCH_3$, 5), 103 (M^+-$COOCH_3$ - CH_3OH, 9), 76 (M^+-2

$COOCH_3$, 8), 59 (M^+-$C_6H_4^+$- $COOCH_3$,1.5)

3.5.0 **C, H - ANALYSIS**

$C_{10}H_{10}O_4$: Required: C. = 61.85%; H.= 5.19%

Found: C. = 61.84%; H.= 5.25%.

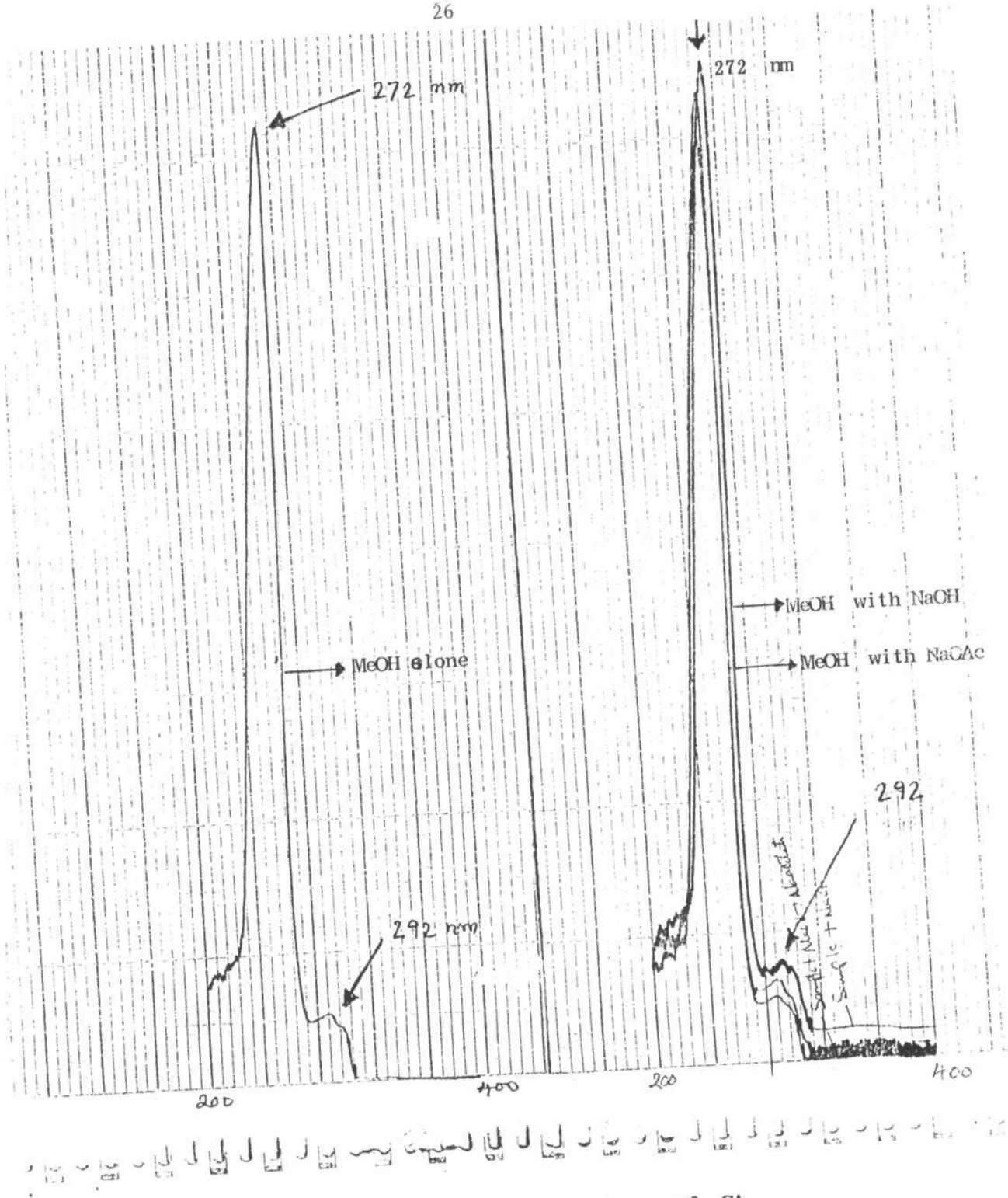


FIG 3b UV Spectrum of CA

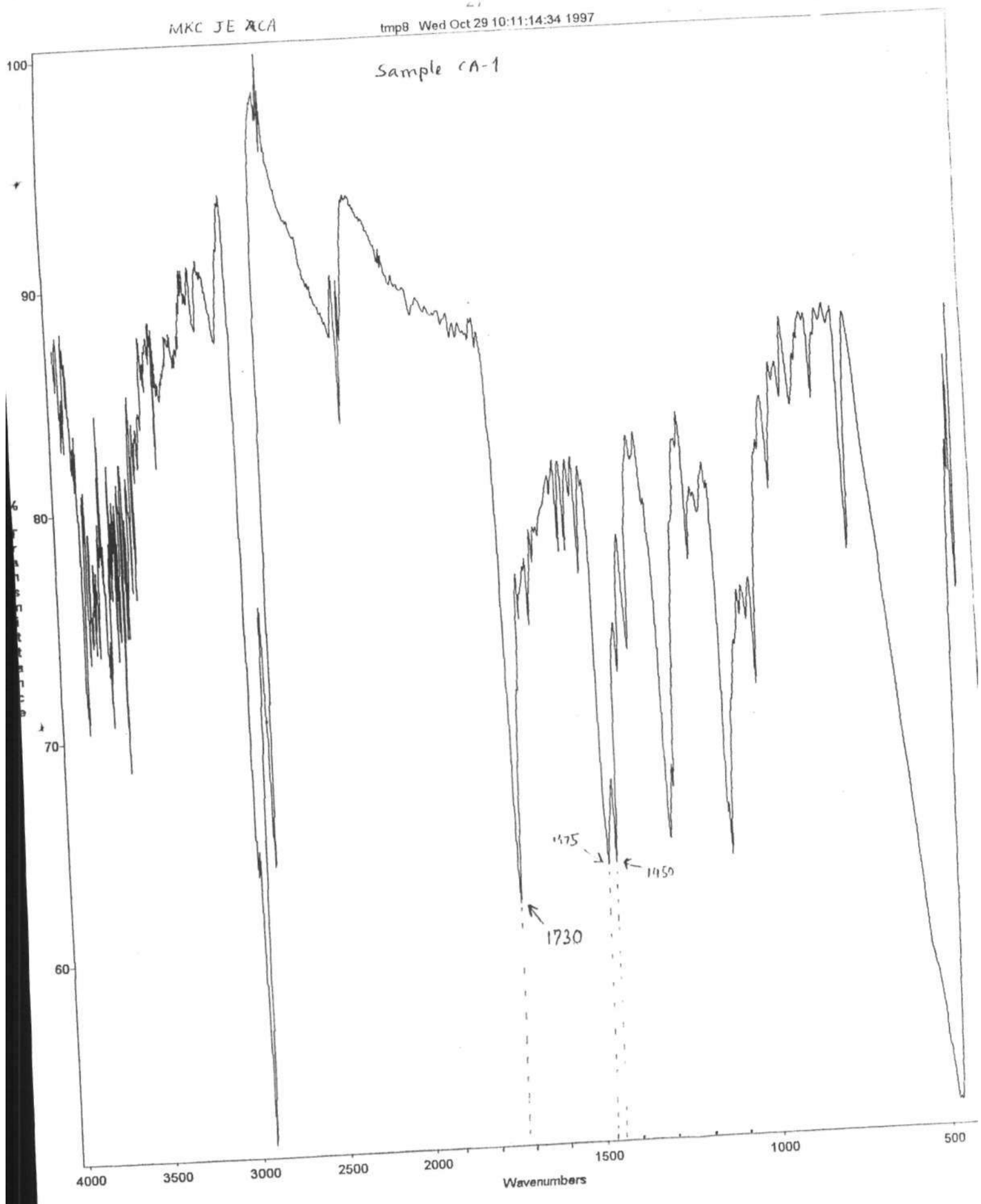


Fig. 3c IR Spectrum of CA

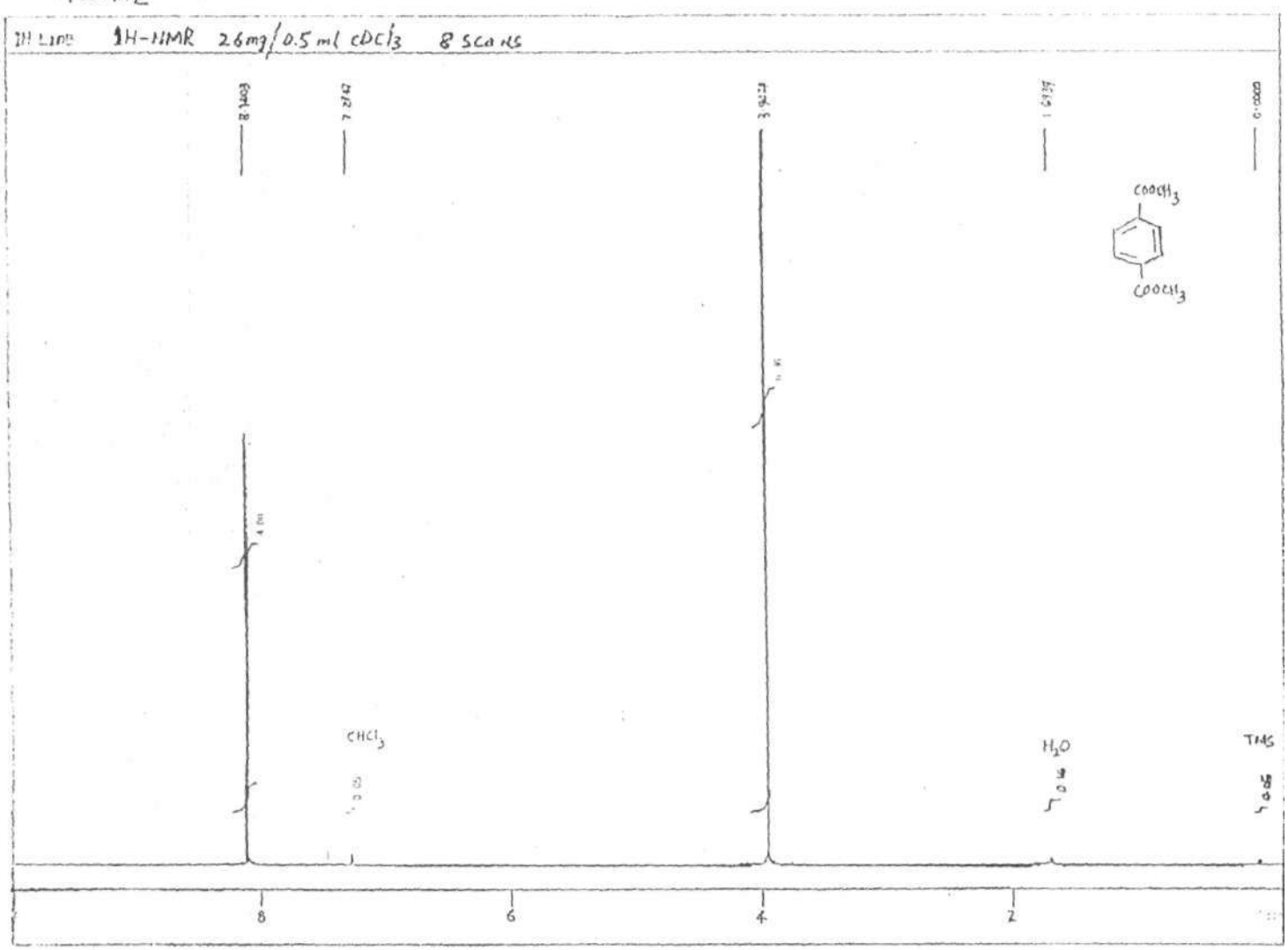


Fig. 3d: ^{1}H - NMR Spectrum of CA

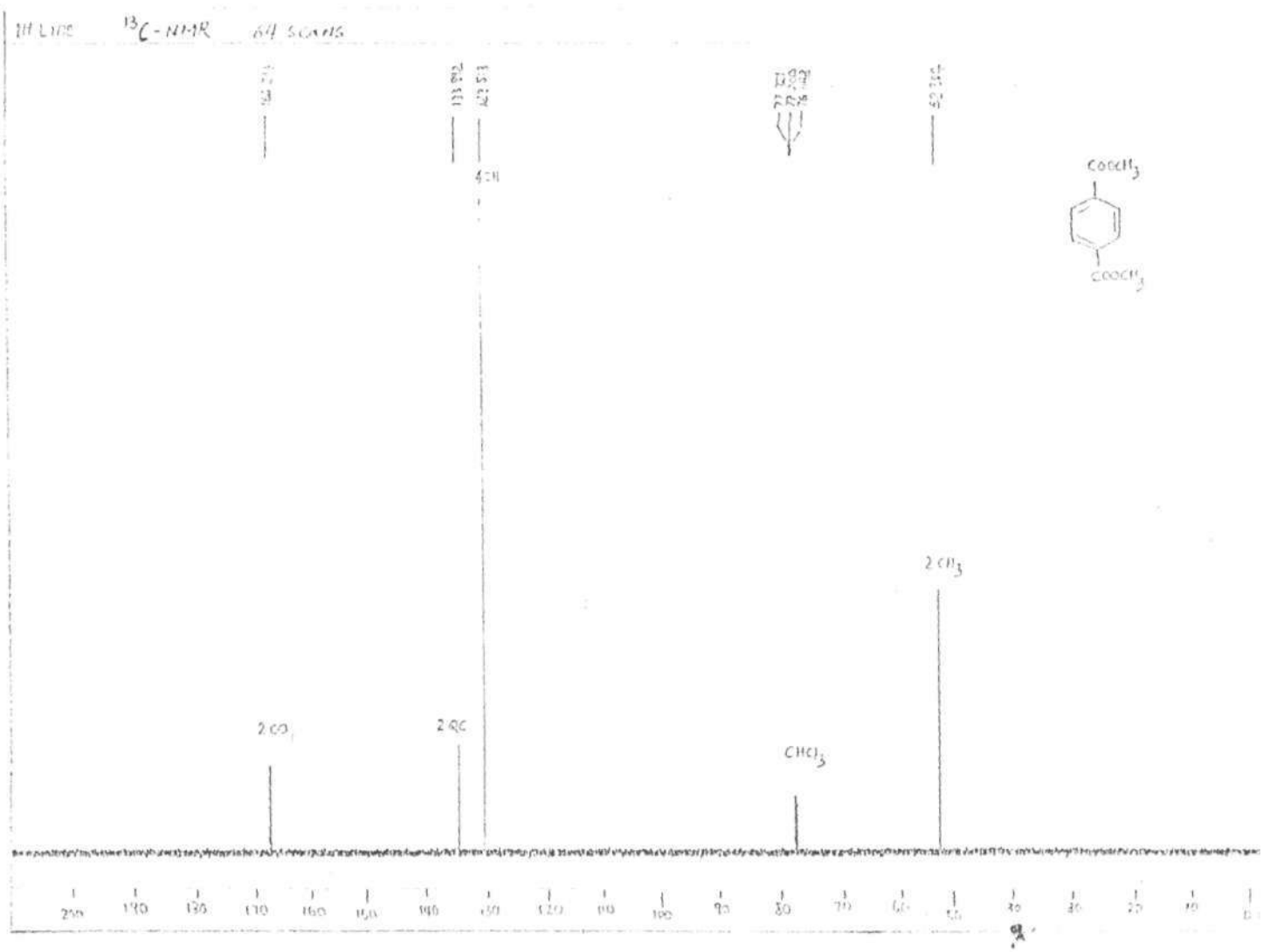


Fig. 3e ^{13}C – NMR Spectrum of CA

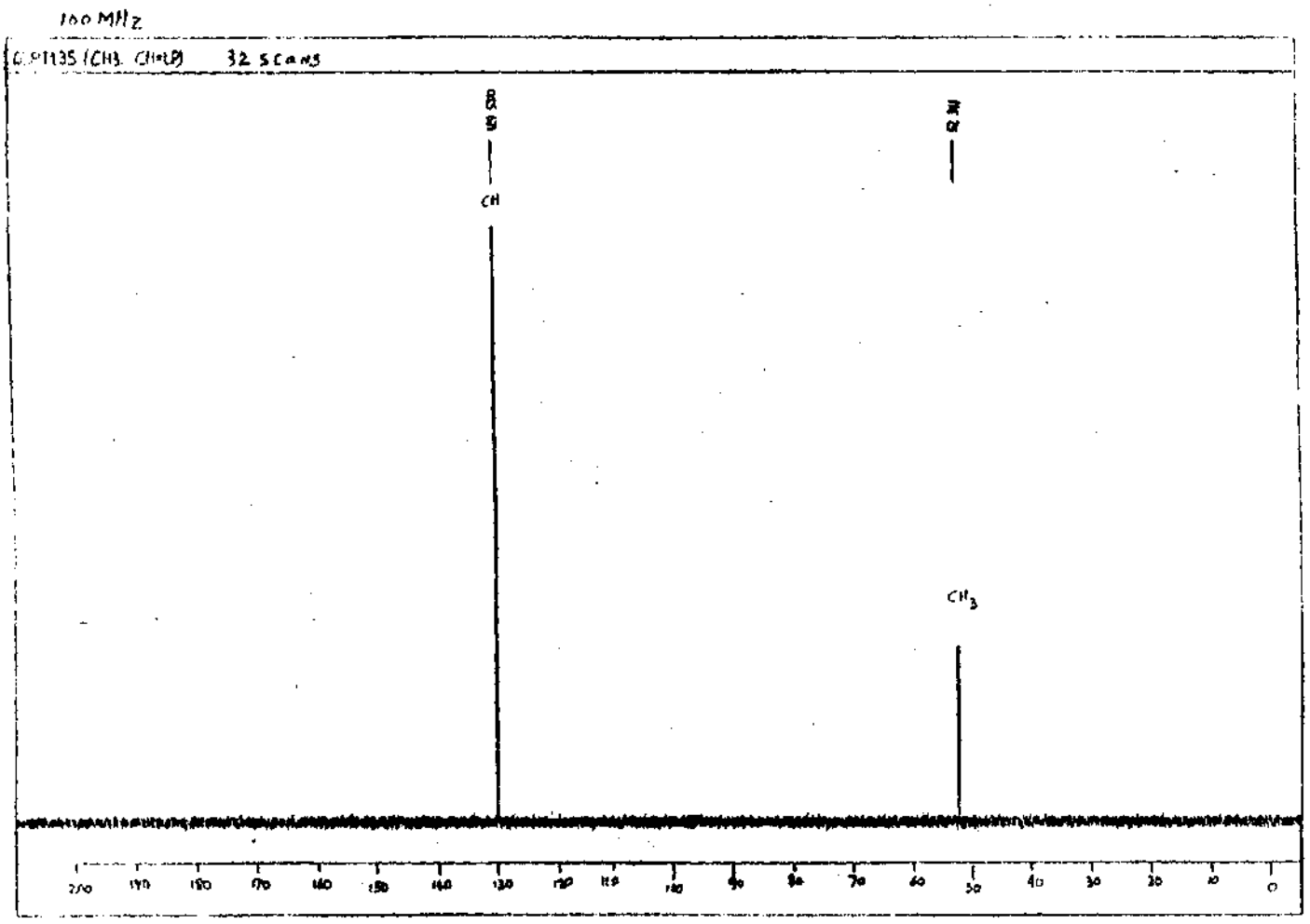


Fig. 3f: ^{13}C - NMR Spectrum (DEPT) of CA

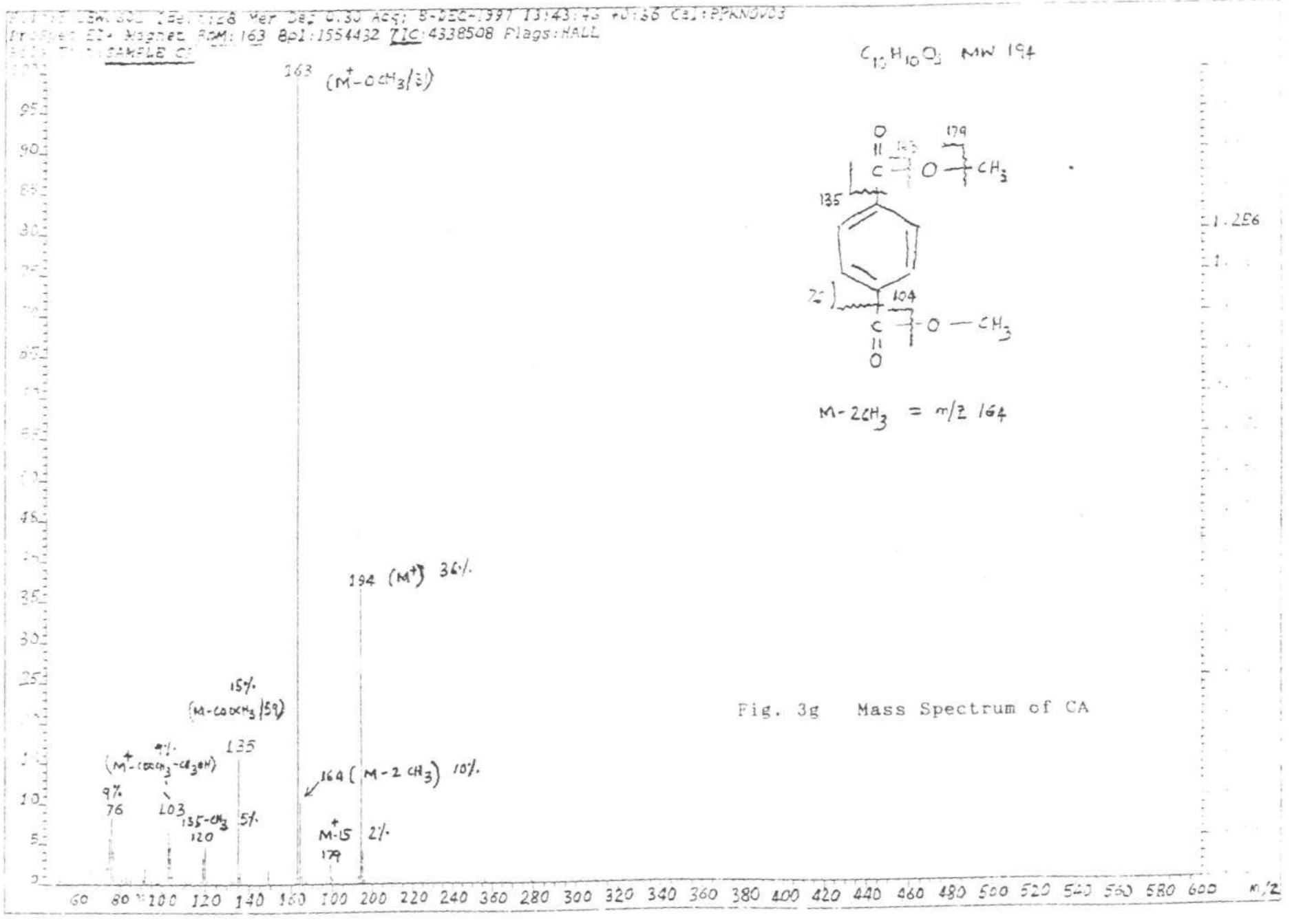


Fig. 3g Mass Spectrum of CA

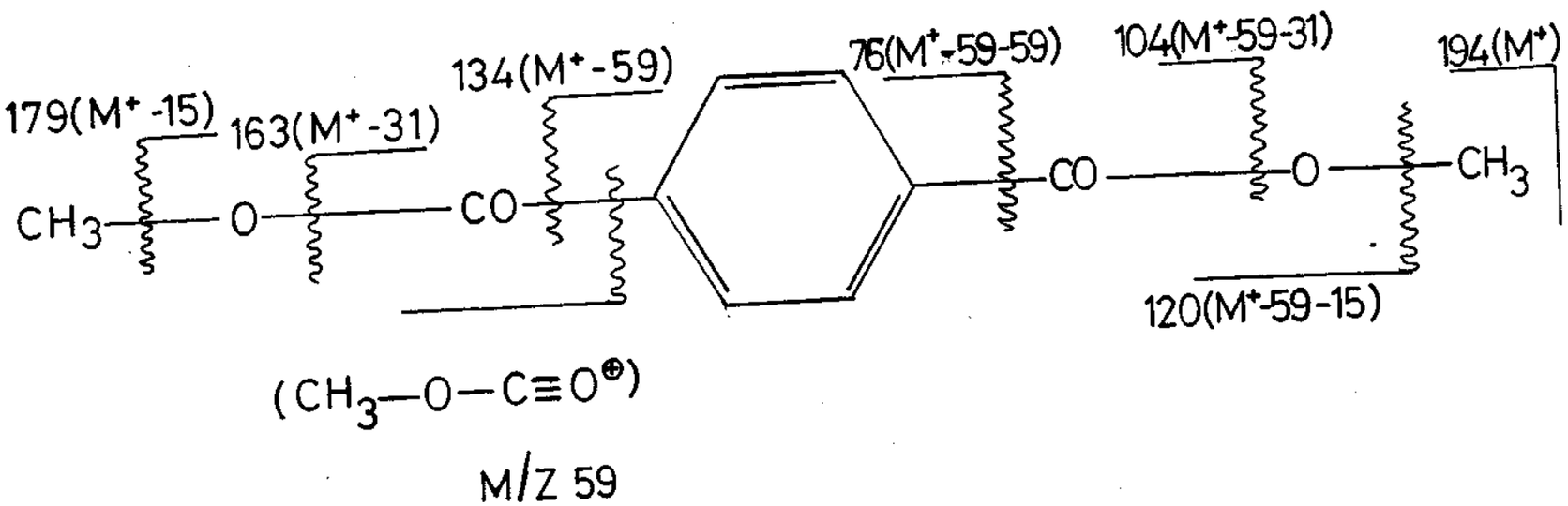
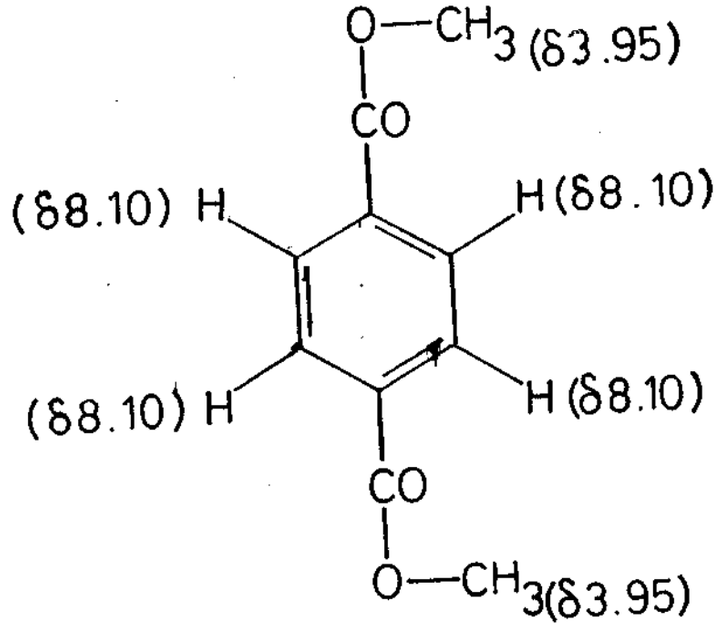
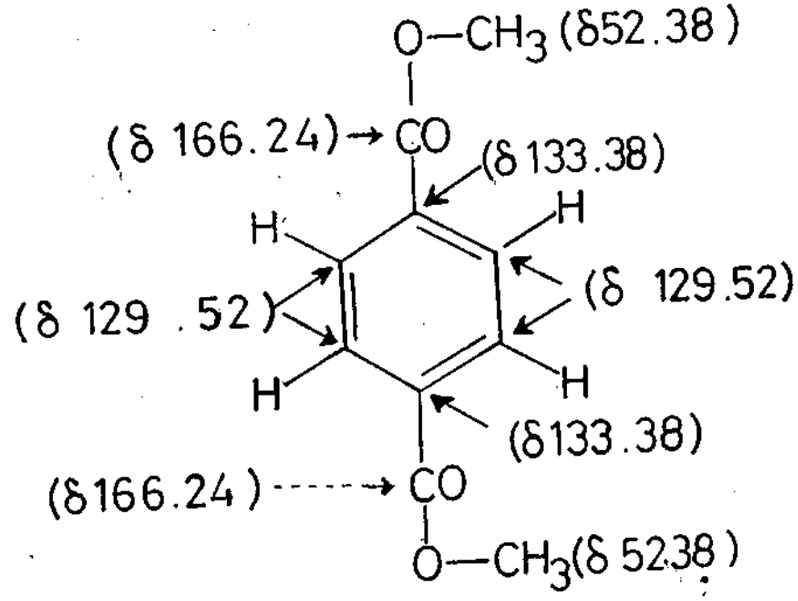


Fig. 3h: MASS SPECTRAL FRAGMENTATION PATTERNS OF CA

NMR SPECTRAL ANALYSIS OF CA



^{1}H-NMR(CDCl$_3$)



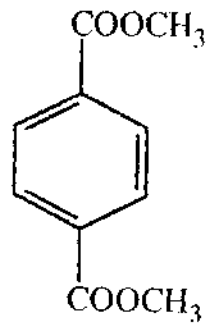


Fig 3j: Structure of 'CA'

3.6 DISCUSSION

The TLC chromatogram of the crude methanolic extract of *C africana* showed six spots which were both LBR and Iodine (I_2) positive. When the plate was viewed under UV - light (366nm) two more additional spots were revealed (spots d and e; Fig. 3a). Spot d (Rf 0.60; $CHCl_3$:MeOH,4:1) appeared as pink while e (Rf 0.70; $CHCl_3$ MeOH,4:1) was bluish flourescent. These two spots did not appear in Iodine and LBR. Spot e was suspected to be CA.

The Preliminary Phytochemical screening showed the presence of carbohydrate (sugars), alkaloids, saponins, tannins, flavonoids,

steroids and cardiac glucoside. The tannins, saponins steroids and the cardiac glycoside are classified as terpenoids and this plant by the result of phytochemical screening is richly endowed with terpenoids considering the strong positive result of LBR test (Robinson, 1967; Harbone, 1973).

The literatures also confirmed the strong presence of terpenes in the genus, *Commiphora*. Terpenes are the major constituents of gums and resins which are the chief products of the species of the genus, e.g:- *C. mukul* (Malhotra, and Ahuja, 1971; Patil, etal, 1972); *C. Myrrha* (Enrico, and Carlo, 1972) and *C. africana* (Jessene et al, 1977).

(a) **UV Spectrum of CA**

The UV (MeOH) absorption at 272 nm was typical of aromatic nucleus. There was no shift when dilute NaOH and fused NaOAc were added to the solvent. This confirmed the absence of free hydroxyl group in the ring.

(b) **IR Spectrum of CA**

The absorption at 1730 cm^{-1} indicated the presence of an ester group while those at 1540, 1520 1475, and 1450 cm^{-1} could be due to C - H Vibration in - CH_2 (in benzene).

(c) **NMR Spectrum of CA**

^1H - NMR showed only two signals at δ 3.95 due to-OCH_3 (methoxy1 group) which came from -$COOCH_3$ (ester function) and at δ .8.10 which appeared from the protons present in the aromatic ring. The two ester functions gave signals at the same position because they were situated at 1,4 - positions in the benzene ring which made them equivalent.

This was supported by ^{13}C-NMR where the two methyl groups (2 CH_3) of the methyl ester ($COOCH_3$), appeared as a single line at δ 53.38. The other signals at δ 129.52, 133.88 and 166.24 were assigned to four equivalent methines (4CH), two equivalent quartenary carbons (2QC) of benzene nucleus and two carbonyls (2CO) of the two ester functions respectively.

(d) **Mass Spectrum of CA**

The mass spectrum showed the molecular ion peak (M^+) of CA at m/z 194 (36%) corresponding to the formula $C_{10}H_{10}O_4$. The mass spectrum also indicated the presence of two methylester groups attached to benzene ring.

The loss of a methyl group from the molecular ion accounted for the peak at m/z 179 (M^+-CH_3/15, 3%), while the more prominent peak at m/z 164 (M^+- $2CH_3$/30, 10%) could be assigned to the loss of two methyl groups from the two ester functions present in the molecule. The base peak at m/z 163 (M^+-OCH_3/31, 100%) was due to the loss of a methoxy function from the methylester group. The peak at m/z, 135 (M^+-$COOCH_3$/59,5%) was due to the loss of -$COOCH_3$ from the molecular ion. The peak at m/z (103 (M^+-$COOCH_3$/59,CH_3OH/32, 9%) corresponded to the successive loss of a -$COOCH_3$ group and CH_3OH moiety. The signal at m/z 76

(M^+-$2COOCH_3$,8%) was due to the loss of two ester groups from the molecule, while the one at m/z, 59(1.5%) was due to -$COOCH_3$. The mass spectrum pattern is represented in Fig 3h.

3.7 CONCLUSION

The mass spectrum indicated the presence of two methylester groups attached to benzene ring but their respective positions were

confirmed by NMR (Proton and ^{13}C) spectra. The presence of ester group was supported by IR spectrum at 1730, 1300 and 1100 cm^{-1}. Two methylester functions situated at 1, 4 positions in the benzene nucleus made the ester groups and four aromatic protons equivalent. This fact was revealed in the proton NMR spectrum which showed two signals at δ 3.95 (OCH_3 from $COOCH_3$) and δ 8.10 (aromatic). It was further supported by the ^{13}C NMR spectrum, where two equivalent methyl ($2CH_3$) groups of methylesters ($COOCH_3$), four equivalent methines (4CH), two equivalent quartenary carbons (2QC) of benzene nucleus and two equivalent carbonyl groups (2CO) of the two ester functions appeared as single line at 53.38, 129.52, 133.88 and 166.24 respectively. The compound isolated from the plant was found to be identical with the synthetic dimethylterepthalate (prepared from terephthalic acid with dry methanol in presence of conc. H_2SO_4), in all respect (same R_f values, co-TLC, mixed mp, super-imposable IR) which gave an unambigous proof for the structure (Fig 3j).

This is the first report of ^{13}C NMR of dimethyltere-pthalate. The isolation of dimethylterephthalate was reported earlier from other plants (Hörhammer et al, 1966; Rajani and Sarma, 1988; Reddy et al, 1990), and to the best of our knowledge no phytochemical and pharmacological studies have been reported so far on this plant.

CHAPTER FOUR

PHARMACOLOGICAL STUDIES OF COMMIPHORA AFRICANA

4.1 OBJECTIVE OF THE STUDIES

The objective of this work is to evaluate the pharmacological effect of the aqueous extract of the bark of *Commiphora africana* on isolated rabbit ileum, rabbit uterus and rabbit heart in order to establish the basis for the use of the plant in treating stomach disorders and some heart related diseases.

4.2 MATERIALS AND METHODS

4.3 MATERIALS

The test animal, adult female (non-pregnant) rabbit (weighing 1.30kg) was purchased from Sabon Gari Market, Zaria, and kept in the Animal House, Faculty of Pharmaceutical Sciences, A.B.U. Zaria for seven days under standard environmental conditions of temperature, relative humidity, light; and fed on standard diet and water (Pfizer feeds, Lagos).

(a) **Apparatus**

i. Recording microdynamometer and transducer (Model No. 7050, ugo basile, Italy).

ii. Thermostatically controlled tissue bath (25 ml)

iii. Perspex chamber

iv. Dissecting kits

(b) **Standard Drugs and Sample Used.**

The standard drugs and aqueous extract of the plant were prepared by dissolving the known weight of the drug and solid extract (after evaporation of water) in the required volume of de-ionized water.

The following standard drugs were obtained from BDH, Chemical Ltd, England and concentrations of the stock solutions used for the experiments are indicated below.

NAME OF COMPUND	STOCK SOLUTION
i. Acetylcholine (Ach).....	10 μg/ml
ii. Adrenaline (Adr).....	10 μg/ml
iii. Atropine (Atr).....	10 μg/ml
iv. Carbachol (Carb).....	10 μg/ml
v. Diethylstilbestrol (Des).....	10 μg/ml
vi. Heparine.....	10 μg/ml
vii. Isoprenaline (Isopr).....	10 μg/ml
viii. Propranolol (Propr.....	10 μg/ml

The concentration of stock solution of the aqueous extract was

10 mg/ml.

(c) **The Physiological Solutions**

The physiological solutions used were

i. Dejalon solution

ii. Ringer - Locke Solution

iii. Tyrode Solution

(d) Preparation of Physiological Salt Solutions (Kitchen, 1984)

SALT	QUANTITIES FOR 10 LITRES OF		
	TYRODE	RINGER-LOCKE	DE-JALON
NaCl	90 g	90 g	90 g
KCl (10% solution)	20 ml	42 ml	42 ml
NaH_2PO_4 $2H_2O$ (10%):	5 ml	-	-15 g
D- Glucose	10 g	10 g	5 g
$NaHCO_3$	10 g	5 g	2.7 ml
$CaCl_2$ (Molar Soln)	-	10.8 ml	-
$CaCl_2$ (10% soln)	20 ml	-	-
$MgCl_2.6H_2O$	1 ml	-	95%O_2+5%
Aerating gas	O_2 or Air	Pure O_2	CO_2

(e) **The Animal Isolated Tissues**

The following isolated tissues of the rabbit were used for the experiments:

i. Rabbit ileum

ii. rabbit ileum

iii. rabbit heart.

4.4 METHODS

4.4.1 Preparation of the isolated tissues from the animal

Adult female non pregnant rabbits locally bred (Weight: 1.30kg) were pretreated with 0.1 mg/kg diethylstilboestrol, 24 hours before killing to induce the state of oestrous. 0.1 mg/kg heparine was injected through the vein in the ear lope to prevent blood clotting in the heart, just before killing the animal.

The rabbit was killed by a blow on the head and exsanguinated. The abdomen was cut open to reveal the uterine horn; the lower intestine and thoracic region.

(a) **The ileum:**

The ileum part of the small intestine of the rabbit was removed and cut into 3 cm long pieces. The tissue was placed in a dish containing Tyrode solution. A thread was sewn through the wall of the lower end of the tissue and tied to the oxygenator and at the other end a long thread was used to attach the tissue to the lever. This was then fixed in the bath, washed with Tyrode solution and allowed to

stabilize for ten minutes. The effects of the following drugs were compared with that of the aqueous extract of *Commiphora africana,* acetylcholine, atropine and adrenaline.

(b) **The uterus**

The uterine horns with attached fat and mesentery were removed from both right and left side and placed in a petri dish containing De-jalon solution. The residual fat and mesentery were detached from the uterus. The uterus was cut into left and right horns. Each horn was mounted in an organ bath (25 ml), well supplied with oxygen and the temperature maintained at 37°C. The set up was allowed to equilibrate for 30 minutes before comparing the effects of carbachol and Acetylcholine with that of aqueous extract of *Commiphora africana* on the tissue.

(c) **The heart**

The heart with 1 cm of the aorta attached was removed as quickly as possible. It was placed in a dish of Ringer-Locke solution at room temperature. The residual fat and adherent tissues were trimmed off. The heart was then attached to the transducer by means of a thread through the aorta. The set up was allowed to equilibrate for about 30 minutes, after which the normal rate and the amplitude of the beats were recorded.

The effects of the various drugs namely; normal saline, acetylcholine, isoprenaline and propranolol were compared with that of the aqueous extract of *Commiphora africana.*

4.4.2 Recording Techniques

Various concentrations of the standard drugs and the aqueous extract of *Commiphora africana* were added to the organ bath and the responses recorded isometrically using transducer connected to a recording microdyanometer.

4.5 RESULTS

4.5.1 Rabbit ileum

Acetylcholine (0.04-0.64 μg/ml) produced a concentration-dependent contraction of the isolated rabbit ileum (Table 4a, Fig. 4a). Atropine (0.04-0.64 μg/ml) inhibited the contractions produced by acetylcholine (0.16 μg/ml). The responses are shown on Table 4b and Fig. 4b. The log-dose response curves are represented in Figs. 4q. and 4r Adrenaline (0.04-0.32 μg/ml) produced a relaxation effect on the isolated rabbit ileum (Table 4c, Fig. 4c). The aqueous extract of *Commiphora africana* relaxed the rabbit ileum, and dose dependently (40-320 μg/ml) antagonized the effect of acethylcholine (0.16 μg/ml) on the ileum (Tables 4d and 4e, Figs. 4d and 4e).

4.5.2 Rabbit uterus

Carbachol (0.04-0.16 μg/ml) contracted the isolated rabbit uterus (Table 4f, Fig 4f). Acetylcholine (0.04-0.32 μg/ml) also contracted the isolated rabbit uterus (Table 4g, Fig 4g. Atropine (0.04-0.16 μg/ml) antagonized effect of acetylcholine (0.16 μg/ml) on the tissue. (Table 4h, Fig 4h).

The aqueous extract of *C. africana* (40-320 μg/ml) did not show any observable response on the uterus. (Table 4i, Fig. 4i).

The aqueous extract of the test plant (40-320 μg/ml) did not change the response of acetylcholine (0.16 μg/ml) (Table 4j, Fig. 4j) on the tissue.

4.5.3 Rabbit Heart

Acetylcholine (0.04-0.16 μg/ml) slowed down the rate of the rabbit heart beat (Table 4k Fig. 4k). Isoprenaline (0.04-0.16 μg/ml) increased the rate of the heart beat (Table 4l, Fig. 4l). Propranolol (0.04-0.16 μg/ml) slowed down the rate of the isolated rabbit heart beat (Table 4m, Fig. 4m). The aqueous extract of *C. africana* (40-320 μg/ml) also slowed down the rate of the heart beat of the rabbit (Table 4n, Fig. 4n).

The effect of isoprenaline (0.16 μg/ml) was antagonized by both propranolol (0.04-0.32 μg/ml) and aqueous extract of *C. africana* (40-160 μg/ml) in a comparable manner (Table 4o, and 4p Fig. 4o and 4p respectively).

Table 4a:

Effect of contraction produced by Acetylcholine on isolated rabbit ileum (conc. of stock soln 10 μg/ml)

Vol. of Ach administered (ml)	Conc. of Ach (μg/ml)	Average Response (mm) S.D.	Log-Dose	% Response
01.	0.04	18 ± 0.10	- 1.40	18
0.2	0.08	33 ± 0.25	- 1.10	33
0.4	0.16	75 ± 0.11	- 0.80	75
0.8	0.32	100 ± 0.20	- 0.50	100
1.6	0.64	100 + 0.10	- 0.20	100

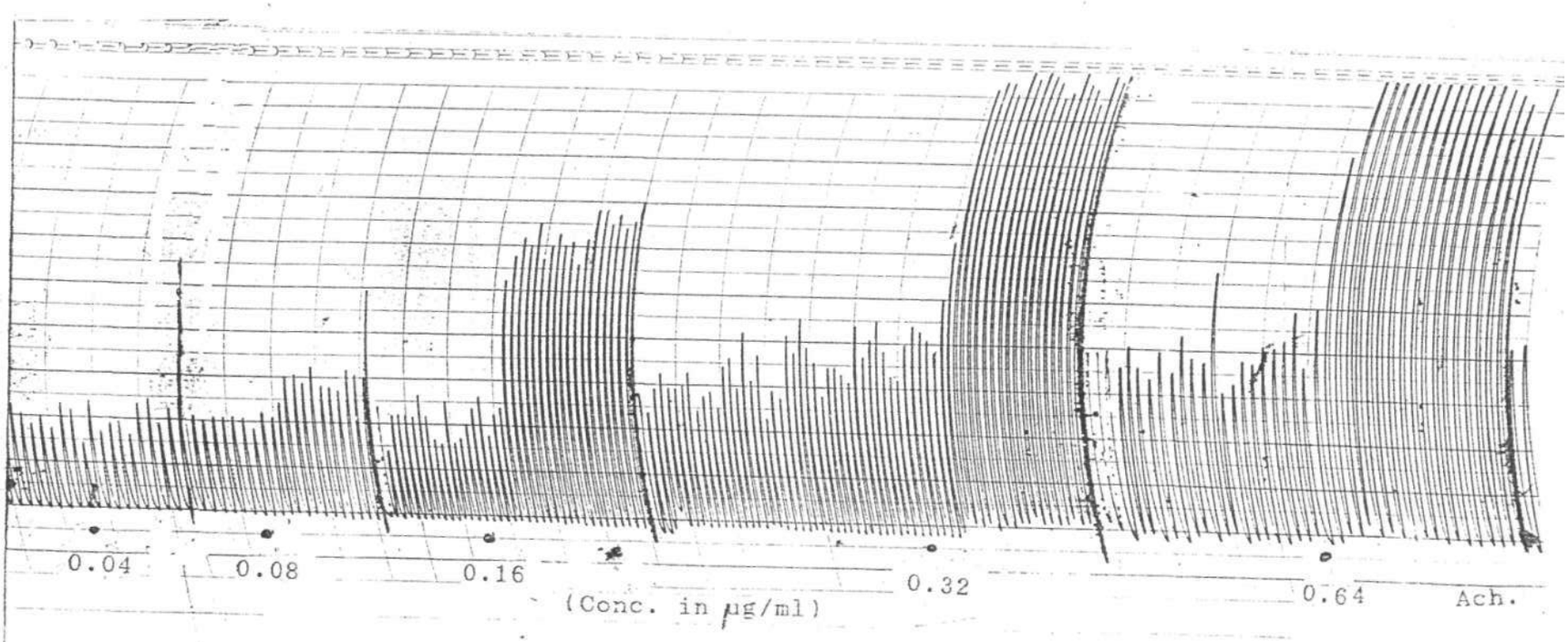
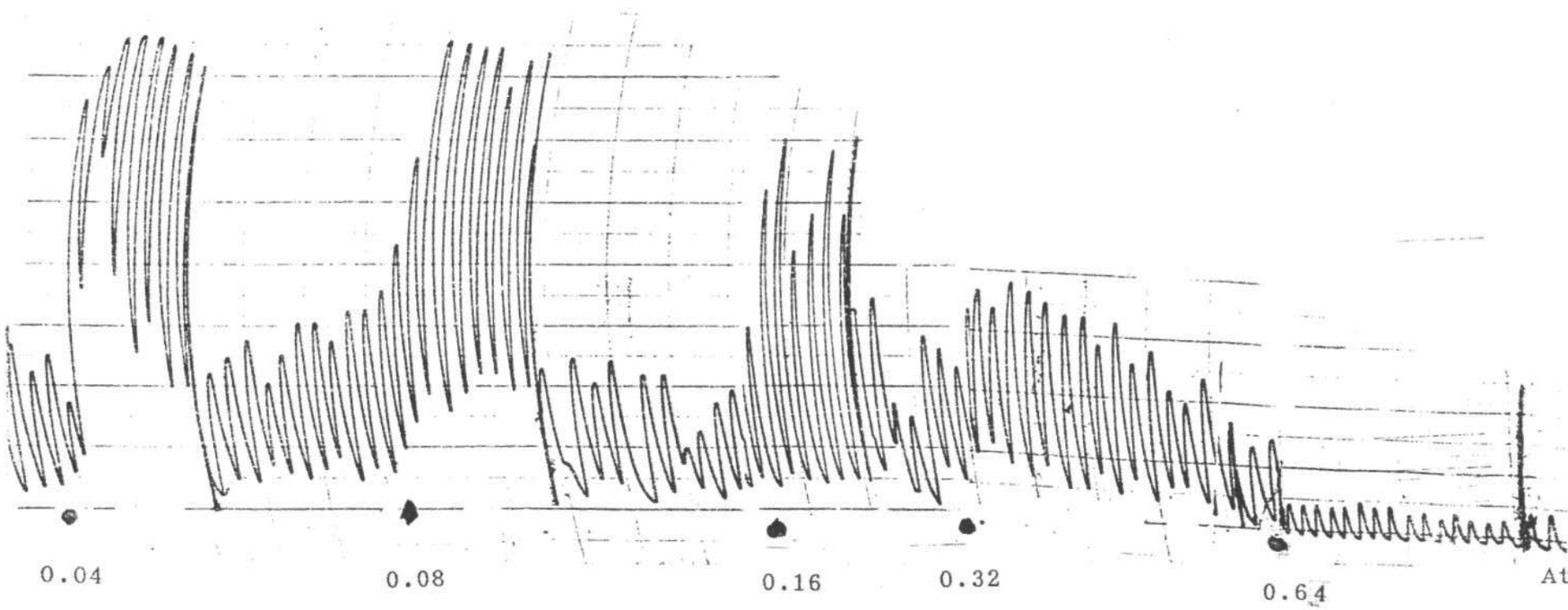


Fig. 4a Effect of Contraction Produced by acetylcholine on isolated rabbit ileum



(Conc. in µg/ml)

Fig. 4b Effect of acetylcholine (0.16 µg/ml) in the presence of atropine on the isolated rabbit ileum

Table 4b:

Effect of Acetylcholine (0.16 μg/ml) in the presence of Atropine on the isolated rabbit ileum.

Vol. of Atr administered (ml)	Conc. of Atr (μg/ml)	Average Response (mm) S.D.	Log-Dose	% Response
0.1	0.04	75 $\pm$ 0.55	- 1.40	100
0.2	0.08	60 $\pm$ 0.20	- 1.10	80
0.4	0.16	38 $\pm$ 0.40	- 0.80	51
0.8	0.32	15 $\pm$ 0.41	- 0.50	20
1.6	0.64	5 + 0.10	- 0.20	7

Table 4c:

Effect of relaxation produced by adrenaline on the isolated rabbit ileum

Vol. of Atr administered (ml)	Conc. of Atr (μg/ml)	Average Response (mm) S.D.	Log-Dose	% Response
01.	0.04	75 $\pm$ 0.55	- 1.40	100
0.2	0.08	60 $\pm$ 0.20	- 1.10	62
0.4	0.16	38 $\pm$ 0.40	- 0.80	39
0.8	0.32	15 $\pm$ 0.41	- 0.50	15
1.6	0.64	5 + 0.10	- 0.20	100

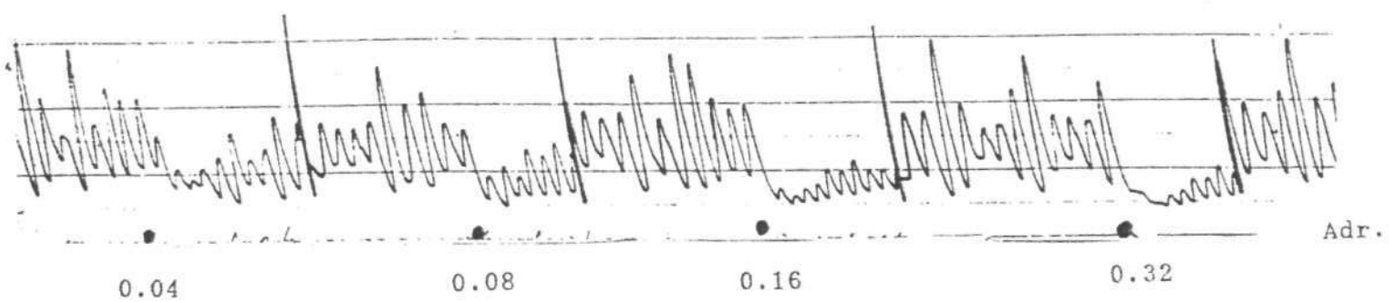


Fig. 4c Effect of adrenaline on isolated rabbit ileum (relaxation)

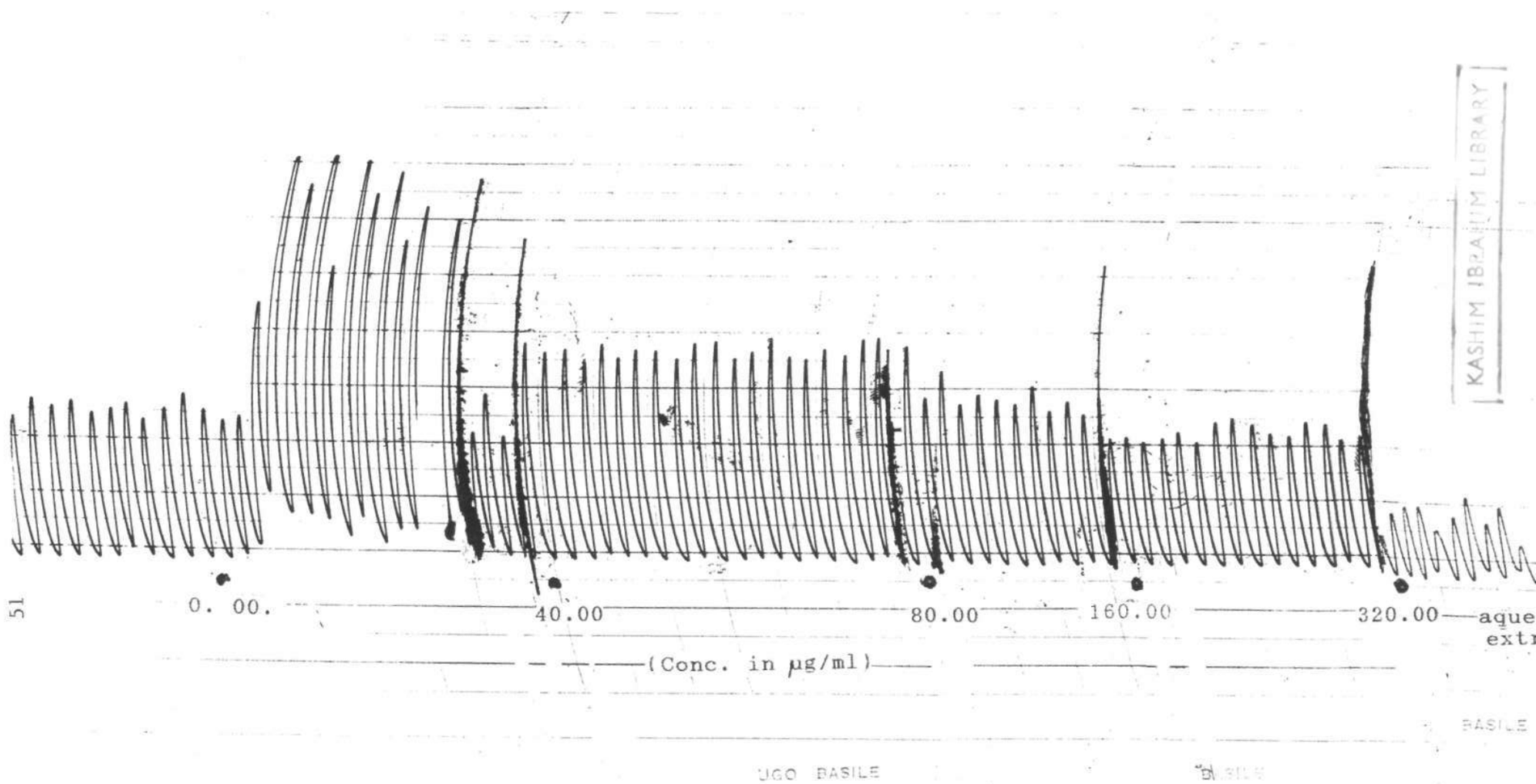


Fig. 4d Effect of aqueous extract of C africana on isolated rabbit ileum

Table 4d:

Effect of relaxation produced by aqueous extract of *C. africana* on the isolated rabbit ileum.

Vol. of extract administered (ml)	Conc. of extract (μg/ml)	Average Response (mm) S.D.	Log-Dose	% Response
01.	40	75 ± 0.31	1.60	100
0.2	80	70 ± 0.32	1.90	93
0.4	160	57 ± 0.29	2.20	76
0.8	320	15 ± 0.45	2.50	20

Table 4e:

Effect of Acetylcholine (0.16 μg/ml) in the presence of aqueous extract of *C. africana* on isolated rabbit ileum

Vol. of extract administered (ml)	Conc. ofextract (μg/ml)	Average Response (mm) S.D.	Log-Dose	% Response
0.00	0.00	75 ± 0.41	0.00	100
0.10	40	70 ± 0.22	1.60	93
0.20	80	45 ± 0.01	1.90	60
0.40	160	20 ± 0.20	2.20	27
0.80	320	4 + 0.10	2.50	5

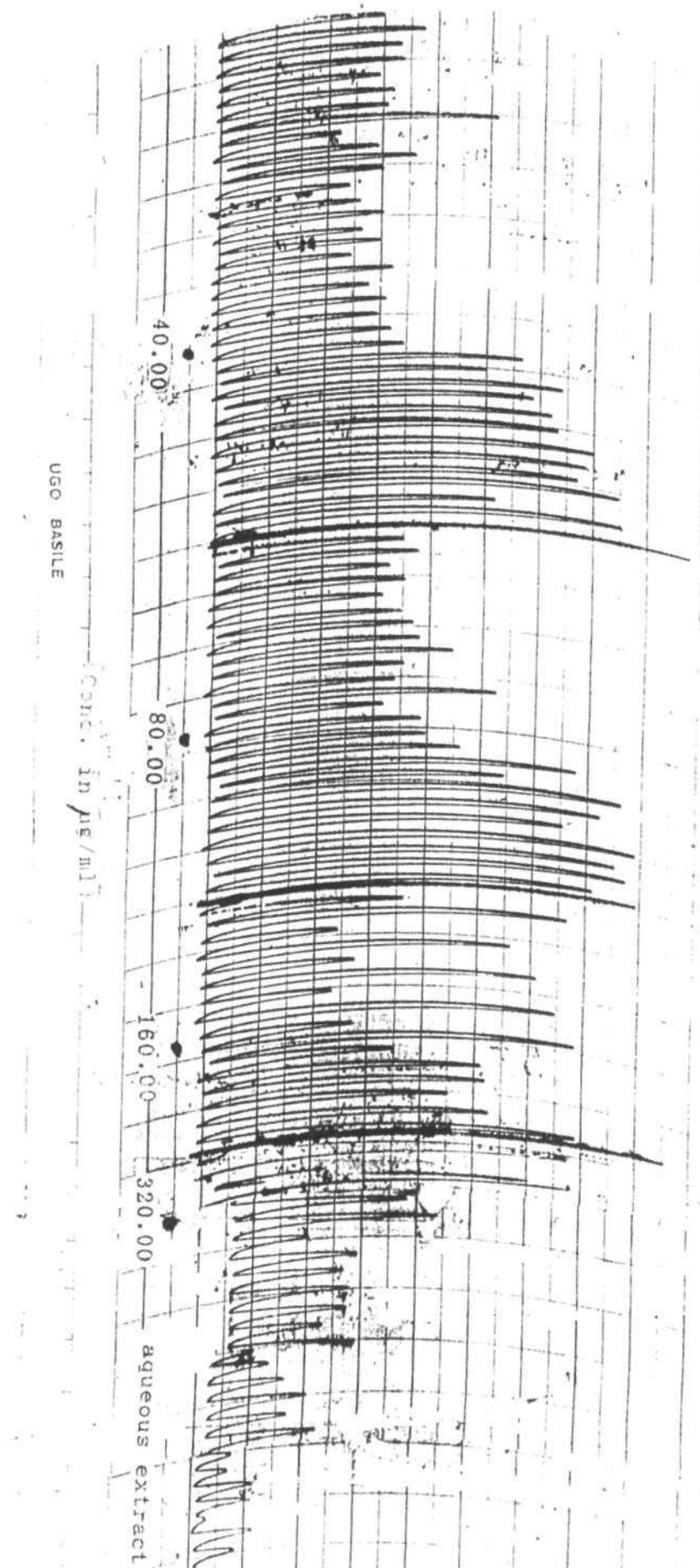


Fig. 1e Effect of acetylcholine (0.16 µg/ml) in the presence of aqueous extract of C. africana on isolated rabbit ileum

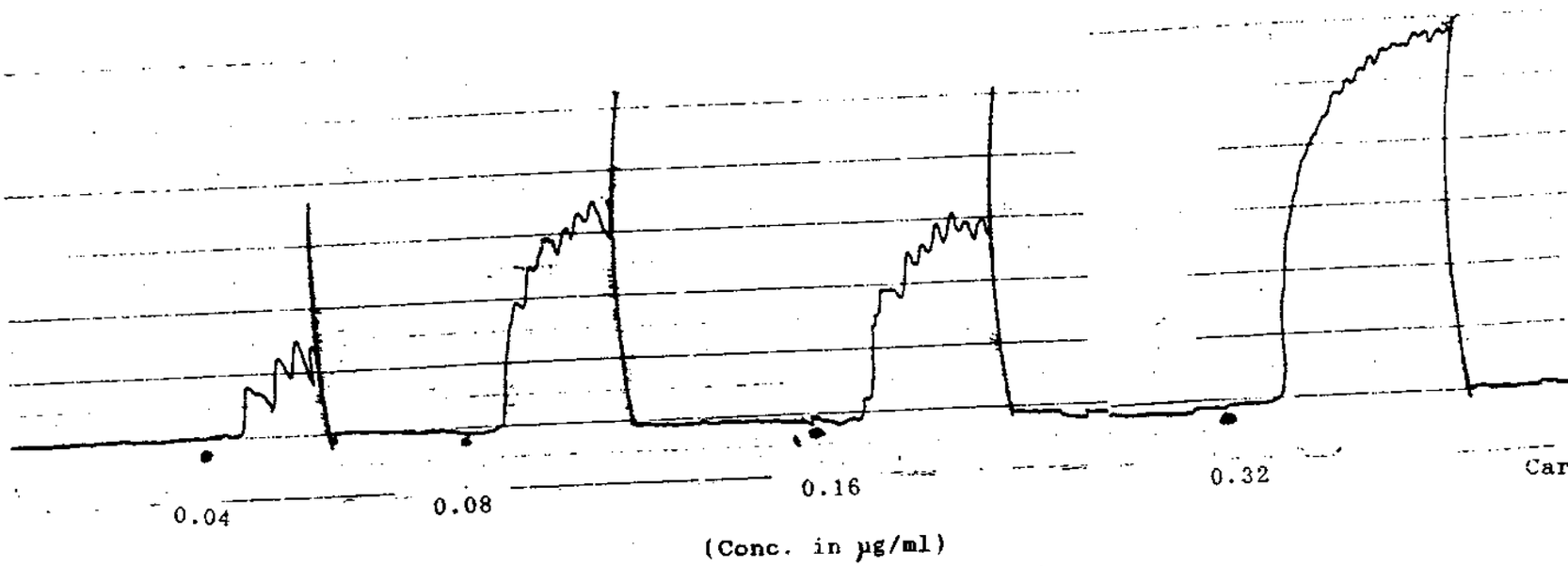


Fig. 4f Effect of carbachol on isolated rabbit uterus (contraction)

Table 4f:

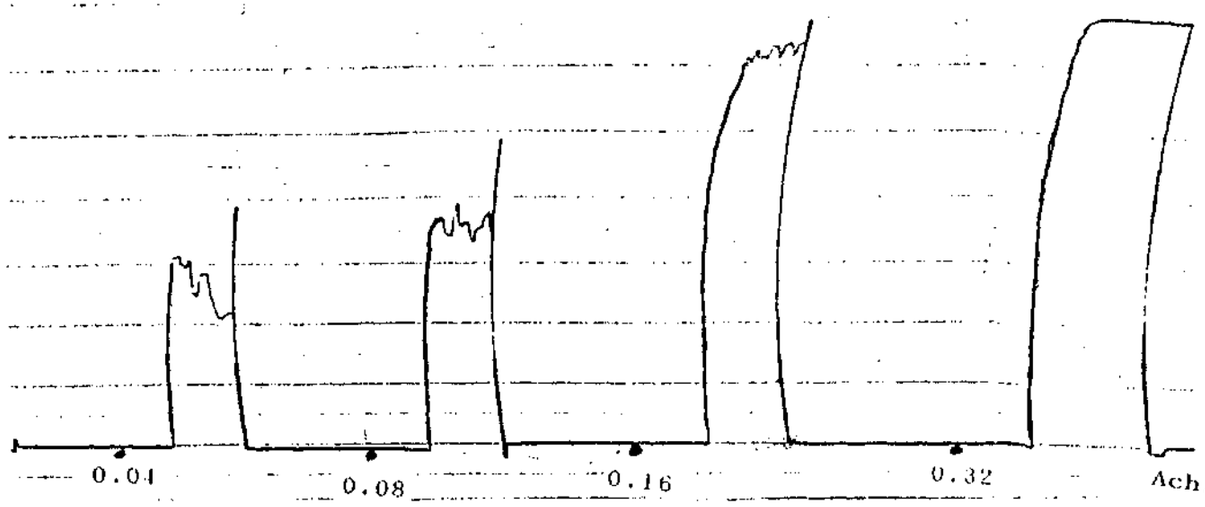
Effect of contraction produced by Carbachol on the isolated rabbit uterus

Vol. of Carb administered (ml)	Conc. of Carb (μg/ml)	Average Response (mm) S.D.	Log-Dose	% Response
0.10	0.04	25 ± 0.10	- 1.40	40
0.20	0.08	34 ± 1.00	- 1.10	54
0.40	0.16	35 ± 1.10	- 0.80	56
0.80	0.32	63 ± 0.90	- 0.50	100

Table 4g:

Effect of contraction produced by Acetylcholine on the isolated rabbit uterus

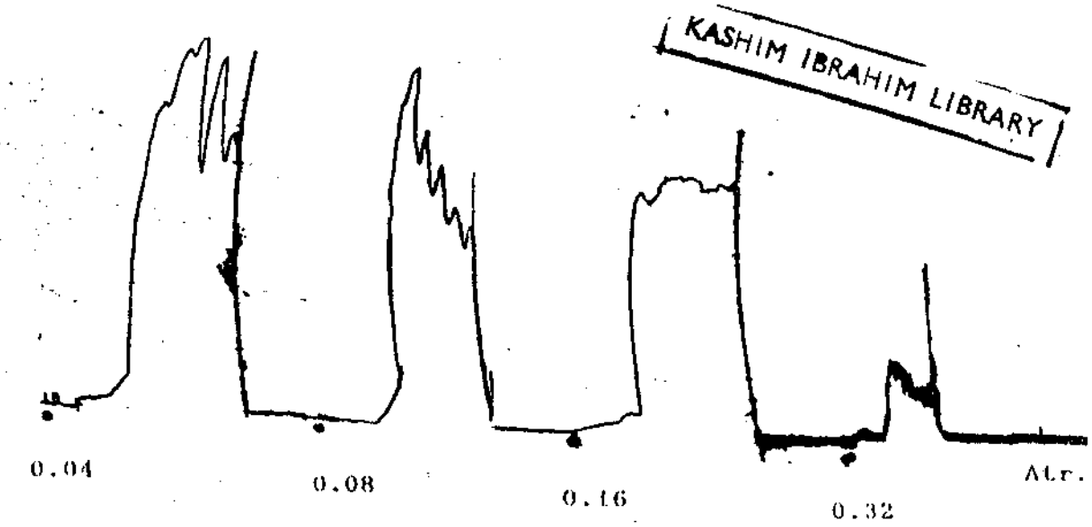
Vol. of Ach administered (ml)	Conc. of Ach (μg/ml)	Average Response (mm) S.D.	Log-Dose	% Response
0.1	0.04	32 ± 1.00	- 1.40	44
0.2	0.08	42 ± 1.40	- 1.10	58
0.4	0.16	65 ± 1.30	- 0.80	90
0.8	0.32	72 ± 1.2	- 0.50	100



(Conc. in µg/ml)

Fig. 4g Effect of acetylcholine on isolated rabbit uterus. (contraction)

KASHIM IBRAHIM LIBRARY



(Conc. in µg/ml)

Fig. 4h Effect of acetylcholine (0.16 µg/ml) in the presence of atropine on isolated rabbit uterus

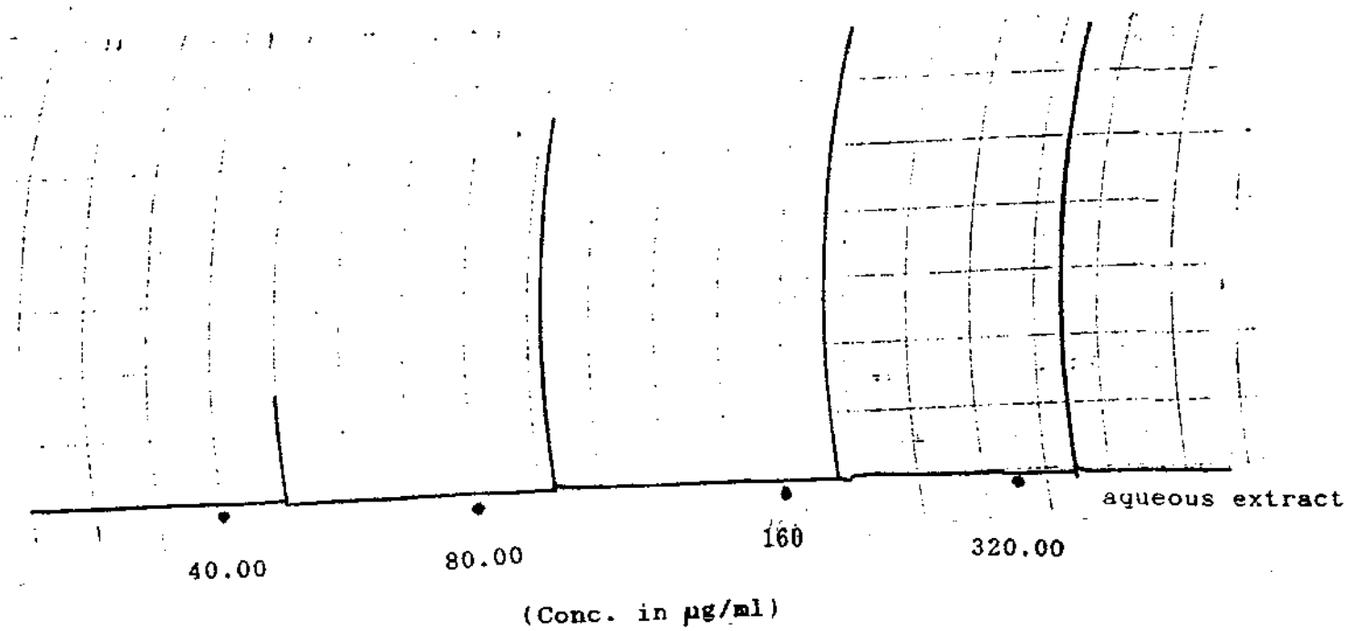


Fig. 4i Effect of aqueous extract of C. africana on the isolated rabbit uterus (No effect)

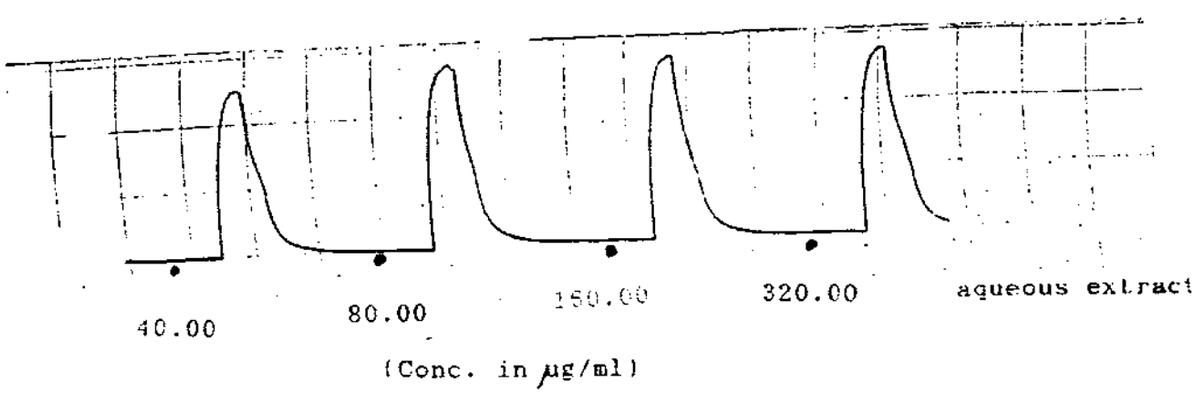


Fig. 4j Effect of acetylcholine (0.16 µg/ml) in the presence of aqueous extract of C. africana on isolated rabbit uterus.

Table 4h:

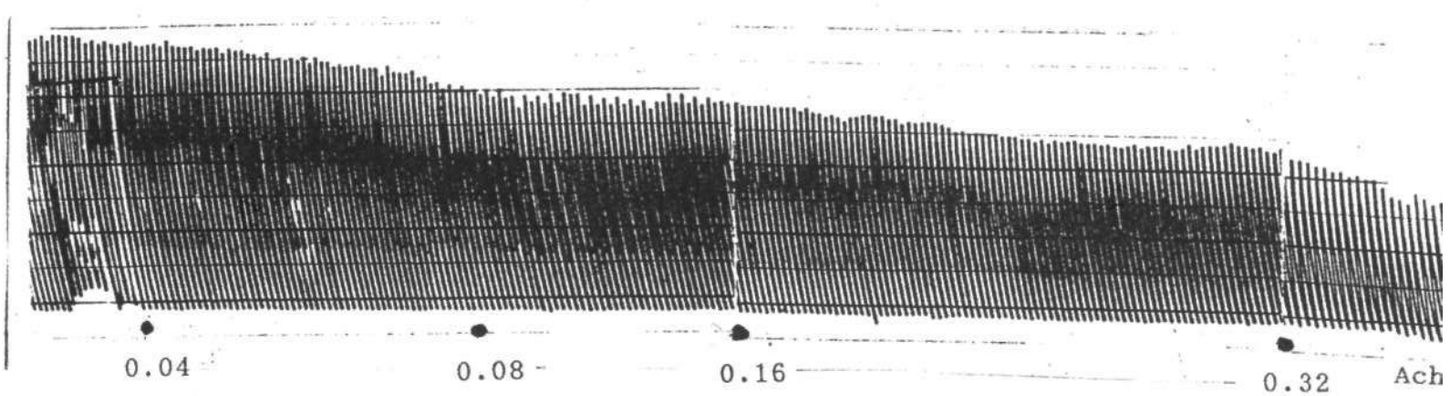
Effect of Acetylcholine (0.16 μg/ml) on the isolated rabbit uterus in the presence of Atropine.

Vol. of Atr administered (ml)	Conc. of Atr (μg/ml)	Average Response (mm) S.D.	Log-Dose	% Response
0.1	0.04	70 $\pm$ 0.00	- 1.40	100
0.2	0.08	60 $\pm$ 1.00	- 1.10	86
0.4	0.16	40 $\pm$ 1.10	- 0.80	57
0.8	0.32	15 + 0.55	- 0.50	21

Table 4i:

Effect of aqueous extract of *C. africana* on the isolated rabbit uterus.

Vol. of Aq extract administered (ml)	Conc. of Aq extract (μg/ml)	Average Response (mm)	Log-Dose	% Response
0.1	40	0.0	1.60	0.0
0.2	80	0.0	1.90	0.0
0.4	160	0.0	2.20	0.0
0.8	320	0.0	2.5	0.0



(Conc. in µg/ml)

Fig. 4k Effect of acetylcholine on isolated rabbit heart

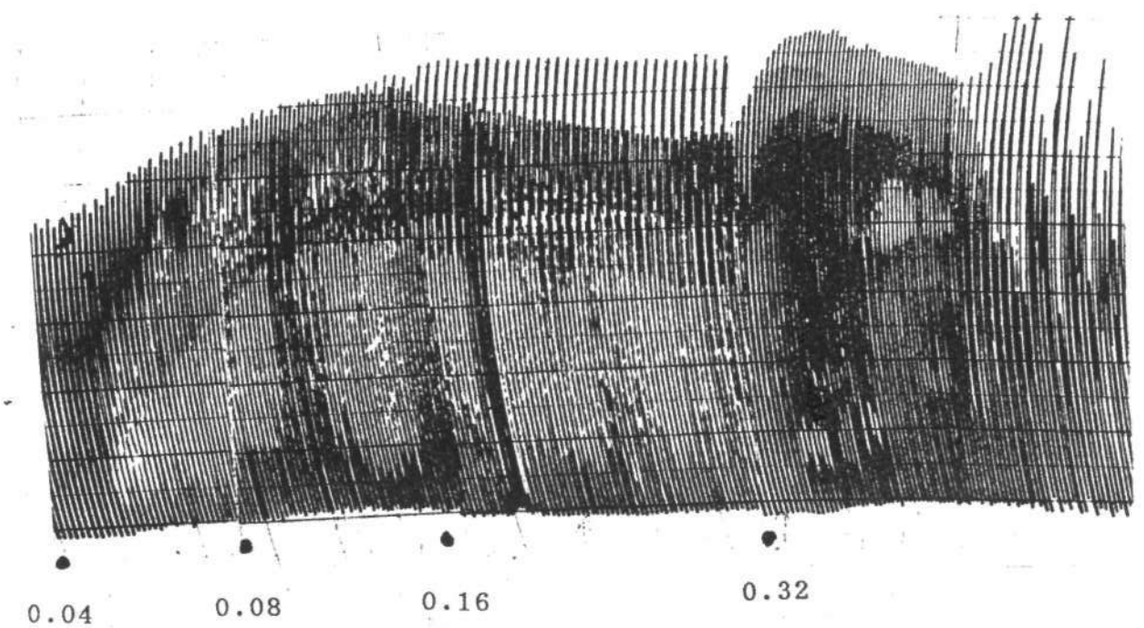


Fig. 41 Effect of isoprenaline on isolated rabbit heart

Table 4j:

Effect of Acetylcholine (0.16 μg/ml) in the presence of aqueous extract of *C. africana* on isolated rabbit uterus

Vol. of Aq. extract administered (ml)	Conc. of extract (μg/ml)	Average Response (mm)	Log-Dose	% Response
0.1	40	30	1.60	100
0.2	80	30	1.90	100
0.4	160	30	2.20	100
0.8	320	30	2.50	100

Table 4k:

Effect of relaxation produced by Acetylcholine on the isolated rabbit heart

Vol. of Ach administered (ml)	Conc. of Ach (μg/ml)	Average Response (mm)	Log-Dose	% Response
0.1	0.04	40 $\pm$ 1.30	- 1.40	100
0.2	0.08	35 $\pm$ 1.10	- 1.10	88
0.4	0.16	25 $\pm$ 0.80	- 0.80	63
0.8	0.32	20 + 0.50	- 0.50	50

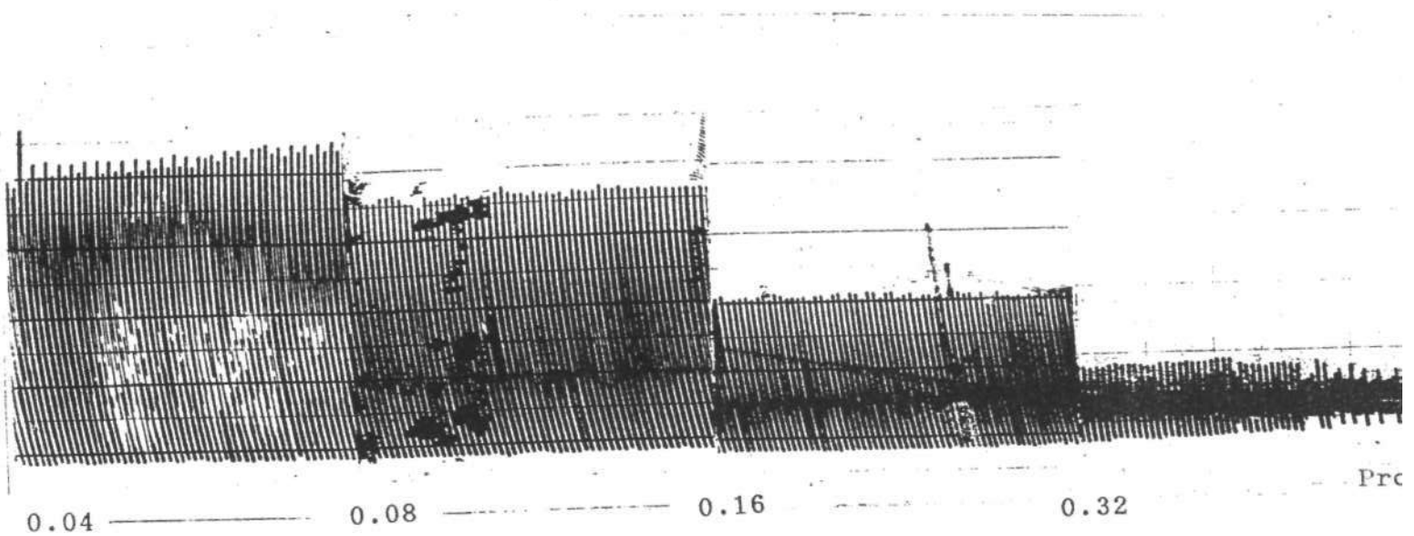
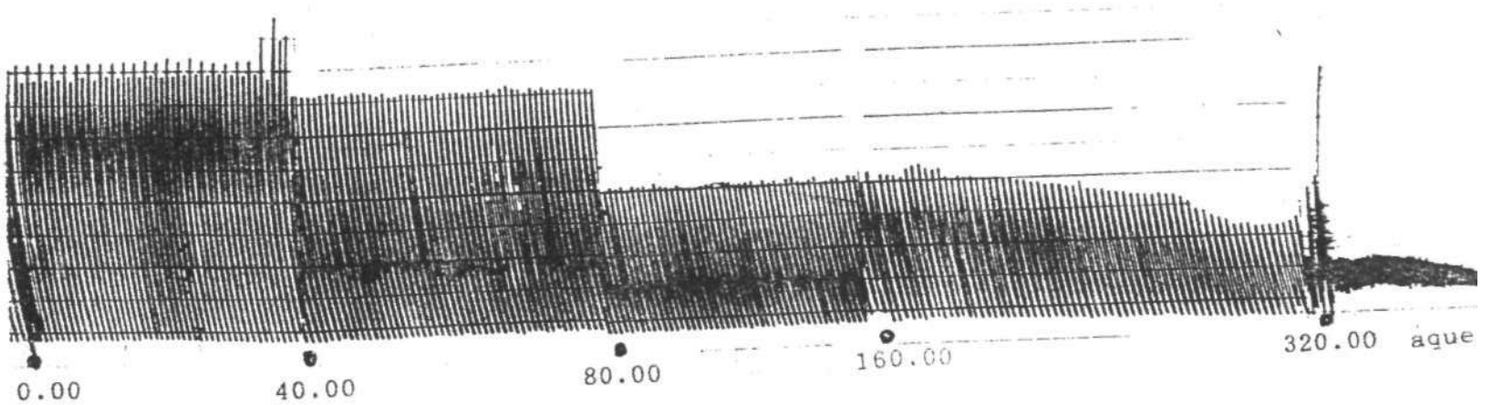


Fig. 4m Effect of propranolol on isolated rabbit heart



(Conc. in µg/ml)

Fig. 4n Effect of aqueous extract of C africana on isolated rabbit heart (relaxation)

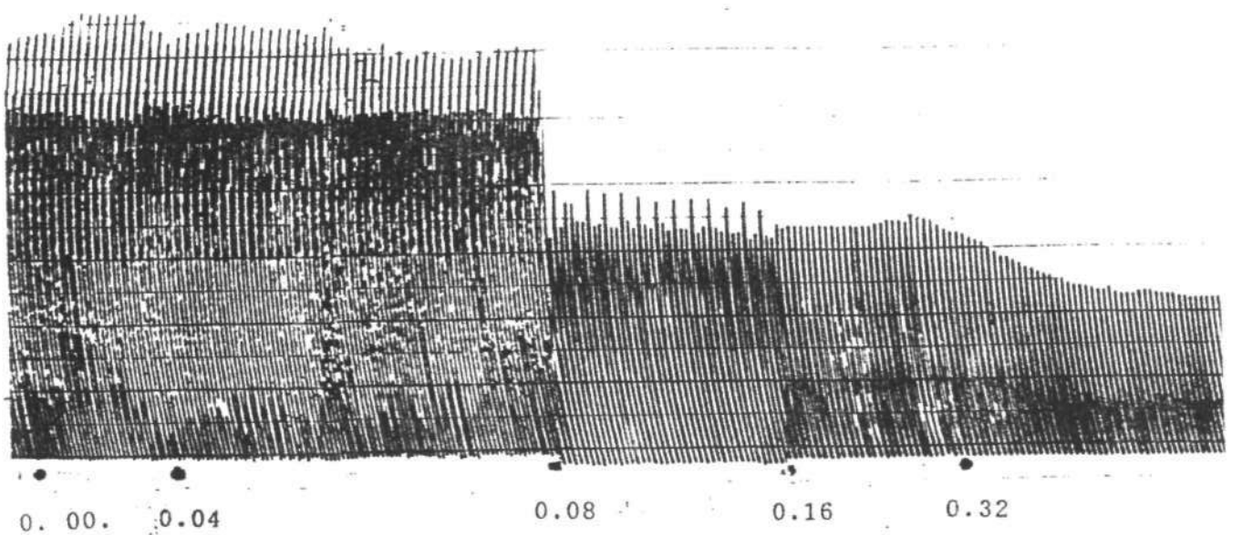


Fig. 4o Effect of isoprenaline (0.16 µg/ml) in the presence of propranolol on isolated rabbit heart (relaxation)

Table 4l:

Effect of contraction produced by isoprenaline on the isolated rabbit heart

Vol. of isopr administered (ml)	Conc. of isopr. (μg/ml)	Average Response (mm)	Log-Dose	% Response
0.1	0.04	55 ± 1.25	- 1.40	73
0.2	0.08	65 ± 0.20	- 1.10	87
0.4	0.16	70 ± 0.20	- 0.80	93
0.8	0.32	75 + 1.70	- 0.50	100

Table 4m:

Effect of relaxation produced by propranolol on the isolated rabbit heart

Vol. of propr administered (ml)	Conc. of propr. (μg/ml)	Average Response (mm)	Log-Dose	% Response
0.1	0.04	45 ± 0.61	- 1.40	100
0.2	0.08	35 ± 0.55	- 1.10	77
0.4	0.16	22 ± 0.50	- 0.80	49
0.8	0.32	5 + 0.25	- 0.50	11

Table 4n:

Effect of relaxation produced by aqueous extract of *C. africana* on the isolated rabbit heart

Vol. of extract administered (ml)	Conc. of extract (μg/ml)	Average Response (mm)	Log-Dose	% Response
0.00	00	45 ± 1.05	0.00	100
0.10	40	40 ± 0.10	- 1.40	89
0.20	80	25 ± 0.20	- 1.10	56
0.40	160	20 ± 1.00	- 0.80	44
0.80	320	3 + 0.50	- 0.50	7

Table 4o:

Effect of isoprenaline (0.16 μg/ml) in the presence propranolol on the isolated rabbit heart

Vol. of propr administered (ml)	Conc. of propr (μg/ml)	Average Response (mm)	Log-Dose	% Response
0.00	00.00	70 ± 0.50	0.00	100
0.10	0.04	65 ± 0.50	- 1.40	93
0.20	0.08	45 ± 0.40	- 1.10	75
0.40	0.16	42 ± 0.20	- 0.80	70
0.80	0.32	25 + 0.10	- 0.50	42

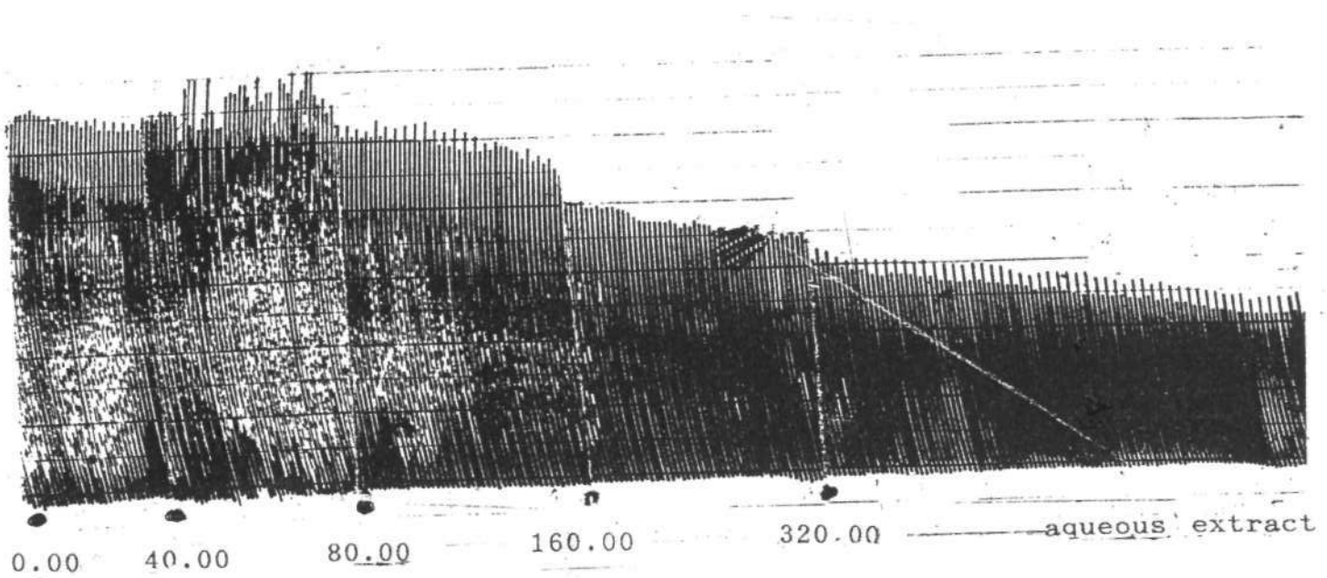


Fig. 4p Effect of isoprenaline (0.16 µg/ml) in the presence of aqueous extract of C africana on isolated rabbit heart

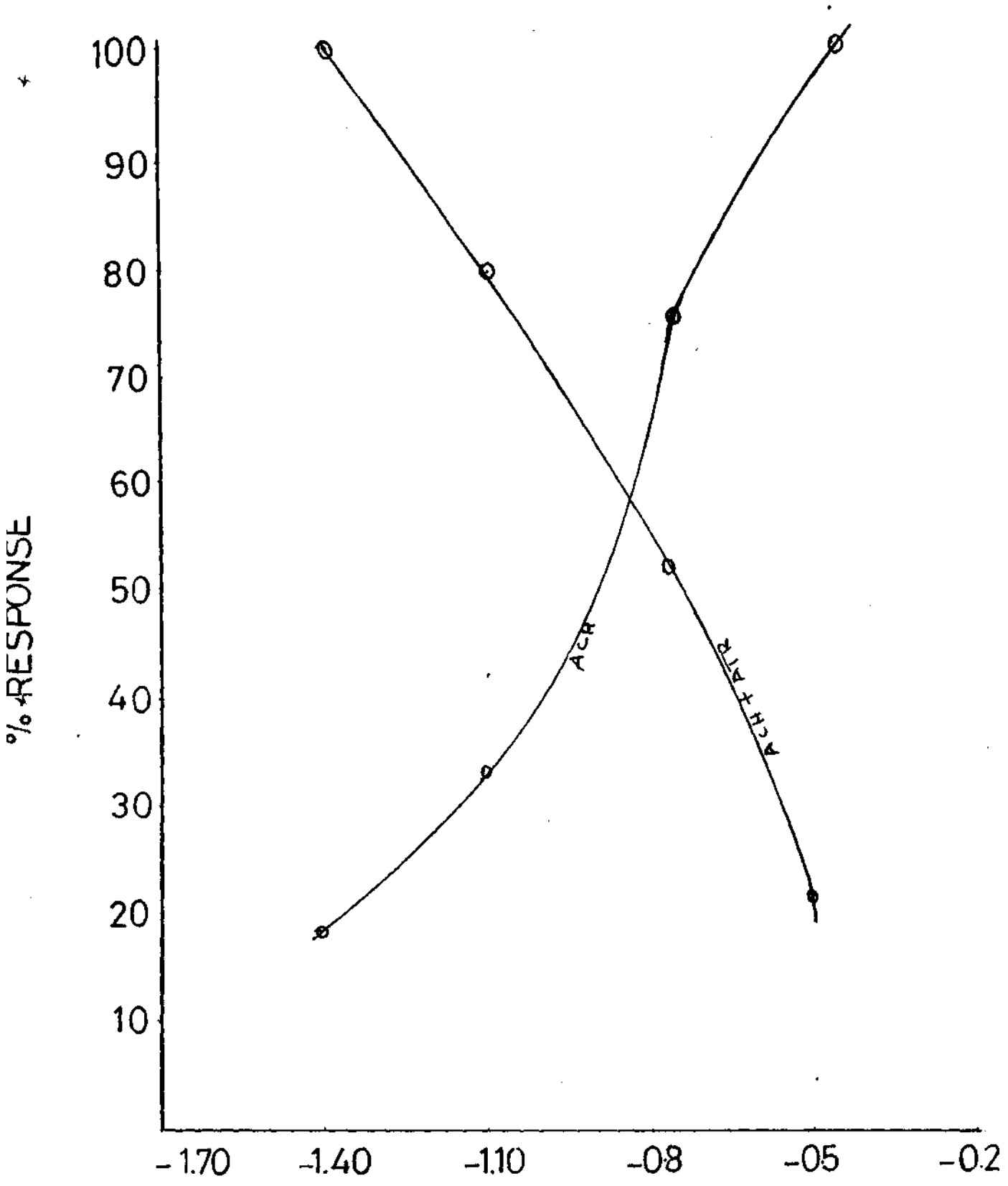
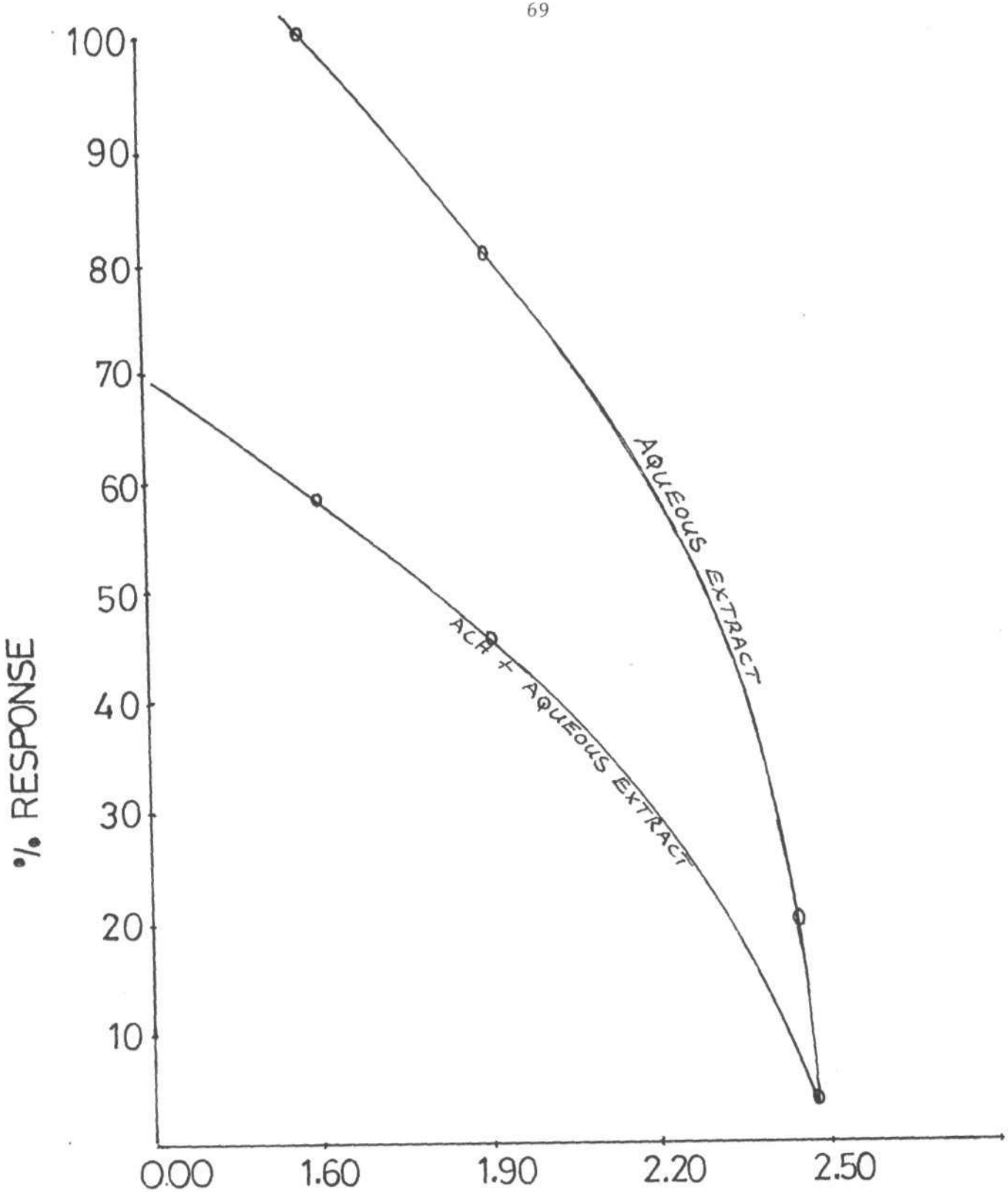
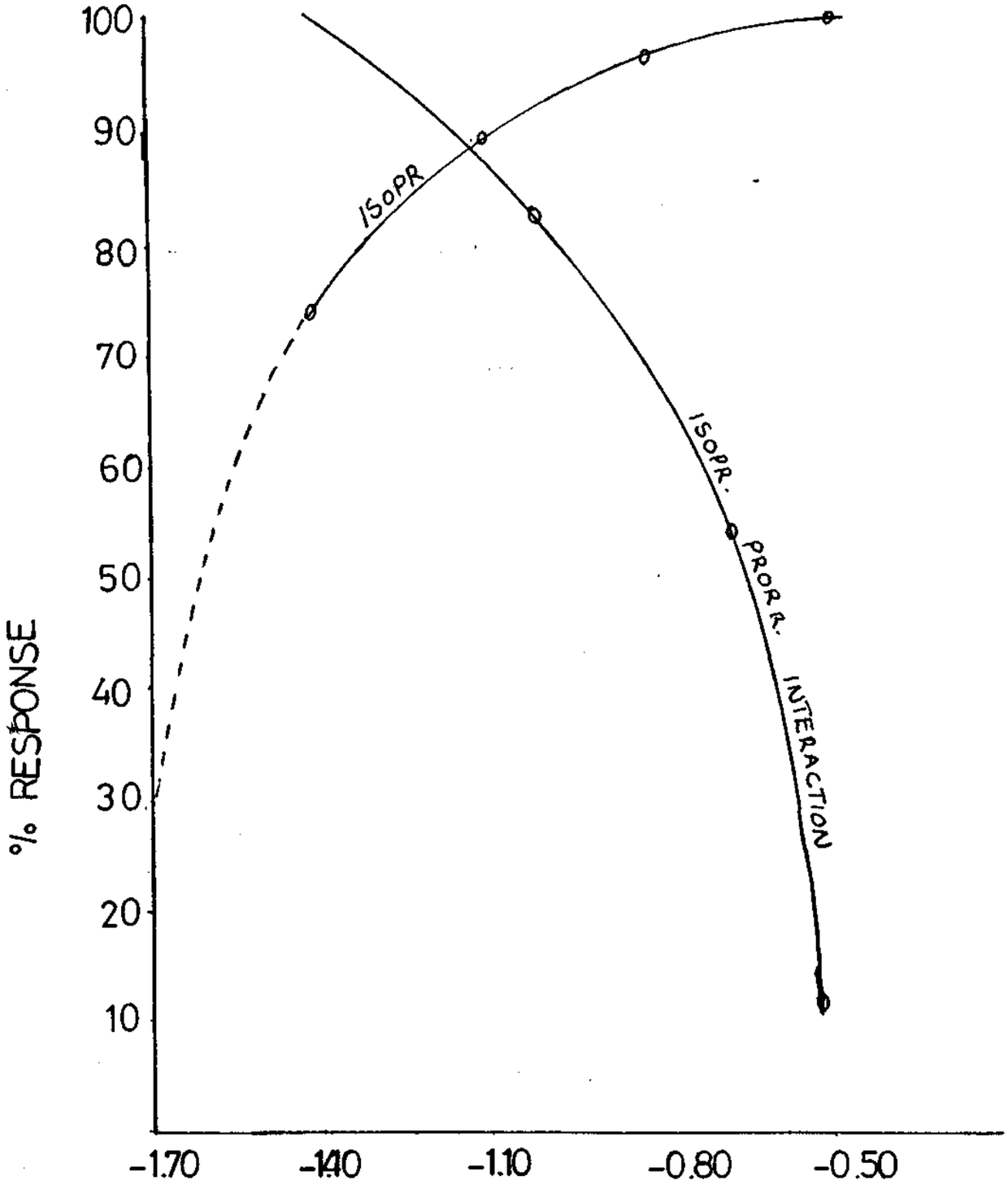


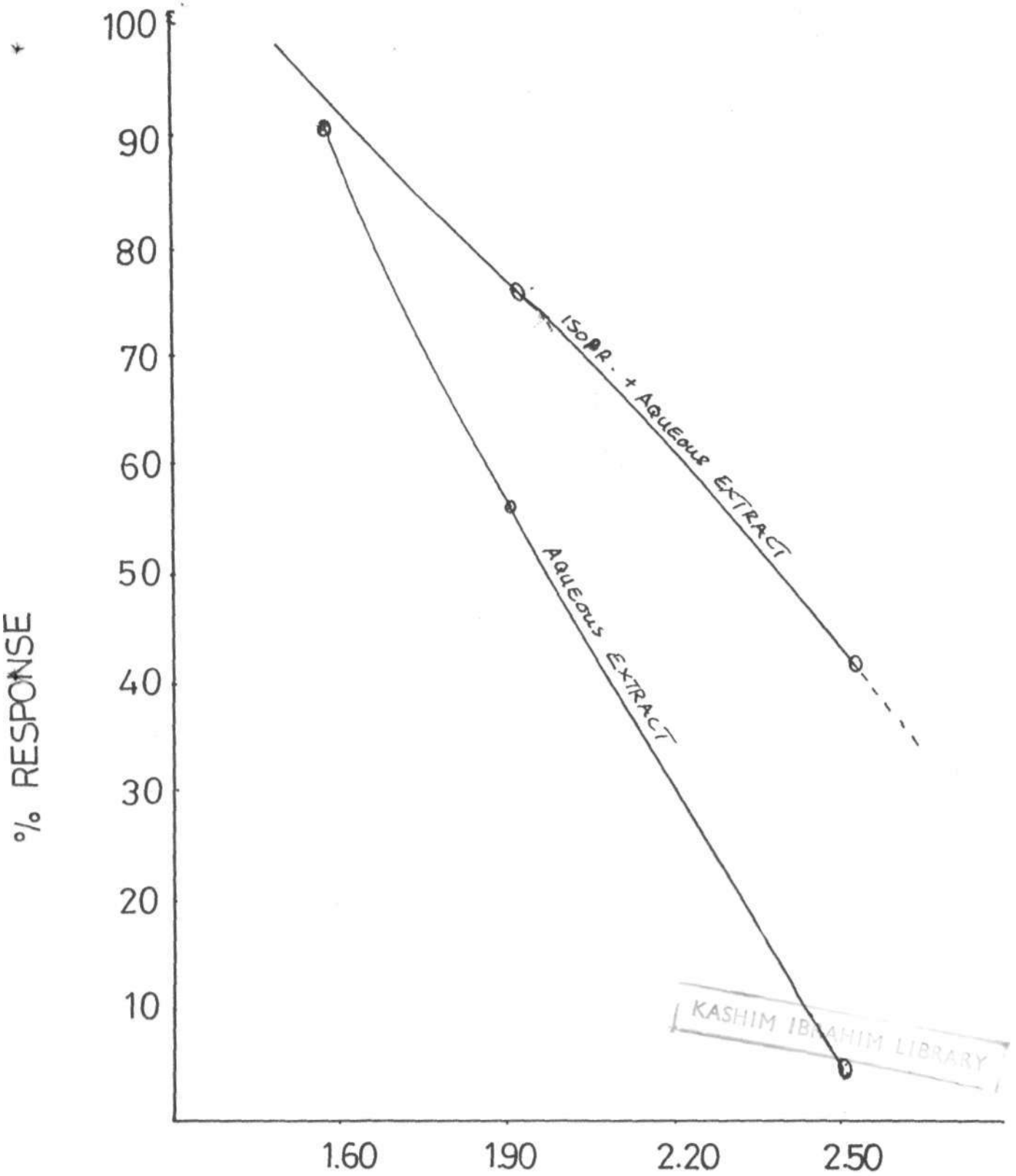
Fig 4q: LOG. DOSE RESPONSE CURVES FOR ACH AND ACH + ATR ON ISOLATED RABBIT ILEUM.



F,g.4r: LOG DOSE RESPONSE CURVES FOR AQUEOUS EXTRACT OF C. AFRICANA AND ACH. + AQUEOUS EXTRACT OF C. AFRICANA ON ISOLATED RABBIT ILEUM.



Fg. 4S: LOG. DOSE CURVES OF ISOPR. AND ISOPR. + PROPR. ON ISOLATED RABBIT HEART HEART. (NO POINTS TO JUSTIFY THE DOTTED LINES)



KASHIM IBRAHIM LIBRARY

Fig. 4t: LOG DOSE RESPONSE CURVES FOR AQUEOUS EXTRACT OF C AFRICANA AND ISOPR. + AQUEOUS EXTRACT OF C AFRICANA ON ISOLATED RABBIT HEART (no points to

Table 4p:

Effect of isoprenaline (0.16 μg/ml) in the presence of *C. africana* on isolated rabbit heart.

Vol. of aq. extract administered (ml)	Conc. of aq.extract (μg/ml)	Average Response (mm)	Log-Dose	% Response
0.00	00	60 $\pm$ 0.71	0.00	100
0.10	40.0	55 $\pm$ 0.28	1.60	92
0.20	80.0	48 $\pm$ 0.28	1.90	80
0.40	160.0	35 $\pm$ 0.41	2.20	58
0.80	320.0	20 $\pm$ 0.22	2.50	33

4.6 DISCUSSION

4.6.1 RABBIT ILEUM

The standard drugs used were Acetylchololine (Ach), Atropine (Atr), and Adrenaline (Adr) Acetylcholine (a quartenary ammonium compound) is an acetyl ester of choline. It serves as a neurotransmitter in a number of organs, including neuro-muscular junctions, post ganglionic fibres of both sympathetic and parasympathetic systems. (Elliot et al., 1977). The two major actions of acetylcholine on the nerves system are:-

(i) to stimulate the ganglia, adrenal medulla and skeletal muscle.

(ii) to stimulate the post ganglionic nerve endings in cardiac muscles, smooth muscles and the glands.

The first action resembles the effect of nicotine and therefore is referred to as the nicotinic effect of acetylcholine which is elucidated via nicotinic receptors. The second action resembles that of muscarine (an alkaloid from poisonous toad stool-*Amanita muscara*) and therefore is referred to as the muscarinic effect of acetylcholine which is elucidated via the muscarinic receptors.

Acetylcholine caused contraction of smooth muscles (Elliot et al, 1977) and in the experiment it produced contraction of the rabbit ileum. Similarly, atropine a known muscarinic antagonist to acetylcholine (Rang and Dale, 1987) was found to inhibit the contraction evolved by acetylcholine. The aqueous extract of *C. africana* exhibited a dose dependent relaxation of the ileum. This effect is comparable to those of atropine and adrenaline on the ileum.

The extract of *C. africana* might be mediating muscarinic inhibition similar to atropine or inhibition caused by adrenaline on adrenergic receptors. The extract of *C. africana* dose dependently inhibited the contractions evolved by acetylcholine. This is similar to atropine, suggesting that, the extract might be mediating its relaxant effect through muscarinic receptors. This effect is beneficial in the management of gastro intestinal disorders thus justifying the use of the drug in stomach disorders in traditional medicine.

4.6.2 RABBIT UTERUS

The standard drugs used were carbachol and Acetylcholine, carbachol has similar effect as acetylcholine on both nicotinic and muscarinic receptors but it is not readily hydrolysed (Rang and Dale 1989; Remington Pharm. Scs., 1980) as is the case with acetylcholine. Carbachol produced dose dependent contractions of the smooth muscle of the isolated rabbit uterus just like acetylcholine. The aqueous extract of *C. africana* was found from this present study to have no significant action on the contracting activity of acetylcholine on the isolated rabbit uterus (fig. 4h) and did not exhibit any agonistic or antagonistic effect of its own on the uterus, (Fig. 4i).

4.6.3 RABBIT HEART

The standard drugs used were acetylcholine isoprenaline and propranolol.

Both sympathetic and the parasympathetic systems normally exert a tonic effect on the heart at rest. The main effects that sympathetic activity produces are:-

(i) increased heart rate i.e positive chronotropic effect

(ii) increased force of contraction i.e positive inotropic effect

affecting all parts of the heart.

(iii) increased automaticity

(iv) facilitation of conduction in the atrio-ventricular (A.V.) node

(v) reduced cardiac efficiency i.e oxygen consumption is increased more than cardiac work.

These effects all result from activation ß- receptor (Rang and Dale, 1987). Catecholamine acting on $ß_1$- receptors exert a powerful stimulant effect on the heart. Both the chronotropic and inotropic effects are increased.

Catecholamine is a compound possessing a catechol nucleus i.e. benzene ring with two adjacent hydroxyl groups; and an amine containing side chain (Fig 4u).

This include:

(i) **Noradrenaline:** a transmitter substance released by the post ganglionic sympathetic neurons i.e. adrenergic neurone

(ii) **Adrenaline:** hormone secreted along with noradrenaline by adrenal medulla

(iii) Dopamine: metabolic precursor of nor adrenaline.

(iv) Isoprenaline: a synthetic derivative of noradrenaline which is not produced in the body. It acts selectively on ß-drenoceptors. (Rang and Dale, 1987).

In the heart, each cardiac impulse is preceded by a local depolarization in the region of the sino-arterial (S.A) node called pacemaker potential. The most characteristic effect of the catecholamines (i.e. Adrenaline, Noradrenaline and Isoprenaline) is to increase the slope of the pacemaker potential so that the firing threshold is reached faster. The heart beats interval becomes shorter and the heart rate increases. The effect of isoprenaline in increasing the rate of pacemaker activity is most spectacular in isolated heart. It increases the rate strength of contraction of the heart partly by increasing the rate at which the contractile elements are activated in both auricle and ventricle (Schild, 1980)

Drugs which reduce the activity of adrenergic receptors, reduce automacity and improve certain types of heart dys function. Two classes of adrenergic blockers are used in this context.

(i) **ß-adrenergic blockers** such as propranolol

(ii) **adrenergic neurone blockers** such as bretylium.

Propranolol is a ß-adrenoceptor blocking agent. It is a competitive antagonist of isoprenaline with identical side chain which allows it to interact with ß-receptors and thereby block the effects of adrenergic stimulation mediated through these receptors. Propranolol is

an unselective ß-blocker with equal affinity for B-receptors in the heart and smooth muscle.

It acts by blocking the cardiac chronotropic and inotropic ß-receptors resulting in a decrease in heart rate and myocardial contractility.

From the results shown in table 4l, (Fig. 4l), propranolol produced a dose - dependent relaxing effect on the isolated rabbit heart, while isoprenaline showed a dose dependent contraction on the isolated rabbit heart (Table 4k and Fig. 4k). The aqueous extract of *C. africana* mimicked the actions of propranolol on the isolated rabbit heart.

The effect of isoprenaline on the isolated heart was blocked in the same manner by both propranolol and aqueous extract of *C. africana* (tables 4n and 4o figs.4n and 4o).

From the results it could be seen that the effect of propanolol on the rate and the amplitude of the heart beat i.e. frequency and force of contraction were comparable to those of the aqueous extract of *C. africana*. Since the rate the function of the pacemaker potential and the amplitude the function of the force and energy process, which were potentiated by the isoprenaline (a selective $ß_1$ - receptor) it could be inferred that, $ß_1$ receptor activity predominates and that ß-

receptors are involved in the mechanism. This could be supported by the result of propranolol - isoprenaline interaction. By way of extension of the same argument it could also be said that since aqueous extract of *C. africana* interactions with isoprenaline were almost the same as those of propanolol - isoprenaline, the extract is showing propranolol - like actions. Thus it mediates through $ß_1$ receptors and it may be regarded as a competitive selective blocking agent of the adrenergic ß-receptors, just like propranolol. (Black et al, 1965; Brunner et.al, 1966; Scriabine and Sweet, 1976)

4.7 CONCLUSION

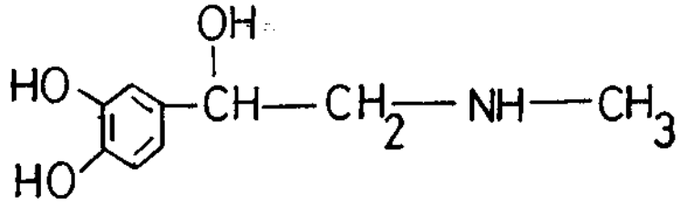
The effects of the aqueous extract of *C. africana* on three isolated tissues of a adult female non-pregnant rabbit (namely; ileum, uterus and whole heart) were investigated and compared with those of same standard drugs. The result showed that the extract acts as muscarinic blocking agent in the ileum, which justifies its traditional use as remedy for stomach pains and dysentery. The extract however has no significant effect on the uterus.

Studies in the heart revealed that the aqueous extract of the bark of *C. africana* mimicks the action of a typical ß-adrenergic antagonist like propranolol, on the isolated heart. This

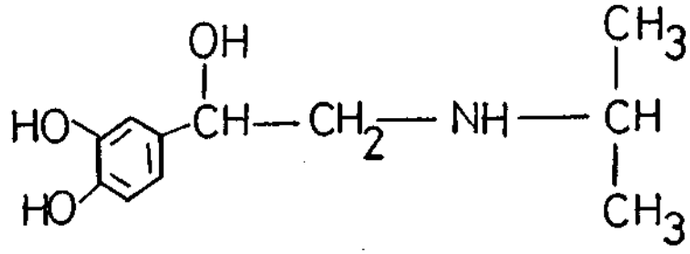
justifies to an extent the use of the plant for the treatment of heart burns and opens a new area of further reseach on how to use the plant in the management of other cardiac related problems like antidysrythmic actions and hypertension.

STRUCTURES OF STANDARD DRUGS USED

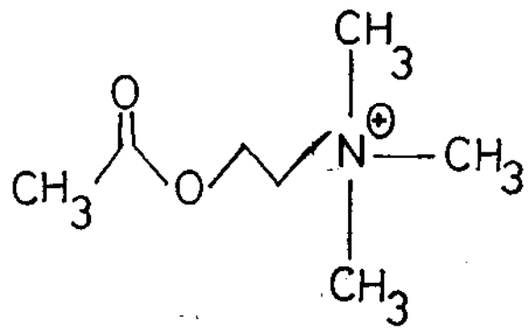
ADRENALINE



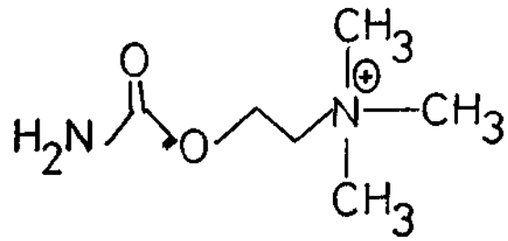
ISOPRENALINE



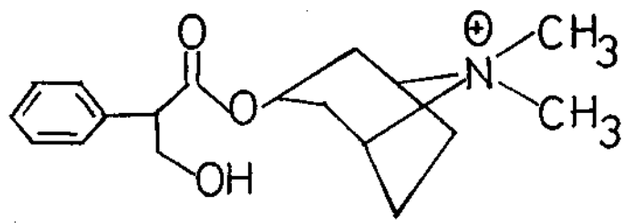
ACETYLCHOLINE



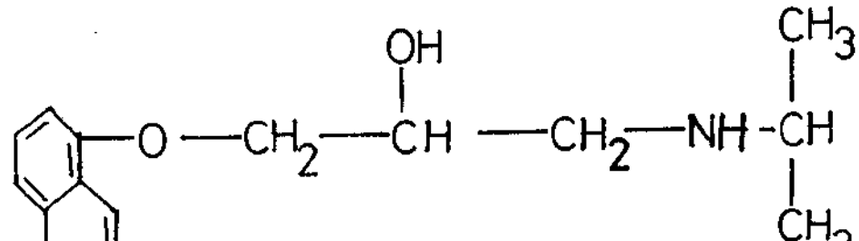
CARBACHOL



ATROPINE



PROPRANOLOL



REFERENCES

Anderson, A.W. (1956). The Plants of the Bible, Crosby Lockwood & Sons Ltd, London, p.40 - 41.

Bhatnagar, L.S; Singh, V.K. and Pandey, G. (1973). Survey of Medicinal Plants. *Indian J. Med. Res.* 8 (1), 69.

Black, J.W; Duncan, W.A.N. and Shanks, R.G. (1965). Comparison of Some Properties of Pronethalol and Propranolol. *Br. J. Pharmacol*, p.25 - 57

Brain, K.R. and Turner, T.D. (1975). The Practical Evaluation of Phytopharmaceuticals. Wright Scientechica, Bristol, p.140 - 144; 152 - 154.

Brunner, H.H. Maitre, L.; and Meier P. (1966) Beeinflussung des Prosocrischen Eserine- Effekts durch Propranolol. Naunyn *Schmudebergs Arch - Pharmakol* p. 254

Dalziel, J.M. (1937). Useful Plants Of Tropical West Africa. Crown Agents For Oversea Governments and Administrations, London, p. 316 317.

Dazeley, G.H. (1979). Organic Chemistry, Cambridge University Press, Cambridge, New edn. p. 29

Dipalma, J.R. (1971). Drills Pharmacology in Medicine, McGraw -

Hill Book Corpon. p. 863 - 364.

Driver, J.E. (1951). Bentley and Drivers Textbook of Pharmaceutical Chemistry, Oxford University, Press, London 5th edn, p. 519 - 520.

Elliot, G.R. Edelman, A.M., Bensan, J.F. and Berger, P.A. (1977). Indoleamines and Other Neuroregulators, in, Psychopharmacology From Theory to Practice (Brachas, J.D. BERGER, P.A., Claranello, R.D. and Elliot, G.R. eds) Oxford Univeristy Press London.

Enrico, M.I. and Carlo, L. (1972). Terpenes From Arabian *Commiphora. Myrrha, Chim Ind* (Milan) **54** (5), p.423-425. (Ital.)

Ghani, A. (1990). Introduction to Pharmacognosy. Ahmadu Bello University Press Ltd, Zaria, Nigeria, 1st edn, p.109,145

Ghazanfar, S.A. (1989). Savannah Plants of Africa (An Illustrated Guide) Macmillan Publishers, 1st edn, p. 101 - 102.

Harborne, J.B. (1973). Phytochemical Methods. A Guide To Modern Techniques Of Plant Analysis, Charpman and Hall, London, p.279.

Harris, G. Dictionary of Organic Compounds, Eyre and SpottiswoodPublishers Ltd, E & F.N Spon Ltd, London, Vol.5, p.2949.

Herbert, B.E.; and Ellery, E.W. (1948). Textbook of Practical

Pharmacognosy; Bailliere, Tindall and Cox, London, p.313-314, 363.

Higazy, S.A.L Abdil-Akher, M.A.O.; Elwakeil, F.A.; Loutfy, M.K. (1973). extracted by Benzene from Arabic Olibanum Gum. *J. Food. Sci.* **1** 203 -214

Hörhammer, L., Wagner, H. and Kramer-Heydweiller, D. (1966). Terephthalsauredimethylester in der Bluten von *Sarothamnus scoparious L. Naturwissenschaften,* **53**, 584.

Hutchinson, J. and Dalziel, J.M. (1937) Flora Of West Tropical Africa. The Crown Agents for the Colonies London Vol. 1, 2nd edn. p. 695

Hutchinson, J. (1959). The Families of Flowering Plants. Dicotyledons. Vol. 1, 2nd edn, Macmillan and Co. Ltd., London, p. 356.

Jessene, M.G.; Bezanger-Beauquesne L.; Pinkas, M. and Trotin, F., (1977). Gums of Two Resins. Bdellium and Galbanum. *Plant Med. Phytother.* **8** (4), p. 241 - 249 (Fr.).

Jinju, M.H. (1990). African Traditional Medicine. A case Study of Hausa Medicinal Plants and Therapy. Gaskiya Corpn. Ltd. Zaria. Nigeria p.43,50

Keay, R.W.J. (1989). Trees Of Nigeria Clarendon Press,

Oxford, p.333 - 334.

Kitchen, I. (1984). Texbook of In Vitro Practical Pharmacology.

Blackwell Scientific Publication, London, 1st edn., p.52-60. Laurence,

D.R. (1973). Clinical Pharmacology. Longman,

London, 4th edn., p. 171 - 200

Malthotra, S.C. and Ahuja, M.M.S. (1971). Comparative Hypolipidemic

Effectiveness of Gum Guggulu (*Commiphora Mukul). Indian*

*J.*Med. *Res*. **59** (10), 162 - 163.

Olaniyi, A.A. and Ogungbamila, F.O. (1991) Experimental

Pharmaceutical Chemistry, Shanesan C.I.Ltd, Ibadan,

Nigeria. p. 229.

Patil, V.D., Nayak, U.R. and Dev, S. (1972) Chemistry of

Ayurvedic Crude Drugs Guggulu (Resin from *C. mukul*);

Steroidal Constituents. *Tetrahedron*, **28** (2), 2341-2352

Pernet, R. (1972). Phytochemistry of Burseraceae. *Lloydia*,

35 (3), 280 - 287.

Rajani, P. and Sarma, P.N. (1988). Chemical Examination

of *Tephrosia hamiltonii. J. Indian. Chem. Soc.* **65,**

148 - 149.

Rang, H.P. and Dale, M.M. (1987) Pharmacology Longman Group,

Singapore, p. 161 - 176.

Reddy, P.P.; Ashok, D. and Sarma P.N. (1990). Chemical Examination of the Pods of *Cassia roxburghii* *J. Indian Chem Soc.* **67**, 783 - 784.

Remington, J.P. (1980). Remington's Pharmaceutical Sciences. Mack Publishing Co., Pensylvania. 15th edn., p. 837

Rendle, A.B., (1952). The Classification Of Flowering Plants. Dicotyledons. Vol. 2, Cambridge University Press, London, p. 290.

Robinson, T. (1967). Organic Constituents of Higer Plants. Burgess Publications USA, p.178 - 205

Scheinmann, F. (1970). An introduction to Spectroscopic Methods For Identification of Organic Compounds. Nuclear Magnetic Resonance and Infrared Sepectroscopy. Vol. 1, Pergamon Press, Oxford, p. 169, 181.

Schild, H.O. (1980). Applied Pharmacognosy Churchill Livingstone 12th edn., Edinburgh, p. 115, 147

Seriabine, A. and Sweet S.C. (1976). New Antihypertensive Drugs. Spectrum Publ. Inc., New York, p. 275.

Shoppee. C.W. (1964). Chemistry of Steriods. Butterworths, London p. 74 - 76

Sofowora, A. (1982) Medicinal Plants and Traditional Medicine in Africa. John Wiley & Sons Ltd, Chichester, U.K, p. 142-145.

Sofowora, A (1986). State of Medicinal Plants Research in Nigeria. Ibadan University Press, Ibadan, Nigeria P. 109

Trease, G.E. and Evans, W.C., (1983). A Textbook of Pharmcognosy, 12th edn., Bailliere Tindall, London, p. 658-682.

Venkataram, K. (1962). Methods For Determining the Structures of Flavoniods Compound in Chemistry of Flavonoids. Geissman, T.A. Ed. Pergamon, New York, p. 72 - 75.

Vogel, A.I. (1974). A Textbook Of Practical Organic Chemistry, Including Qualitative Organic Analysis, Longman Group Ltd, London, p. 781.

Wall, M.E., Eddy, C.R., Mcclennan, M.L. and Klump, M.E. (1952). Detection and Estimation of Steroids and Sapogenins in Plant Tissue. *Anal. Chem.*, **24**, 1337.

Wall, M.E.; Krider, M.M.; Krewson, C.F. Eddy, C.R.; Wilaman. J.J.; Cornell, D.S. and Gentry, H.S. (1954). Steroidal Sapogenins XIII. Supplementary Table of Data For Steroidal Sapogenins VII: *Agr. Research Service Circ.* Aic, **363** p. 17