PHYTOCHEMICAL INVESTIGATIONS

ON

STRIGA SENEGALENSIS BENTH

(Family- Scrophulariaceae)

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In partial fulfillment of the requirement for the award of

Master of Science Degree in Pharmaceutical Chemistry

(Pharmaceutical and Medicinal Chemistry).

August 1998

CERTIFICATION

This is to certify that research on the Phytochemical and Pharmacological Investigations of Striga senegalensis Benth (Family: Scrophulariaceae) conforms to acceptable standard of scholarly presentation and is fully adequate in scope and quality as a dissertation for the award of the degree of Master of Science (Pharmaceutical and Medicinal Chemistry) of the Ahmadu Bello University, Zaria, Nigeria.

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Praise the Lord, O my soul and forget not all His benefits (Psalm 103:2).

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Rarely do supervisors, for instance, visit and discuss academic and other relevant matters in their students' rooms, but Dr. M.K. Choudhury has done this times without number. Whatever I am now and would be in the world of sciences, Dr. M.K. Choudhury has had a significant impact on me, an impact that cannot be erased easily.

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DECLARATION

I declare that the phytochemical (and pharmacological) Investigations on Striga senegalensis has been conducted by me,

Mr. Zechariah Garba Marting, under the supervisions of **Dr. M.K. Choundhury** (A.B.U.)

Dr. K.S.Gamaliel (NIPRD, Abuja) and **Dr. M.Garba** (A.B.U.)

And that no similar work has been submitted elsewhere for the same degree.

Also, appreciative recognition of past and present researchers in this and allied fields have been referred to.

DEDICATION

I dedicate this piece of work to my family, namely: **Mrs. Hannatu Garba** (Wife), **Retle Paul Garba** (Son) **Retnan Patience Garba** (Daughter) , **Retmun Priscilla Garba** (Daughter) and to all researchers (past and Present) in the field of Natural products Chemistry of medicinal value.

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ABSTRACT

Striga Senegalensis Benth is among the worst weeds of the world, but locally, it is an ethnomedicinal plant.

Its antifertility and abortifacient activities have been published however little on its Chemistry is known.

ST-1 , an isolate from its (the plant) ethylacetate exract was physically (spectroscopic methods) and chemically examined. The information obtained led to the proposed structure: 2- Carbonamide-6-hydroxy-9H-carbazole (a carbazole alkaloid).

The ethanolic extract of the plant had marked effects on the isolated heart artria and ileum of the rat and rabbit respectively . Its mechanism of action on the uterus appeared to be that of interfering with calcium ingress and / or calcium release from intracellular store.

CHAPTER ONE

INTRODUCTION

Striga , one of the genera of the family Scrophulariaceae is a noted worldwide weed. The family, although a large cosmopolitan one, with good representation in tropical Africa, is of little economic importance except for the negative effects of Striga on crop yields.
Over thirty species of the plant, Striga , are known, however, only seven are of economic value, with Striga senegalensis Benth top on the least.

Striga senegalensis Benth is a widespread parasite of Sorghum and millet. Agriculturists have considered it the world's worst weed among others. Immense difficulty have been encountered while targeting its eradication .Of the approximately 200,000 or more plant Species in the world, only about 0.10% of the worldwide taxa are sufficiently and universally troublesome to be called weeds, and Striga alone threatens the lives of over 100 million people in Africa (Kim, 1988).
With this, can anything useful come out of the plant? The recent pharmacological investigations on the plant (Choudhury et at , 1998), based on the ethnomedicinal information on its antifertility property (when chewed as such) has placed Striga senegalensis Benth on further pharmacological and ***phytochemical*** investigations. Coupled with the fact that the plant is also used as a dyestuff, animal fodder and in treatment of liver diseases in herbivorous animals locally, the importance of its phytochemical investigations cannot be overemphasized.

Little is actually known about the chemistry of Striga senegalensis Benth. General preliminary phytochemical screening has shown the presence of flavoniods, tannins and sugars. No documented literature on the active constituents of the plant or related species exist .

These phytochemical investigations are aimed at establishing some of the possible active constituents(where available) . These involve the plant collection, its authentication and extraction. The proceeds of the extract will be appropriately chromatographed for subsequent purification, separation and isolation. By application of chemical tests where helpful and the use of spectral means and other physical methods such as melting point determination, the isolated (if obtained) will be molecularly determined and the likely structure subsequently established.

The local people take this plant orally, it would be acceptable to investigate the possible pharmacological effects of the extract on the ileum. Furthermore, the heart is among the major organs in the body, the effects of the extract on the isolated heart atria has also been investigated. Isoprenaline has been used in conjunction with the ethanolic extract in this study.

On the uterus, however, having established its antifertilty activity ,the probable mechanism of the plant extract has been investigated utilising drugs of known mechanisms. Verapamil, a calcium channel blocker, and salbutamol, a β_2- adrenoceptor agonist, have been used in this investigsation.

CHAPTER TWO

2.0 ..... LITERATURE REVIEW

2.1 THE ROLE OF NATURAL PRODUCTS IN MEDICINAL CHEMISTRY

The early medicines of man were obtained from natural sources, especially the vegetable kingdom. Empiricism, superstition and traditional medicinal folklore have guided man to find healing in herbs and barks, fruit, leaves, roots, seeds and stems of plants in many different climates and parts of the world. Lichens and fungi have also had their share as contributive sources for drugs for centuries.

The science of pharmacognosy refined the principles of empirical drug extraction from plant sources and put them on a quantitative basis. Pharmacognosy built a bridge to the chemistry and pharmacology of natural medicinal products. Medicinal chemistry attempts to establish relationships between chemical structure and biological activity and to link biodynamic behavior to the chemical reactivity and physical properties of therapeutic drugs. It also involves the isolation, characterization and synthesis of compounds that can be used in medicine for the prevention, treatment and cure of diseases. Medicinal chemistry thus provides the chemical basis for the interdisciplinary field of therapeutics.

The study of natural products of established of potential therapeutics value has been and is of great fascination not only because of the medicinal applications of the active principles involved, but equally as an intellectual exercise in understanding ***nature's*** processes and

purpose. To be able to understand nature to imitate and perhaps improve on it, has been man's oldest thought and most cherished dream among others.

Questions that excite the thoughts of man and propel him further and further into research depths about natural products include:

- What is the nature of organic compounds one finds in higher and lower forms of plants and Animals?
- Why are they there, and how did they get there?
- Do they serve a purpose in the cells and tissues where they occur; and have they been placed there for the use of animals and man?
- Or do they need any further modification for instance, molecular alterations, to become of better use or application to man and animals?

History has it that interests in natural products declined considerably until the discovery of the important uses of reserpine, which revived medicinal product chemistry.

The purpose(s) of medicinally active natural products cannot be formulated in a sweeping way but known to include protection and biocatalyst. Some toxic substances may protect their plant or animal host from pedators or parasites. Hormones that have "evolved" over endless generations, undoubtedly serve the animal or plant as biocatalysts to achieve and maintain health, homeostasis, and suitable conditions for reproduction. These and other purposes are subject to debate.

The lengthy modern extraction procedures by which plants with or without medicinal history are screened for alkaloids, saponins, terpenoids, etc, seems to yield only negligible numbers of candidate compounds for serious clinical trials, have always frustrated wishes in natural products. Despite this and other factors, natural products have retained the mission of presentation to us novel and unexpected structures, and these structures serve as welcome "leads" for molecular modification.

Natural products offer clues on configurations and conformational information concerning steric restrictions on a given biological activity and as multifunctional compounds, give us a hint as to which function be varied to attain greater potency, medicinal specificity, or lower toxicity.

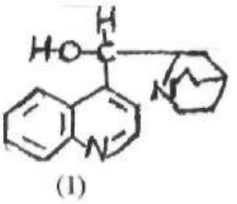
Today, natural products especially of medicinal remedies, are receiving stronger and firmer attention, thus research in them is expected to continue with intensification in the elucidation of structures, via ***phytochemical*** screening, biochemical screening, pharmacological screening, etc.

2.1.1 MEDICINAL HERBS

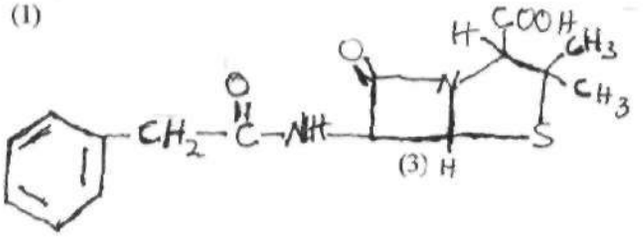
Plants are immensely complicated factories, which turn the relatively simple ingredients of air and water into enzymes, sugar, proteins, solid cellulose, liquid oils and scents to attract pollinating insects and poisons to kill off predators.

No wonder that what are arguably the four most early drugs in the history of medicine were all derived from plants:

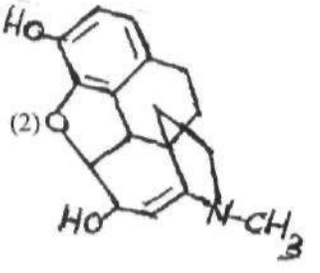
quinine(1),from the back of cinchonna, morphine (2), from seed capulses of the Opiom poppy, penicillin (3), from the mould Penicillium notatum



(1)



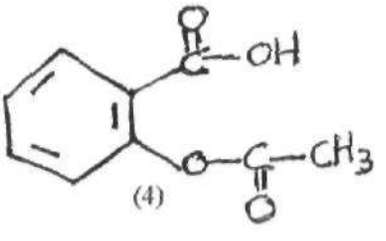
(3)



(2)

Even the "pill" would be a commercial impossibility if some of the complex chemicals in South American yams were not available as starting materials.

Over 50% contemporary prescriptions are still used as medicines which contain plant derivatives. Even our native flora has given us remedies as diverse acetysalicylic (4) derived from the willow bark, used originally for headaches, and has both analgesic and antipyretic properties; and colchicine(from the root of meadow saffron) (Mabey, 1977).



(4)

The discovery of a new drug may have its origin in the modification of natural product that has a desired therapeutic effect or, on the other hand, in rational concepts of the nature of a diseases and of the metabolic fate of a therapeutic agent. Between these two extremes lies the research based on the new lead. A lead is the often-accidental discovery that a compound has

a desired activity in an animal or in man. To be prepared for unusual discoveries in science, we must continue to test the biological effects of compounds in whole animal as well as isolated tissues.

Of the thousands of new compounds prepared and evaluated each year, only a handful ever find actual use in patients. Something very special is required to succeed. This is one of the reasons why the research for new medicines is important and challenging.

Successful drug research requires an interdisciplinary approach involving clinicians, biologists, chemists, mathematicians, pharmacologists, biochemists, etc.- a unique effort with few parallels in the world of science.

2.1.2 COLLECTION OF MEDICINAL HERBS

Collection of any plant material of medicinal value can be and is known to be affected by the following factors: season (whether annual, biannual or perennial), plant portion (whether it's flowers, flowering tops, roots, whole plant, seeds, fruits, bark, etc) and time of the day (especially when materials are flowers). Almost all of these factors have little effects on Striga senegalensis except that it is a seasonal plant and the flowering tops are preferred.

The collected pant must be authenticated by a herbarium center.

Striga senegalensis is usually air- dried on collection under shade which colour usually changes to purple and sometimes dark violet.

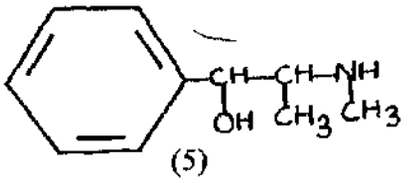
The dried plant (if drying the medicinal herb is part of the method) is then milled to acceptable reduced size. Different solvent systems can then be used for the extraction using the soxhlet apparatus or other most suitable extraction technique.

2.1.3 VALUING THE BIODIVERSITY OF MEDICINAL PLANTS

For the purpose of trying to assess their value, plants can be placed in one of the following four categories:

a. Those that have not been investigated or that have been investigated but have not been found to be pharmacologically active.;

b. Those that have been found to be pharmacologically active and whose active ingredient(s) can be economically synthesised for example, ephedrine (5)



(5)

c. Those that have been found to be pharmacologically active and whose active ingredient(s) have not been made synthetically; and

d. Those that have been investigated and found to have useful information(structure or mechanism) that is used pharmacologically.

Plants have always played a major role in the treatment of human traumas and diseases. India's use of plants for medicinal treatment dates back over 5,000 years, and has become codified in the Ayurveda, which contains over 8,000 herbal remedies. This same system of treatment is still used in over 14,000 dispensaries(Huxley,1984).

At about the same time, a Chinese emperor was describing 365 herbal remedies including ginseng, opium(source of codeine and morphine) and ephedra (source of ephedrine).

During the next millennium, the Assyrians listed 250 medicinal plants and the Sumerians recorded 1000 plants. The later civilizations of Egypt, Greece, Rome, Arabia, Europe, and America all recorded their use of plants as medicine.

Within the realm of pharmacology, there are three major ways in which plants are used (Hanse,1972).

a. Constituents isolate from plants which are used directly as therapeutic agents, examples, atropine and morphine(2)

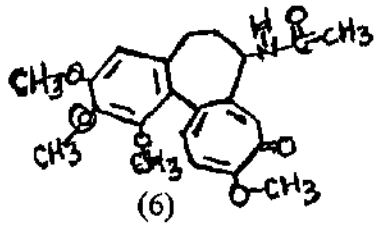
These constituents are still unsurpassed in their respective fields.

b. plant constituents which are used as starting materials for the synthesis of useful drugs. Examples include adrenal cortex and other steriodal hormones which are normally synthesised from plant sterodal sapogenin and the synthetic from natural penicillin (penicillin G).

c. Natural products which serve as models for pharmacologically active compounds in the field of drug synthesis.

d. Their use in research of chemicals which side effects are too strong to permit use as prescription drug (Suffness and Douros, 1979).

❖ Frequently, the side effects of a natural product often prevent its use in medicine and can be solved only by preparation of a synthetic derivative, for example, cocaine which led to the development of modern local anaesthetics; the coumarins as precursors of modern antithrombins; and modification of colchicine(6) and podophyllotoxin to obtain antitumor preparations.



(6)

In particular, natural products are used to investigate and characterize biochemical process and their mechanisms. More than 50 drugs that have aniticancer properties are not marketed because of their adverse side effects, but they are widely used in research. An example is taxol, a drug derived from the Northwest Yew, currently being used by National Institute of Health (NIH) for anticancer research purpose(Pricinpe,1992).

2.2 DEFINATIONS: ETHINOMEDICINE AND TRADITIONAL MEDICINE

Ethnomedicine is the use of plants by members of an indigenous culture for which there is no organized system or formal training by the prescriber.

Traditional medicine on the other hand refers to the use of plants in the treatment or amelioration of disease within an organized indigenous system.

2.2.1 ETHNIC VALUE OF STRIGA SENEGALENSIS BENTH

Locally, Striga senegalensis Benth is known to be used:-

(a) as an antifertility agent. The flowering tops are chewed and swallowed as such.

(b) In treating liver diseases in herbivorous animals. The diseased animal is encouraged to eat the plant as a fodder.

(c) As a dyestuff. Its aqueous extract is concentrated to dryness and the dried material so obtained is used as a dye.

(d) As a fodder. The wet or dried plant is gathered as such, usually along with other feeds, and the animal simply feeds on it.

These assumed properties of Striga senegalensis render its scienctific research a worthwhile venture.

2.2.2. UPDATE ON PHARMACOLOGICAL PROPERTIES OF STRIGA SENEGALENSIS BENTH

Recent pharmacological investigations on Striga senegalensis have revealed its antifertility activity and that it is also known with abortifacient activity (Choudhury et al, 1998a, 1998b). Earlier on, its antibacterial activity (against pneumonia) was reported use as the crude extract (Kokwaro, 1976).

2.2.3 UPDATE ON THE CHEMICAL PROPERTIES OF STRIGA SENEGALENSIS BENTH

Preliminary phytochemical screening of the aqueous extract of the Striga senegalensis Benth has shown the presence of flavonoids, sugars and tannins.

No documented literature on the active constituents of the plant or its related species has existed (such as in Chemical Abstracts, Cdrom,Medline Search, Napralet,etc). Documents,

however, are much available as per its effects as weed on crops such as rice, maize, wheat, sorghum, cowpeas, sugarcane, etc. Thus, the ***phytochemical*** investigation on this plant is a need.

2.3 STRIGA – A WORLDWIDE WEED

Weeds have been man's constant companion. He is accustomed to seeing them everywhere. It is understandable therefore that hand- weeding, cultivation, and toleration were the systems employed in the fields ages back. Weeds rank as one of the most powerful forces to be reckoned with when planning for food production, and yet strangely a word almost without dimension in common use (Holm et al,1977).

The word Striga, erected by the Italian botanist, Lourerio in 1790, signifies a small straight, hair-like scale; semi-parasite and troublesome when crops are attacked (Howest, 1931). Striga is a worldwide weed and thus have earned different names from continent to continent, region to region, country to country and even within the same country from locality to locality. For instance, it is called "witchweed" in United States of America; "bile kasa" (India);

"red witchweed" (East Africa); "gwin bin" (Burma); "Fireweed" (South Africa) "Wuta-wuta", "Kudiji", "Makasar dawa", i.e; Guinea corn killer, or "kashen dawa" in Hausa language (Nigeria); and the Indonesians call it "radja tawa" (Holm et al, 1977).

As a weed, Striga possesses the following properties:

- A concept of growing in undesired location
- Competitive and aggressive habits
- Of wild and rank growth
- Persistence and resistance to control or eradicate

- Consisting often of large proportions, with abundant, rank and extensive growths.
- Harmful to man, animals and crops: directly or indirectly, economically, physiologically, etc.
- Spontaneous growth, appearing without being sown or cultivated.
- Of high reproductive capacity
- Unsightly, with disfigurement of the landscape
- Useless, unwanted and undesirable (especially when the virtues of the plant have not been discovered (King, 1966). Appendix I contains the biological characteristics of weeds and their expressions.

Striga like any other weed, is harmful to crops principally in two ways:-

- Competitive for space
- Competition for nutrient (Frankton, 1955).

As the world's worst weed. Striga is well noted to have caused drastic reduction in crop production with consequent serious hunger in the history of India.

There are over thirty known different species of Striga, however, only seven are of economic value as named in this order of importance: Striga senegalensis Benth; S. asiatica Kuntze; S gesneriodes Vatke; S densiflora Benth; S eupharasioides Benth; S. aspera Benth; S. forbestil (Musselman, 1987; Kim, 1988).

1 shows Striga senegalensis Benth (family: Scrophulariaceae) as weed of sorghum.



Fig 1. Striga senegalensis (Scrophulariaceae)

2.3.1 SYNOPSIS OF WITCHWEEDS OF THE WORLD

Witchweed is the common name applied to members of the genus Striga of the large cosmopolitan family Scrophulariaceae.

Table 1 (Hutchinson and Daziel, 1954 and 1963; Andrews, 1956; King, 1966; Hepper, 1976; Musselman, 1987; and Mohammed, 1989) is a tentative listing of some species of Striga. It also attempted at listing some major synonyms. Distribution data is very general.

TABLE 1: CHARACTERISTICS OF SOME IMPORTANT STRIGA SPECIES

NAME	SYNONYM(S)	DISTRIBUTION	CULTIVATED HOST
Striga asiatica (L)	Striga luteu Lour	India South Africa	sorghum,maize,millet,
	S. zangebarica Klotzsch	Burma,Mozambique	sugarcane, rice.
	S. pusilla Hochst.	Sumatra, Mauritius,	Nicotiana tobacum.
	S. coccinea Benth	United States, Saudi Arabia	
	S. hirsuta Benth	China.	
Striga densiflora Benth	- - -	India, Indonesia	sorghum, millet, sugarcane
Striga cuvifolia	Striga aspera Willd	Sahara, Australia, Sudan	sugarcane
Striga euphrasioides Benth	S.angustifolia (Don),Saldanha		
	S.glabrata Benth	India,Burma,Rhodesia,Malawi , Tanzania, Oman	sugarcane, rice, sorghum.
Striga hermonthica Benth	S.senegalensis Benth	Egypt, Sudan, Rhodesia, West and Central Africa	sugarcane, maize
Striga ofobanchoides Benth	S. gesnerioides Willd	Central Africa, Khodesia,	Nicotiana,
	S. orchidea Hochst	South Africa, India, Australia,	Arachis hypogaea,
		Saudi Arabia	Nicotiana rustica, Epomoea batatas
Striga perrtflora Benth	- - -	Australia	sugarcane
Striga fulgens Hepper	- - -	Tanzania (only)	
Striga suphurea Datz	S. angustifolia (Don)?	India (only)	
Striga primuloides Chev		Ivory coast, Nigeria	
Striga linearifolia Hepper	S.strictissina Skan	West Africa	
	S. conescens Engl		

2.3.2 STRIGA SPECIES: GENRAL CHARACTERS

Striga species are annual, semi-parasitic herbs. Stems are usually erect, oppositely or alternatively arranged. The flowers are in terminal spikes. The Calyx is tubular and lobes linear inflated about the middle with 5 lobes spreading. They have 4 stamens. The capsule is loculicidal, surrounded by the calyx. Seeds are minutes, many and striate.

2.3.3 STRIGA SENEGALENSIS BENTH: DIAGNOSTIC CHARACTERS

Family: Scrophulariaceae

Erect: herb

Stems: branching, scabrid

Leaves: linear to narrowly lanceolate

Flowers: purple in racemes

Bracts: ciliate

Calyx: 4-5 ribbed

Corolla tube: almost glabrous.

S. senegalensis is a widespread parasite of sorghum (fig 2) and millet. The plant becomes purple on drying (Ghazanfar,1989).



Fig 2: Striga senegalensis as Weed of Sorghum

2.4 THE FAMILY: SCROPHULA RIACEAE

Scrophulariaceae is a large cosmopolitan family, chiefly distributed in the north temperate zones. It is also well represented in tropical Africa.

The leaves of Digitalis, a member of the family, are used for the extraction of the drugs digoxin and digitalin. Some species such as Snapdragon (Antirrhinum) and Foxglove (Digitalis) are cultivated as garden ornamentals.

Other genera besides Striga are Buchnera, Veronica, Rhamnicarpa, Alectra, etc (Ghazanfar,1989).

2.4.1 SCROPHULARIACEAE: GENERAL CHARACTERS

Scrophulariaceae are annual or perennial herbs and shrubs, rarely trees (Paulownia- E.Asia), semi-parasite plants (Striga, Euphrasia, Pedicularis) or Xerophytes (Hebe, New Zealand).

Leaves: simple, opposite, sometimes alternate or whorled.

Flowers: bisexual, zygomorphic, solitary or in racemes or cymes

Bracts/Bractioles: present, sometimes-brightly coloured.

2.4.2 SCROPHULARIACEAE: DIAGNOSTIC CHARACTERS

Leaves: opposite, exstipulate

Flowers: usually zygomorphic

Stamen: 4

Sepal: 5 united, persistent

Petals: 5 united,usual 2-lobed or 2-lipped.

Stamens: 4(2 in some genera), didynamous, epipetalous,alternating with corolla lobes, the fifth is sometimes represented by staminods.

Carpels: 2 united

Ovary: Superior, bilocular

Style: Simple or bilobed

Fruit: A capsule or berry

Seeds: many, small (Ghazanfar,1989).

2.5 CHARACTERISATION TECHNIQUES

Characterisation of the constituent isolates is possible utilizing chromatograhic, spectrophotometric(physical), chemical and/or combination of several of these methods.

2.5.1 CHROMATOGRAHIC METHODS

These afford information on the homogeneity, molecular size and structure of the isolate and provide useful data such as proof values. Separation and purification are better achieved with chromatographic procedures. Chromatographic procedures may generally be grouped thus:

a. Partition Chromatography- examples are paper, high liquid performance and gas-liquid techniques.

b. Adsorption Chromatography- examples are column and thin layer techniques.

c. Ion Exchange Chromatography.

d. Gel filtration and affinity Chromatography

e. Zone electrophoresis

f. Droplet countercurrent chromatographty (DCC)

This grouping is not exclusive. Several factors determine which of the type of the techniques should be employed and these include the cost, availability of technical manpower, availability of the instrument,, ease of the use of the instrument, time etc.

Partition chromatography involves the distribution of the substance being chromatographed between two solvent phases-a stationary phase and moving phase, with the water rich phase usually the former.

It is a commonly employed technique in isolation, purification and separation.

Adsorption chromatolography on columns of thin layer plates is also a very useful method for the separation and purification of isolates. Column separation is achieved either by elution or extrution.

Thin- layer in particular, due to its very rapid and easy to perform, most powerful and versatile tool, and very small quality of sample can be used, has rendered adsorption chromatolgraphy the most utilized method. Column eluates or extrusates are usually by the thin- layer method.

Droplet counter chromatography, a simple and very efficient all-liquid separation technique was developed almost thirty years ago. It is method based on the partitioning of solutes between a steady stream of droplets of mobile phase and a column of surrounding stationary phase. It is particularly indicated for the preparative scale separation of polar compounds and has been applied to the isolation of various types natural products. In many cases, the separations were achieved far more readily than by conventional chromatography and with a smaller solvent consumption. Quick for the selection of the solvent systems is usually done.

2.5.2 PHYSICAL (SPECTROSCOPIC) METHODS

The advantage of physical methods (spectrophotometric) cannot be over stressed as they include:

- Ability to give much more precise information about molecular structure than a classical method.
- Amount of sample required is small and it can generally be recovered.
- Information is obtained rapidly (usually), often in the form of permanent record on a chart.
- Analytical techniques are of high selectively and sensitivity, thus invaluable in the analysis of complex mixtures and in the detection of impuries.
- System can be examined without disturbing their composition (unlike chemical methods) in the study of tautomeric, conformational and other equilibria.
- Continuous analysis is often possible, facilitating kinetic work and the automatic control of process variable in industry .

The effective and economic use of physical tools can not be over emphasized, however, their limitations should also be appreciated as choice of method depends on:

- The information required and the types and complexity of the compound under investigation;
- The amount of relevant empirical data already in the literature and/or the availability of relevant reference compound;
- The quantity of sample available;
- Certain physical characteristics of sample e.g its solubility, cost staff specialist, etc.
- The importance of the problem, that is, the amount of time and effort which it justifies; and

- The accessibility, cost staff specialist, etc of the apparatus required (Shwarz, 1965)

Table 2 (Shwarz, 1965) attempts to summarize the salient features of a selected few physical methods, of most common application and availability (in some cases)

TABLE 2: A SURVEY OF FEW PHYSICAL METHODS

Method	PRINCIPLE	APPROXIMATE AMOUNT OF SAMPLE	TIME REQUIRED	STATE OF SAMPLE	INFORMATION OBTAINED AND APPLICATIONS
infrared spectroscopy	quantised absorption of infrared radiation leading to vibrational excitation of molecules	1-10mg	Mins.	solid(usually mull), liquid film, dilute solution in suitable solvent (not, in general, water) alkali halide disc or vapor	a. presence and environment of functional groups, especially those containing X-H (e.g. O-H) or multiple bonds (e.g.C=O) b. Diagnosis of finer structural detail e.g conformation, intramolecular hydrogen bounding, and *cis-trans* isomerism, etc. **analytical applications** a. Qualitative and quantitative analysis b. Investigation of reaction rates and equilibria (including intermolecular H-bounding) c. Examination of crude reaction

TABLE 2 Cont.

					product d. Detection of impurities e. Industrial production control.

Table 2 Cont.

Ulravoilet and visible spectrosopy	Quantised absorption of ultavoilet or visible radiation leading to electronic excitation	0-1-10mg	Mins.	Dilute solution in transparent solvent	a. presence and nature of unsaturation, particularly conjugated double bonds and aromatic rings. b. Stereochemistry of conjugated systems. c. Detection of certain (relatively few) functional groups e.g ketonic or aldehydic (C=O) **ANALTICAL APPLICATION** Similar to ir, except that: a. Restricted to conjugated and aromatic compounds mainly. b. An aqueous solution can be used. c. Often more sensitive, i.e, very dilute solutions can

TABLE 2 cont.

					be examined.

TABLE 2 Cont.

Nuclear magnetic resource (POTON)	Quantised absorption of radiation giving rise to transitions between different spin orientations of nuclei (commonly protons in a magnetic field)	Ca 0.5 ml of 0-1-1m solution, i.e, ca 5-100mg	Mins, hrs, dayOs.	Fairly concentrated solution, commonly in CCl_4 $CDC1_3$ or D_20	a. Environment of nuclear hence unambiguous detection of certain functional groups and information about the environment of the environment of many others. b. Counting of protons of various types in a molecule. c. Determination of proton sequence and hence of relative points of attachment of functional groups. d. Assignment of configuration and conformation. e. Can be used for quantitative analysis including studying of reaction rates and equilibria, but requires more material than uv or ir. f. Studying of fast reactions and of H-bounding

TABLE 2 Cont.

X-Ray Diffraction	Interference between scattered X-rays (scattering caused by electrons of atoms)	1-10mg	Hrs, days, months, years.	Single crystal (structure analysis, also molecular weight determination); crystalline power (identification only); oriented fibre.	a. Determination of complete molecular sructure and stereochemistry (but location of H-atoms is difficult). b. Such structure analysis gives bond lengths and angles as well as distances between non-bounded atoms in crystals. c. Molecular weight determination. d. Investigation of fibres. e. Powder photographs useful for identification purposes, especially when ir and uv spectra are not characteristic (e.g for *di-* and *tri-* saccharides)
Mass spectrometry	Determination of the mass/charge ratio and relative abundance of the	10 ag-1mg	Mins, hrs.	Vapour (compounds must usually be volatile)	a. Accurate molecular weight determination. b. Determination of

TABLE 2 Cont.

	ions (molecular and fragment) formed on electron bombardment of sample.				molecular formulae. c. Elucidation of structure (functional groups, length of side chains, nature of skeleton, etc); in favorable cases, complete structure can be deducted. d. Isotopic abundance measurements, (tracer studies, Isotope dilution analysis). e. Quantitative analysis (including detection of impurities). f. Ionization potentials and bond dissociation energies.

TABLE 2 Cont.

Carbon-13 NMR	Quantised absorption of radiation giving rise to transition between different spins orientations of nuclei (commonly C-atom and its environment in a magnetic field.	5- 100mg (0-.5ml of 0.1-1m solution)	Mins, hrs days	Fairly concentrated solution, commonly in CCl_4,D_2O,$CDCl_3$,etc.	a. Enables the analyst to directly observe he number of magnetically non-equivalent carbons in an organic structure. b. Being a sensitive and eligible instrumentation capable of producing high quality natural abundance 13C nuclear magnetic reonance thus improves characterisation of organic molecules. c. It defines the number of hydrogen atoms attached to each of these carbons (CH_1,CH_2,CH_3)

TABLE 2 Cont.

Electron Diffraction	Interference between scattered electron beam "wavelets" (scattering caused by electrostatic field,depends on distribution of both nuclei and electrons)	1-10mg	Months	Vapor, very thin single crystal.	a. Determination of complete molecular structure (bond lengths and angles) of fairly simple molecules. b. Indicates preferred conformation in gas phase. c. Location of H-atoms easier than in X-ray diffraction.

2.5.3 CHEMICAL METHODS:

Chemical methods are usually confirmatory in nature at stages of the characterization processes of a pure isolate. They suffer the disadvantage of being destructive in the process (usually) of the compound during identification.

2.6 PHARMACOLOGICAL CONSIDERATIONS

The collection of a natural product followed by its correct identification and "treatment", and in some cases characterization, is a necessary step to screening.

Drug screening refers to the initial testing of chemical compounds (or crude extract) for pharmacological activity. Compounds that survive primary tests provide leads to a more intensive subsequent study of those compounds and other structures related to them.

Before a new drug (or crude extract) is tested in man, most especially with no previous human remedies, the researcher must have some logical basis for assuming that it will have the desired activity. The most convenient animals for initial test (screening) are usually small rodents such as mice, rats, guinea pigs, rabbits. Also small dogs and monkeys are used as well. The smaller animals are economical and relatively easy to handle. Simple and sophisticated models have been used for screening. Whole new areas of research are emerging as molecular structures enzymes are mapped and details of their mechanisms studied.

Screening can either be targeted or untargeted in its approach. The untargeted suffers the disadvantages of having a chance probability succeeding and does not intellectually have general acceptability by scientists since it may not be related to only logically ordered information regarding the system being used as a test object, and, furthermore, the compounds being screened may not be analogous of the known structures of substrates, agonist, and / or antagonist, of the system.

The targeted approach is usually considered a "rational" way as positive information exists regarding the way in which the compounds that are chemically (or pharmacologically) related closely to the one being screened may influence the system under study.

However, both targeted and untargeted screening have offered very significant drug information, for instance, propranolol, verapamil, nifedipine,indomethacin, etc, were obtained through the untargeted way; while methotrexate, azathioprine, etc, have been obtained from targeted form.

Target screening is appealing to chemist because it gives them a starting point for the synthesis of active analogues (Maxwell and Eckhardt, 1990).

Drugs screening may also be viewed as:

a. Simple screening: only one or two tests are conducted, with the objective of finding drugs with specific pharmacological action;

b. Blind screening: when a series of routine tests developed to provide clues to potential activity of different types;

c. Programmed screening: this has greater precision and designed for a drug with specific pharmacological action (Gourley,1970).

2.6.1 THE INFLUENCE OF VARIATIONS IN SPECIES AND STRAINS OF EXPERIMENTAL ANIMALS AND DRUG RESPONSES

By definition, animals species and strains differs from each other by distinctive biochemical, functional and morphological features. As a corollary, responses of drugs vary among species and strains, often quantitatively and some time qualitatively. These variation interfere with the evaluation of drugs, since they limit the extrapolation of pharmacological observations from species to species, especially from experimental animals to man, and from strain to

strain, as from one human being to another. To asses the extent of permissible extrapolation, it is necessary not only to recognize these variations but also to understood; some however; are at least partially explained.

Interspecies differences in drug responses may result from variations in the fate of the drug, that is, its absorption, distribution, excretion, and biotransformation (pharmacokinetic factors), and /or from variations in the responding system themselves (pharmacodynamic factors).

Trains variations involve the same general mechanisms as species differences, for example, some strains of rabbits resist atropine and this has long been described and ascribed to the hydrolysis of the drug by an atropinestrase. The gene responsible called **As**, belongs to the group containing the pigmentation gene (Sawin and Glick, 1943).

Systematic exploration of species and strain differences has provided animal models for the preclinical evaluation of drugs and has also disclosed the fallacies of other models. some analysis is also appropriate to clinical pharmacology, at least to the extent that ethical considerations permit its application in man. The pattern of inter-and inter-species differences and similarities in drug response has already proved its value for contributing to a better understanding of the metabolic fate of drugs, their mechanisms of action, and the underling biochemical and fundamental features of living organism (Jacob, 1968).

2.6.2 PROBLEMS ASSOCIATED WITH THE EXTRAPOLATION OF DATA FROM *IN VITRO* EXPERIMENTS TO EXPERIMENTS IN INTACT ANIMALS

Owing to complex interrelationships of physiological and biochemical processes in living animals, studies on the mechanisms of drug action are frequently carried out in vitro. However, attempts to relate the results such studies to therapeutic and toxic actions observed in vivo are fraught with difficulties, for most drugs can interfere with almost any biochemical system provide that their concentration is high enough. To prevent misinterpretation of in vitro studies, it generally assumed that such studies should be carried out at drug concentrations approximating those present at receptor sites in living animals receiving therapeutic does of the drug

(Gillette, 1968).

2.6.3 A CRITICAL APPRAISAL OF THE USE OF ISOLATED ORGAN SYSTEMS FOR THE ASSESMENT OF DRUG ACTION ON THE RECEPTOR LEVEL

The pharmacologist often attempts to interpret the effects of drugs on organs in terms of drug action at the receptor level. He seeks answers to questions such as the following:

- When different agonists (active drugs) produce the same qualitative response in an organ, which of them do so by acting on the same receptor?
- What are the relative potencies of different agonists acting on the same type of receptor in a given organ?
- What contribution to potency of as agonists is made by its affinity for a receptor, and what contribution is made by its efficacy in provoking a stimulus on reacting with the receptor?
- If the second drug antagonises the responses produced by an agonist, does the second drug do so by interacting with the receptor for the agonists?

- If the second drug does interact with the receptor, is it reversible competitive antagonist or an irreversible (non -equilibrium) antagonist?
- What fraction of receptors of a given type are inactivated when an organ is exposed to an irreversible antagonist under fixed experimental conditions?
- What is the affinity of a given competitive antagonist for a given type of receptor?

For obtaining experimental data useful in answering basic pharmacology question such as listed, the pharmacologist generally uses as test preparation a surviving isolated tissues or organ. Some of the advantages of the isolated preparation over the organ in vivo are:

a. Quantitative measurement of response can usually be made more readily and more accurately.

b. The concentration of drug in the fluid perfusing or bathing the isolated preparation can be easily brought to a desired level, and maintained at that level for the desired time. (The variables encountered *in vivo* such as drug disposition and distribution are largely avoided).

c. With the isolated preparation one eliminates response that may be indirectly mediated in vivo by processes initiated by action of the drug at sites outside of the organ itself.

d. With the isolted preparation one eliminates the nervous reflexes and humoral "feed back" mechanism which may modify in vivo response which the drug would produce by acting on the organ only. Using an isolated test preparation, dose response data for the agonists can be obtained. Often the data is obtained before and after treatment of the tissue with other drugs, particularly antagonist. The pharmacologist then attempts to analyse the data with the use of concepts and cautions from receptor theory of drug

action. It is important to emphasis that both anatagonists and agonists are employed in any experimental program designed to assess drug action at the receptor level. Only the appropriate antagonist and agonist usage that the development and application of receptor theory can stand (Furchgott, 1968).

2.6.4 AUTONOMIC RECEPTORS

A great deal has been learned about the chemical nature of autonomic receptors during the last decade, and this field is one of the most active research areas in pharmacology.

Historically, structure-activity analysis, with careful comparisons of the potency of a series of autonomic analogues, led to the definition of different autonomic subtypes, including nicotinic and muscarinic cholinceptors, B-adrenceptors; and dopaminergic receptors.

Molecular biology has provided a new approach by making possible the discovery and expression of genes that code for related receptors within these sub-groups.

Adrenoceptor is widely used to describe receptors that respond to catecholamines such as norepinephrine (noradrenaline), epinephrine (adrenaline); cholinoceptor denotes recepros (both muscarinic and nicotinic) that respond to acetylcholine (ACh); and dopaminergic receptors denote those that respond to dopamine.

Besides, it has been recognized in recent years that most autonomic nerves also release transmitter substances or co-transmitters in addition to the primary transmitter. Examples are ATP, galanin, enkephalin and related opioid peptides, GABA(gama amino butyric acid)

subtance P, vasoactive intestinal peptide (VIP), Nitric oxide (NO), Serotonin (5-HT), neuropeptide Y (NPY).

A basic understanding of the interaction of autonomic components with each other and with their effector organs is essential for the appreciation of the actions of autonomic drugs, especially because of their significant "reflex" effects (Katzung, 1995).

Based on distinct actions of certain antagonist and agonist that interact with nicotinic receptors from skeletal muscle and ganglia, it has long been evident that nicotinic receptors (receptors that respond to nicotine) are not identical. The same applies to muscarinic receptors(that respond to muscarine). Through molecular cloning, nicotinic and five muscarinic subtype receptors have been detected.

Anatomically, the autonomic nervous system (ANS) lends itself to two divisions the sympathetic (thoracolumbar) divisions and the parasympathetic (craniosacral division). Both divisions originate in the nuclei within the central nervous system (CNS) (Katzung,1995).

TABLE 3. shows drug effects of autonomic nerve activity and autonomic drugs on a very few selected organs of importance and relevance to this study on the ethanolic extract of Striga senegalenesis Benth.

This is meant to enhance the appreciation of the pharmacological screening of the extract from the mechanistic and other view points.

TABLE 3: DRUG EFFECTS OF AUTONOMIC NERVE ACTIVITY AND AUTONOMIC DRUGS ON A FEW SELECTED ORGANS.

	EFFECT OF							
	SYMPATHETIC			**PARASYMPATHETIC**				
Organ	*Action*	*Receptor*	Agonist example	Antagonist example	Action	Receptor	Agonist example	Antagon example
accelerates		β_1	isoprenaline	betaxolol	decelerates	M_2	oxotremorine	gallamine
-		β_1	isoprenaline	betaxolol	.....	.....		
increases		β_1	isoprenaline	betaxolol	decreases	M_2	oxotremorine	gallamine
				-	(atria)			
relaxes		$\alpha_2\beta_2$	clonidine, albuterol	yohimbine. Butoxamine	contracts	M_3	muscarine	HHSD*
contracts		α_2	phenylephrine noradrenaline	prazosin	relaxes	M_3	muscarine	HHSD*
.....		.....			increases	M_3	muscarine	HHSD*
inhibits		α	noradrenaline	rauwolscine	activates	M_1	acetylcholine	Pirenzepin
relaxes			albuterrol	betazolol				
contracts			noradrenaline	rauwolscine	contracts	M_3		
							muscarine	HHSD*

* Hexahyhdrosiladifenidol

The effort to understand the mechanisms by which catecholamines act has a long and rich history. Ahlquist in 1984 rationalized a large body of observations by his conjecture that catecholamines acted via two principal receptors. He termed them alpha (α) and beta (β). Alpha receptors are those that exhibit the potency series epinephrine> norepinephrine>> isoproterenol; beta receptors have isoproterenol> epinephrine≥ norepinphrine series. Ahlquist hypothesis was dramatically confirmed when drugs that selectively antagonised beta but not alpha-receptors were developed. Today, a cascade of alpha and beta receptor-subtypes have been identified (Hoffman,1995).

2.6.4.1 THE UTERUS

The uterine smooth muscle is characterized by a degree of spontaneous electrical and contractile activity (Kao, 1977). Waves of decreased membrane potential with superimposed spike activity are associated with contraction. Cell –to-cell spread of excitation occurs, but electrical condition is slow and decrement in nature. Low- resistance contacts between cells (gap junctions) greatly facilitate the spread of excitation. The numbers of such junctions are regulated by steroid hormones and increases in the later stage of pregnancy. Increased frequency and duration of spike activity in "pacemaker" areas and more extensive, spread of excitation are associated with increase in force of contraction in most species (including human female), the influx of Na^+ appears to play the primary role of depolarization. However, the duration of spike potentials is relatively long, and depolarization is not affected by tetrodoxin, meaning that the participation of the so-called fast Na^+ channels is lacking.

Ca^{2+}that crosses the plasma during excitation is insufficient to cause contraction. It is sufficient, however, to trigger the release of much larger amounts of Ca^{2+} from the sarcoplasmic reticulum .Hence, the availability of extracelluar Ca^{2+} (or the presence of Ca^{2+}-channel blockers) strongly influences the response of uterine smooth muscle to various physiological and pharmacological stimuli; Example of Ca^{2+} – channel blockers include verapamail, nifedipine, diltiazem etc.

The uterus has parasympathetic innervation by way of the pelvic nerve and postganglionic fibres from the inferior mesenteric and hypogastric ganglia respectively. Both α1-(exitatory) and β2- (inhibiting, hyperpolarising) adrenrgic receptors are clearly demostrable in the myometrium of mammal. As in other smooth muscles, the inhibitory effects of β2-adrenergic agonists on uterine contractility are thought to be mediated by cAMP. Examples of B2 agonist are albuterol (salbutamol), ritodrin, terbutaline, etc.

In addition to such factors as endocrinological status, contractile response of uterine smooth muscle are strongly influenced by variables such as the period of gestation, the degree of stretch, and the region of the uterus under investigation. Thus, it is not surprising that there are many conflicting reports of the effects of drugs on this organ.

2.6.4.2 THE HEART

Each minute, the heart of a resting adult pumps about 5 litres of blood, or approximately the person's total blood volume. This works out to at least 7.200 litres/day; this volume of blood weight about 100 times more than the body. Moreover, the rate of pumping may increase to 20-30 litres/min, during strenous exercise. The relentless work of the heart uses 5%-10% of the body's total energy budget.

Regulation of the heart to meet the demands of the body is exceedingly complex; thus it has been difficult to construct mechanism hearts. The heart has a large measure of intrinsic regulation-its basic rhythm is established by its own pacemaker cells, and the force of its contraction is largely self-adjusting. Inputs to the heart from both branches of automatic nervous system (sympathetic and parasympathetic) cause the changes in the activity that accompany physical exertion, excitement, and emotion (Moffet *et al* 1993).

The heart receives both parasympathetic (cholinergic) and sympathetic (adrenergic) impulses, the adrenergic impulses are mediated by the β_1-receptors which influence the heart thus:

SA node ..... Increase in heart rate

Atria ..... Increase in contractility and conduction velocity

AV node ..... Increase in automaticity and conduction velocity

His-Purkinje system ..... Increase in automaticity and conduction velocity

Ventricles ..... Increase in contractility and conduction velocity, and in automaticity and rate of idioventricular pacemakers.

2.6.4.3 THE INTESTINE

The gastrointestinal (Gl) system is a tubular system the surface of which, like the skin and lung surface forms one of the boundaries between the physiological interior and the outside world. There are four major operations of gastrointesinal tract (GIT) and its gland:

a. Transport and mixing of food within the tube system;

b. Secretion of fluid, salts and digestive enzymes;

c. Digestion, and

d. Absorption,

c & d largely take place in the intestine.

The intestine has a length of about 22ft (6.5m) from the stomach onwards to its broadest point of about 35mm ($1_{1/2}$) in width. It is further divided, rather arbitrarily, into the duodenum, jejunum (the longest portion of the intestine, when on death is found empty) and the ileum (the shortest portion of the intestine).

Several receptors are present in the intestine as both cholinergic and adrenergic impulses innervate it. The adrenergic impulses are mediated by receptors such as α_1, α_2, β_1- and β_2-, causing various influences such as decrease in motility and tonicity of the intestine; the α_2-receptors cause contraction of the sphincters while they tend to inhibit secretion.

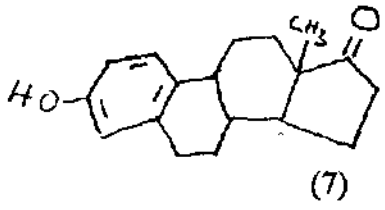
The muscarinic receptors mediate the cholinergic impulses, which are known to increase the motility and tonicity of the intestine, relax the sphincters and increase (stimulate) secretion.

2.6.5 SOME DRUGS THAT AFFECT UTERINE MOTILITY

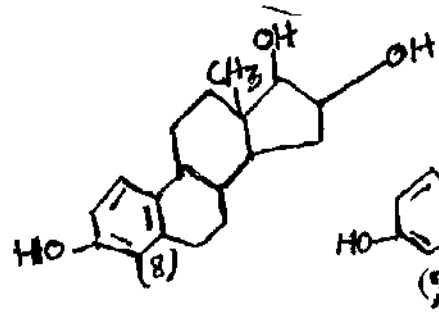
2.6.5.1 THE ESTROGENS(DIETHYLSTILBESTEROL)

Uterine smooth muscle is usually susceptible to endocrine influence, especially that of estrogen. Estrogens are produced by the ovary, placenta and adrenal cortex in the female while in male, the testis and the adrenal cortex. The ovary is the main site of estrogen production under normal conditions. During pregnancy the placenta takes over. The classical estrogens: estrone, 17β - estradiol, and estriol are the three major estrogens in man and frequently designated as E^1, E^2 and E^3 respectively (Sawhney, 1970).

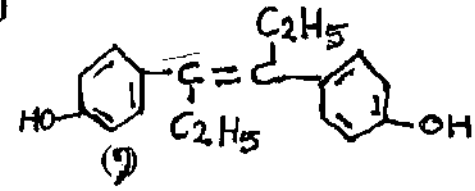
Diethylstilbestrol was the first totally synthetic estrogen to be used therapeutically. Its high potency and low cost (and being cheaper than the natural estrogens or their derivatives) have made it an important estrogen.



(7)



(8)



(9)

The *trans*-isomer of diethylstilbesterol is at least 10 times more active than the corresponding *cis*-isomer.

The biological effects of estrogens on various target organs of the female reproductive system may be summarized in Table 4.

TABLE 4: EFFECTS OF ESTROGENS ON THE FEMALE REPRODUCTIVE ORGANS.

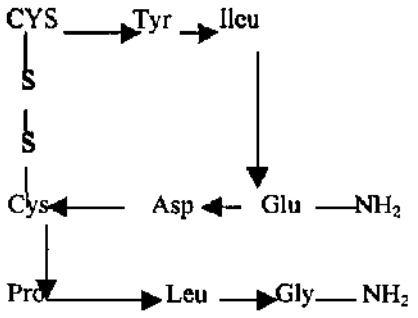
Ovary	Stimulate follicular growth; small doses cause weight increase of the ovary whereas large doses cause atrophy.
Uterus	Endometrial growth.
Vagina	Cornificatioin of epithelica cells, accompanied by thickening and stratification of epithelium.
Cervix	Increased amount of cervical mucus with lowered viscosity, thus favoring sperm penetration and motility.
Pituitary	Small doses promote secretion of pituitary gonadotrophins (supprotive effect); Larger doses cause inhibition as well as general atrophy of this gland.

2.6.5.2 OXYTOCIN

Oxytocics are drugs that stimulate uterine motility. By promoting the contractility of the uterus they hasten labour and are therefore useful clinically in the management of labour, particularly if they are selective for the uterus (Sawhney, 1970).

Oxytocin (9) , a neurohypophyseal hormone, is the main principle responsible for oxytocic, avian depressor and milk-ejecting activities of the posterior pituitary extracts. The effects of oxytocin on the uterus are quite complex and differ among various animals and man. Oxytocin contracts human, guinea pig, rabbit and albino rat uteri. Moreover, the responses of the human uterus vary in non-pregnant and pregnant states and also during the various stages of pregnancy. Oxytocin is most effective in late pregnancy thus the preferred agent in the third stage of labour

(Sawhney, 1970).



(9)

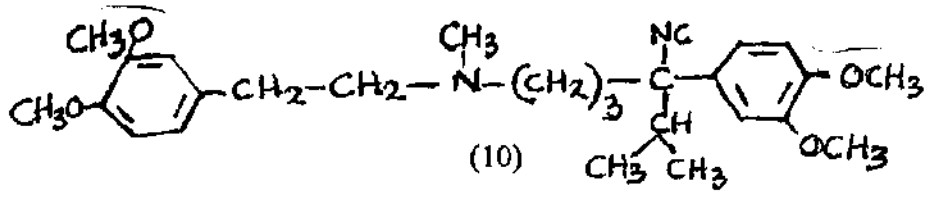
Mechanism of action: The mechanism for translation of receptor binding into increase frequency and force of contraction is poorly understood. Although there is evidence for oxytocin-induced hydrolysis of phosphoinositides (Fuchs. 1987), the marked inhibitory effects of Ca^{2+} channel blockers on contracile responses suggest that release of intracellular $Ca^{2+}$by inositol – 1,4,5- triphosphate plays a relatively minor role. Direct or depolarization-induced activation of voltage- sensitive Ca^{2+} –channels by oxytocin may be of greater importance.

Oxytocin causes the release of prostaglandins in several species, however, it is unclear whether this effect is primary or is a result of uterine contraction.

Oxytocin stimulates both frequency and force of contractile activity in the uterine smooth muscle. With higher concentrations, sustained decreases in resting membrane potential occur. At a threshold concentration where there is no change in membrane potential, Oxytocin initiates spike discharges as well as increase the amplitude. These effects are highly dependent on the presence of estrogen and the immature uterus is resistant (Rall,1990).

2.6.5.3 VERAPAMIL

Hass and Hartfielder (1972) reported that verapamil (10), a putative coronary vasodilator, possessed negative ionotropic and chronotropic effects that have not been seen with other vasodilators such as nitroglycerin.



(10)

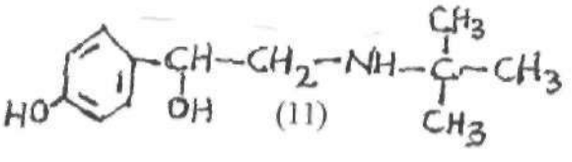
Fleckenstein and others subsequently suggested that the negative ionotropic effect resulted from inhibition of excitation- contraction coupling and that the mechanism involved reduction of the movement of Ca^{2+} into cardiac myocytes (Fleckenstein et al, 1967).

Smooth muscle effects: Most types of smooth muscle are dependent on transmembrane calcium influx for normal resting tone and contractile responses.These cells are relaxed by

the calcium channel blockers. Vascular smooth muscle appears to be the most sensitive, but similar relaxation can be shown for bronchiolar, gastrointestinal and uterine smooth muscles.

2.6.5.4 ALBULEROL (SALBUTAMOL)

Albuterol (11) has a strong β_2- weak β_1- and no α- adrenergic agonist activity, β_2- agonist relax smooth muscle and induce the activation and muscle glycogenolysis through the activation of the adenylcyclase system and increasing the intracellular levels of cyclic AMP. Albulerol, a typical β_2 agonist relaxes both the smooth muscles of the uterus and intestine.



(11)

2.6.6 SOME DRUGS THAT AFFECT INTESTINAL MOTILITY

2.6.6.1 ACETYLCHOLINE

Acetylcholine, (ACh) (12) is a muscarinic – receptor agonist although it is usually a short-lived agent. The muscarinic drugs stimulate gatrointestinal smooth muscle and increase persistalsis, thus decreasing bowel transit time and promoting defecation.

ACh is readily hydrolysed to choline and acetic acid by the enzyme acetycholinesterase at or near the site of liberation of the neurohumor.

$$CH_3 \text{———} CO_2 \text{—} CH_2 \text{—} CH_2 \text{—} N(CH_3)_3$$

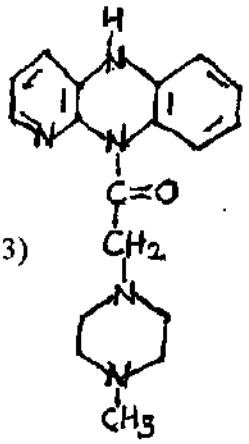
(12)

2.6.6.2 PIRENZEPINE

Pirenzepine (13) (Gastrozepine by Boehringer Ingelhelm) is a tricylic benzodiazepine antiulcer agent comparable to standard antiucler agents such as cimetidine and ranitidine, although with a different mechanism of action, being with a selective antimuscarinic activity for gastric acid secretory cells, known to be mediated by M_1- muscarinic subtype receptor.

Pirzepine selectively suppresses both basal and stimulated acid and pepsin secretion with lesser effect on other muscarinic sites (eg salivary secretion) compared to atropine and other classic anticholinergic agents (Kastrup, 1989).

(13)



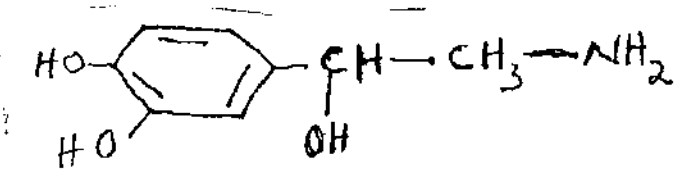
2.6.6.3 ATROPINE

Atropine is a tertiary amine antimucarinic drug with all of the actions, most uses, and adverse effects of antimuscarinic drugs such as decreased GI motility, decrease gastric secretion, dry mouth, drying of the mucous membranes in general mydriasis, loss of accommodation (and *pari passu* tendency to increased intraocular pressure), urinary retention, decreased sweating, tachycardia, etc. Atropine blocks all muscarinic subtype receptors (M_1,M_2,M_3). It competitively antagonises the effects of ACh.

2.6.7 ISOPROTERENOL (ISOPRENALINE) EFFECTS ON THE HEART

Isoproterenol (14) is mainly a β_2-and β_1- agonist but lacks α – activity. The β_1- agonists increase the heart rate, enhance atrioventricular conduction, and increase the strength of the heart beat (positive ionotropic action). These effects are achieved, in part, through the activation of the adenylcyclase and the intermediation of 3', 5'- cyclic adenosine monophosphate (cAMP). In the heart, especially, β_1- agonists also increase calcium ingress and storage , in part, the result of mediation by cAMP (Harvey, 1985).

(14)



CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 *COLLECTION OF THE PLANT*: Striga senegalensis Benth

The flowering tops of the plant Striga senegalensis Benth (family: Scrophulariaceae) was collected between the months of August and November 1997, near the Senior Staff Club, Area-A, Ahmadu Bello University, Samaru Campus, Zaria, Nigeria (Fig.3).



Fig. 3: Collection of Striga senegalensis

3.1.1 AUTHENTICATION OF THE PLANT

The collected plant has been authenticated at the herberium center, Department of Biological Sciences, of the Ahmadu Bello University, Zaria, Nigeria.

3.1.2 DRYING THE PLANT

The plant collected was air-dried under shade and further cleaned from dust particles. The dried was then ground in a milling machine and weighed.

Weight of paper 1.35g

Weight of paper and milled material 40.54g

Weight of milled material ..... 39.19 g

3.2 SOLVENTS AND EXTRACTION

All Solvents were distilled before use in extraction. The solvents- hexane, ethanol and ethlacelate were obtained from BDH Chemicals ltd, Poole, England.

3.2.1. n-HEXANE EXTRACTION

The milled material (39.19g) was extracted with 100ml of n-hexane in a soxhlet apparatus for seven hours. The solvent was recovered by simple distillation and extract concentrated over waterbath.

Observations: The extract had yellow colour with scanty, irregularly shaped and ill-defined crystals, sparingly dispersed in an oily material.

Weight of flask + Extract + beads..... 44.270g

Weight of flask + beads 44.030g

Weight of Extract ..... 0.240g(240mg)

3.2.2 ETHYLACETATE EXTRACTION

The marc above (in 3.2.1.) was extracted with 100ml ethylacetate in a Soxhlet apparatus for six hours. The solvent was recovered by simple distillation and the extract concentrated over waterbath.

Observations: Colour of extract in the flask changed from yellow to pale green as extraction continued. Well defined, irregularly shaped crystals were seen stacked to the walls of the flask. Voluminous precipitate was formed on precipitation.

Weight of flask + extract + beads 52.110g

Weight of flask + beads 50.830g

Weight of extract ..... 1.28g(1280mg)

3.2.3 ETHYL ALCOHOL EXTRACTION

The marc above (in 3.2.2.) was extracted with 100ml of ethyl alcohol for six hours in the soxhlet apparatus. The solvent was recovered by simple distillation and extract concentrated on waterbath.

Observations: The colour in the flask changed from pale green to yellowish brown with continued extraction. Semi-solid fatty materials stacked to flask surfaces.

Weight of flask + extract + beads 45.02g

Weight of flask + beads 42.81g

Weight of extract ..... 2.21g.

3.2.4 DISTILLED WATER EXTRACTION

The marc (in 3.2.3) was extracted with 300ml distilled water for three hours in a 500ml conical flask and again repeated. In each case, the extract was filtered onto a large open porcelain dish and evaporated on the waterbath to its concentrate.

Observations: The dark violet aqueous extract was a waxy, stiff material, which stacked to the dish's surface.

Weight of dish + extract ..... 150 .60g

Weight of dish 141.60g

Weight of extract 9.00g

3.2.5 THE ETHYLACETATE EXTRACT

The ethylacetate extract was considered further for possible isolation as there were well-defined crystals, with voluminous precipitate.

3.3 MATERIALS

Silica gel from M&B Laboratory Chemicals ltd, Degenham, England and silica gel G from E-Merck, Germany were used for the preparation of the column chromatography and thin layer chromatography respectively. Others included the glass column, 40 microscopic slides, glaze cottonwool, glassware, spatulas and test tubes.

3.3.1. PREPARATION OF COLUMN CHROMATOGRAPHY

The wet method of column filling was used. 20g of silica gel was triturated with dissolved ethylacetate extract (in ethylacetate) in a small petri dish and allowed to dry gently under air – conditioner while being strirred from time to time. No heat was applied.

A 40g slurry of the silica gel was prepared in n-hexane (the least polar solvent available) and transferred into the dry column, followed by the dried triturated extract. Thus, the total amount of silica gel used was 60g. Some cotton wool was placed on top of column content to cushion any disturbance due addition of solvent systems.

3.3.2 PREPARATION OF THIN LAYER CHROMATOGRAPHY

An amount of silica gel G was transferred into a conical flask (beaker could well be used) and sufficient distilled water added to make a slurry.

The slurry was uniformly (and thinly) spread on the microscope slide (many slides were used) and allowed to settle before placing in an oven at 105^0C for further drying.

3.3.2 ELUTING THE COLUMN AND DETECTION

The column chromatography was eluted using various solvent systems between two extremes: 100% n-hexane and 100% methanol. Between these extremes were mixtures of solvent systems including 100% ethylacetate.

The detection of eluates was effected with the thin layer plates. A 1:1 benzene/ethylacetate ratio solvent system was used throughout to develop the tlc plates. Spots on developed and air-dried plates were first observed under UV lamp at 365nm and then exposed to Iodine vapour in an Iodine tank. Various fractions were mixed up base on tlc report.

See table 5.

3.3.4. CRYSTALL ISATION

Fractions (19-23), (24-32) and (86-105) had well defined crystals, thus separately crystallized from methanol. Tlc report indicted they had the same R_f values hence were considered the same sample, thus they were pulled together to form sample A .

3.3.5 RECHROMATOGRAPHY

The mother liquors of the fractions in 3.3.4 were mixed up and rechromatographed using a mixture of solvent systems. Various fractions were mixed up based on tlc report.

Fraction (22-30) had crystals and these were crystallized from methanol. It R_f value was found to be similar to that in 3.3.4 which formed sample B.

3.3.6 RECRYSTALLISATION

The products obtained in 3.3.4 and 3.3.5 were pulled together and recrystallized from methanol, then dried and weighed.

Weight of paper 0.2000g

Weight of paper +product 0.2100g

Weight of product ..... .100g(100mg)

3.4 SOME CHEMICAL TEST ON ST-1

A few chemical tests were effected on ST-1

a. To a methanolic solution of ST-1, a few drops of dilute HCl was added. The solution was then boiled and its R_f value compared with the untreated ST-1 . Observation were noted.

b. To a methanolic solution of ST-1 was added a few drops of ferric chloride. Changes were noted.

c. ST-1 solution was spotted on a tlc plate. The developed and air – dried plate was exposed to ammonia vapour instead of iodine. Observation were inferred to.

3.5 INSTRUMENTATION

A few number of instruments were employed to further assist in characterizing ST-1.

3.5.1 MELTING POINT DETERMINATION

The melting point of ST-1 was determined using the Gallenkamp Melting Point Apparatus, No. 889339, England. Observations were noted.

3.5.2 ULTRAVOILET (UV) SPECTROSCOPY

5.0mg of ST-1 was dissolved in 10ml of methanol to produce a stock solution. Serial dilutions were prepared from the stock solution. A 1 in 100 dilution gave good data when ran in methanol alone in a 1cm cuvette, and again ran in the presence of sodium hydroxide (NaOH). The instrument used was SP8- 100 Ultravoilet Spectrophotometer, PYE UNICAM. See fig 4.

3.5.3 INFRARED (IR) SPECTROSCOPY

A few crystals of ST-1 were transferred into a small pestle/ mortar and crushed. 1 drop of Nujol was added to produce a slurry. A very small amount of the slurry was thinly spread on a surface of one of the potassium bromide discs and rubbed together gently. On insertion in the disc's compartment and operation, the recorder showed the data.

ST-1 was analyzed in ATI Mattson, Genesis series FTIR UNICAM. See fig. 5.

3.5.4 ^{1}H-NUCLEAR MAGNETIC RESONANCE(NMR) SPECTROSCOPY

ST-1 was dissolved in DMSO-d_6 and analyzed using amx400 proton-NMR. Details of the parameters on current data, F2- Acquisition and NMR plot are as defined on the spectral sheet. See fig. 6.

3.5.5 ^{13} C-NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

ST-1 was dissolved in DMSO-d_6 and analyzed amx400 ^{13}C-NMR instrument. Details of the parameters on current data,F2- acquisition and NMR plots are as defined on the spectral sheets.See fig. 7.

3.6 THE PHARMACOLOGICAL ASPECTS

3.6.1. THE ETHANOLIC EXTRACT

The ethanolic extract of the plant Striga senegalensis Benth was used for the pharmacolgical investigations. 600mg of the ethanolic extract (EE) was weighed and triturated with three drops of Tween 80 to improve solubility. This was then dissolved in 6m1 of distilled water to produce a 100mg/ m1 stock solution. Serial dilutions were prepared using distilled water as follows:

EE_1 = 10mg 1mL

EE_2 = 1mg/mL.

3.6.2 DRUGS, CHEMICALS AND ANIMALS USED IN THE PHARMACOLOGICAL INVESTIGATIONS.

The drugs used in the investigations were isoprenaline (from Sigma Llaboratory), verapamil (Sigma), diethylstilbesterol dipropionate (M&B), pirenzepine (Sigma), atropine (Sigma), acetylcholine (Sigma) and oxytocin (Ranbaxy, Pakistan).

A 100mg/m1 stock solution of each of the drugs (except diethylsilbesterol dipropionate) was prepared and serial dilutions made from the stock for the investigations.

The chemicals used were mainly in the preparation of the physiological salt solutions needed in the screening. Three different physiological solutions were prepared according to formula

(Crossland, 1980) viz: Tyrode solution for the ileum, Ringer Lock's for the heart and De jalon's for the uterus. The chemicals were obtained from various manufactures. See Appendix II.

Two different species of animals were used. The pretreated female rats with 2ml (equivalent to 20mg) diethylstilbesterol dipropionate were for the uterine investigation. The drugs used in these investigations were verapamil (a calcium-channel blocker), oxytocin (a tocolytic agent) and salbutamol (β_2 . adrenoceptor agonist). The effects of the ethanolic extract were screened on the uterus alone and in the presence of these drugs.

The isolated heart artria of the rat were also used to investigate the effects of the ethanolic extract alone and in the presence of Isoprenaline (a β_1- adrenoceptor agonist).

Finally, the effects of the ethanolic extract were screened alone and in the presence of acetylcholine (a muscarinic –receptor agonist), atropine (a muscarinic – receptor antagonist) and pirenzepine (M_1-receptor specific antagonist) on the isolated ileum of the rabbit.

The animals were killed by a blow on the head and the abdomen (for ulterine horns or ileum) and the chest (for the heart) were opened immediately. Isolated organ was immediately placed in a well aerated petri dish containing the required physiological solution before transference to the organ bath.

3.6.3 THE INVESTIGATIONAL INSTRUMENT

The Isolated Tissue Set – Up Unirecord 7050, Ugo Basile, Italy, was used. The Isolated organ (under investigation) was placed in the organ bath containing the required physiological solution and aerated with the appropriate air. Temperature of 37 ^{0}C was maintained by the

thermoregulator. Responses resulting from drug, extract and extract/ drug were recorded by the unirecorder. New substance was added only when the organ had been washed out four times. Time of contact was maintained at 15 seconds.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 *COLUMN ELUTION AND DETECTION OF ETHYLACETATE EXTRACT.*

Table 5 shows the fractions eluted, volume collected in each case and the thin layer chromatography report.

TABLE 5: COLUMN ELUATES AND TLC REPORT OF ETHYLACETE EXTRACT

Fraction	Solvent system	Vol. Collected	Remark	Tle Report
1-12	Hexane, 100%	10ml	Fr 1-5 yellow 6-8 light yellow 9-12 colourless	No observable spots in fractions 1-12 mixed up solvent system Hex:EtoAc=2:1
13-41	Hex:EtoAc=4:1	10ml	Fr 13-18 colourless 19-20 light yellow 24-32 colourless 33-41 colourless	Solvent system BZ:EtoAc=1.1, Separate spots observed. 19-23 mixed up 24-32 mixed up 33-41 mixed up 13-18 mixed up.
42-53	Hex:EtoAc=3:1	10ml	41-53 colourless	42-53 mixed up
54-73	Hex: EtoAc=2:1	10ml	54-73 colourless	54-73 mixed up
74-85	Hex: EtoAc=1:1	10ml	74-85 colourless	74-85 mixed up
86-105	Hex: EtoAc=1:2	10ml	86-105 pale yellow	86-105 mixed up
106-125	Hex: EtoAc= 1:3	10ml	106-125 colourless	106-125 mixed up
126-140	Hex: EtoAc= 1:4	10ml	126-140 colourless	126-140 mixed up

141-149	EtoAc,100%	10ml	141-149 colourless	141-149 mixed up
150-155	EtoAc:Meoll=1:1	10ml	150-155 colourless	150-155 mixed up
156-164	EtoAc:Meoll=1:2	10ml	156-164 colourless	156-164 mixed up
165-166	EtoAc: Meoll=1:3	10ml	165-166 colourles	165-166 mixed up
167-170	MeoH,100%	10ml	167-170 colourless	167-170 mixed up

Fractions (19-23), (24-32) and (86-105) contained crystals which on filtering, the obtained in each case was crystalised from methanol. They all had similar R_f values indicating the same sample, hence were merged to form sample A.

Their mother liquors, having been combined was rechromatographed.

Table 6 shows the fractions eluted, volume collected and the tlc report of the mother liquors.

TABLE 6: COLUMN ELUATES AND TLC REPORT OF THE MOTHER LIQOURS

Fraction	Solvent system	Vol. Collected	Remarks	Tlc Report
1-15	Hex:EtoAc=4:1	5ml	1-3 colourless 4-15 pale yellow	4-6 mixed up
16-30	Hex:EtoAc=3:1	5ml	16-20 pale yellow 22-30 yellow	16-20 mixed up 22-30 mixed up
31-36	Hex:EtoAc=2:1	5ml	31-36 colourless	No spot with I_2 vapour
37-39	Hex:EtoAc=1:1	5ml	37-39 colourless	No spot with I_2 vapour
40-51	Hex:EtoAc= 1:2	5ml	40-48 pale yellow 49-51 colourless	40-51 mixed up

52-57	Hex:EtoAc=1:3	5ml	52-57 pale yellow	
58-63	Hex:EtoAc=1:4	5ml		
64-70	Eto Ac,100%	5ml	58-70 pale yellow	
71-77	MeOH,100%	5ml	71-73,75-77 yellow	52-77 mixed up

Key:

EtoAc = Ethylacetate

Hex = Hexane

BZ = Benzene

MeOH = Methanol

Fraction (22-30) continued crystals and was crystallized from methanol. The obtained was named sample B.

The R_f values of Sample A and Sample B were compared and found to be the same. Thus, samples A and B are supposedly considered same hence merged.

The product obtained was weighted (100mg) and coded ST-1. It had a pale yellow colour,

4.2 THE MELTING POINT OF ST-1

The melting point is > 360°C. No sharp change was observed,. At this point the colour of the material was ashy.

The high me ting point of ST-1 cannot be explained. However, chemical compounds of high melting poin s do exist, for instance, among flavonoids, alkaloids (e.g. carbazole alkaloids), etc.

The presence of "impurity" can cause discrepancy in melting point reading as well.

4.3. ***THE CI. EMICAL TESTS ON ST-1***

Some of the chemical tests effected on ST-1 are as tabulated. The probable inferences are also made. Se e table 7:

TABLE 7: C HEMICAL TESTS FOR ST-1

TEST	OBSERVATION	INFERENCE
ST-1+few drops of HC1,heated on water bath, then checked on tlc against original ST-1	No spot other than ST-1 was observed	No glycosidic linkage present.
ST-1 in Methano + very dilute solution of ferric chloride	Colour changed from light yellow to deep voilet	Presence of – OH attached to an aromatic ring (phenolic compound)
Developed tlc sp t of ST-1 + ammonia vapour	No colour change on spots.	No more than a – OH phenolic group.

4.4 ***THE U LTRAVIOLET (uv) SPECTROSCOPY OF ST-1***

In methanol al ne: λmax values – 220nm, 276nm and 348nm

In methanol + drop of dilute NaOH: λmax values-214nm, 282nm, and 400nm.

All \max in icate a $\pi \rightarrow \pi^*$ transition type (220nm, 276nm) This type is particularly marked for a omatic compounds. n $\rightarrow \pi^*$ due to \ max 348nm may indicate the presence of C=O.

The increas wavelength of absorption for conjugated molecules, that is, the chromophores present are a jacent may be noticed clearly in the uv spectra of ST-1, suggesting that ST-1 is an aromatic ompound.

In dilute Na H, the shift in λmax to higher (bathochromic shift) tends to support the presence of a – OH g up attached to an aromatic nucleus. See figure 4.

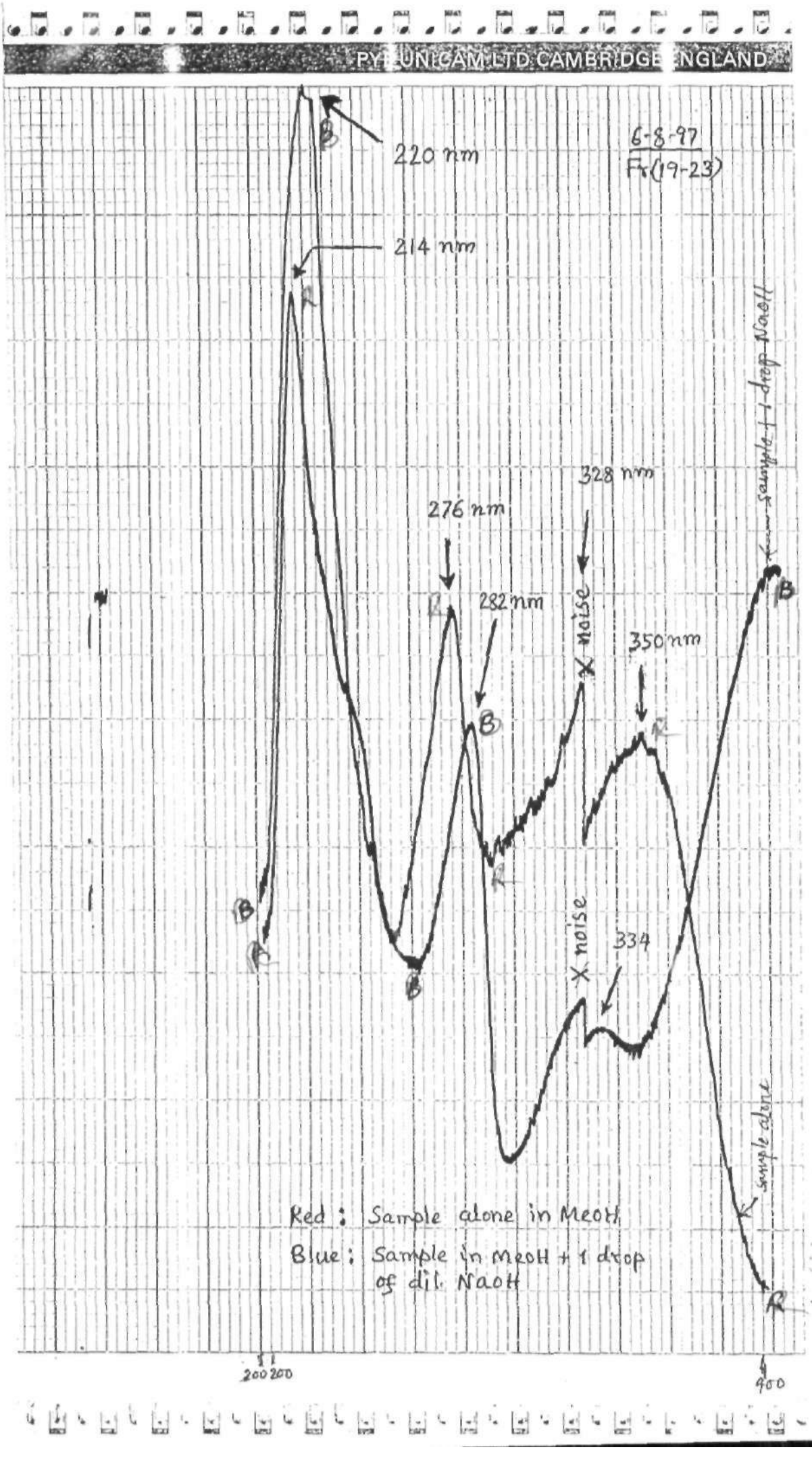


Fig. 4: UV SPECTRA OF ST.1

4.5 INFRARED (1.R.) SPECTRASCOPY OF ST-1

The broad peak at Ca 3303cm^{-1} in this spectrum is indicative of a phenolic –OH, 3083 cm-1 depicts an N-H stretching of an amide (or amine). 829 cm^{-1} and 1031 cm^{-1} are C-- H out-of – plane bending and stretching modes, respectively. 1651cm^{-1} and 1354 cm^{-1} confirms the presence of an aromatic nucleus. See figure 5.

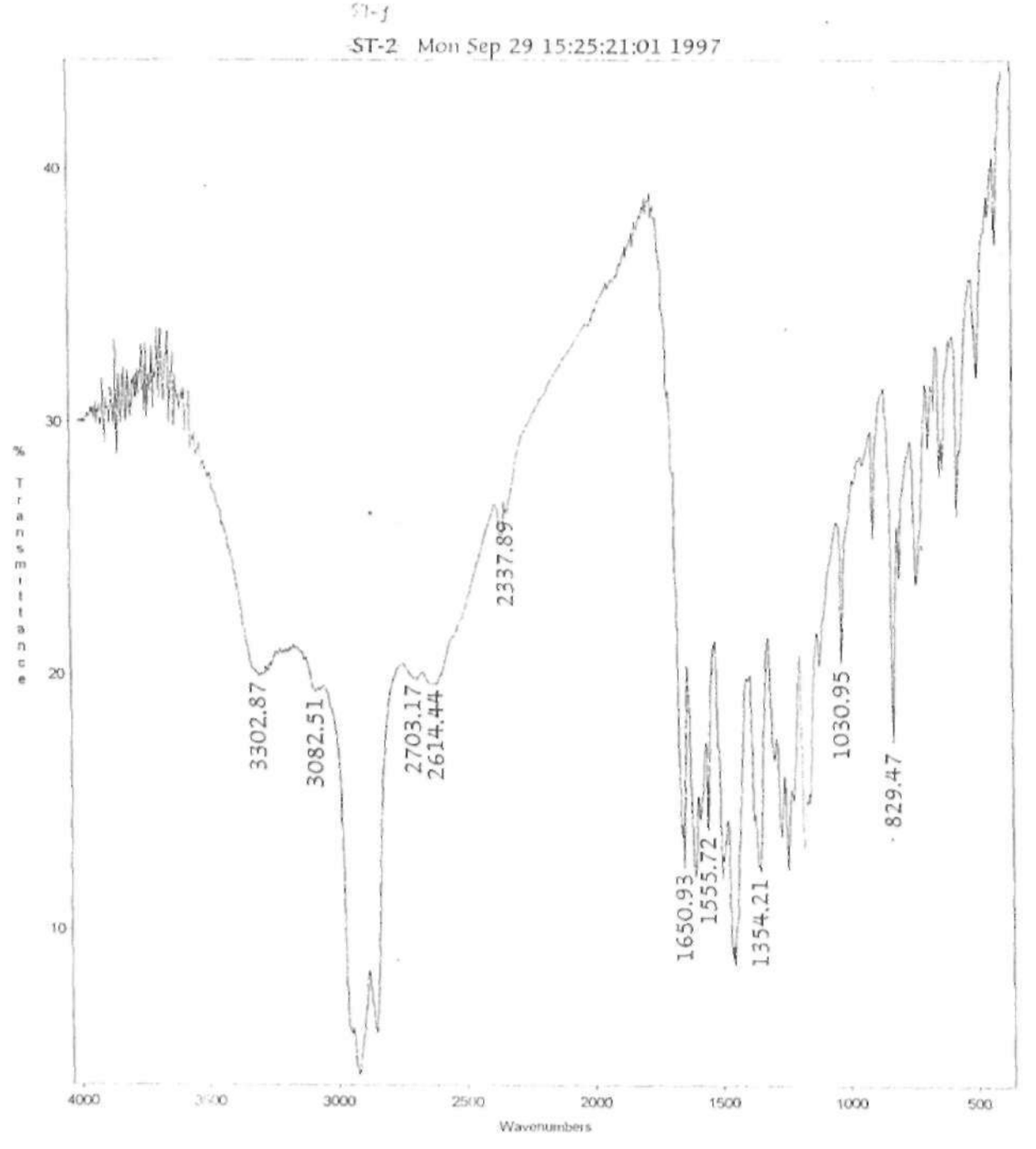


FIG. 5: IR SPECTRA OF ST-1

4.6 ^1H- NUCLEAR MAGNETIC RESONANCE OF ST-1

The values had been recorded in δ-values. There are clearly seven types of hydrogen atoms in the molecule, the number (s) in each group being in the ratio of the integrated intensities.

The low δ- value (12.98) for the single phenolic hydrogen atom reflects the negative inductive effect of the oxygen atom. The δ-value 10.58 suggests a carboxamide

 - NH_2 protons are present. Thus, number of protons present is eleven.See fig.6.

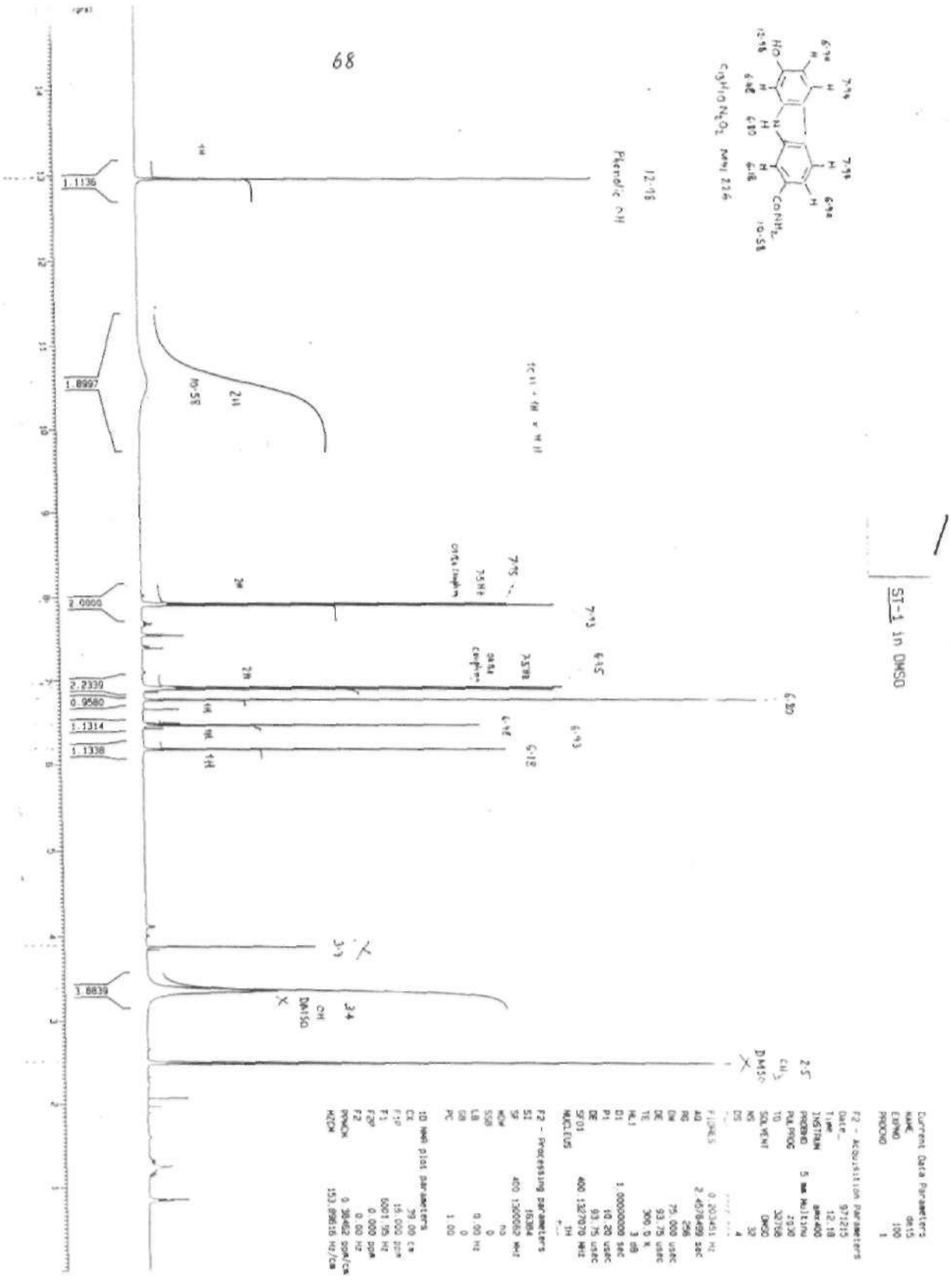


FIG. 6: ^{1}H- NMR SPECTRA OF ST.1

4.7 ^{13}C – NUCLEAR MAGNETIC RESONANCE OF ST-1

The ^{13}C- NMR indicated that there are thirteen carbon atoms in ST-1. However, the environments of these carbon atoms are not well defined. The needed information to determine their environments is not available. Further work needs to be done to ascertain these environments hence structure of ST-1. However, the NMR shows the following δ-values of: 93.99 , 98.85, 102.85, 103.70, 115.97, 121.18, 128.51, 157.32, 161.19, 161.47, 163. 74, 164. 18, 181. 77 (CO from $CONH_2$).See Fig.7

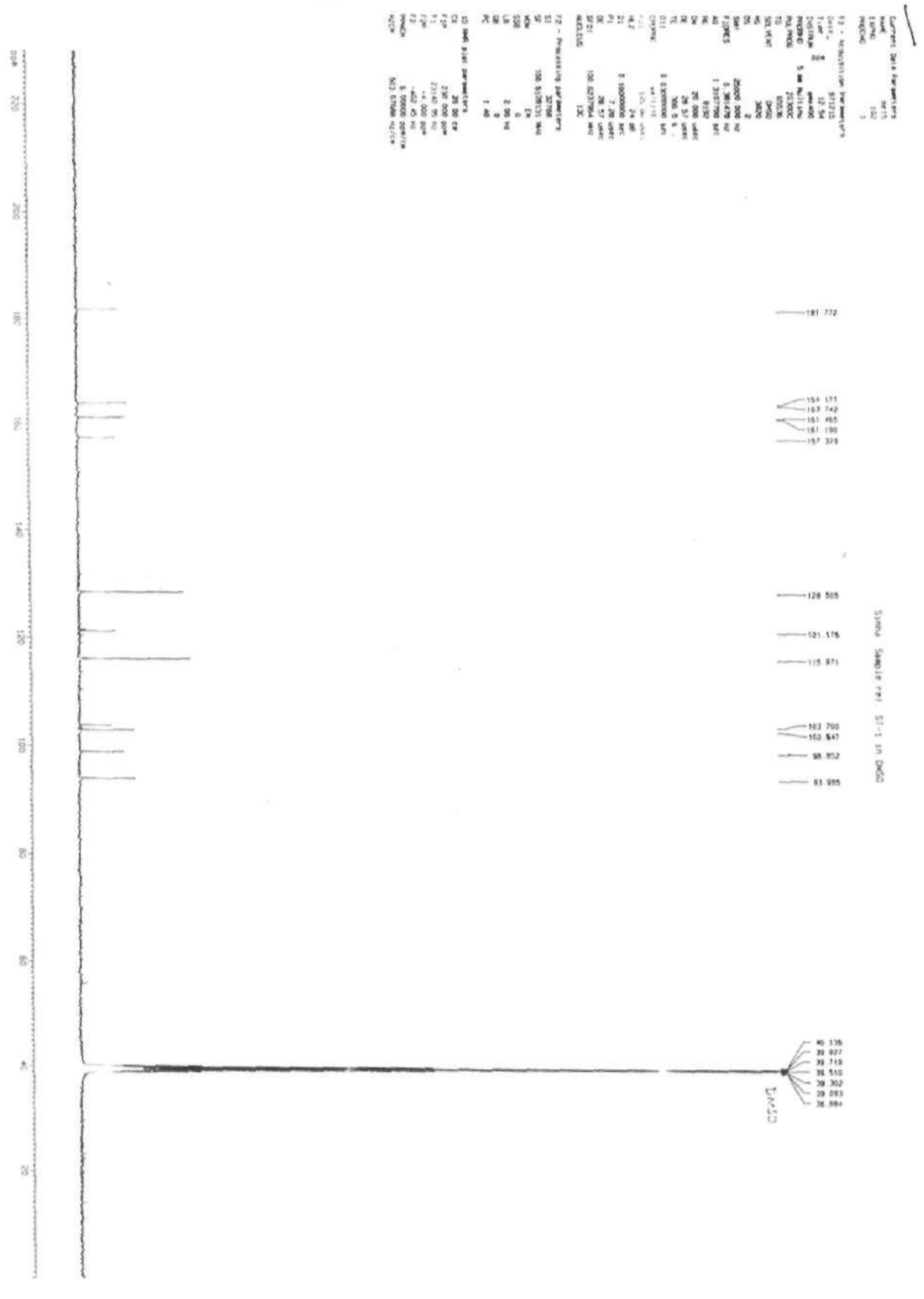


FIG. 7: 13 C-NMR SPECTRA OF ST 1

4.8 THE ASPECTS OF PHARMACOLOGY (discussion)

4.8.1 EFFECTS OF THE ETHANOLIC EXTRACT ,OXYTOCIN, VERAPAMIL AND SALBUTAMOL ON THE ISOLATED UTERUS OF THE NON-PREGNANT FEMALE RAT.

4.8.1.1 OXYTOCIN, ETHANOLIC EXTRACT AND VERAPAMIL

The effects of the ethanolic extract on the uterus of the rat is comparable to that of oxytocin in the presence of verapamil, a calcium – channel blocker, the effects of the extract ceased while that due to oxytocin reduced (depending on the concentration).

Calcium ion is involved in the genesis of the action potentials. In contractile cells, it links excitation to conduction and control energy storage and use. The contractile processes of vascular and non-vascular smooth muscle depend upon the movement of extracellular calcium ions into these cells through specific channels. (Kastrup,1989).

Calcium channel blocking agents, also called slow channel blockers or calcium anatagonist, share the ability to inhibit the movement of calcium ions across the cell membrane. The resultant pharmacological effect on the smooth muscle is depression of both impulse formation (automaticity) and conduction velocity. The resulting effects depends on the direct activity of the drug, reflex physiological responses (primarily β- adrenergic response to dilatation) and the status and type of the smooth muscle (Kastrup, 1989).

A useful working description of how these drugs act is not known in detail. Calcium enters cells through specialized pores in the membrane wall called channels. There are two types of channels:

a. Voltage - Operated (membrane is activated by depolarisation)

b. Receptor – Operated (membrane is activated by the activation of a receptor).

Calcium entry blocking drugs decrease calcium ion entry in both voltage-operated and receptor-operated channels, but the voltage – operated is the more sensitive to drug blockade. Exactly how the entry blockers " plug" the channel is still unknown.

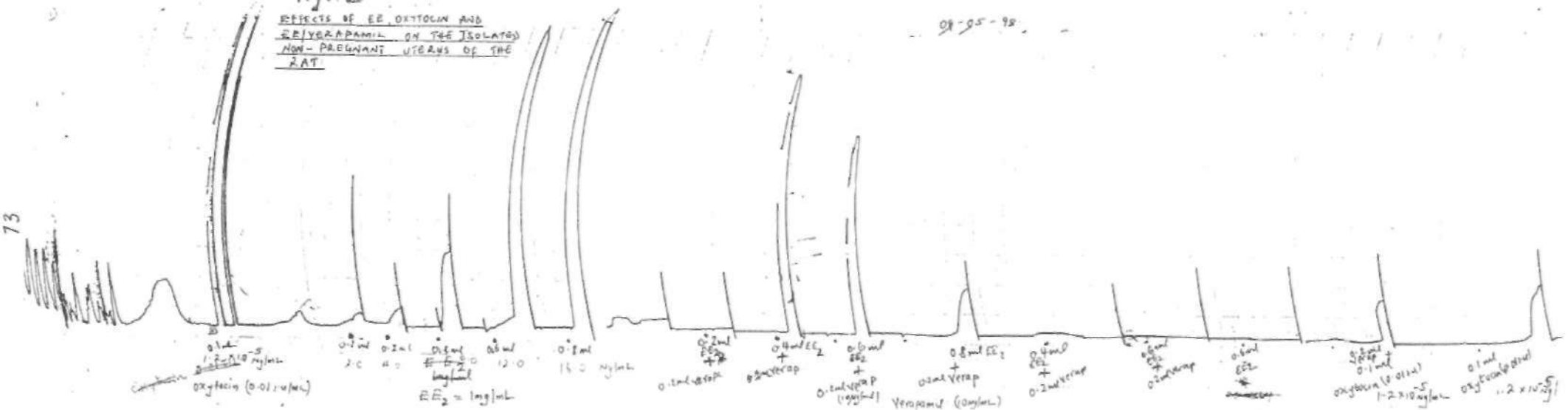
Although all calcium entry-blocking drugs have in common the ability to decrease calcium entry into cells, drug effects vary from site to site and from drug to drug. (Harvey and Withrow, 1985).

Verapamil is able to block pre-and post –junctional calcium channels and usually exhibits negative ionotropic effect and in the presence of a β- adrenergic blocker, both negative chronotropic and ionotopics effects are produced (Kastrup,1989).

The effect of the ethanolic extract was examined in the presence of verapamil (4×10^{-2} ug/ml) and there was total blockade on the contraction and conduction velocity of the isolted uterus of the rat. However, for verapamil together with oxytocin, the decrease was gradual. See fig.8.

Fig. 8

EFFECTS OF EE, OXYTOCIN AND EE/VERAPAMIL ON THE ISOLATED NON-PREGNANT UTERUS OF THE RAT



The indication is that the ethanolic extract appears to exhibit its action more as B- adrenergic blocking.

4.8.1.2 OXYTOCIN, ETHANOLIC EXTRACT AND SALBUTAMOL

The oxytocin has been used to ascertain the sensitivity of the isolated uterus of the female rat. Salbutamol has a strong β_2-, weak β_1-and no α- adrenergic agonist activity. β_2- agonists relax smooth muscle and induce the activation and muscle glycogenolysis through the activation of the adenylcyclase system and increasing the intracelluar levels of cyclic AMP. Salbutamol, a typical β_2- agonist relaxes the smooth muscle of the uterus.

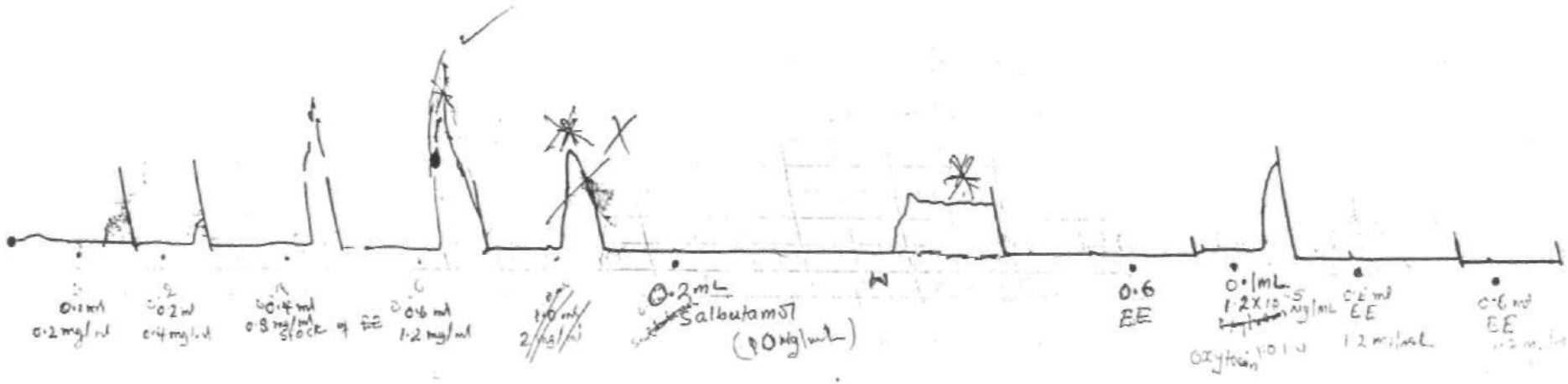
The effects of the ethanolic extract was investigated on the isolated non -pregnant uterus of the rat in the presence of salbutamol (albuterol). There was gradual reduction in the contraction of the uterine smooth muscle. This further suggests that the action of the extract is mediated likely by adrenergic – blockade.See fig. 9.

Fig 9. EFFECTS OF EE AND EE/SALBUTAMOL ON THE ISOLATED NON-PREGNANT UTERUS OF THE RAT.

EE = 100 mg/ml

EE = 10 mg/ml

EE = 1 mg/ml.



The uterine smooth muscle is a complexed structure and can be unpredictable. In addition to such factors as endocrinological status, contractile response of smooth muscle are strongly influenced by variables such as the period of gestation, the degree of stretch, and the region of the uterus under investigation. Thus, it is not surprising that there are many conflicting reports of the effects drugs (and other agents) on the organ (Rall, 1990).

4.8.2 EFFECTS OF ETHANOLIC EXTRACT, ACETYLCHOLINE, ATROPINE AND PIRENZEPINE ON THE ISOLATED ILEUM OF THE RABBIT.

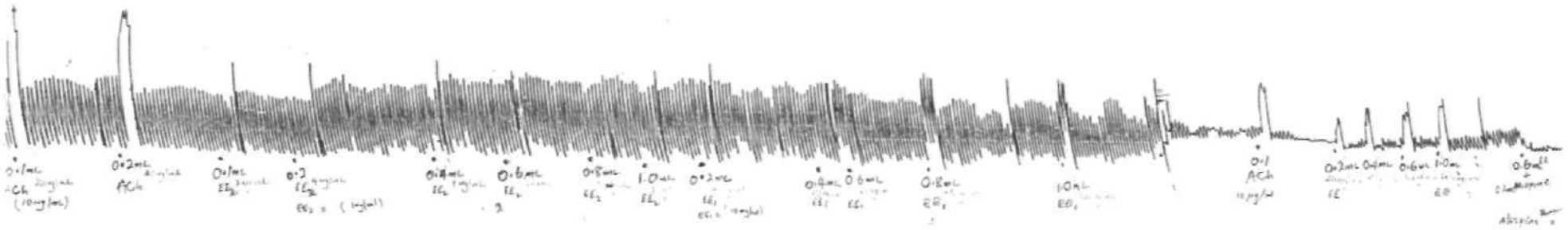
4.8.2.1 EHTANOLIC EXTRACT, ACETYLCHOLINE AND ATROPINE.

The dose- response curve obtained with the ethanolic extract is comparable to that of acetylcholine.

The effects of the ehtanolic extract of the plant was investigated in the presence of atropine (4×10^{-2} ug/mL). The effects of the extract were immediately blocked without recovery. However, acetylcholine effects in the presence of atropine were gradually reduced with recovery.

See fig. 10.

Fig. 10: EFFECTS OF EE, ACh, ATROPINE AND EE/ATROPINE ON THE ISOLATED JEJUNUM OF THE RABBIT



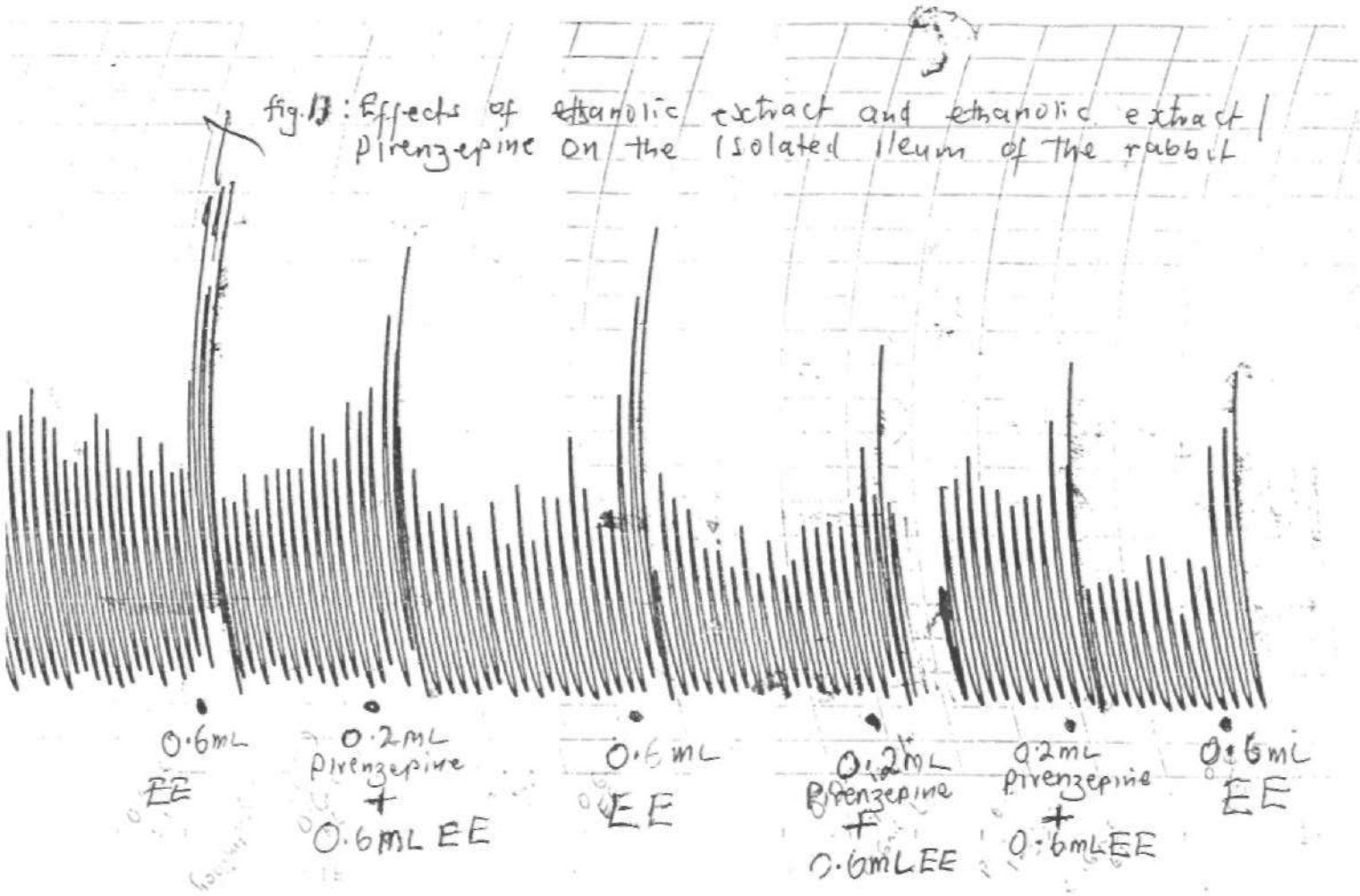
Atropine blocks all muscarinic subtype receptors (M_1,M_2 and M_3) and all its total blockade of the extract's effect can suggest that the extract mediates its action on the ileum via muscarinic receptors.

4.8.2.2 *ETYANOLIC EXTRACT, ACETYLCHOLINE AND PIRENZEPINE.*

Acetylcholine was used to ascertain the sensitivity of the ileum. The effects of the ethanolic extract was investigated in the presence of pirenzepine (4×10^{-2} ug/mL) and there was marked decrease in contraction of the isolated ileum of the rabbit.

Although the dose of the drug was maintained, the increase dose of the extract did not bring the contractility to a halt. See fig. 11.

fig. 13: Effects of ethanolic extract and ethanolic extract / pirenzepine on the isolated ileum of the rabbit



If it is assumed that pirenzepine effectively blocked M_1- receptors, the other known, M_2 and M_3 would have been available for the extract to mediate its action on the intestine, thus block was slow. On the other hand, type of antagonism between the extract and pirenziepine may be described as competitive, that is, dose-dependent.

The extract further indicted it is capable of having a negative effect on the GIT at substantially high doses.

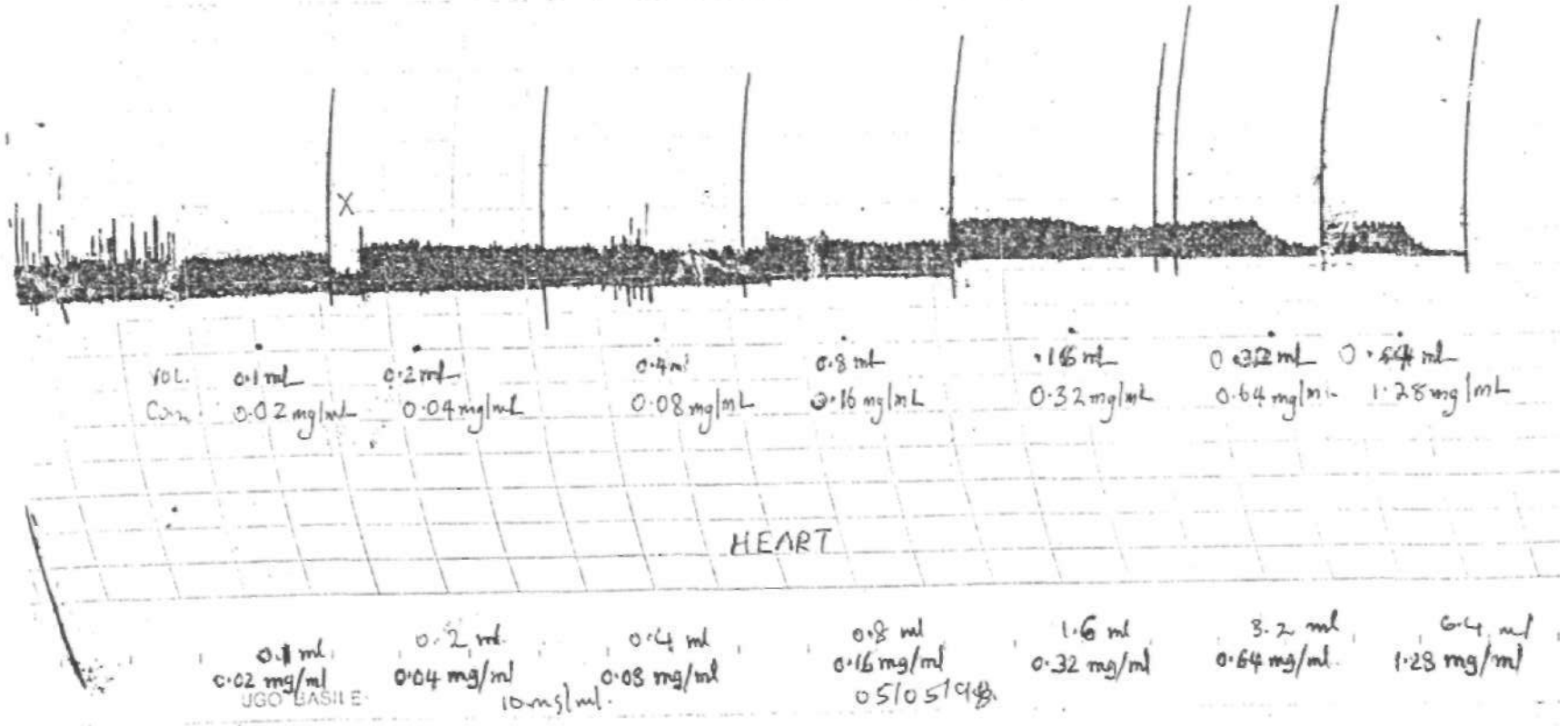
4.8.3. EFFECTS OF ETHANOLIC EXTRACT AND ISOPRENALINE ON THE ISOLATED HEART ATRIA OF THE FEMALE RAT.

4.8.3.1 ETHANOLIC EXTRACT

The heart atria of the rat has been exploited for the investigational studies of the ethanolic extract of the plant Striga senegalensis Benth . a dose-response relationship of the extract was done. There was marked and gradual decrease in the contraction and conduction velocity of the atria. See fig. 12.

Fig. 12: EFFECTS OF ~~EE~~ Ethanolic extract ON THE ISOLATED HEART of A RAT (EE dissolved in Tween-80 and distilled water)

Stock soln: 100 mg EE/ml H_2O followed by a few drops (2-3) of Tween-80, which gave a clear soln



4.8.3.2 ETHANOLIC EXTRACT AND ISOPRENALINE

On subjecting the heart atria to effects of the ethanolic extract , the reduction in conduction velocity and contraction were noticed. On adding the Isoprenaline the increase in contraction and conduction velocity became quite higher than the normal.

Isoproterenol is mainly a β_2- and β_1- agonist but lacks α- activity. The β_1-agonist increases the heart rate, enhances atrioventricular conduction, and increases the strength of the heart beat (positive ionotropic action). These effects are achieved, in part, through the activation of the adenylcyclase and the intermediation of 3', 5'- cyclic Adenosine monophosphate (cAMP).

In the heart, especially, β_1- agonist also increases calcium ingress and storage, in part the result of mediation by cAMP (Harvey, S.C. 1985).

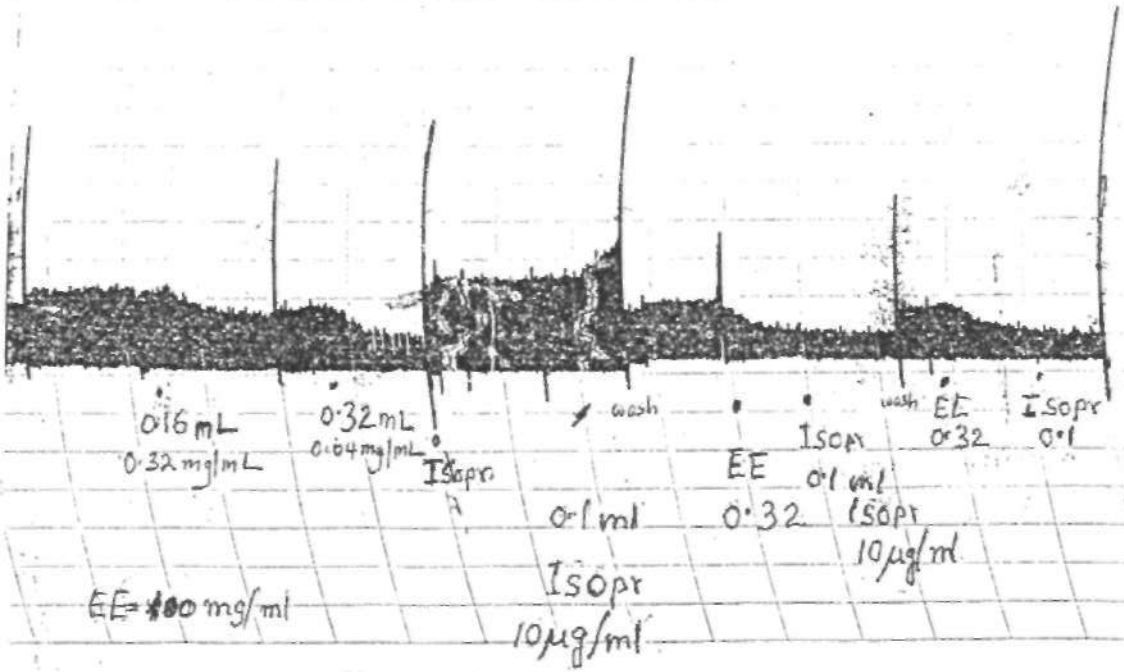
Isoproterenol exhibited these effects on the heart when 2×10^{-2} *ug*/mL was injected.

At a maintained concentration of both Isoproterenol and ethanolic extract, there was no significant change in the contraction and conduction velocity of the heart atria. Thus, actual drug interaction has occurred. See fig. 13.

Ethanol extract

fig 13: EE / ISOPRENALINE INTERACTION ON HEART OF THE RAT. 06-05-98

HEART ATRIA



4.9. CONCLUSION

4.9.1 PHYTOCHEMISTRY

The Spectral information obtained on ST-1 suggests that it probably have the proposed structure below. Fig. 14.

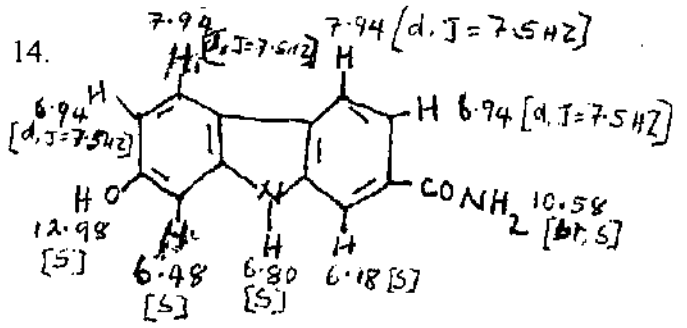
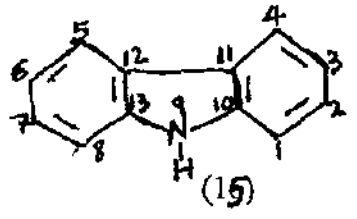


Fig.14: Proposed Structure of ST-1

The proposed structure of ST-1 indicates it is a carbozole alkaloid.

Carbozole alkaloids are extremely weak bases which exhibit strong fluorescence and long phosphorescence on exposure to uv They are insoluble in water and dissolve in conc.H_2SO_4 without decomposition. Their N- potassium salts are formed when fused with KOH.

Carbazole (15) was discovered in cool far by Graebe and Glaser in 1872. The major development in the chemistry of carbozoles up to 1920 took place in Europe due to its importance in the European dyestuff industry (chacraborty, 1977).

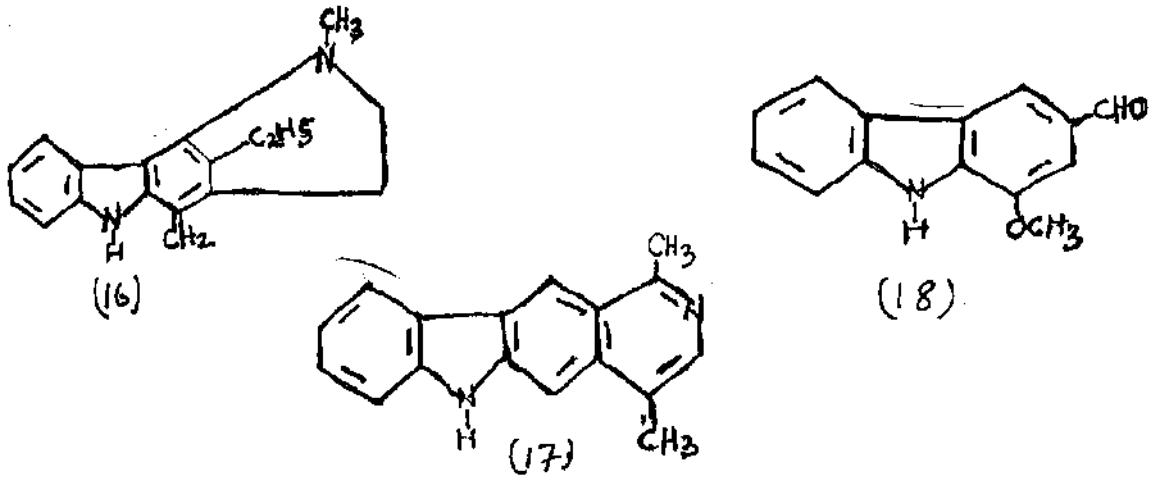


(15)

Carbazoles were unkown as plant products except as degradation products of strychnine and aspidospermine until the discovery of ulein (16) and olividine (17) (Marini-Bettolo et al, 1959) these two novel pyrido-carboles from the genus Aspidosperma (family: Apocynaceae constitute a novel group of indole bases lacking the conventional tryptamine fragment.

Murrayamine (Chakraborty et al, 1965), the first member of the carbozole alkaloids without any basic nitrogen and distinctly different from the pyrido-carbozoles was reported in 1965.

Carbazole alkaloids are neutral or phenolic but this group of nitrogenous plant constituents originates from anthranilic acid like many alkaloids of Rutaceae. This justifies its inclusion in the alkaloidal group. Although the carbozole alkaloids are few in number, there is considerable structural complexity. A review of carbazole alkaloids was first presented by Kapil (1971).



Carbozole alkaloids were first reported from the stem bark of Murraya koenigi Spreng, family Rutaceae (Kirtikar et al, 1933) which is the richest source of phytocarbozoles so far reported. Subsequently, they were isolated from other genera.

Today, it is has been (supposedly) Isolated from Striga senegalensis Benth (family: Scrophulariaceae).

4.9.2 PHARMACOLOGY

The effects to the ethanolic extract of Striga senegalensis Benth have been known on the uterus. The mechanism of action appears to be mediated through B- adrenergic blocking, which is subsequently related to the interference of calcium influx and / or calcium release from intracellular store.

The effects of the ethanolic extract are marked and opens for further investigation.

Clue of its mechanism of action on the ileum appears to be known as the extract certainly can have pronouced effect on the GIT. The extract appears to have similar receptor site as acetylcholine.

Thus, we cannot afford to ignore the emerging chemistry, pharmaceutical and pharmacological importance of Striga senegalensis, although a worldwide weed.

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APPENDIX I

BIOLOGICAL CHARACTERISTICS OF WEEDS AND THEIR EXRESSIONS		
PRINCIPAL CHARACTERISTIC	MORPHOLOGICAL EXPRESSION	PHYSIOLOGICAL EXPRESSION
Undesirable location	Objectionable Size	Ability to thrive Almost everywhere
Competitive; aggressive	Divers forms of reproduction	Rapid establishment, growth under adverse conditions
Wild; rank growth	Sizeable plants covering large areas	Rapid growth; highly efficient use of habitat, nitrophiaus
Persistence; resistance	Diverse forms of propagules; structural protection from herbicides.	Survival of plants fragments; biochemical resistance.
High reproductive capacity	Structures aiding dispersal; heterocomorphy; apomixes.	Varying levels of seed dormancy; food attractants.
Large populations	High seed numbers; dispersal near mother plants	Growing thickly; not anatagonistic no teletoxicity.
Useless, and undesirable	Thorns; trichomes	No Known contemporary value (s)
Harmful to man, animals, crops.	Thorns; indigestible fibers	Contains poisons; secret teleoxins; parasitic habit.
Not sown; spontaneous growth	Propagules small, obscure, buried in soil.	Dormancy; sensitive to tillage.
Unsightly	Flowers and foliage not attractive	Rank growth and establishment; replacing desirable forms.

APPENDIX II

DESCRIPTION OF SOME PHYSIOLOGICAL SALT SOLUTIONS

S/N	SALT	TYRODE'S (g)	De JALON'S (g)	RINGER LOCK'S (s)
1.	Sodium Chloride (M & B)	40.00	45.00	45.00
2.	Sodium Bicarbonate (Dalton)	5.00	2.50	1.00
3.	D. Glucose (BDH)	5.00	2.50	5.00
4.	Sodium Dihydrophosphate (E.Anglia)	0.25	-	-
5.	Potasium Chloride (BDH)	1.00	2.10	2.10
6.	Magnesgesium Chloride (Vieckers)	0.50	-	-
7.	Calcium Chloride Dihydrate (BDH)	1.32	0.40	1.60
8.	AERATION	AIR	95%O_2 5% CO_2	O_2

N.B Salts are dissolved in distilled water to make 5 litres solution for each physiological solution.