PHARMACOGNOSTIC STUDIES AND INVESTIGATION OF ANTI-INSECT ACTIVITY OF THE STEM BARK OF STEGANOTAENIA ARAL1ACEA Hochst (F. Umbelliferae)

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTER OF SCIENCE (PHARMACOGNOSY) IN THE DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

BY

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CERTIFICATION

This thesis entitled "THE PHARMACOGNOSTIC STUDIES AND INVESTIGATION OF THE ANTI-INSECT ACTIVITY OF *STEGANOTAENIA ARALIACEA* Hoscht" F. umbelliferae by Mujitaba, Sulaiman Abubakar meets the regulations governing the award of the degree of **Masters of Science in Pharmacognosy** of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DECLARATION

I hereby declare that this thesis has been composed by myself and that it is a record of my own research work. It has not been submitted in any previous application for a higher degree. All quotations are distinguished by quotation marks and the sources of informations are specially acknowledged by means of references.

M. S. ABUBAKAR

DEDICATION

TO

MY WIFE SAFIYA FOR HER ENDURANCE, PATIENCE AND

PERSEVERANCE AND TO OUR TWO NATURAL PRODUCTS

SAKINA AND ABDUL-JALAL

ACKNOWLDGEMENT

I wish to express my sincere gratitude to Dr. E.M. ABDURAHMAN for his help, guidance and unending constructive criticism throughout the course of this work.

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ABSTRACT

This study deals with the phamacognostic characters and anti-insect activity of Steganotaenia araliacea Hochst family umbelliferae, a family not otherwise found in tropical West Africa. This plant usually occurs as a shrub in tropical Trees. The stem bark of (his plani is used locally in insect pest control practices. The stem bark is slightly curved and breaks easily with short fractures leaving smooth surfaces. The bark was collected from the plant by making longitudenal incissions on the stem.

S. araliacea selected from among ten plants used traditionally in insect pest control because of its singnificant larvicidal activity against the larva of Culex quinquefasiatus, with highest activity exhibited by the

n-Hexane extract with LC50 = 6 lug/ml comparable to 5.7ug/ml of nicotine a contact insecticide

A bioassay-guided chromatographic studies of the

n-Hexane extract of S. araliacea reveals that the compounds responsible for activity lies in the non polar fractions. By a combination of column chromatography and preparative thin layer chromatography compound "a" the substance responsible for activity was isolated. This was shown to have an appreciable activity against adult Culex quinquefasciatus with LC50 56.2ug/cm^2. The compound has no growth regulating effect on all stages of C. quinquefasicatus life cycle but adults which emerged from a medium containing the compound were smaller than those from the controls. Chemical and spectroscopic studies were earned out on the active compound "a", it was found to be steroid or terpenoid.

A preliminary macroscopical and microscopical examination of S. araliacea were carried out to establish phamiacognostic parameters for the identification of the plant.

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CHAPTER ONE

1. INTRODUCTION

1.1 Relationship Between Insects And Man

The ways in which insects affect man are many and varied. Those insects that are in some way harmful are referred to as pests, but as important as they are, they make up only a small minority of insect species. Among the millions of insect species available, only a few thousands qualify as pests. Many insects are distinctly useful to man. The bulk of them, however fall into neither being distinctly harmful nor beneficial, however, these groups are important components of the ecosystem.

1.1.1 Beneficial insect activities

There are three main ways in which insects are beneficial to man.

a) NATURAL ENEMIES OF PEST SPECIES

Although insects like other organisms are limited by environmental factors, often the main regulators of population are other insects which attack and feed on them as parasites and predators. Almost all insects are affected this way to some extent, some much more than others, so that without

natural insect enemies most pest problems would be much more severe and many species that are now unimportant would qualify as pests.

b) POLLINATORS OF CULTIVATED PLANTS

Many cultivated plants are dependent on insects for pollination and effective crop production. This applies virtually to all fruits and also to many of those plants which are grown for their seeds. Many other crops also require insect pollination for seed production. Naturally occurring insect population brings about some degree of pollination but often this is quite inadequate for practical purposes. In such a case it is necessary to supplement natural pollinators with colonies of insect specifically bred for that purpose, e.g. lucerne and other species of bees can be managed to improve seed yields.

c) PRODUCERS OF USEFUL MATERIALS

Many insect derived products are important in human economy. Honey bees have been managed (in agriculture) for the production of honey and bees wax, silk worm for the production of silk and other scale insects for the production of shellac and cochineal (useful in making capsule shells of some drugs) (Atkins, 1978). In addition some insects are eaten by man, they are nutritionally valuable and can enliven poor and restricted diets.

1.1.2 **Harmful insect activities**

Although the number of insect species that are harmful to man are very limited, their activities have major impacts on human welfare. These harmful insects are usually referred to as Insect-pests. There are 2 main harmful effects of insects.

a) EFFECTS OF INSECT IN AGRICULTURE AND HORTICULTURE

Insects may cause outrageous loses of food crops in field and during storage after harvest. In a world in which many people are hungry, the consequence of food loss due to insect is very significant. Since such insects are claiming more than their fair share, there is a great urge to control them by using convenient pest control procedures.

Some plant feeding insects are important not because of the amount of food they eat but because they serve as vectors of many plant diseases thereby reducing the quality and crop yield.

Livestock farming is also under serious threat by biting flies, blow-flies, lice, ticks etc. These insects reduce growth rate, lowers productivity and may cause significant mortality of animals, slaughter skins and hides may be of inferior quality. In addition these insects may act as vectors of many domestic animals diseases.

b) Insects as house hold and industrial pests

Insect pests attack stored food and other house hold items such as clothes, furniture etc. They are aesthetically offensive, their simple presence can constitute a lot of nuisance in the house and industries as they are generally seen as potential health hazard. In industries they may cause significant damage to raw material and finished product especially during storage, thus a lot of money may be spent in combating their menace and so may reduce profit.

Insects also act as vectors of many human diseases such as yellow fever, malaria, elephantiasis etc.

1.1.3 Neutral insect activities

Many insect species are infact not directly beneficial nor harmful to man, but this does not mean to say that they are not important in the total realm of nature. Some of these neutral species are for instance amongst the early colonisers of dead plants, and remains of animals assisting in decay and recycling in the environment; others form the major links in the food chains of mammals, bird, freshwater fish. Although insects are small, they make up in their numbers to such an extent that their total biomass exceed that of any other animal group in many natural ecosystem. Neutral insects are therefore important component of worlds major ecosystem.

1.2 Utilisation Of Anti-insect Activity In Insect Control

1.2.1 Classification of anti-insect activity

There are many ways of using anti-insect activity in insect control. Anti-insect properties are those activities which when directed towards insect are detrimental to some normal behaviours of insects. These effects may lead to death or expulsion of insect from an area. These effects are categorised into:

a) **INSECTICIDES** - When the effects lead to the death of insects while causing minimal effects on other organisms. Some insecticides are applied as fumigants, others must be ingested to kill and a number act as contact poisons. There are 3 classes of insecticides based on their origin and source.

i) *Inorganic Insecticides* - These are inorganic substances including arsenates, fluorides, sulphur, Paris green etc. They are now obsolete.

ii *Synthetic Organic Chemicals*: These are the most popular insecticides and include organochlorines such as DDT dialdrin, lindane, the organophosphates like parathion, malathion and paraoxon and the carbamates such as aldicarb and carbaryl.

These synthetic chemicals give rise to many environmental problems due to persistence of their residues on plants, animals and

the environment which we live in (Davies, 1973).

iii *Naturally occuring insecticides*: These have their origin from natural sources. These sources include:

1. **MICROBES:** Such as fungi (mycohemical insecticides) that produces piericidin and dextruxin e.g. from *Metarrhizum anisopliae* (Wright *et al*, 1982) products such as the toxins from *Bacillus thurigiensis* are effective against the larvae of many *Diptera* and *Lepideptera*.

2. **ATHROPODS:** Arthropod toxins for biting and stinging are effective against some insects. Venoms from the insectivorous *Hemiptera, Diptera, Hymenoptera Neuroptera* and *Coleoptera* produces insecticidal venoms. Male beetles produce monoterpenoids called cantharidins which act as nerve poisons to many insects.

3. **PLANTS:** These contain many insecticidal secondary metabolites like pyrethrins, Nicotine, Rotenone, insecticidal alkaloids, terpenoids and flavonoids. For very long period farmers in Africa, Asia and Latin America have been using plants to control insect. This they use in the form of crude formulations, semi-purified extracts and dry powders or dried whole plant part. Plant derived insecticides are usually much safer, biodegradable and environmentally safer than other forms of insecticides. Advances in techniques of extraction, isolation and

analysis have led to the discovery of many new compound. However, the knowledge of how these affect insect is only beginning to advance (Jacobson, 1989).

b) ***INSECT REPELLENTS***: These are chemicals which cause insects to make oriented movement away from its source. Plants, plant extracts and chemicals that have repellent properties are usually strong smelling, odouriferous and pungent. There are many synthetic insect repellents available. However, their presence in plants is parallel with the insect defences. example of repellents are formic acid, Hydrogen cyanide,

β-pinene, β-selenin and Dendrolysin all found to be present in plants.

c) ***INSECT ATTRACTANTS*** These are chemicals which cause insects to make oriented movements towards source. Chemical communication among insect is becoming evidently an important means of attracting pollinators of plants. In addition to synthetic chemical attractants, plants provide the most reliable source of Certain secondary metabolites attractants are found in both plants and insects. Floral perfumes are very important in the attraction of bees, flies, carrion beetle, moth, hawk moth and butterflies (Baker and Hurd 1968). There are some structured relationship between flower odourants and some insects attractants. Example of attractants are linalol, myrcenol, β-pinene, benzaldehyde, vanillin. There are three types of attractants:

i *Feeding attractants* - When the stimulus guides the insects to its food. All insects have feeding attractants.

ii *Sex attractants* - when the stimulus leads to sex. All types of insect shave specific sex attractants.

iii *Trail attractants* - when the stimulus (odour trail) makes members of the species to follow a trail to and from nest and their food sources. This is usually present in social insects such as bees, ants termites.

d) ***INSECT ANTI-FEEDANTS***: (FEEDING DETERRENTS)

These are substances which prevent or limit the feeding habit of an insect on the source of the compound. A wide range of substances have anti-feedant properties example terpenoids, alkaloids, quinones and flavonoids.

e) ***INSECT GROWTH REGULATORS*** (IGR'S)

The growth and development of insect is under the influence of two type of homones; juvenile homones (JH), and Molting homones (MH). These homones control the process of metamorphosis

1. JUVENILE HOMONES: These are produced by special glands, the copora alata associated with insect's brain; these are structurally terpenoids. They produce in conjunction with molting hormones instars of larva of insect. They act in a negative fashion, their

continuous presence suppresses metamorphosis but just before production of instars their concentration markedly falls. An insect like cockroach can produce as much as five instars in one metamorphosis, some insects as little as two instars. There are analogues of juvenile homones, from synthesis or plants. A number of plant contains juvenile homones and their mimics, principally JHI, JHO, JHII, JHIII, juvabione, Juvocimene 2 and Juvadecene. (Fig 1.2)

ii) MOLTING HOMONES: This is produced in the fore part of insect thorax (prothoracic gland) and released into the insect blood (haemolymph). Between moults the concentration of molting hormones are low but just prior to moulting it is released in large quantities. Moulting hormones are steroids usually called ecdysones, they cause resorption of old cuticle, hardening and thickening of the new one. Plants are important sources of steroids, those that have molting hormone activities are referred to as phytoecdysteroids of which there are many naturally occurring active forms.

1.2.2 **Anti-insect Substances In Pest Management**

Pest management is an advanced practice aimed at achieving appreciable effect on insect while minimizing loss due to the pest and a little or preferably no effect on the ecosystem. It is therefore a collection of various pest control practices. During the past 60 years vast improvement have been

made in insect control with the discovery of dichlorodiphenyl trichloroethane. (DDT) between 1936-1937, and other synthetic chemicals (Ordish, 1976). In the beginning the effect of these chemicals on the environment have been neglected until significant residue, were found to be present in both mammalian and plant tissues and the accompanied serious health and environmental hazards. (Robinson and Roberts, 1967). The practice now is to incorporate into pest management programmes insecticides or chemicals with less residual effect on the environment as such substances like repellents, attractants, growth regulators and anti-feedants have become important ingredient of pest management practice.

a) Use of insecticides in pest control

There are a variety of insecticides to choose from, however the choice is based on the following criteria:

i. Reliability: For an insecticide to be considered effective, it should kill a maximum number of insect pest. It should therefore reduce the population of viable insects to inappreciable number.

ii) Selectivity: The insecticides should have restricted activity on a single specie of insect, however, this property is very rare in conventional insecticides.

iii) Human Safety: Maximum kill is required for insects while causing very negligible untoward effects on the human and the environment.

iv) Rate of Use: As insecticides are applied to cover large areas, better insecticides are those that are applied only a minimal number of times to achieve the desired effect. Those that have to be applied several times makes the procedure cumbersome, ineffective and extremely expensive.

v) Economy: The insecticides should be cheap, effective and easy to apply, requiring minimum equipment for application, storage and handling.

b) Use of repellents in pest control

Insect pest damage may be minimised by the use of repellent as this do not necessarily kill them. However, most repellents are used in pest management together with some cultural and traditional practices in the control of crop pest. Repellents are now important in minimising the effect of most household pest like mosquitoes. Though mosquito repellents are now popular, their use are limited because they may not significantly alter the population of mosquitoes.

c) Use of attractants in pest control

These chemicals have very limited use in insect pest control, they are mainly used in luring insects to traps a process aimed at reducing the population of pests attacking crops or animals. Although significant number of insects may be attracted to the traps, the quantity may not be sufficient to alter the course of pest attack, they are mainly used together with other pest control procedures. The use of attractants is limited mostly to sampling of pest attacking crops for experimental purposes.

d) Use of anti-feedants in pest control

Anti-feedants are now becoming important ingredient of pest management. They are certainly effective in preventing significant damage to crops. The use of neem and neem products as anti-feedant on many crops have been well documented (Jacobson, 1988). Although anti-feedants are effective in pest control, they are usually applied in large quantities or require frequent application for any significant effect. It is certainly less expensive and safer to use anti-feedants on crops than insecticide as most anti-feedant are the same secondary metabolites found in the plants.

e) The use of insect growth regulators (IGRS) in pest control

IGRS in small concentration are able to arrest embryonic development of insect with lethal consequences. They are now opening new and very

promising avenues of pest control. Juvenile hormones act by causing abnormal supernumery metamorphosis instars arising may lack some vital insect structures. Molting hormones acts by producing moults (larva and pupa) which are smaller, thinner and with weaker cuticle, these moults rarely produces adults and when they eventually does, the adult is extremely weak and unable to support itself. The combination of juvenile hormones and molting hormone may therefore serve as a reliable armoury in pest management. A lot of research on the consequences of IGRS on Different insects is going on. The advantage of using IGR is that they are quite safe in the environment, usually cheap and small quantities are required for massive effects, there is therefore a lot of hope on this chemicals. They are present in plants in massive quantities and their extraction and isolation are simple, cheap and plants containing them are abundant. (Robins *et al* ., 1968).

1.3 Useful Plants In Africa For The Traditional Control of Insect pests.

In tropical Africa plants have been used for a long time for insect control both in household and crop protection practices. Irvine, 1961 had cited a number of plants used traditionally in insect control in addition to a number of plants mentioned by Dalziel, 1955. (Table 1)

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Table 1: Plants used in West Africa for the Traditional Control of insect pests (Dalziel, 1955)

PLANT	PLANT PART	METHOD OF APPLICATION	REMARKS
1. *Adansonia digitata* Bombaceae	Pulp	The pulp burns with an irritating smoke used by the Fulanis of Northern Nigeria to drive off flying insects from their cattle	Repellent properties
2. *Balanites aegyptica* Zygophyllaceae	fruits, seeds and bark	The seed is incinerated in rooms to drive away insects	Repellent properties
3. *Banisteria leona* Malpighiaceae	Seeds, fruits and leaves	Used as a salve on the head to kill lice	Insecticidal properties
4. *Berlina glandiflora* Caesalpiniaceae	Wood	A gum exuding from the bark is repellent to termites	Repellent properties
5. *Boswellia dalzielli* Burseraceae	Bark	The fragrant resin from the bark is used for fumigating rooms and clothings to remove flies, mosquitoes etc.	Repellent properties
6. *Calotropis procera* Asclepiadaceae	Leaves	The leaves is used in Senegal to destroy fowl lice	Insecticidal properties
7. *Clausena anisata* Rutaceae	Whole plant	The dried plant is believed to drive off mosquitoes	Repellant properties
8. *Carica papaya* Caricaceae	Whole plant	It is planted around dwellings to prevent mosquitoes entering rooms, the few that pass this defense are in a state of vertigo and exhaustion.	Repellent properties

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9. *Cyperus articulatus* Cyperaceae	Rhizomes	The dried rhizome is incinerated in rooms to drive off mosquitoes	Repellant properties
10. *Grewa molis* Tiliaceae	Whole plant	When soaked in water used for washing hair by women to remove lice.	Insecticidal properties
11. *Guiera senegalense* Combretaceae	Leaves and twigs	Burnt in cattle pens and horse stables to drive away insects.	Repellant properties
12. *Hyptis suaveolens* Labiatae	Aerial part	The strongly scented plant is burnt in rooms to drive away mosquitoes	Repellent properties
13. *Mundulea sericea* Papilionaceae	Bark and roots	Used generally as a household insecticides.	Insecticidal properties substantiated by the presence of rotenoids.
14. *Oldfieldia africana* Euphorbiaceae	Bark and leaves	The bark boiled or leaves powdered and mixed with palm oil is used to make a salve	Insecticidal properties
15. *Psorospermum guinense* Gutliferae	Bark	Bark is boiled in water and mixed with seeds before planting to kill insects and birds. When the bark extract is mixed with oil, it is used to anoint the skin of animals to keep flies away.	Insecticidal properties.
16. *Ricinus cuminis* Euphobiaceae	Seed oil	In South Africa the seed oil is mixed with kerosine as a cheap mosquito larvicide	Larvicidal properties.
17. *Sesbania sesbans* Papilionaceae	Leaves	Animals washed in water soaked with the powdered leaves can safely transverse tsetsefly belts	Used as a preventive of tse-tse bites.

1.4 Plant Families which contain Compounds with Anti-insect Activity

The continued interest in new types of chemicals for insect control has resulted in extensive efforts to seek out hitherto unsuspected sources of plant derived insecticides and several thousands species of higher plant have been screened (Nakanishi, 1994). Many exhibited limited activity, while a few have sufficient activity against a variety of insects. Active principle may occur in any part of the plant. The families of Leguminoceae, Solanaceae and Apocynaceae have high frequency of activity. According to Grainge and Ahmed, 1988 the following plant families have significant number of plants which contain active principles with potent anti-insect activities.

1. AMARANTHACEA

Species of *Amaranthus* contain compounds with anti-feedant activity against *Leptinotarsa decemlineata* (Jermy, 1966), *Cyatula* species contain ecdysteroids which have IGR effects (Camps, 1991).

2. AMARYLLIDACEAE

Many species of *Allium* have been shown to have insecticidal and anti-feedant activity against a wide range of insects due to high levels of saponins in the flowers. Species of *Crinum* and *Hymenocallis littoralis* contain anti-feedant alkaloid against *Spodoptera exempta.* (Grainge and Ahmed, 1988).

3. ANNONACEAE

Ethanolic extracts of the fruits of *Annona* sp. (*Annona reticulata* and *A. glabra*) contains kaurene diterpenes which have IGR activity against *Diabrotica* (Jacobson, 1989).

4. APOCYNACEAE

Holorrhena antidysentrica contains a steroidal alkaloid, conessine which is a potent growth disrupter, sterilant and anti-feedant, other genera *Acokanthera, Apocynum, Mandevilla* and *Nerium* have potent anti-feedant compounds. (Grainge and Ahmed, 1988).

5. ARISTOLOCHIACEAE

Asclepias contain cardenolides and alkaloids which are utilised by the monarch butterfly *Danaus plexipus* for its protection from bird predation. These compounds are toxic to other insect species, however toxic to mammals (Harborne, 1991). *Calotrpis* also contain compounds that have potent IGR and anti-feedant activity (Grainge and Ahmed, 1988).

6. ASTERACEAE

This family contains many genera with compounds that affect insect growth, such as ecdysteroids (Camps, 1991), Pyrethrins in *Crysanthemum cinerariafolium* (Elliot, 1977) and photo-activated thiopenes, that could have potentials as insecticides. When alpha

terthienyl (alpha-l) is subjected to U.V light it becomes very toxic. However, there appears to be no cross-resistance with other insecticides (Arnason *et al*, 1989). Chromenes, such as precocenes isolated from the ornamental plant *Ageratum houstonianum* differ in their activity and have insect molting activity. However precocenes are very toxic to rats.

Other insecticidal compounds in these families include sesquiterpene lactones from *Encelia*, triterpenoids like momordicine from *Momordica*

7. BIGNONACEAE

Catalpa speciosa contains iridoid glycosides in its nectar. These includes catapol and cataposiol which prevents skipper insect from feeding on the nectar, the iridoid specionin has anti-feedant action against *Choristoneura fumiferana* (Chan *et al*, 1990).

8. CAESALPINACEAE

Cassia species have compounds with IGR and anti-feedant effects. (Grainge and Ahmed, 1988).

9. CELESTRACEAE

Celastrus angulatus and *C. glaucophyllus* have been used for pest control by farmers for a long time. Active principles are sesquiterpenoid, dihydro-beta-agrofuran alkaloids viz celongalin,

maytansine, tripolide, mayfoline and evonine. These cause narcosis and death in insects (Chiu, 1989).

Other species with insecticidal properties include *Maytenus* species, *Triptergium wilfodii* used by the Chinese as insecticide. The study of Celasteracea for insecticidal wilforine which may occur in other species is very necessary.

10. CHENOPODIACEAE

Spinacia specie contains phytoecdysteroids that have potential IGR activity. Extracts from *Beta* and *Chenopodium* are potent anti-feedant on *Pieris brassica.*

11. COMBRETACEAE

Combretum contains compounds with anti-feedant activity against *Locusta migratoria.*

12. COMPOSITAE

These are known to contain sesquiterpenes, however, only a few have been tested for insect anti-feedant property. Many of these sesquiterpenes occur at high concentrations and function in the plant as defensive agents. Those with activity incudes taginin, lachipicrin, 8-deoxylactucin and 8-hydroxy coumarin cichorin. Others include Diketones, tenulin and diterpenoid kaurenoic and trachybanoic acid. (Grainge and Ahmed, 1988).

13. CONVOLVULACEAE

Species of this family contains phytoecdysteroids with IGR effect and anti-feedant properties. These include *Ipomea Calstergia* and *Convolvulus* (Grainge and Ahmed, 1988).

14. CRUCIFERAE

This family contain a range of sulphur containing indole alkaloids which have potent anti-feedant activity against *Spodoptera frugiperda* and *Ostrinia nubilis* (Powel *et al* 1991). *Brassica* contains phytoecdysteroids with IGR activity.

15. CUCUBITACEAE

Momordica contains compounds which are insecticidal in addition to anti-feedant substances in *Momordica charantia.* (Grainge and Ahmed, 1988).

16. DIOSCOREACEAE

Dioscorea contains unidentified insecticidal and anti-feedant compounds. (Grainge and Ahmed, 1988).

17. EUPHOBIACEAE

Euphobia, Alchornea and *Aleurites* contain anti-feedants. The Diterpenoid (-) hard wickiic acid from *Croton aromaticus* has insecticidal activity. (Bandara *et al*, 1987).

18. GERANIACEAE

Geranium spp contains compounds with potent IGR activity, *Pelargonium* contains limonene which has anti-feedant activity. (Grainge and Ahmed, 1988)

19. LABIATAE

Ajuga, Scuttelaria and *Ocimum* contains anti-feedant and IGR clerodanes. Insecticidal and anti-feedant monoterpenes are found in *Mentha* and *Eridoma saucia* Koul *et al.*, 1990. Some genera contain attractant terpenoids and can be used to trap pest insects or increase the number of beneficial insects to an area. Nepetalactones obtained from sp. of *Nepeta* have been shown to be pheromones like in many aphid species (Dawson *et al*, 1990).

20. LEGUMINOCEAE

Many species contain allelochemicals that have potential use as insecticides. Species of *Tephrosia* and *Lonchocarpus* contains Rotenone and derivatives which have insecticidal effects (Hassanali and Lwande, 1989). They also contain insecticidal flavonoids, non protein amino acids and polyhydroxyalkaloids. The genera *Medicago, Lotus* and *Lupinus* contain phyto alexins active against both fungi and insects (Sutherland, *et al*, 1986).

Species within this family have already provided novel compounds that have been used as pesticides and are providing new leads for biotechnological crop protection.

21. LILIACEAE

Some species contain anti-feedant and insecticidal compounds against a wide range of insects. The anti-feedant activity of extracts of *Urginea miritima* could be due to the alkaloid caffeine. (Grainge and Ahmed, 1988).

22. MALVACEAE

Gossypol from *Gossipium hirsuitum* alone and together with condesed tannins and flavonoid, deters many insect species from feeding. (Jacobson, 1989). The genera *Hibiscus* and *Sphaeralcea* contain insecticidal and anti-feedant compounds.

23. MELIACEA

The seeds and leaves of neem tree *Azadirachta indica* contain terpenoids with potent anti-insect activity. Neem products have been shown to have a multitude of anti insect activity including repellent, anti-feedant activity, growth inhibition, suppression of reproduction, mating disruption and ovicidal activity. One of the most active terpenoids in neem seed is azadirachtin which acts as anti-feedants and growth disrupter against a wide range of insects at microgram levels. Other active terpenoids includes nimbin, deacetyl nimbin, and thiomene. An extensive review of the neem tree has been given by Jacobson, 1989.

Other genera within this family also have potent anti insect activity example *Cedrela odorata*, *Acalymma vitatum* and *Melia azedarach* (Grainge and Ahmed, 1988).

24. MYRTACEAE

Eucalyptus spp. contain alkaloids which may be responsible for anti-feedant activity of the plants. (Grainge and Ahmed, 1988)

25. PAPAVERACEAE

The alkaloids protopine, sparteine and stylopine in *Chelodonium majus* are anti-feedants to *Spodoptera exempta* (Grainge and Ahmed, 1988).

26. PIPERACEAE

Over 50 unsaturated amides have been isolated from this family, however, only a few have been evaluated for anti-insect activity. Those examined like Guineensine (LD_{50} = 0.11µg/insect) was more active than pipercide (LD_{50} = 0.56µg/insect) against *Callosobruchus chinesis*, it is nearly as active as pyrethrin (LD_{50} = 0.1 µg/insect). Miyakado *et al*, 1989.

Piperecea family may provide new leads for Agrochemical industry.

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27. RUTACEA

Atalantia racemosa contain anti-feedant coumarins xanthoxin and racemosin which deters *Spodoptera litura* from feeding. The limonoid (Limonin) has been isolated from many *Rutaceae* species, it is very active against *Leptinotarsa decemlineata* (Jacobson, 1989). Anti-feedant alkaloids have been isolated from this family. These include zanthophylline from *Zanthopylum monophyllum*, n-methyl-flindesine from *Fagara chalybea* and Herculin an insecticide from *Zanthoxyllum clava-herculis*.

28. SIMAROUBACEAE

Genera within this family contains Quassinoids which are potent anti-feedants against some insects. Quassin from *Quassia* spp. is an effective insecticide. (Grainge and Ahmed, 1988).

29. SOLANACEAE

The genera are known to have specific type of alkaloids e.g. *Lycopersilon solanum* and *Physalis* contain steroidal glyco alkaloids (tomatine and solanine); *Nicotiana* contains pyridine alkaloid (Nicotine), *Atropa* and *Hyoscyamus* contains tropane alkaloids (Atropine) Nicotine is a well known insecticidal compound also tropane alkaloids are used as insecticides. Other alkaloids from solanaceae may have anti-feedant properties. (Grainge and Ahmed, 1988).

30. UMBELLIFERAE

Angelica silvestris contain a sesquiterpene bisabolangelone which is anti-feedant to *Leptisotarsa decemlineata* at 1000p.p.m and myrthimna unipuncta (Van Beek and de Goot, 1986).

31. VERBANACAE

Stachytarpeta mutabilis contains bitter iridoids glycosides ipolamiide wich has anti-feedant propertics on some grass hopers. Phytoecdysteroids, vitexiron, 20-hydroxyecdysone, ajugasteronc and turkesterone from *Vitex fisherii* all have IGR activity (Kubo *et al*, 1990). The clerodane diterpenes from *Clerodendron* and *Caryopteris* have anti-feedant and IGR activity.

1.5 Plant Secondary Metabolities With Anti-insect Activity

Plant secondary metabolites have a variety of effects on insects, these effects includes: insecticidal, repellent attractants, insect growth regulating and anti-feedant properties.

The classes of compound which have been reported to have effects on insects are: (Jacobson and Crosby, 1971).

1. Terpenes
2. Steroids
3. Flavonoids and other polyphcnolic compounds
4. Alkaloids
5. Isobutylamides

1.5.1 **Effects of terpenes on insects**

I. **MONOTERPENES**: These have varying effects on insects.

A. *Defensive substances*: A cyclic monoterpenes such as Geraniol, Citronellal, citral, limonene and pinene are usually present in insect as defensive substances. They are also present in volatile oil containing plants classically *Labiatae* and *Umbelliferae*. (Harborne, 1991). (Figure 1; 3a.)

B. *Insecticidal iridoids*. Iridoids are cyclopentanoid monoterpenes related to iridomyrmecin first isolated from the Argentinian ant *Iridomyrmex humilis*. These include iridodial (Fig 1:3a), iridolactone, dolichodial and Nepatalactone. Compounds of these group have both insecticidal and antibiotic property. They are widely distributed in plants belonging to the families *Rubiacea, Cornaceae, Plantaginaceae* and *Labiatae* and several others. The catmint oil *Nepata cataria* contains Nepatalactone. (Eisner, 1970).

C. *Sex attractants* - Monoterpenes that posses this activity belongs to a great variety of structures from the chemically simple ones like n-valeric acid to the complex types like Bombykol. Most of them are however aliphatic hydrocarbons. Similar substances have been found in both insects and their plant diets, it is believed that some insect sequesters these from their diets (Eloy and Donald, 1976).

D. *Feeding repellents and attractants*: Monoterpenes are known to posses important functions on the feeding habits of insects either as attractants or deterrents (Dethier, 1957). Monoterpenes in this group have a wide range of structures. The effects of these compounds occur mostly on phytophagous insects.

(Fig 1;3)

II. SESQUITERPENES These also posses variable effects on insects.

A. *Defensive substances*: The structure of the defensive substance depends on the insect from the simple structure like Farnesol to the complex furanosesquiterpene dendrolysins. There is a parallel relationship between insects and their plant diets.

B. *Insect anti-feedants*: Sesquiterpenes are known to have anti-feedant effects on insects, the plant which contain these prevents the plants from attack by phytophagous insects.

C. *Insecticides*: Insecticidal compounds of the piericidin type (Piericidin A and B) have been isolated from lower plants (fungi). These were shown to have side chain resembling sesquiterpenes (Wright *et al*, 1982).

D. *Insect Hormones*; A number of plants are known to contain juvenile hormones (JH) and their mimics. (Bowers, 1968). Juvenile hormone activity is due to four hormones JHO, JH1, JHII and JHIII and their mimics

juvabione, juvocimen-2 and juvadecene. These have been found in plants. (Fig 1; 2a and 2b).

III DITERPENES. Except for very few, these have limited effects on insects. The diterpene hydrocarbon(-)-kaurene, a tetracyclic diterpene found in compositae has anti-feedant activity against insects. Also the diterpenoid (-)- Hardwickiic acid from *Croton aromaticus* has potent insecticidal property (Bandara *et al.*, 1987).

IV. TRITERPENES: The effect on most triterpenes on insect is anti-feedants. The anti-feedant effect of Azachirachtins and related compounds from the family Meliaceae e.g. *Azadirachta indica* and *Melia azedarach* is well documented (Jacobson, 1988). These compounds are known to be unpalatable to many insects.

1.5.2 The effects of steroids on insects

It has been known for many years how steroids affect insects. These includes:

1. MOLTING HORMONES: These hormones are necessary for the initiation of metamorphosis, their release into the insect haemolymph (insect blood) causes molting. Molting hormones are steroids otherwise known as ecdysones Fig 1; 2c. These steroids in contrast to other steroids are usually water soluble as such their presence and isolation from plants have been neglected for a long time. (Takemoto *et al.*, 1967). Massive

quantities of steroids possessing enormous Hormonal activity are found in plants when compared to that present naturally in insects. (Table 2) Plants that contain as much as 0.1% of their dry weight of phytoecdysteroids (plant steroids) must present a great barrier against insects which feed on them. (Nakanishi, 1969) had shown the activity of these steroids, as much as 100% mortality of instars of *Culex quinquifasciatus* molestus mosquito was found when dipped in aquaoes solution of ponasterone A.

TABLE 2: OCCURENCE OF PHYTOECDYSTEROIDS IN VARIOUSQUANTITY OF DRIED PLANTS (JACOBSON AND CROSBY, 1971)

PLANT	PART	PHYTOECDYSTEROIDS	QUANTITY (mg/kg)
1. *Podocarpus elatus*	Bark	Crustecdysone	500
		Podoecdysone A	1
2. *Morus* spp.	Leaves	Crustecdysone	1
		Inokosterone	10
3. *Taxus baccata*	Leaves	Crustecdysone	220
4. *Bosea yervamora*	Root	Crustecdysone	200
5. *Polypodium vulgare*	Root	Crustecdysone	880
		Polypodine B	370
		Ecdysone	8
6. *Vitex megapotamica*	Leaves	Crustecdysone	880
7. *Ajuga decumens*	Leaves	Crustecdysone	120
		Cyasterone	80
		Ajugasterone C.	15
8. *Polypodium japonicum*	Root	Crustecdysone	50

Certainly the discovery of ecdysones and analogues 6-keto steroids from plants have opened a new and interesting field of research in the control of insect pests.

2. **Defence substances:** Some insect sequester steroids from plants and utilise them for their defence, these are usually cardenolides (Fig 1; 3c).

1.5.3 The effect of mixed terpenoids on insects

Mixed terpenoids are miscellaneous class of terpenoids which are built predominantly of isoprene residues but have additional carbon atoms or lack the required number of carbon. Classically represented by the pyrethrins. These pyrethrins are very valuable insecticides found only in the flower of some members of the genus *Crysanthemum*. The chief commercial source being *C cinerariafolium*. Four substances of very similar structures have been isolated. These are pyrethin I and II, and cinerin I and II. These compounds have a rapid knock down and widespread insecticidal activity. (Figure 1; 1a).

1.5.4 The effects of flavonoids and other poly-phenolic compounds on insects

A. Effect on insect feeding: Flavonoids and Tannins provide the greatest barrier against insects feeding habits, other polyphenols with similar

effects include quinones and glycosides. The effect of these compounds is probably related to their astrigency which makes them unpalatable to many insects.

B. Insecticidal: This is represented by the Rotenoids. (Fig 1. 1b) Rotenoids are related to the isoflavones. They are found in the plants belonging to the family Leguminocea principally genera of *Derris, Tephosia* and *Lonchocarpus.*

1.5.5 The effects of Alkaloids on Insects

1. **Anti-feedant Effect:** There are many anti-feedant alkaloids from plants with varying spectrum of activity. The anti-feedant property of alkaloid is classically represented by *Solanum* alkaloid solanine and demissine which are anti-feedant to the colarado beetle.

2. **Insecticidal effect**: Nicotine and related alkaloids (Fig 1.1c) from *Nicotiana tobaccum* have been known as insecticides for a long time. Other insecticidal alkaloid includes veratrum alkaloids from *Veratrum album* wilforine and related compounds from *Triptergium wilfodii* Benzoisoquinoline alkaloids from *Anona reticulata* and Haplophytine and related alkaloid from *Haployphyton cinicidum*

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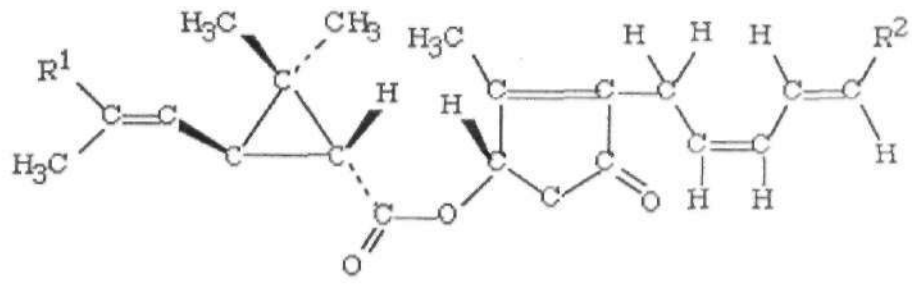
1.5.6 The effect of unsaturated isobutylamides on insects

A number of insecticidal isobutylamides have been isolated from plants belonging to the families *Rutacea* and *Compositae*. Insecticidal isobutylamides posses both pungency and rapid knock down of flying insect, a desirable property they share with pyrethrines. Example of insecticidal isobutylamides are pellitorine from *Anacyclus pyrethrum*, spilanthol from *Spilanthes oleracea*, affinin and scabrin from *Heliopses longipipes* and herculin and neoherculin from *Zanthoxylum clava-herculis*. (Fig 1; 1d.)

FIGURE 1: Structuresof Plant Derived Compounds With Anti-Insect Properties. (Jacobson and crosby, 1971)

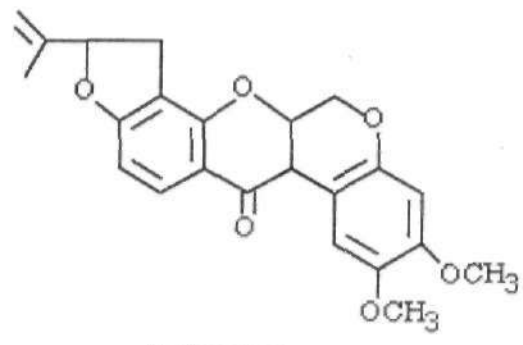
1. INSECTICIDES

a. Pyrethroids from *Crysanthemum cineraria folium*

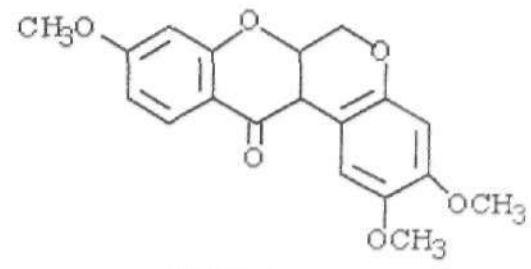


$R^1 = CH_3$, $R^2 = H$	Pyrethrin I
$R^1 = CO\text{-}OCH_3$, $R^2 = H$	Pyrethrin II
$R^1 = R^2 = CH_3$	Cinerin I
$R^1 = CH_3$, $R^2 = CO\text{-}OCH_3$	Cinerin II
$R^1 = CH_3$, $R^2 = CH_2CH_3$	Jasmolin I
$R^1 = CO\text{-}OCH_3$, $R^2 = CH_2CH_3$	Jasmolin II

b. Rotenoids from *Derris* species



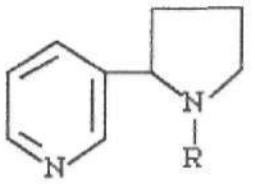
Rotenone



Muduserone

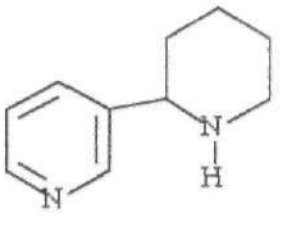
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c. Nicotinoids from *Nicotiana tobaccum*



R = CH_3 Nicotine

R = H Nornicotine



Anabasine

d. Unsaturated Isobutylamides

i. Pellitorine from *Anacyclus pyrethrum*

$CH_3(CH_2)_4CH{=}CHCH{=}CHCONHCH_2CH(CH_3)_2$

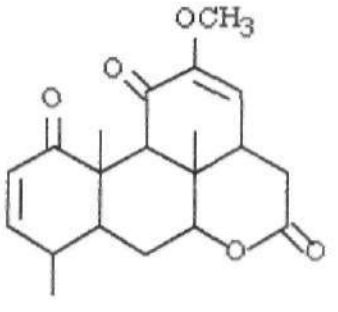
N-isobutyl-trans-2-trans-4-decadienamide

ii. Spiranthol from *Spilanthes oleracese*

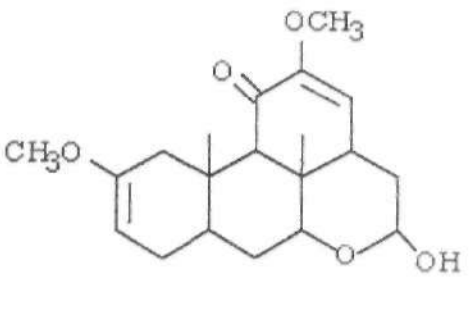
$CH_3CH{=}CHCH(CH_2)_2CH{=}CHCONHCH_2CH(CH_3)_2$

N-isobutyl-2,6,8-decadienamide

e. Quassinoids from Quassia wood (*Picrasma excelsa*)



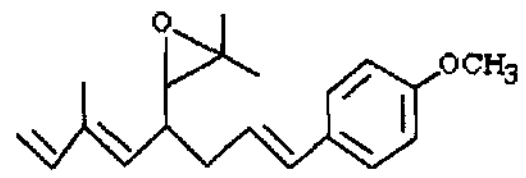
Quassin



Neoquassin

2. INSECT GROWTH REGULATORS

a. Juvenile Hormones

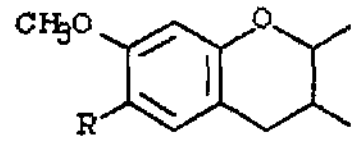


Juvocimene from *Ocimum bacilicum*



Juvadecene from *Micropiper excelsum*

b. Anti-Juvenile Hormones

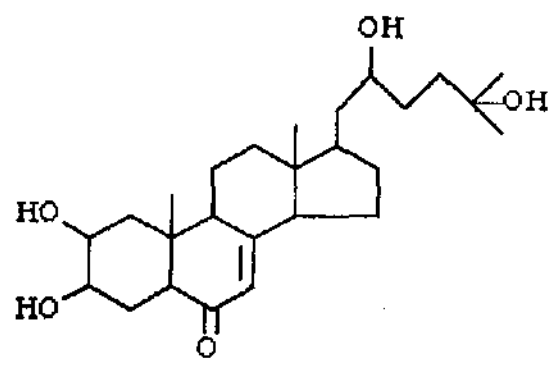


R = H Precocene 1

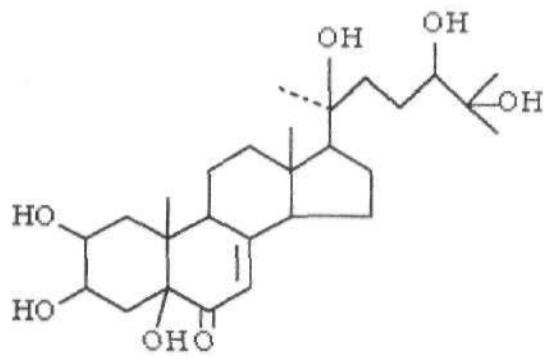
R = OCH_3 Precocene 2

Precocene from *Ageratum houstoniatum*

c. Molting Hormones



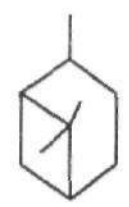
Ecdysone from *Polypodium vulgare*



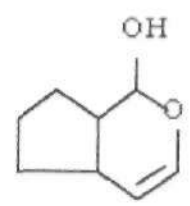
Polypodine B from *Vitex megapotamica*

3. INSECT SEMIOCHEMICALS

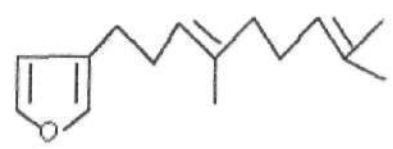
a. Repellants



∂,ß-pinene in Umbelliferae

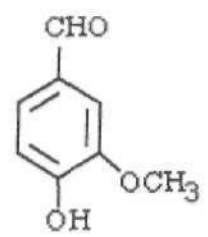


Iridodial from *Nepata cataria*



Dendrolysin in Convulvulacea

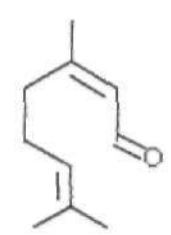
b. Attractants



Vanillin found in Vanilla

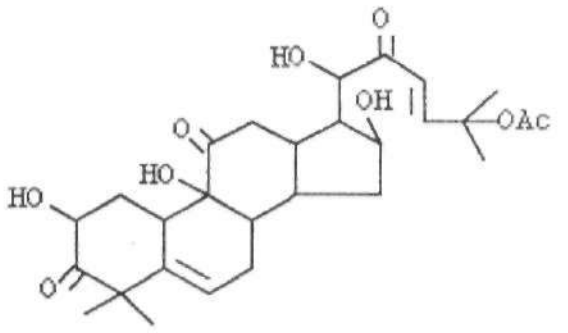


Myrcenol found in Labaite



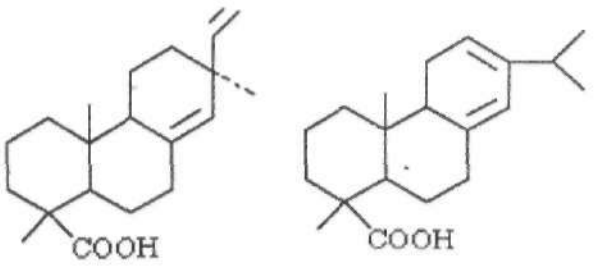
Citral found in Citrus fruits

c. **Antifeedants**



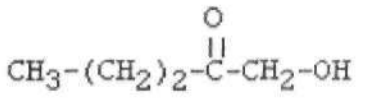
Curcubitacin A from Curcubita specie

d. **Allomones**



Resin acids aboundant in Umbelliferous Resins

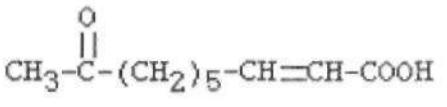
e. Kairomones



Heptanoic acid from Irish potato serve as Kiromone in potato tuberworm moth Vs *Orgilus lipidus* interraction.

f. Pheromones

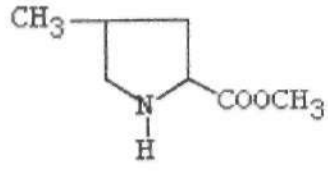
i. Sex Pheromones



trans-9-keto-2-decenoic acid

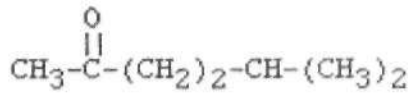
Sex pheromones on Honey bee (*Apis mellifera*)

ii. Insect Trail Pheromones



Methyl-4-methylpyrolle-2-Carboxylate,
trail pheromone in *Atta cephalotis*

iii. Alarm Pheromones



Isoamyl acetate found in bees (*Apis mellifera*),
present also in some plants

1.6 *Techniques For Screening Insecticides*

Bioassay of insecticides is the estimation of the effects of small traces of insecticides on insects exposed to them. The advantages of bioassay is the extreme sensitivity of some insects to the minute quantities of toxicant, these insects usually respond only to actual poison present. The insects used in bioassay of insecticide includes: mosquito larva, Musca, Tribolium beetles and *Macrocentrus ancylivorus.* (Busvine, 1957).

1.6.1 Bioassay using mosquito larva

Mosquito larva is the most commonly used insect in bioassay of insecticides. Several workers (Buchfield, 1952) have used this on traces of insecticide as a result of the extreme minute quantity which affects the larva. Most work is on *Aedes aegypti* some work also has been done on *Anopheles qadrimaculatus* and *Culex quinquifasciatus.* The residue of extraction of insecticide is made into a series of concentration and tested against the larva. Several workers used fourth instar larva (Hartzell and Stores 1950). The final volume of test suspension or solution is 1ml, 2ml, 10ml, 25ml or 100ml containing 5 or 10 larvae with several replicates. The result is usually estimated as percentage mortalities observed after 20 to 40 hours. Buchfield and Wilcoxon (1954) used time as the critical variable and determined the time required for 50% of the larva to fail to respond to a light stimulus.

ACCURACY AND LIMITATION OF BIOASSAY

Theoretically it is possible to detect minute quantities of insecticides by using mosquito larvae. Larvae may show critical mortalities between 0.03 - 0.1 ppm, and in 1ml suspension this corresponds to 0.03 - 0.1µg of insecticides. (Busvine, 1957).

There are four disadvantage to using mosquito larva bioassay.

1. INTERFERENCE BY CONTAMINANTS

The presence of fatty substances may mask the effects of insecticides and reduce their toxicity. (Fletcher, 1951).

2. VARIABILITY OF MOSQUITO LARVAE

There may be differences in susceptibility of larva due to physiological ages (different instars have different susceptibility) or between different generations. To avoid such differences 4th instar larva have been used by Buchfield, 1952.

3. ASSESMENT OF MORTALITY

Intoxicated mosquito larvae pass through several stages before death. Some workers include even moribund individuals among the dead, this allows differences in estimation between workers. (Buchfield, 1952) devised an apparatus which segregates larva which are so badly affected that they cannot respond to a light stimulus.

4. INSENSITIVE RESPONSE TO INCREASE CONCENTRATION

It is sometimes found that a rather large increase in concentration of insecticide is necessary to produce an increase in mortality, this decreases the sensitivity of the determination of critical concentrations. However, it is not clear how to avoid this as it is characteristic of aqueous suspension used in this way.

1.6.2 **Screening methods for insect repellents**

Repellency of a substance can be used to the advantage of protection of man other animals and crops against insects. Basically, the methods used depend on application of the repellent to food, oviposition site or to counteract a source of insect attraction. Effectiveness is indicated by the ability of the particular repellent to prevent alighting, feeding, egg laying or movement of the insect to other sites. Insect species differ in their requirements therefore details of screening methods differ considerably with different insects.

METHODS

1. CAGE TESTS AGAINST BITING INSECTS

i) With human baits: This is done by exposing the treated fore-arm in a cage of hungry mosquitoes, stable flies or fleas. There is no standardization of the type of cage used. (Busbey, 1939).

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ii) Cage test with animal baits: This is done by shaving parts of the animal body, this is then treated with the repellent to be tested. The animal is then confined to test cage with restricted movement.

Most of the repellent tests are ambiguous and statistically unsound due to the limitations of the above tests (Busbey, 1939).

2. REPELLENT TEST BY CONFINING WITH FOOD

This is done by confining a group of the insects with a treated supply of food for a standard time and subsequently the level of feeding by the insect is determined. There are basically 2 types of this method:

i) PLANT-FEEDING INSECTS: A large flat leaf is cut from a bean plant and flattened by pressing. It is treated by spraying or dusting the repellent. The leaf is placed flat on a damp surface with petiole stuck into vial of water for turgidity and then exposed to a batch of 5-fourth instar larva of *Epilachna verivestis* (Mexican Bean beetle) or any suitable leaf feeding insect and the amount eaten is determined after a suitable interval of time. (Srivastava and Proksch, 1993).

ii) BLOOD FEEDING INSECTS: The blood feeding are confined for short periods on skin treated with repellents and the numbers of insects prevented from feeding is determined. (Kubo, 1993).

iii) OLFACTOMETER TEST

Olfactometers are equipment used in testing the odour stimulus in insects.

Various types of olfactometers have been used which includes those with air currents and those without.

Gilbert *et al.*, 1957 have designed a method of comparing repellents and they used the following formula.

$$\text{Average } T_i = \frac{2T_i - B_i}{rn} + m$$

where

T_i = Total protection time for all tests with repellent i.

B_i = Total protection time for both repellent in all pair in which repellent occurred.

n = Total number of repellents

r = The number of times each pair was replicated

m = Grand mean of all protection times in the series.

1.6.3 **Screening methods for insect attractants**

The evaluation of attractants is made by observing and recording the behavioural responses of insects to the test substance (Busvine, 1957). Insects posses a highly developed olfactory sense for chemical substances. Based on this, attractants are selected based on their behaviour responses to (1) food (2) Sex and (3) Oviposition. Terms used to describe these reponses are:

1. kineses for random movements
2. Klino-taxis for regular alternating deviations.
3. Tropo-taxis for movements on a straight path.

Behaviour may be studied individually or collectively in the laboratory or field, methods used includes:

1. FEEDING METHODS

Low concentrations of attractants are sprayed on filter paper sheet paraffin or other suitable substrate. The amount of the substrate consumed by the insect serve as index of attractiveness. (Dethier, 1957).

2. CONDITIONING METHODS

This is done by conditioning the test insect to the test substance and then responses to different concentrations of the substance is determined.

3. OLFACTOMETER METHODS

Attractants are measured in the laboratory by means of olfactometers. This can quickly and accurately determine qualitative responses to odours. The procedure involves attracting insects towards a light stimulus through forks, just as they are about to pass the fork they are influenced by odours drawn through the forks, another fork serves as control.

4. BAITING

Baiting is the practice of utilising attractants to lure insects. This has been used for sampling insects and also in insect control. Common baits includes food, fermenting solid, poison, chemical and sex baits depending on the stimulant. The attractivencss is determined by the

numbers of insects attracted to the baits after a given period. Fleming *et al.*, (1951) described an index for attractants using Japanese beetle.

Coefficient of attractiveness =

$$\frac{\text{Number of beetles in trap with test attractant}}{\text{Number of beetles in trap with Eugenol.}} \times 100$$

1.7 **Toxicological Statistics in Insecticide Screening**

The use of toxicological statistics provides a reliable method of assessing the toxicity of a substance against insects. Method of insecticidal assay includes:

i) Direct assay: This involves the measuring of the exact doses necessary to kill individual insect. This is determined by gradual increase in doses up to the critical point. The comparison of agents is usually done using LD50 which represents the dose needed to kill 50% of the test insects.

ii) Indirect Assay: This is determined by giving standard doses of test agent to batches of insects. Comparison is done using LC_{50}. This is known as the concentration required to kill 50% of the test insects.

Usually LD_{50} and LC_{50} are determined from regression lines of results obtained by plotting a graph of probit mortalities against logarithm of dose or logarithm of concentrations.

1.8 Botanical Classification, Ethnobotany and Chemistry of *Steganotania araliacea* Hochst

Steganotaenia araliacea Hochst belongs to the family *Umbelliferae*, a family not otherwise prominent in West tropical Africa. It is a small tree with compound umbel of tiny white flowers in the dry season (Uphof, 1968). The plant belongs to the subfamily peucedaneae (Hutchison and Dalziel, 1955).

1.8.1 Description of the family Umbelliferae

They are usually shrubs and rarely trees; stem is usually fistular. Leaves are alternate simple, often much divided, sometimes decompound, petiole usually sheathing at the base. Inflorescence forms an umbel usually compound or in a few cases simple. Flowers are regular (actinomorphic) or sometimes zygomorphic, epigynous, bisexual or polygamous, outer flowers sometimes rayed; mostly protandrous, bracts in the form of an involucre. Calyx has 5 sepals, free adnate to the ovary, often considerably reduced in size. Corolla has 5 petals, rarely absent, free, adnate to the ovary, sometimes unequal, margin often incurved, valvate or imbricate. The Androecium has 5 stamens, alternating with the petals, epigynous; filaments bent inwards in the bud; anthers introrse. Gynoecium has 2 syncarpous carpels; an inferior ovary -2 celled, anteroposterior crowned by 2-lobbed epigynous disc called the stylopodium, with two free styles arising from it; stigmas capitate; ovules

are 2 in number solitary in each cell and pendulous. Fruit is a cremocarp consisting of two indehiscent carpels laterally or dorsally compressed, breaking up into two parts, called mericarps, which remain attached to a slender, often forked, axis (carpophore); each mericarp usually shows five longitudenal ridges and oil canals (vittae) in the furrows. Seeds are two, solitary in each mericarp and albuminous (Dutta 1979a).

Anatomically they also present characteristic development of collenchyma support the presence of secretory ducts in spatial relation to the vascular bundles, and the multilacunar nodes. The family is divided into subfamilies.

1. Hydrocortyloideae
2. Saniculoideae
3. Apioideae
4. Peucedaneae - ferulineae
5. Aminineae - carineae

These subfamilies are well differentiated by the structure of the fruit. In all these occur independently different degree of dissection of the leaf blade, different degrees of petal infloding and lobbing of the Apex; comparable modification of the infloresence, presence of woody, shrubby or aboresscent species, such woody species shows similar general characteristics, pores diffuse, scanty, vasicentric parenchyma, heterogenous medullally rays, vessel elements with almost exclusively simple perforations and mostly wide angles and in most cases, Further modification like helical thickenings. The examples of genera in this family are (*Erygium* (200sp), *Pimpinella* (200sp) ferula (over 100sp). *Peucedanum*

or *Steganotaenia* (over 100sp) *Hydrocotyle* (100sp).; *Daucus* (60sp) *Centella* (40sp), *Oenanthe* (40sp) and *Carum* (30sp). Examples of important species are:

i) Medicinal plants, Condiments and spices: Fennel (*Foenuculum vulgare*, coriander (*Coriandum sativum*) Caraway (*Carum carvi*), Dill (*Anethum graveolens*) Parseley (*Petroselenum crispum*), Anise (*Pimpinella anisum*) Asafoetida (*Ferula asafoetida*) etcetera.

ii) Vegetables: Carrot (*Daucus carota*), Parsnip (*Pastinaca sativa* and Oeleny (*Apium graveolens*)

1.8.2 **Description of the subfamily peucedaneae**

Conspicuous here are the high mountain central and south American genus *Myrrhidendron wulter* and Rose and the African *Steganotaenia* Hochst, both showing scalariform borderd side wall pitting (rather primitive) and exclusively simple perforation (advanced). The structure of the xylem is that of normal dicotyledonous wood with no indication of secondary derivation (Rodriguez, 1957).

1.8.3 **Description of the genus *Steganotaenia***

There is probably only one specie of plant belonging to this genus in West Africa and this is *Steganotaenia araliacea* Hochst otherwise known as *Peucedanum flaxinifolium* Hiem exoliv. It is a small tree up to 12m high, leaves simply pinnate, leaflets, sub entire to tooted 5-15cm long, 2-7cm broad glaborous, primary umbels about 12 rayed, ultimate, peduncles about 3cm long, pedicel 5mm long, fruit obovate, 1cm long, 7mm broad

and 3-ribbed. Flowers are white. The plant is well distributed throughout tropical West Africa.

1.8.4 **Ethnobotanical uses of *Steganotaenia araliacea* Hochst**

The plant has a lot of ethnobotanical uses ascribed to it. A decoction of the plant is used for gonororhea, the root is used for sore throat, externally for snake bites, rheumatism and swelling caused by allergies, for stomach pain in women (Herdberg, 1983). The plant is believed to cause abortion in goats, liver and lung diseases in cows (a disease characterised by sweating and difficulty in breathing) and in humans for asthma. (Wild and Gelfand, 1959). The plant is also believed to increase the size of the penis when wrapped around it, used as an analgesic for malaria and in Bilhaziasis (Watt and Breyer - Brandwick, 1962).

1.8.5 **Chemical constituents of *Steganotaenia araliacea* Hochst**

The plant is known for the presence of cytotoxic lignans and bioactive triterpenes.

A. **LIGNANS**: The plant is known for the presence of cytotoxic lignans. The first to be isolated were steganacin and steganangin, antileukaemic lignan lactones It is also the plant that furnishes the first Bisbenzocyclooctadiene lignan called Neoisostegane (Wickramoratne *et al.*, 1993). As much as 17 cytotoxic lignans have been isolated from the plant and these includes prestegnane B Steganolide A, B and C (Robin et al, 1986) and a host of other cytotoxic lignans.

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B. **TRITERPENES**: The triterpenes of *S. araliacea* include Barrigenol, Steganogenin and related glycosides and others found in the saponin extract of the leaves (Lavaud *et al.*, 1992).

1.9 Aims And Objectives Of The Study

The plant *S. araliacea* is used locally in insect control. This study is designed to investigate the rationale of this traditional usage of the plant with the objective of identifying and isolating the active constituent responsible for the actions against insects.

CHAPTER TWO

2.0 EXPERIMENTAL

2.1 MATERIALS AND METHODS

2.1.1 Plant Collection and Identification

Fresh plant materials (parts used are listed in table 3) were collected from one location within Zaria, Kaduna state, Nigeria. They were identified by the herbarium keeper of the department of Biological Sciences Ahmadu Bello University, Zaria Nigeria. The plant material were dried at room temperature powdered and sieved to a suitable size (sieve no. 44) and stored at room temperature in closed vessels before use.

TABLE 3 PLANTS USED IN THE CONTROL OF INSECT PESTS IN KADUNA STATE OF NIGERIA

S/NO	PLANTS AND THEIR LOCAL NAMES	PLANT PART USED	PLACE OF COLLECTION	LOCAL USES AGAINST INSECTS
1.	*Steganotaenia araliacea* family: Umbelliferae (Hanno) Voucher No. 21191TCN Baker	Bark	Around the university and in some villages in Chikun and Igabi local government area of Kaduna state	The stem bark is claimed to contain resins which are repellent to mosquitoes and other insects.
2.	*Cyperus rotundus* Family: cyperacea (Ayaa-Ayaa)	Rhizome	Around the banks of river Kaduna	The fragrant rhizome when inginerated in rooms repel flying insects
3.	*Vitex doniana* family: Verbanaeae (Dinya)	Bark	Around the University Dam, Samaru Zaria	Bark is believed to contain insecticidal latex
4.	*Psorospermum guineense* family: Hypericaceae (Hudu-tukunya)	Bark	from a village in Chukun local *government* area of Kaduna state	The water extract is *mixed with* seeds to be sown a method believed to discourage insects and birds.

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TABLE 3 CONTD.

S/NO	PLANTS AND THEIR LOCAL NAMES	PLANT PART USED	PLACE OF COLLECTION	LOCAL USES AGAINST INSECTS
5.	*Anogeissus leiocarpus* family: Combretaceae (Marke)	Leaves	Around the University Dam, Samaru Zaria	The leaves are used as fumigarnt against flying insects
6	*Securidaca longepedunculata* family: Polygalaceae (Uwar-Magunguna	Leaves	Around a village in Chikun local government area of Kaduna state	Leaves are used as fumigants for flying insects
7.	*Striga senegalense* family: Scrophulariaceae (Wutaa-Wataa	Aerial parts	Around the University Samaru Zaria	The aerial parts is incinerated in worms to repel mosquitoes
8.	*Parkia clappertonia* family: Mimosaceae (Dorawa)	Fruits	Around the University Samaru Zaria	The plant is a known fish poison and may kill insects
9.	*Newbouldia laevis* family: Bignoniaceae (Aduruku)	Leaves	Around the University Samaru Zaria.	The leaves when boiled produces lethal vapours. Dry leaves used in barns also prevent insect infestation
	Mangifera indica family: Anacardiaceae (Mangoro)	Oil from the fruits	Around the University Samaru Zaria	Oil is believed to be toxic to aphids.

Names in brackets are the Hausa names of the plants in Kaduna state of Nigeria.

2.1.2 **Materials for Biological experiment**

Culex quinquefasciatus larva and adult raised according to specifications by WHO, 1985. The following materials were used for experiment with mosquitoes.

1. Sucrose for feeding adults
2. Sucking tubes
3. Yeast powder for feeding larvae
4. Mosquito rearing cage. (WHO specification) 1985.
5. Petri dishes
6. Plastic tubes (3cm width x 10cm depth)

2.1.3 **Equipment, Solvents And Reagents**

All solvents and reagents used in these work were of analytical grade and refers to specification of B.P. Among the equipment used for this work were a compound microscope (wild MII) with Camera lucida fitted to it was used for the microscopical work; pye-Unicam SP8-100 for recording the UV spectra and FTR Pye Unicam IR spectrophotometer for IR spectra of compound.

2.2 **Evaluation of Larvicidal Activity of Some Nigerian Plants**

Some Nigerian plants which are commonly used in Kaduna state for their anti-insect activity were subjected to an evaluation procedure for larvicidal activity. This is done to select the most active plant among them.

Early Fourth instar larva of *Culex quinquefasciatus* (5-6 days) old were selected from the colony, washed with water to remove debris and transferred into a beaker containing a minimum quantity of water. With the aid of a dropping pipette, five (5) larvae were added to each test tube form of solution, suspension or emulsion were added in such a way and that the final volume in the test tube is 10ml (Pal and Sharma, 1952) five replicates of each test dilution were made. These were left undisturbed for 24 hours after which mortalities were determined. The criteria for death was failure to respond to prodding with a glass rod or inability of larvae to rise to the surface of water.

a) **Rearing of *Culex quinquefasciatus* larva**

The larvae were reared from eggs collected from wild mosquitoes. (Eggs of *Culex quinquefasciatus* are peculiar because they are laid in Raft form).

i. collection of eggs: polythene bowls were filled with water and placed in a suitable breeding area for mosquitoes. After 2-3 days eggs laid were harvested.

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ii. Hatching of eggs: The eggs of *Culex quinquefasciatus* collected were transferred into polythene pans (20cm width x 4cm depth and fresh distilled water was added. More water was added at interval of every 24 hours to maintain depth and keep the temperature approximately 28°C. With these condition incubation lasts 2 days within which larvae emerged. The newly hatched larvae were transferred into another polythene breeding pan each containing between 100-200 larvae to avoid over crowding. These are allowed to grow and with each moult, larva were collected based on their sizes and transferred to separate polythene breeding pans.

iii) **Feeding of Larvae**: Larvae were fed on dry baker's yeast (WHO, 1985) about 0.1g added to each breeding pan after 24 hours. Addition of food is done in such a way to coincide with changing of water. This is to prevent fermentation and fungal growth.

b) Extraction of plant material with various solvents

The plant material were extracted using water, ethanol and n-Hexane separately.

i. Water extraction: 10g of powdered plant material was mixed with 100ml of distilled water for 24 hours with occasional shaking. The extract was filtered and marc pressed. The extract was reduced to dryness under vacuum using a rotatory evaporator.

ii. Ethanol extraction: 10g of powdered plant material was mixed with 100ml ethanol with occasional shaking and left for 24 hours. The extract was filtered and marc pressed. The solvent was evaporated to dryness under vacuum using a rotatory evaporator.

iii. n-Hexane extraction: 10g of powdered plant material was mixed with 100ml of n-Hexane with occasional shaking for 24 hours. The extract was filtered and marc pressed. The solvent was evaporated to dryness under vacuum using rotatory evaporator.

c) Evaluation of larvicidal activity of Nicotine

Nicotine is a naturally occurring insecticide obtained from *Nicotiana tobaccum* linn. its toxicity, against insects has been documented. (Jacobson and Crosby, 1972). It is used here as a standard to evaluate larvicidal activity.

The larvicidal activity of sub critical doses of Nicotine (Doses which do not cause prompt and immediate death of larvae) were determined in the rang of 1-0.0001%v/v of Nicotine using the method described in 2.2 above.

d) Evaluation of larvicidal activity of various plant of extracts in relation to Nicotine

Serial dilutions of the three different extracts of each of the ten plants were made to get concentrations of 10mg/ml, 1mg/ml 0.1mg/ml, 0.01mg/ml and 0.001mg/ml activity determined as in 2.2 above.

This serial dilutions were made based on the consistency of the stock solution of each extract. The water extracts which easily go into solution in water are formulated without modification. Stock solutions of ethanolic extracts were formulated in the form of suspension by blending thoroughly in a vortex mixer while the oily petroleum ether extracts, are formulated as emulsions by mixing with tween 40 and this mixture blended in a vortex mixer. Serial dilutions were made from the stock.

2.3. **Preliminary Phytochemical Screening of the n-Hexane extracts of stem bark of *S. araliacea***

The stem bark was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described by Sofowora, (1982). The stem bark was examined for tannins, Alkaloids, Glycosides ectetera.

2.4 **Activity Guided Chromatographic Studies of *S. araliacea* n-Hexane extract of the Bark.**

An activity guided chromatographic Studies was carried out in order to isolate and identify the compound responsible for activity. The larvicidal activity was used to monitor the column fractions with larvicidal constituents.

In the evaluation tests carried out in 2.2 (d) above the n-Hexane extract of stem bark of *S. araliacea* was found to have the most potent

activity against the larva of *C. quinquefascisatus* at conc. tested hence further work was concentrated on this extract.

2.4.1 **Bulk Extraction of *S. araliacea* powdered bark with n-Hexane**

500g of the dried and powdered bark of *S. araliacea* was extracted with n-Hexane by maceration over a period of seven days. This was done by adding sufficient quantities of the solvent to the bark and leaving overnight, after every 24 hours the solvent was removed and fresh solvent added. All the n-Hexane extracts were combined and solvent removed by vacuum evaporation.

2.4.2 **Thin layer chromatographic studies of n-Hexane extract of *S. araliacea***

The extracts and fractions from column chromatographic studies, were monitored by TLC. The systems used were as follows.

1. Adsorbent (Merck) silica gel G thickness 0.25mm activated at 105°C for 1 hr.
2. Solvent
   - a) n-Hexane : Di ethyl ether
     10 : 1
   - b) n-Hexane : Acetone
     8 : 1
   - c) Chloroform : Acetone

5 : 1

3. Detection techniques

a) Daylight

b) Spectrophotometric using U.V 366nm

Reagents

c). Iodine vapour

d) Spray reagents

1. Potassium permanganate for reducing substances

(Stahl, 1969).

2. Antimony III chloride for terpenes and steroids. (Stahl, 1969).

(Antimony III Chloride: $CHCl_3$

1 : 3

TLC result of extract is shown in table 9,

2.4.3 **Activity -directed fractionation of the n-Hexane extract of stem bark of *S. araliacea***

The n-Hexane extract obtained in section 2.4.1 above was then subjected to column chromatography using the larvicidal activity to monitor the activity and TLC to monitor column fractions.

The following column conditions were employed:

a) Column

i. Dimension : 36cm length x 3cm width

ii. Adsorbent - 200g of silica gel 60/120 mesh. (Merck)

iii. Column packing - By wet (slurry) method

50ml of n-Hexane was added to 200g of silica gel and stirred with a glass rod. The slurry was poured slowly and steadily into the column. The column is allowed to continue to drip, so that is is properly drained and allow the silica gel to settle.

b) Solvents for Elution

By gradient elution using. The column was eluted first with pure n-Hexane followed by a mixture of n-Hexane and Ethyl acetate and finally with pure ethyl acetate.

i. n-Hexane

ii. n-Hexane: Ethylacetate (1:1)

iii. Ethylacetate

c) Sample - 11.6g of n-Hexane extract was dissolved in n-Hexane and mixed thoroughly with a small quantity of silica gel. This was allowed to dry before applying it to the column.

d) Flow rate 1ml/min

e) Fractions - 5ml fraction collected, a total of 100 were collected for all the solvents. The fractions were monitored on TLC using silica gel with n-Hexane Acetone 8:1.

The fractions were monitored on TLC on silica gel using n-Hexane acetate 8:1 The conditions of the TLC are described in 2.4.2 above. Similar spots in terms of colour or R_f were combined as below and subjected to bioassay to determined activity:

i. Fractions 1-7 contains 2 majors spots a and b and very minor c.

ii. Fractions 8-55 contains 8 spots

iii. Fractions 56-75 contains 6 spots

iv. Fractions 76-85 contains 2 spots

Fractions 86-92 contains 2 spots

Fraction 93-96 contains 1 spot

Fraction 97-100 No spot

Fractions 76-96 were also observed on TLC using

chloroform : Acetone and this system.

5 : 1

resolved other compounds (Table 11).

The larvicidal activity of these fraction were monitored and evaluated as follows:

Evaluation of larvicidal activity of all fractions. An emulsifible concentrate was prepared for each fractions by dissolving the required quantity in 0.2ml of n-Hexane the volume was made up to 10ml with 0.1% tween 40 (emulsifying agent). Appropriate dilutions were made with distilled water to get a concentration of 0.01mg/ml. The larvicidal activity was

determined in a series of tubes each containing 10 larvae, and 5 replicates were made for each sample. An appropriate n-Hexane 10ml and 0.1% tween 40. 1ml of this was diluted to 10ml and set aside as controls.

Fraction 1-7 containing 2 spots on silica gel using n-hexane: acetone (8:1) as solvent) was the most active.

Fractions, 1-7 (containing 2 spots) was the most active. However the quantity was small and used for determination of

2.4.4 **Isolation of larvicidal constituents from the n-Hexane extract of *S. araliacea***

i) Fractions 1-7 obtained from the column were combined and resolved on another column of silica gel 120/60 mesh

Adsorbent - silica gel 120/60 mesh 5g packed by slurry method Column dimension - 1cm x 5cm Solvent - by continuous elution with n-Hexane to resolve the non polar fractions.

On monitoring this on TLC using n-Hexane: Acetone (8:1) 2 spots were observed containing spot "a" as a major spot and spot "b" as a minor spot. On evaluation of larvicidal activity of all the column fractions these spots shown to be more active than all other fractions.

The combined n-Hexane eluate were subjected to preparative Thin Layer chromatograpy on silica gel in order to isolate the larvicidal constituents. The conditions of this separation are as stated below:

Preparative thin layer chromatography

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Adsorbent

Silica gel G, 0.5mm thick.

Solvent

n-Hexane : Acetone

8 : 1

Detection technique: U.V absorbtion at 366nm. The compound a (R_f = 0.93) off from the TLC plates and eluted with

n-Hexane. It was then discovered in ethylacetate by slow heating. After cooling crystalls begin to form. This was filtered by means of Buckner apparatus. The compound was then evaluated for biological activity against Culex quinquefascatus mosquito and subjected to chemical and spectroscopic studies to identify the compound.

iii. Biological activity of compound "a" The activity of compound "a" against the larva and adult of Culex quinquefasciatus were determined.

a) Evaluation of sub-lethal dose of compound "a"

An emulsifiable concentrate was prepared in 0.1% tween 40 to get a concentration of 0.01mg/ml. This is serially diluted to 0.001mg/ml and 0.0001mg/ml. Larvicidal activities of these dilutions were determined in a series of tubes, each containing 10 larvae and in 5 replicates. Results in table 15.

b) Determination of effect of long term exposure to compound "a" on the larvae. The effect of 0.001mg/ml of the fraction was determined on the fourth instar larva of Culex quinquefasciatus after 2, 3, 4 and 5 days to the development of larva, pupation and emergence of normal adults after these exposure periods from the growth media were determined.

c) Determination of the effect of compound "a" on adult Culex quinquifasciatus mosquitoes. The newly emerged mosquitoes were raised in a colony cage maintained on a diet of 10% sucrose in test tubes covered with cotton. The fraction "a" was dissolved in 10ml of n-Hexane to get a suitable stock solution, required quantities of the solution was taken and mixed with 1ml portion of n-Hexane before applying to petri dishes.

This was allowed to dry and leave an even film of the substance. First generation adult Culex quinquefasciatus mosquitoes were added to each petri dish by means of sucking tubes and maintained on the surface by means of a funnel shaped retainer. After a 60 second exposure, the mosquitoes were transferred into plastic recovery tubes (3cm width x 10 cm depth). Mortalities were determined after 4 hours (Table 18). Effects were compared to those due to Nicotine. Criteria for effects on mosquitoes.

1. All mosquitoes that showed no sign of movement of wings and limbs were considered dead.
2. All mosquitoes that showed only slight movement of limbs and/or wings on prodding with a blunt needle were considered to be adversely affected.

2.5 Chemical and Spectroscopic Studies of Compounds (a) the isolated larvicidal active constituent of *S. araliacea*

i) Physical properties: The properties of compound observed were the physical appearance and solubility.

ii) Thin layer Chromatographic studies of compound "a".

The system used were the same as those used for the extracts as described in 2.4.2. These indicated compound "a" as a single compound and hence the compound was subjected to spectroscopic studies.

iii) Spectroscopic Studies of compound "a".

The compound "a" was subjected to the following spectroscopic studies.

a) Ultraviolet Spectroscopy

The UV spectra was recorded using chloroform (BDH spectroscopic grade) as solvent on a Pye unicam SP8-100 spectrophotometer from the range of 450-250nm.

b) Infra red spectroscopy

The IR spectra was recorded using WinFIRST FTR pye-unicam spectrophotometer from 4000cm^{-1} to 500cm^{-1}. The sample was prepared in nujol mull.

2.6 **Preliminary Pharmacognostic Studies of *S. araliacea* leave and Bark**

Preliminary pharmacognostic studies were carried out on the leaves and Stem Bark of this plant by examining the macroscopical, microscopical and chemomicroscopical characteristics. Quantitative microscopy was carried out on the leaves to establish some pharmcapoeal standards.

2.6.1 **Pharmacognostic studies of *S araliacea* leaves**

A) **MACROSCOPICAL EXAMINATION**

A macromorphological examination of the leave was carried out to establish some characteristics of the leaves.

B. **MICROSCOPICAL EXAMINATION**

Different anatomical sections and powder of the leaves were examined and described using normal terms of Dutta, 1979b.

C. **CHEMOMICROSCOPICAL EXAMINATION**

Transverse section of the leaves were treated with appropriate chemicals on slides and reactions observed under the microscope

for the presence of oils, cellulose, tannins, mucillages etc. Using standard procedures of Trease and Evans, (1983).

D. **QUANTITATIVE MICROSCOPY**

Quantitative microscopical studies were carried on the leaves to establish various constants like stomatal number, stomatal index; palisade ratio, vein islet number and vein termination number using standard methods of Trease and Evans, (1983).

2.6.2 Pharmacognostic examination of powdered Stem bark of *S araliacea*

A. **MACROSCOPICAL EXAMINATION**

The macromorphological examination of the powdered bark was carried out to establish the organoleptic properties.

B. **MICROSCOPICAL EXAMINATION**

The powdered bark was observed to describe the different anatomical structure. (Fig. 5).

CHAPTER THREE

3.0. RESULTS AND DISCUSSIONS

3.1 MATERIALS

3.1.1 PLANT COLLECTION AND IDENTIFICATIONS

The different plants collected were identified using Taxonomic data in the herbarium of Department of Biological Sciences Ahmadu Bello University, Zaria Nigeria.

A herbarium specimen of each plant was deposited in the herbarium as reference. The plants used are along with information about the plant part used in insect control, the place of collection and other traditional uses of the plants are listed in Table 3. Chapter 2.

3.1.2 Materials for Biological Experiment

The larva of *Culex quinquefasciatus* thrive well on yeast powder while the adults could live up to a period of 10-14 days on sucrose alone.

3.1.3 Equipment, Solvents and Reagents

Equipment were used according to standard. procedure. All solvents are as referred to in materials and methods.

3.2 **Evaluation of Larvicidal Activity of Some Nigerian Plants**

a) Rearing of *Culex quinquefasciatus* larva.

The larva were raised on a diet of yeast powder which was seen to support growth of the larva at (26°C - 30°C)

b) Extraction of plant materials with various solvents

The n-Hexane extracts were dried to an oily residue the ethanol extracts to dark green or black gummy residue while the water extract to syrupy semi solid residue.

c) Evaluation of larvicidal activity of Nicotine

The larvicidal activity of Nicotine was high, prior to death the larvae were seen to undergo spiral and telescopic movement. The result of activity recorded in Table 4 below.

Table 4: Larvicidal activity of Nicotine against the larva of *Culex quinquefasciatus*

Dilution v/v	1	0.1	0.01	0.001	0.0001	0.00001
Percent mortalities	100	100	96	48	12	4

From graph of probit mortality against log concentration

LC_{50} = 0.00056%v/v as 1ml of Nicotine = 1.02g

LC_{50} = 5.71µg/ml

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d) Evaluation of larvicidal activity of various plant extracts in comparison to Nicotine

The selected plants were evaluated for larvicidal activity. The larvicidal activities of the various Extracts of some Nigerian plants used in traditional insect control practices reported in table 5, 6, 7 and 8.

The plant extracts killed the mosquito larvae within the first 12 hours of exposure. Prior to death there is telescopic and spiral movement. The petroleum ether extract of *S. araliacea* is especially interesting as even those that survive the 24 hour exposure of the lowest concentration have difficulty in rising to the surface of water.

Table 5: Percentage mortalities produced by dilutions of the water extracts of some Nigerian Plants.

PLANTS	CONCENTRATIONS mg/ml				
	10	1	0.1	0.01	0.001
1. *Steganotaenia araliacea*	4	0	0	0	0
2. *Cyperus rotundus*	8	0	0	0	0
3. *Striga senegalense*	8	0	0	0	0
4. *Vitex doniana*	8	0	0	0	0
5. *Psorospermum guineense*	28	12	8	4	0
6. *Anogeissus leoicarpus*	48	12	8	4	0
7. *Securidaca longepedunculata*	5	0	0	0	0
8. *Parkia clappertonia*	55	15	10	0	0
9. *Newbouldia laevis*	8	4	0	0	0
10. *Mangifera indica*	76	28	20	16	8

For *Mangifera indica* dilutions were made directly from the latex collected by plucking the fruits.

Table 6: Percentage mortalities produced by ethanol extracts of some Nigerian plants.

PLANTS	CONCENTRATIONS mg/ml				
	10	1	0.1	0.01	0.001
1. *Steganotaenia araliacea*	88	56	24	12	0
2. *Cyperus rotundus*	32	20	12	8	4
3. *Striga senegalense*	60	20	16	16	8
4. *Vitex doniana*	56	13.4	12	6.6	6.6
5. *Psorospermum guineense**	-	-	-	-	-
6. *Anogeissus leoicarpus*	12	4	0	0	0
7. *Securidaca longepedunculata*	28	12	0	0	0
8. *Parkia clappertonia*	12	8	4	0	0
9. *Newbouldia laevis*	16	12	8	4	0

* The dry extract is insoluble in water and inspersible in water. Results were erratic.

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Table 7: Percentage mortalities produced by n-Hexane ether extracts of some Nigerian plants.

PLANTS	CONCENTRATIONS mg/ml				
	10	1	0.1	0.01	0.001
1. *Steganotaenia araliacea*	100	92	52	24	0
2. *Cyperus rotundus*	100	92	64	16	0
3. *Striga senegalense*	8	4	0	0	0
4. *Vitex doniana*	8	0	0	0	0
5. *Psorospermum guineense*	8	0	0	0	0
6. *Anogeissus leoicarpus*	8	4	0	0	0
7. *Securidaca longepedunculata*	8	0	0	0	0
8. *Parkia clappertonia*	4	0	0	0	0
9. *Newbouldia laevis*	12	0	0	0	0

Table 8: **The activities of extracts of some Nigerian plants against the larva of *Culex pipiens* (Activities are given as LC_{50}). (Values of LC_{50} were derived from graphs of probit mortalities Vs Log Concentration.)**

PLANTS	ACTIVITIES OF THE EXTRACTS (mg/ml)		
	WATER	ETHANOL	PET. ETHER
1. *Steganotaenia araliacea*	NA	0.45	0.61
2. *Cyperus rotundus*	NA	100	0.63
3. *Striga senegalense*	NA	15	NA
4. *Vitex doniana*	NA	5.7	NA
5. *Psorospermum guineense*	25.1	NA	NA
6. *Anogeissus leoicarpus*	7.4	NA	NA
7. *Securidaca longepedunculata*	NA	NA	NA
8. *Parkia clappertonia*	1.4	NA	NA
9. *Newbouldia laevis*	NA	NA	Not applicable
10. *Mangifera indica*	0.18	Not Applicable	

KEY:
NA = Not active which represents negligible moralities only at 1 and 0.1mg/ml dilutions of the extracts.

When all these extracts were compared to Nicotine their larvicidal activities were very negligible. However, two extract have shown significant activity

i. *Steganotaenia araliacea* with LC_{50} = 61ug/ml representing 9.3% that of nicotine.

ii. *Cyperus rotundus* with LC_{50} = 63ug/ml representing 9.1% that of Nicotine. Nicotine is a very toxic insecticidal alkaloid (Ordish, 1976) and the above results is not surprising for extracts as the larvicidal constituent is present in the crude and not in pure form as Nicotine. This activity may increase by isolation and purification

3.3 Preliminary Phytochemical Screening of the n-Hexane extract of stem bark of *S. araliacea*

The n-Hexane extract of the plant was examined for the presence of secondary plant metabolites and the following results were obtained.

1. Test for Tannins: There was no black, blue or green colouration with the addition of ferric chloride. This indicates the absence of tannins.

2. Shinoda Test for flavonoids

There was no red colouration on the addition of magnesium tuning) followed

by Conc. HCl. This indicates the absence of flavonoids.

3. Test for alkaloids

There was no precipitation or colour change with the addition of Dragendoff's reagent or Mayer,s reagent, Wagner and Hager. This indicates the absence of alkaloids.

4. Salkowski's Test for Steroids and Terpenes

With the addition of Conc. sulphuric acid slowly to the chloroform solution of the extract, the formation of a brown ring indicates the presence of Terpenes or steroids.

5. Test for steroidal Aglycone.

There was formation of a pink colouration when the extract was boiled with lead acetate, and the filterate shaked with chloroform evaporated and the residue treated with 1% Nitrophenol followed by sodium hydroxide.

3.4 Activity Guided Chromatographic Studies of *S. araliacea* n-Hexane extract of the Bark

3.4.1 Bulk Extraction Of *Steganotaenia Araliacea* with n-Hexane

The bark was extracted by maceration and the solvent evaporated to

obtain the following results.

Weight of powdered bark	500g
Weight of total extract	12.2g
Percentage yield	$\frac{12.2 \times 100}{500} = 2.44\%w/w$

3.4.2 **Thin layer chromatographic studies of n-hexane extract of *S. araliacea***

The n-Hexane extract of *S. araliacea* was subjected to Thin layer chromatography on silica gel.

The n-Hexane extract gave eight (8) spots on TLC on silica gel using n-Hexane Diethyl ether (10:1). The various R_f values and colours with various locating reagents are given below in table 9.

Table 9: Results Of Chromatographic Separation Of The n-Hexane Extract of *Steganotaenia araliacea*

The TLC plates were developed in n:Hexane: Diethyl ether.
10 : 1

Sample	Spots/R_f Value	Colour in Sunlight	Colour in UV. 366nm	Colour in Iodine	Colour with $KMnO_4$	Colour with $SbCl_3$	Spots Identified
1. TOTAL EXTRACT	I-0.93	Nil	Purple	Brown	Yellow	Purple	a
	II-0.71	Nil	Purple	Brown	Yellow	Deep purple	b
	III-0.35	Nil	Purple	Brown	Yellow	Prussian blue	c
	IV-0.27	Nil	Purple	Brown	Yellow	Reddish brown	d
	V-0.17	Nil	Purple	Brown	Yellow	Purple	e
	VI-0.10	Nil	Purple	Brown	Yellow	Blue	f
	VII-0.03	Nil	Purple	Brown	Yellow	Brownish black	g
	Origin	Nil	Purple	Brown	Yellow	Brown	x

3.4.3 **Activity directed fractionation of the n-Hexane extract of stem bark of *S. araliacea* bark**

The n-Hexane extract of the bark was subjected to silica gel column chromatography and fractions were monitored using thin layer chromatography on silica gel the with n-Hexane. DEE as solvent (Table 10) and for 76-96 also with chloroform. Acetone (Table 11).

The column gave many fractions on evaporation of the various solvents fraction 1-7 was white in colour fraction 8-55 was yellow in colour.

Fraction 56-75 was dark green in colour.

86-100 were orange in colour. Colours with various locating reagent given in table 11.

Table 10: Results of chromatographic separation of fractions of n-Hexane extract of *S. araliacea* n:

Hexane : DEE (10:1)

Fraction	Spot R_f Value	Colour in Sunlight	Colour in UV 366nm	Colour in Iodine	Colour with $KMnO_4$	Colour with $SbCl_3$	Spots Identified
1-7	1- 0.93	Nil	Purple	Brown	Yellow	Purple	a
	II- 0.71	Nil	Purple	Brown	Yellow	D. purple	b
	III-0.35	Nil	Purple	Brown	Yellow	Blue	c
	Origin	Yellow	Purple	Brown	Yellow	Red. Brown	x- base line
8-55	I-0.93	Nil	Purple	Brown	Yellow	Purple	a
	II- 0.71	Nil	Purple	Brown	Yellow	D. purple	b
	III- 0.35	Nil	Purple	Brown	Yellow	Blue	c
	IV-0.27	Nil	Purple	Brown	Yellow	Red. Brown	d
	V-0.17	Nil	Purple	Brown	Yellow	Purple	e
	VI- 0.10	Nil	Purple	Brown	Yellow	Blue	f
	VII- 0.03	Nil	Purple	Brown	Yellow	B. black	g
	Origin	Yellow	Purple	Brown	Yellow	Brown	x
56-75	I-0.93	Nil	Purple	Brown	Yellow	Blue	c
	II-0.71	Nil	Purple	Brown	Yellow	Red. Brown	d
	III-0.35	Nil	Purple	Brown	Yellow	Purple	e
	IV-0.27	Nil	Purple	Brown	Yellow	Blue	f
	Origin	Yellow	Purple	Brown	Yellow	B. black	x
76-85	I-0.10	Nil	Purple	Brown	Yellow	Brown	e
	II-0.07	Nil	Purple	Brown	Yellow	Brown	f
	III-0.03	Nil	Purple	Brown	Yellow	Black	g
86-92	I-0.03	Nil	Purple	Brown	Yellow	Brown	h
	Origin	Yellow	Purple	Brown	Yellow	B. black	x
93-96	Origin	Yellow	Purple	Brown	Yellow	Purple	x

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Table 11: Thin Layer Chromatography of Fractions 76-96 Chloroform : Acetone (4:1)

Sample	Spots/R_f Value	Colour in Sunlight	Colour in UV. 366nm	Colour in Iodine	Colour with $KMnO_4$	Colour with $SbCl_3$
76 - 85						
	I-0.91	Orange	Purple	Brown	Yellow	Orange
	II-0.81	Orange	Purple	Brown	Yellow	Black
	III-0.67	Orange	Purple	Brown	Yellow	Green
	IV-0.47	Orange	Purple	Brown	Yellow	Grey
	V-0.40	Orange	Purple	Brown	Yellow	Blue
	VI-0.25	Orange	Purple	Brown	Yellow	Violet
	VII-0.14	Orange	Purple	Brown	Yellow	Brown

Table 11 cont.

Fractions	Spots/R_f Value	Colour in Sunlight	Colour in UV. 366nm	Colour in Iodine	Colour with KMnO4	Colour with $SbCl_3$
86 - 92						
and	I-0.91	Orange	Purple	Brown	Yellow	Orange
93 - 96	II-0.81	Orange	Purple	Brown	Yellow	Black
	III-0.67	Orange	Purple	Brown	Yellow	Green
	IV-0.47	Orange	Purple	Brown	Yellow	Grey
	V-0.40	Orange	Purple	Brown	Yellow	Blue
	VI-0.25	Orange	Purple	Brown	Yellow	Violet
	VII-0.14	Orange	Purple	Brown	Yellow	Brown

3.4.4 **Isolation of larvicidal active constituents from the n-Hexane extract of *S. araliacea***

The active fractions 1-7 resolved in a small column of silica gel was subjected to TLC on silica gel and the following result was obtained (Table 12)

Table 12: Result of Thin layer chromatography of fraction 1-7 resolved on a small column of silica gel.

Solvent : n-Hexane : Diethyl ether
10 : 1

	RF Value	Daylight	UV 366nn	Iodine	$KMnO_4$	$SbCl_3$
a	0.93	-	Purple	Brown	Yellow	Purple
b	0.71	-	Purple	Brown	Yellow	Deep purple

Fraction 1 - 7 contains the non polar compounds "a" and "b" of the extract with larvicidal active constituents. The result together with those of other fractions are recorded in table 13 below. The purple colouration with Antimony III chloride indicates Terpenes and/or steroids this relates to the result of phytochemical screening as given in 3.3 above. However, part of a and b are in fractions 8-55.

Table 13: Larvicidal Activity of Fractions: Effect Of 0.01mg/ml on the Larva of *Culex quinquefasciatus*

SAMPLE	NO OF DEATHS OUT OF 10					AVERAGE MORTALITY	ADJUSTED MORTALITY	% MORTALITY
	1	2	3	4	5			
Control 0.01% tween 40 + 0.02ml n-Hexane	2	1	1	1	1	1.2	-	12%
1 - 7	9	9	9	10	10	9.4	9.32	93.2
8 - 55	5	6	6	5	5	5.4	4.77	47.7
56 - 75*	0	1	1	1	0	0.6	-	
76 - 85*	1	1	0	1	0	0.6	-	-
86 - 92*	0	0	0	0	0	-	-	
93 - 96*	0	0	1	0	0	0.2	-	-

* Adjusted Morality showed negative values indicating probably death were due to natural causes and not due to the extracts

Preparative Thin Layer chromatography

The active fraction 1-7 containing spot a and b was separated using preparative thin layer chromatoraphy. Spot "a" R_f = 0.93 on silica gel using n-Hexane; acetone (8:1) was scrapped off and eluted using n-Hexane gave a white small needles in ethyl acetate. R_f = 0.53. petroleum ether: Methanol (9:1), R_f = 0.94.

Table 14: **Determination of Sublethal Dose of Compound (A) on Larva of *Culex quinquefasciatus***

Concentration	Death out of 10	Percent Dead
0.01mg/ml	9	90
0.001mg/ml	1	10
0.0001mg/ml	0	0

From this result the concentration of 0.001mg/ml of "a" was considered to be the sublethal dose of the compound as it caused death to only 10% of larva after a 24 hour exposure. The larvae were exposed to a sublethal dose of compound "a" to see the effect on larval growth and development. The number of larvae, papae and adults were counted after 2,3,4 and 5 days and the results in table 16.

Table 15: **Effect of long term exposure of sublethal dose (0.001mg/ml) of compound "a" on larva of *Culex quinquefasciatus***

DAYS	DEATHS (Cumlulative					Pupation					Normal adults				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
2	1	0	0	0	1	2	1	2	0	0	0	0	0	0	0
3	1	0	1	1	0	4	2	2	2	1	4	2	1	0	1
4	1	1	1	1	1	0	4	4	6	6	9	5	5	3	3
5	1	1	1	1	1	0	0	0	0	0	9	9	9	9	9
	Average - 1					No pupa left after 5 days					9 adults out of 10 emerged				

Note: The sublethal dose of 0.001 mg/ml did not prevent the emergence of adults from the larvae however in all cases the adults were smaller than control. This indicate that the fraction may not have any growth regulating effect.

The effect of compound "a" was evaluated on adult mosquito, the contact insecticidal effect (Table 18) compared those due to Nicotine (Table 17).

Table 16: Effect Of Nicotine On Adult *Culex quinquefasciatus* mosquito after a 60 Second Exposure followed by Determination Of Mortality after 4 Hours.

Conc µg/cm²	MORTALITIES	OBSERVED PERCENT MORTALITY	ADJUSTED PERCENT MORTALITY
17.2	18/20	90	89.5
3.0	23/26	81.7	80.7
0.30	22/30	59.9	57.8
0.015	8/20	40.0	36.8

CONTROL DEATH = 1/20 = 5%

LC_{50} = 1.29ug/cm² from graph of probit Vs log Conc.

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Table 17: Effect Of Compound "A" on adult *Culex quinquefasciatus* mosquito after a 60 Sec. exposure followed by Determination Of Mortality after 4 Hours

Conc µg/cm²	MORTALITIES	OBSERVED PERCENT MORTALITY	ADJUSTED PERCENT MORTALITY
300.8	17/20	85.0	84.2
172.1	16/26	80.0	78/9
60.2	12/30	34.3	30.8
30.1	7/20	8.3	3.5

CONTROL DEATH = 1/20 = 5%

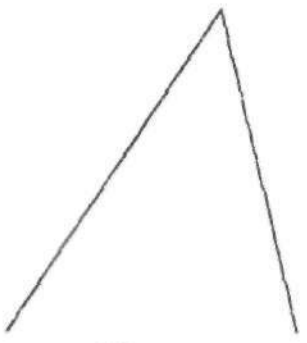
LC_{50} = 56.2ug/cm from graph of probit Vs log Conc.

From the result 56.2µg/cm² of fraction has similar activity to 1.29ug/cm² of Nicotine. The effect is rather small because Nicotine is an extremely poisonous contact insecticide, probably the compound may not have penetrated the cuticle in sufficient quantity. This suggests that compound `a' may not be a good contact insecticide. Further working unveil its potentials as insecticide.

3.5 Chemical and Spectroscopic Studies on the Larvicidal Active Constituent from the n-Hexane extract of *S. araliacea*

Compound "a" gave white needles from ethyl acetate which is soluble in n-Hexane, acetone and chloroform. The compound has been shown to be pure by chromatography on silica gel using n-Hexane: Acetone (8:1), R_f = 0.93, chloroform: Acetone (5:1), R_f = 0.53 and petroleum ether: methanol (9:1), R_f = 0.94. It appears as a dark quenching spot in ultraviolet light (at 366nm), gives a brown spot with iodine a yellow spot against a pink background with potassium permanganate (showing it to be a reducing agent) and purple colour with Antimony III chloride (showing it to be a terpene derivative). It gives no reaction with ferric chloride indicating the absence of phenolic hydroxyl group. The infra red (IR) and ultraviolet spectra of the compound were recorded and the following results were obtained.

U.V: Chloroform



nm 248, 265 280s

S= shoulder

Max Fig. 2

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I.R nujol

max cm^{-1} 3129s, 2727w

1651m, 1462s. 1404s

1137s, 1305w, 1154,

969w and 722s

s = sharp

m = medium

w = weak

Fig: 3 and Fig. 4

The absence of any band in I.R maximum at $3400cm^{-1}$ (fig 2) indicates the absence of free hydroxyl group. The band at $1651cm^{-1}$ shows the presence of carbonyl group together with the band at $1154cm^{-1}$ confirms this to be from an ester. The presence of a band at $722cm^{-1}$ indicates probably an orthomonosubstituted benzene. U.V max are at 248nm and 265nm. (Fig 3)

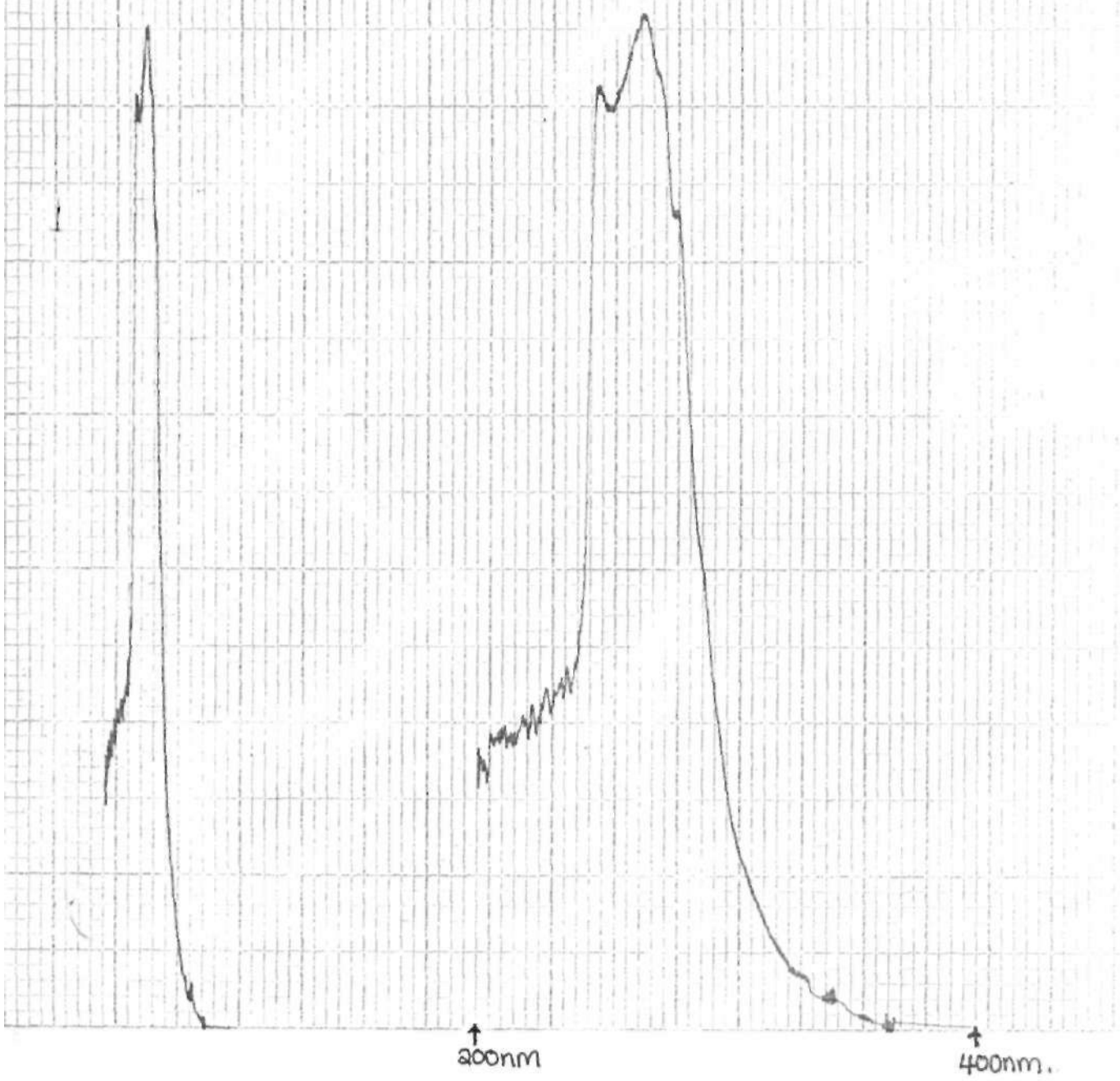
These spectra suggests a steroid or terpenoid. More information is required to completely elucidate the structure of compound "a" such as mass spectroscopic studies and nuclear magnetic resonance spectroscopy. The yield of "a" is very low (about 2mg from 500g of powdered bark) more work is necessary to optimise the yield.

However compound "a" gives a purple colouration with Antimony III chloride (Carr-price reagent). This colouration is characteristic of diterpenes and triterpenes. These terpenoids occur in the non volatile fractions of n-Hexane extracts (non polar extracts). The presence of triterpenes is established in *S. araliacea* (Lavaud *et al.*, 1992). The effects of terpenoids on insects is established (Harborne, 1991).

Typical example of these effects are shown by the triterpenes of *Azadiracta indica* A juss, now patented for the control of many crop pests (Jacobson, 1988). *Steganotaenia araliacea* may therefore be a good potential source of anti insect terpenoids.

Catalogue Number 612206

Fig. 2: Ultraviolet spectra of compound 'a' recorded in Chloroform.



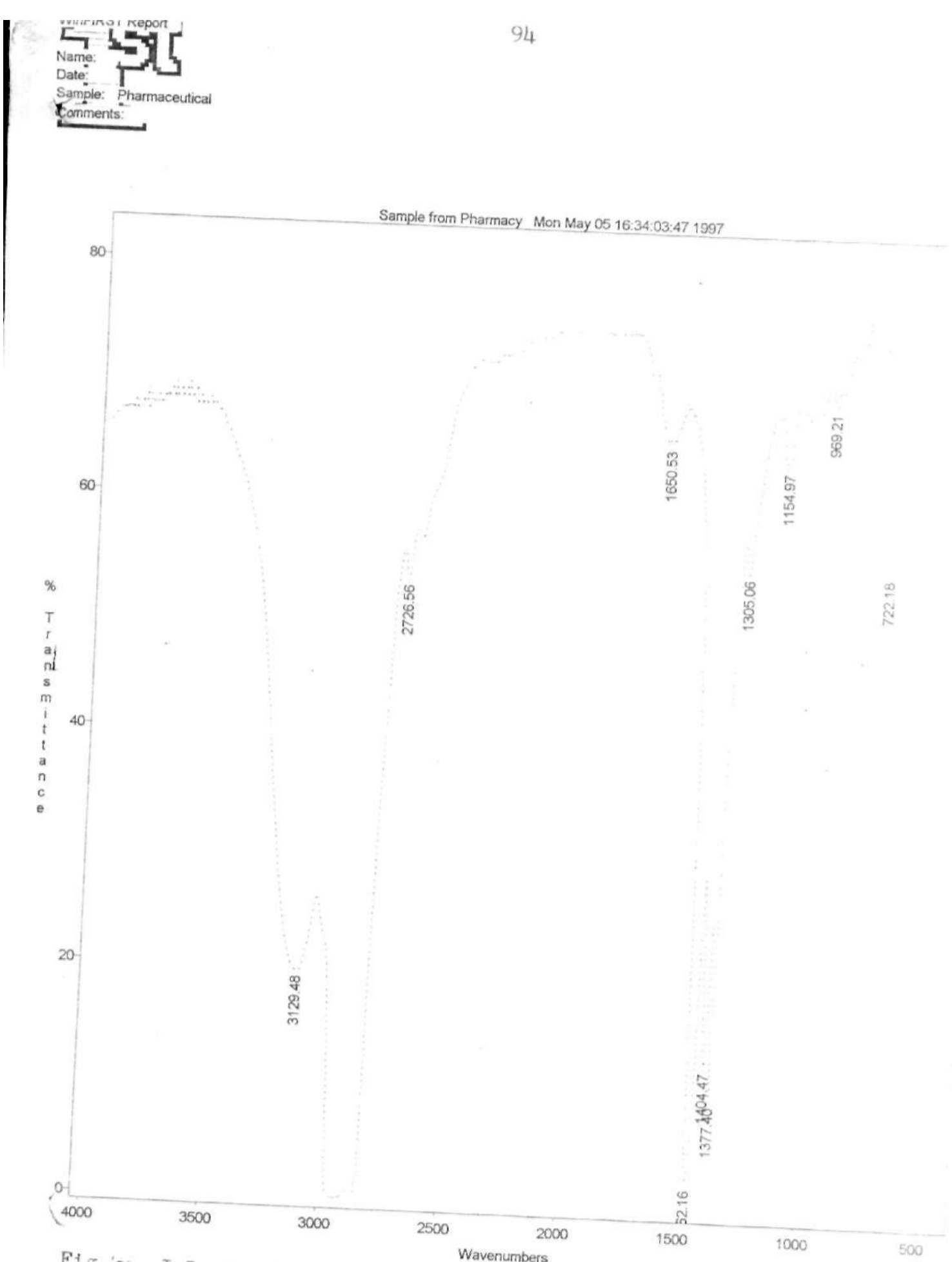


Fig. 3: I.R. Spectra of compound 'a' in nujol mull.

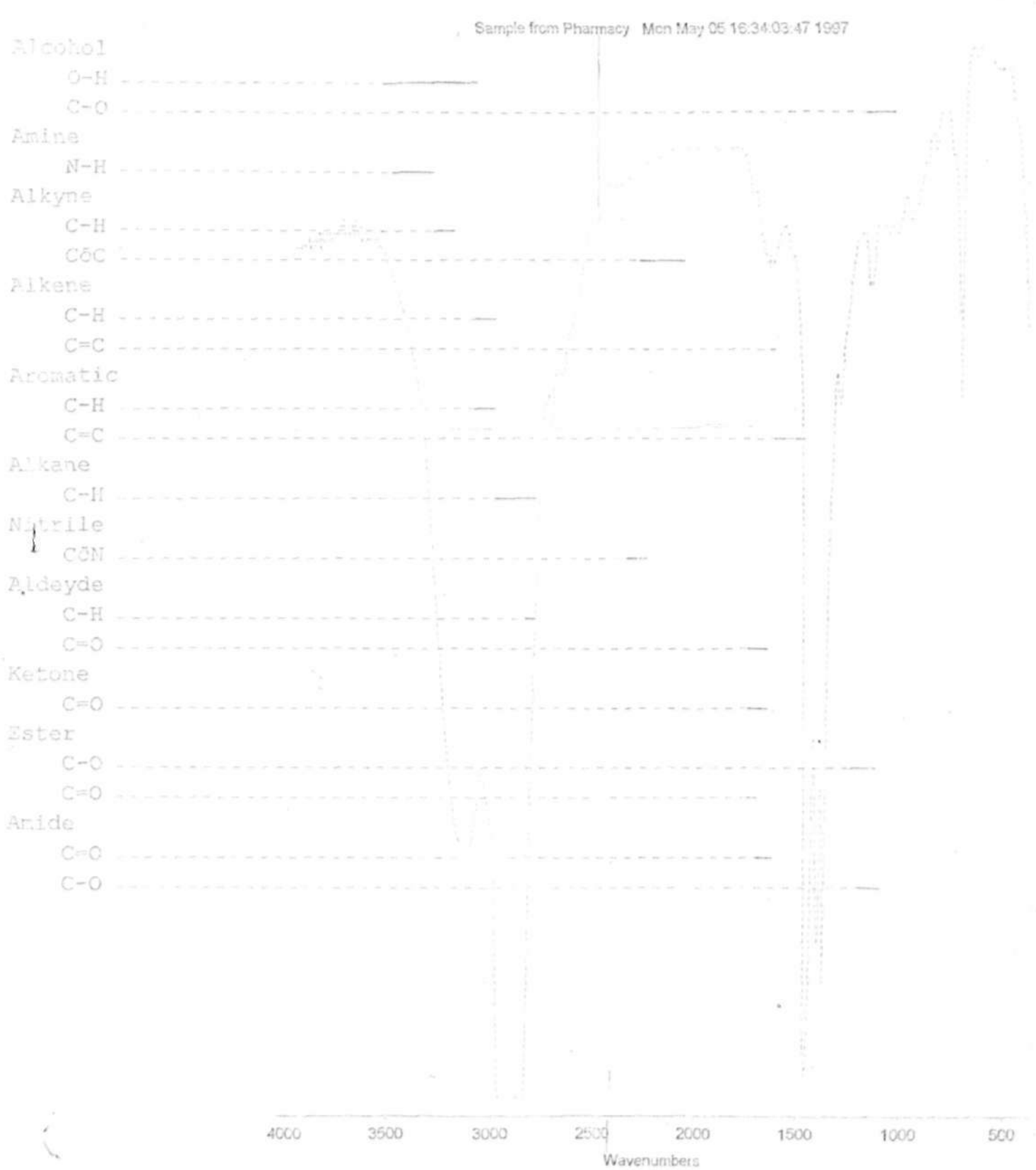


Fig: I.R. spectra of compound 'G' showing characteristic functional groups.

3.6 Pharmacognostic studies of leaves and barks of *S. aralicaea*

3.6.1 Pharmacognostic studies of leaves of *S. araliacea*

MACROSCOPICAL EXAMINATION

WHOLE LEAVES

Botanical source: *Steganotaenia araliacea*

family: Umbelliferae

Geographical source: Zaria Samaru close to the Ahmadu Bello University dam

Form: Entire

Condition: fresh

LAMINA

Shape: Lanceolate

Size: Length: 7.5 - 11.6 - 17.0cm

Width: 3.9 - 4.8 - 8.0 cm

Venation: Reticulate

Margin: Serrated

Apex: Acute

Base: Symmetric

Surface: Coriaceous

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PETIOLE

Size: 0.8 - 1.7 - 4.7cm

Surface: Dark green

SENSORY CHARACTERS

Colour: Pale green

Odour: Strongly aromatic

Taste: Acrid

Texture: Thin and papery

B. MICROSCOPICAL EXAMINATION

Various features of the leaves were examined under the microscope.

i) Surface preparation: The following are the description of epidermal characters of the leaf.

a. Description of the upper epidermal characters. (Fig.5)

Epidermal cells

Shape: polygonal

anticlinal walls: wavy

Thickening: Thick

Papillae: Absent

Cuticle: Smooth

Stomata: Absent

Trichomes: Absent

b. Description of the lower epidermnal characters (Fig.6)

Epidermal cells

Shape: Polygonal

Anticlinal walls: wavy.

Thickening: Thin

Papillae: Absent

Cuticle: Smooth

Trichomes: Absent

Stomata : Present

Type - Anisocytic

Frequency - frequent.

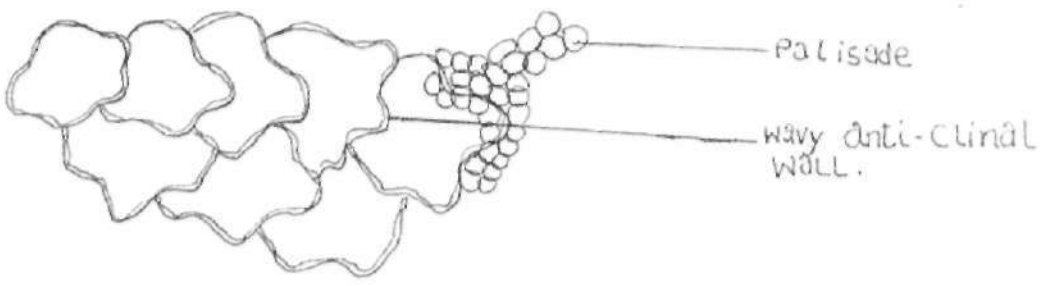


Fig.5: Upper epidermis in surface view with part of the underlying palisade. ×400

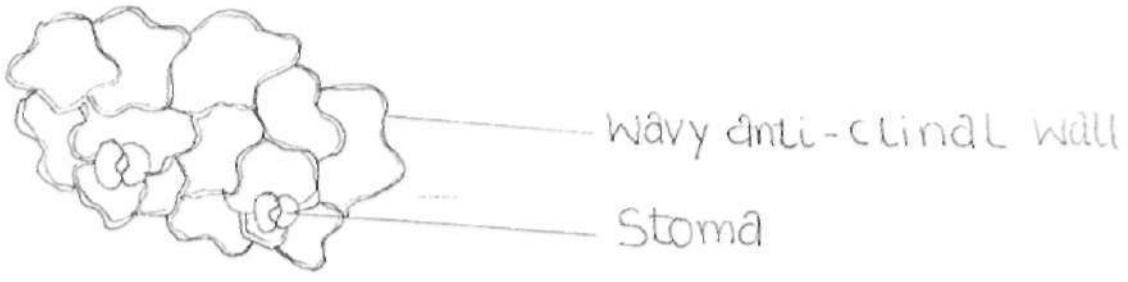
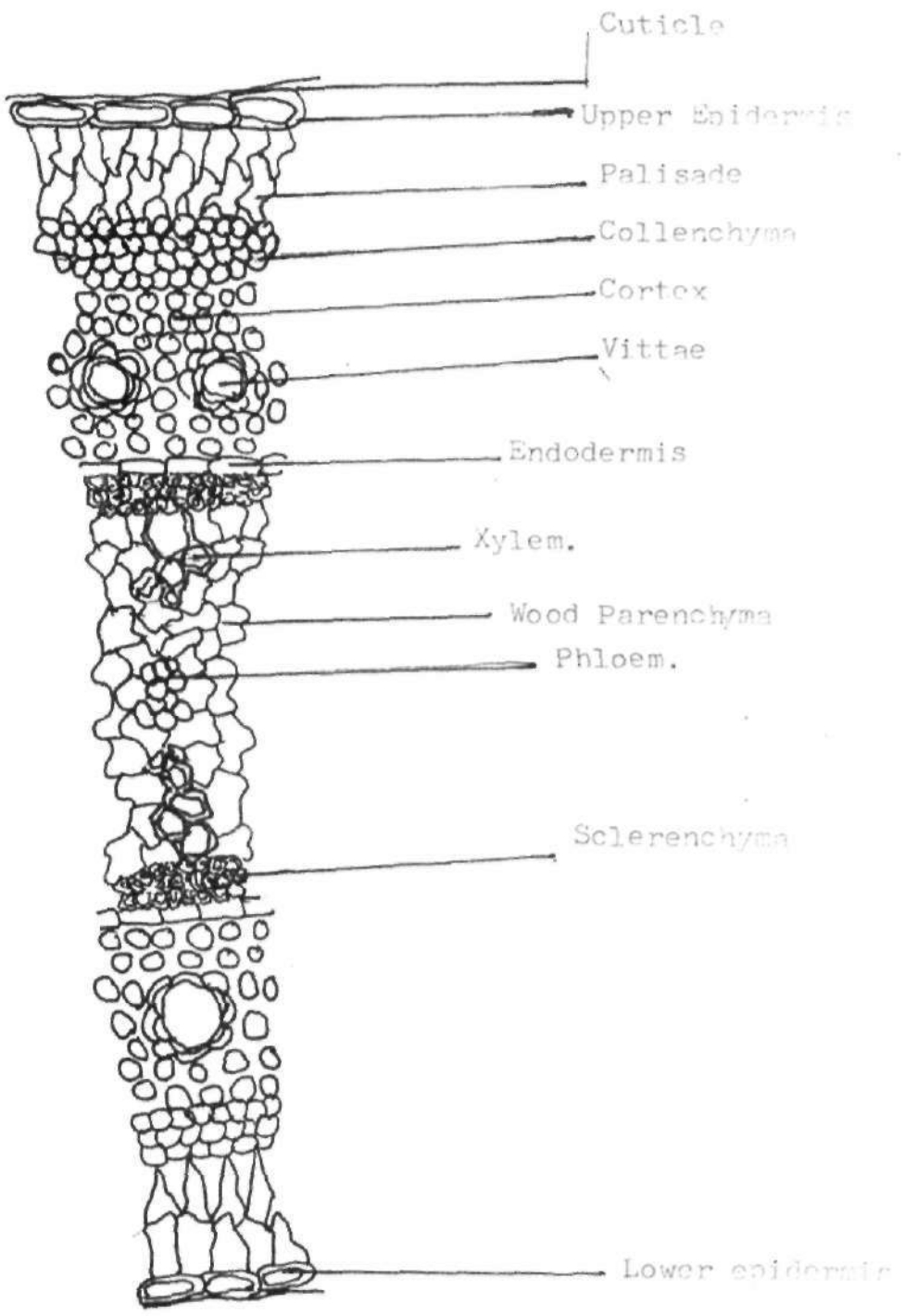


Fig 6: Lower epidermis in surface view showing anisocytic stomata. ×400



Mag. X 400

Fig.7: Transverse Section of Steganotaenia araliacea leaf through the midrib.

ii. Transverse section of the leaf through the lamina and midrib.

Epidermal layer: This consist of a single row of polygonal tubular cells with straight anticlinal walls. Anisocytic stomata are found only on the lower epidermis. Both surfaces were covered with a thin cuticle.

Palisade Layer: The leaf is isolateral consisting of layers of palisade tissues (two layers to each surface). The palisade cells are cylindrical closely packed cells containing chloroplast.

Endodermis: Not distinguishable

Pericycle: Consists of collenchyma cells forming a ring around the vascular bundle secreatory ducts are found in spatial arrangement above the endodermis and usually Nine (9) in number and surrounded by several colllapsed cells.

Vascular System: This appear as a crescent shape structure

with pericyclic fibres surrounding them. The xylem vessels are lignified with acute ends, xylem being mostly primary accompanied by little secondary vessels. (There is no distinct Cambium). Separating the xylem and phloem. The phloem tissue consist of rows of pholem parenchyma companion cells and sieve tubes. (Fig.7)

c: CHEMOMICROSCOPICAL EXAMINATION

The chemomicroscopical examination of the leave was carried out and the following observation were obtained.

a: **Cell Wall Constituents**

1. Cellulose

The parenchyma cells of the cortex, the epidermal cell and sclerenchyma cells were stained blue black when the transerve section was treated with N/50 iodine followed by concentrated sulphuric acid indicating the presence of cellulose.

2. Lignin

A deep red colouration was formed in the xylem elements with the addition of phloroglucinol followed by concentrated hydrochloric acid indicating the presence of lignin.

3. Tannins

A blue black colouration was formed on the walls of the vascular bundles (xylem elements) with the addition of ferric chloride indicating the presence of Tannins.

4. Mucillages

A pink colouration with ruthenium red, blue colouration with chlor-zinc iodine and a violet colour with N50 iodine followed by Conc H_2SO_4 shows the present of mucillages in the palisade parenchyma of the leaf lamina.

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b. **Cell inclusions**

1. Starch

There was a blue black colouration (bands) around the pericycle, with N_{50} iodine the Transverse section of the leaf which indicated the presence of starch grains.

2. Proteins

There was no formation of yellow colouration with the addition of picric acid which indicated the absence of proteins.

3. Calcium oxalate

Spherocyrstals of calcium oxalate were seen after clearing with chloralhydrate, this disappears with the addition of conc. sulphuric acid.

4. Oils

There was a red colouration in the secreatory ducts with Sudan IV, which disappeared on slow heating showing the presence of volatile oils.

Table 18: Summary of Results of Chemomicroscopical Study of *S. araliaecea*

CELL WALL COMPONENTS CELL INCLUSION	RESULT	COMMENTS
CELL WALL COMPONENTS		
1. CELLULOSE	+	Cellulose found in cortical parenchyma sclerechyma and epidermal cells
	+	Lignified xylem vessel with spiral thickening
2. LIGNIN	+	Walls of xylem vessel and fibers contains tannins
3. TANNIN	+	Walls of spongy tissues contain mucillage
4. MUCILLAGES		
CELL INCLUSIONS	+	Abundant starch in the pericyclic area
1. STARCH	-	
2. PROTEINS	+	Spherocrystals of calcium oxalate in parenchyma cells
3. CALCIUM OXALATE	+	Oils in vitae cells
4. OILS		

Table 19: Result Of Quantitative Microscopy Leaves Of *Steganotaenia* araliacea Leaves

PARAMETER	VALUE (AVERAGE UNDERLINED)
1. Palisade number	
a. Upper surface	12 - <u>13.6</u> - 17
b. Lower surface	4 - <u>6.8</u> - 10
2. Stomatal number	
a. Upper surface	- - - -
b. Lower surface	49 - <u>59.4</u> - 69
3. Stomatal index	
a. Upper surface	- - -
b. Lower surface	11.7 - <u>14.4</u> - 18.1
4. Vein islet number	6 - <u>7.8</u> - 9
5. Vein termination number	1 - <u>1.5</u> - 2

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3.6.2 **Pharmacognostic examination of powdered Stem bark of *S. araliacea***

Pharmacognostic studies was carried out using standard procedures and the following results were obtained.

a. MACROSCOPICAL EXAMINATION

The macroscopical features were described using standard parameters and the macromorphological features of the powdered bark are recorded below.

i. Form: Coarse powder (mesh no. 44)

ii. Odour: Characteristic aromatic smell

iii. Colour: Brown

iv. Taste: Sweet bitter taste

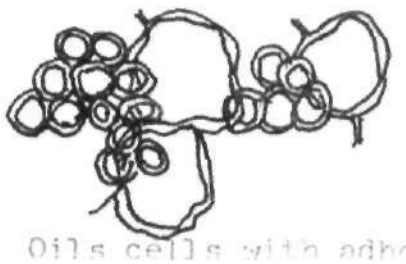
The powdered bark has a striking aromatic smell with bitter taste.

b. MICROSCOPICAL EXAMINATION

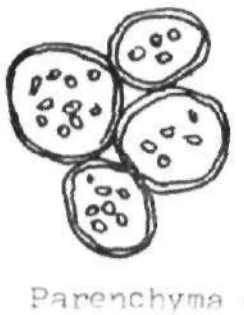
The microscopical features of the powdered bark includes a group of parenchyma cells with starch grains, stone cells, non lignified elongated fibres and secreatory ducts.(Fig. 8)



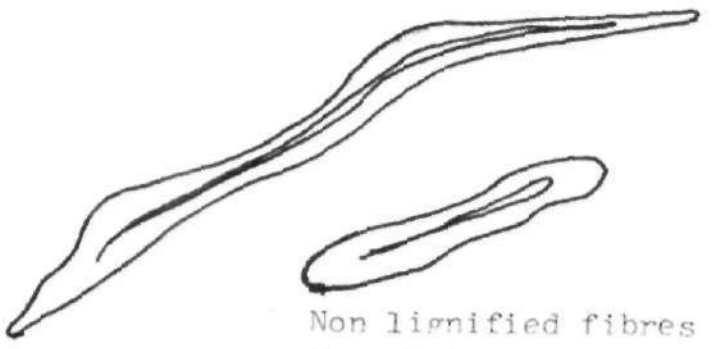
Cork and Phelloderm in Sectional view Mag. X 400



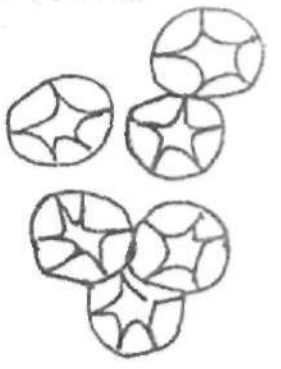
Oils cells with adherent parenchy
Mag. X 400



Parenchyma cells with starch grains
Mag. X 400



Non lignified fibres
Mag. X 400



Sclereids

Fig. 8: Characteristic features of Steganotaenia araliacea Powdered bark.

3.7 **DISCUSSIONS**

Throughout the thousands of years of human history, natural plant insecticides have been sought and used frequently in most part of the world. Many inhabitants of the tropics like Nigeria have an abundant source of powerful plant insecticides, the example of the neem tree is well established (Jacobson, 1988).

Traditional pest control are widely practised in Nigeria, in Kaduna state alone a number of plants belonging to diverse plant families are used in pest control (Table 3). There is therefore the need for designing a bioassay method for identifying potentially useful plants.

Although there are a variety of antiinsect activity (insecticidal, repellent, attractant, antifeedant, larvicidal etcetera); Mosquitoes larva is a reliable organism for screening plants with anti-insect activity. Escoubas, *et al.,* 1994 have screened some Nigerian plants for activity using classicial bioassay with *Aedes aegypti* (mosquito), *Spodiptera speratus* litura (tobacco cutworm) and *Reticulitermis speratus* (termites), they found that the larva of mosquito gives a better and consistent result.

n-Hexane extract of *S. araliacea* was shown to be the most active among the extracts of the plant screened. With an LC_{50} = 6ug/ml, it has a higher activity than similar extracts of neem seeds leaves flowers and stems with LC_{50} = 120ug/ml as reported by Kalanasundaram and Babu, 1982, using the classicial bioassay method on *Culex quinquefasciatus.* However, when compared to nicotine the effect of *S. araliacea* was low due to the very toxic nature of nicotine and probably because they may be acting on different cellular mechanism in the larva. The fractionation of

extract led to identification of activity in the non polar fractions. With further chromatography, the active compound "a" was isolated this was shown to be a terpene derivative (purple with Antimony III chloride, and characteristic U.V and I.R. spectra).

The effects of terpenoids on insects have been documented (Harbone, 1991). The lower terpenoids (mono-and sesquiterpenes) have a no specific toxiciant action and used in insect defences to deter other insect or animal predators. Diterpenes (which occur in resin secretions especially in umbelliferae) have anti-feedant and insect growth regulating activity against insects. (Raymond, 1970).

The complete structure of compound "a" is not conclusive because chemical analysis, U.V and I.R alone cannot give details of the structure. The use of sophisticaed methods such as GC-MS and NMR (^{1}H and ^{13}C) is necessary. Compound "a" has activity against the larva of *Culex quinquefasciatus*, however, the effects on adult is limited. (LC_{50} = 56.2ug/cm^2) with no growth regulating activity.

The plant *S. araliacea* with useful potentials in insect control can be identified using some pharmacognostic characters. Diagnostic characters include lanceolate leaves with serrated margin and reticulate venation. The leaf sizes are characteristic. Microscopically, the transverse section is similar to those of a dicot. leaf, the presence of vittae (containing oil) is characteristic. Quantitative microscopical characters are specific. These parameters can be used in the preparation of pharmacopical standard of the plant. The bark has characteristic features of young bark example,

fibres, sclereids, parenchyma with calcium, oxalate, all cells have abundant oil.

3.8 CONCLUSIONS

The plant *Steganotaenia araliacea* Hochst is used extensively in ethnomedicine among which is the control of insect pests. Considering the fact that mosquito larva is an effective tool for the bioassay of substances active against insects, *S. araliacea* with significant larvicidal activity among the ten plant screened has the highest potentials for active anti insect compound.

A biosassay guided chromatographic studies of n-Hexane extract of *S. araliacea* has shown an appreciable contact insecticidal property, although without any growth regulating activity, the mosquito that emerges in a growth media containing the active compound were weaker than controls.

It is therefore suggested that further work should be directed at establishing the broad anti insect activity of the plant with an extensive phytochemical and spectroscopic studies of the n-Hexane extract of the plant.

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