PHYTOCHEMICAL ANDANTI-INFLAMMATORY STUDIES ON THEHEXANE EXTRACT OF THE STEM BARK OF*STEGANOTAENIA ARALIACEA*HOSCHTS (APIACEAE)

By

Umar FarukSHEHU

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,
FACULTY OF PHARMACEUTICAL SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA - NIGERIA

AUGUST, 2015

PHYTOCHEMICAL AND ANTI-INFLAMMATORY STUDIES ON THEHEXANE EXTRACT OF THE STEM BARK OF *STEGANOTAENIA ARALIACEA*HOSCHTS (APIACEAE)

By

Umar FarukSHEHU (B. Pharm., A.B.U., 2008)
(M.Sc/PHARM-SCI/1923/11 - 12)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE IN PHARMACOGNOSY

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,

FACULTY OF PHARMACEUTICAL SCIENCE,

AHMADU BELLO UNIVERSITY, ZARIA - NIGERIA

AUGUST, 2015

DECLARATION

I declare that the work in this dissertation entitled **Phytochemical and Anti-inflammatory Studies on the Hexane Extract of the Stem Bark of *Steganotaeniaaraliaceae*Hoschts (Apiaceae)** has been carried out by me in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria under the supervision of **Prof. M.S. Abubakar and Dr. U.H.Danmalam**. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis has been previously presented for another higher degree or diploma at any University.

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

Umar FarukShehu **Date**

CERTIFICATION

This Dissertation entitled "**Phytochemical and Anti-inflammatory Studies on the Hexane Extract of the Stem Bark of *Steganotaeniaaraliaceae*Hoschts (Apiaceae),**by Umar FarukShehu 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.

....
Prof. M.S. Abubakar (B. Pharm., M.Sc., Ph.D) Date
Chairman, Supervisory Committee
Department of Pharmacognosy and Drug Development
Ahmadu Bello University, Zaria.

....
Dr. U.H. Danmalam (B. Sc., M.Sc., Ph.D) Date
Member, Supervisory Committee
Department of Pharmacognosy and Drug Development
Ahmadu Bello University, Zaria.

....
Dr. G.Ibrahim(B. Sc, M.Sc., Ph.D) Date
Head,
Department of Pharmacognosy and Drug Development
Ahmadu Bello University, Zaria.

....
Prof. A. Z. Hassan Date
Dean, School of Postgraduate Studies,
Ahmadu Bello University, Zaria.

DEDICATION

To my parents Lt. Col. UsmanShehuAdamu (rtd) and Hajiya Maryam Ahmad for their love, support and understanding .

ACKNOWLEDGEMENT

I acknowledge the grace of Allah for seeing me through this work. To my supervisors Prof. M.S. Abubakar my mentor and Dr. U.H.Danmalam, I say thank you for guiding me through the course of this thesis, may Allah reward you.

I wish thank my wife Rukayya and Muhammad my son for the love and understanding throughout the period of this work. Also,I acknowledge all the Lecturers and Staff of the Department of Pharmacognosy and Drug Development; and Faculty of Pharmaceutical Sciences for their support and encouragement.

To my friends and colleagues Salisu, SalisuUstaz, MalamAbdulmumin, MalamAbdulrahman, Anas, Umar, Jajere, Ibrahim thank you all.

ABSTRACT

Inflammation has been implicated in virtually all human and animal diseases. It has become the focus of global scientific research, more so, since the currently used anti-inflammatory agents both steroidal and non-steroidal are prone to evoking serious adverse reactions. *Steganataeniaaraliacea*(Apiaceae)stem bark has been reported to be used in traditional medicine for the treatment of asthma and rheumatism in East Africa. In this study, thehexane extract of the stem bark of the plant was evaluated for its anti-inflammatory property and with a view to isolating bioactive compounds from it.Methods for evaluating macroscopical features and physico-chemical properties were used for the pharmacognostic studies on the stem bark, chromatographic techniques including the thin layer chromatography (TLC) and column chromatography using silica gel were used for the phytochemical studies on the hexane extract of the plant while the anti-inflammatory study was also carried out on the hexane extractusing formalin induced paw oedema in rats. The macroscopical features of the whole stem bark was described as yellowish-green in colour, waxy and short fractured with an aromatic odour while the powdered stem bark was described as brown in colour, aromatic in odour with a sweet bitter taste. The physico-chemical properties of the bark determined include total ash value 10.67%, acid-insoluble ash value 4.00%, water-soluble ash value 1.25%, moisture content 8%, water-soluble extractive value 2.87%, alcohol-soluble extractive value 6.67% and petroleum ether-soluble extractive value 1.27%. The TLC profiling of the hexane extract of the bark showed the presence of steroids/terpenes. The column chromatography of the hexane extract of the stem bark lead to isolation of Compound UF which was characterized using the ^{1}H-NMR spectroscopy and its physical properties. Compound UF was identified as Hentriacotane ($C_{31}H_{64}$) based on the available spectroscopic data and physical properties which agreed well with those reported in the

literature. The acute toxicity studies of the hexane extract revealed (LD_{50}) of 282.84mg/kg suggesting the extract is moderately toxic. The effect of the hexane extract on formalin induced paw oedema in rats revealed a dose dependant inhibition of the paw oedema when compared to the reference. The concentrations of the extract at 35, 70 and 140 mg/kg of the extract showed 41%, 42% and 50% inhibition respectively at the peak of the paw oedema which were statistically significant at $P<0.05$. This indicates a dose dependent anti-inflammatory activity of the extract.

TABLE OF CONTENTS

COVER PAGE i

TITLE PAGE ii

DECLARATION..... iii

CERTIFICATION iv

DEDICATION..... v

ACKNOWLEDGEMENT..... vi

ABSTRACT..... vii

TABLE OF CONTENTS..... ix

LIST OF FIGURES..... xiv

LIST OF TABLES..... xv

LIST OF PLATES xvi

LIST OF APPENDECIES..... xvii

LIST OF ABBREVIATIONS..... xviii

CHAPTER ONE..... 1

1.0 INTRODUCTION 1

1.1 Definition and Scope of Pharmacognosy..... 1

1.2 Taxonomy of medicinal plants..... 2

1.3 Chemistry and Biological Activity of Plant Extracts..... 3

1.4 Extraction, Isolation and Identification of Bioactive Constituents from Plants..... 4

1.5 Statement of the Research Problem.....6

1.6 Justification of this Study..... 6

1.7 Hypothesis..... 6

1.8 General Aim..... 7

1.8 Specific Aims and Scope of the Study..... 7

CHAPTER TWO..... 8

2.0 LITERATURE REVIEW..... 8

2.1 Taxonomic Classification of the Family Apiaceae..... 8

2.2 Botanical Description of the Plant Family Apiaceae (Umbeliferae)..... 9

2.3 Reported Phytochemical Constituents of the Apiaceae Family..... 10

2.4 Description of the Subfamily Saniculoidae..... 10

2.5 Botanical Description of the Genus *Steganotaenia*..... 11

2.5.1 Ethanobotanical Uses of Species *S. araliacea*..... 13

2.5.2 Phytochemistry and Biological activity of the Species *S. araliacea*..... 13

2.6 Inflammation..... 17

2.6.1 Acute Inflammation..... 17

2.6.2 Chronic Inflammation..... 17

2.7 Plant-Derived Compounds with Anti-Inflammatory Activity..... 18

CHAPTER THREE..... 22

3.0 MATERIALS AND METHODS..... 22

3.1 List of Chemicals and Reagents..... 22

3.1.1List of Chemicals..... …. 22

3.1.2 List of Reagents for Chromatographic Studies..... 23

3.2 List of Materials and Equipments..... 23

3.2.1Chromatographic Materials..... 23

3.2.2 Materials for Anti-inflammatory Studies..... 24

3.2.3 Source of Laboratory Animals Used..... 24

3.3 Plant Collection, Preparation and Identification..... 24

3.4 Pharmacognostic Evaluation of the Stem Bark of *S. araliacea*.....24

3.4.1 Macroscopical evaluation of whole and powdered stem bark of *S. araliacea*..... 24

3.4.2 Physico-Chemical Evaluation of Powdered Stem Bark of *S. araliacea*..... 25

3.5Extraction of Plant Material..... 28

3.6Phytochemical Studies on the Hexane Extract of the Stem Bark of *S. araliacea*.....28

3.6.1 Thin Layer Chromatographic (TLC) Profiling..... 28

3.6.2 Column Chromatography..... 29

3.6.3 Identification of Compound UF..... 30

3.7Acute Toxicity Testing..... 30

3.7.1 Determination of Median Lethal Dose (LD_{50})..... 30

3.8Anti-Inflammatory Studies..... 31

3.9Statistical Analysis..... 32

CHAPTER FOUR……. 33

4.0 RESULT…… 33

4.1Pharmacognostic Evaluation of *S. araliacea* Stem Bark…… 33

4.1.1 Macroscopical Evaluation of *S. araliacea* Stem Bark..... 33

4.1.2 Physico-Chemical Evaluation of the Powdered Stem Bark of *S. araliacea*..... 36

4.2 Extraction Yield of Powdered S. araliacea Stem Bark with Hexane..... 38

4.3Phytochemistry of the Hexane Extract of Stem Bark of *S.araliacea*.....38

4.3.1 Thin Layer Chromatographic Profile..... 38

4.3.2 Result of the Colum Chromatography of the hexane extract of the stem bark……. 41

4.3.3 Isolation of Compound UF..... 43

4.5 Identification of Compound UF..... 45

4.5.1 Physical Properties of Compound UF..... 45

4.5.2 Proton NMR of Compound UF..... 45

4.5.3 Proposed structure of Compound UF..... 48

4.6 Acute Toxicity Testing (Median Lethal Dose LD_{50})..... 49

4.7 Anti-Inflammatory Studies..... 49

CHAPTER FIVE..... 51

5.0 DISCUSSION..... 51

CHAPTER SIX..... 56

6.0 SUMMARY CONCLUSIONS AND RECOMMENDATIONS..... 56

6.1 Summary.....56

6.2 Conclusions..... 57

6.3 Recommendations..... 57

REFERENCES..... 58

APPENDECIES..... 67

LIST OF FIGURES

	Title	Page
Fig. 4.1:	Proton NMR Spectrun of Compound UF	46
Fig. 4.2:	Proposed Structure of Compound UF.....	48

LIST OF TABLES

	Title	Page
Table 2.1:	Some Isolated Compounds from Plants with Anti-Inflammatory Activity.	19
Table 4.1:	Macroscopical Features of *S. araliaceae* Whole Stem Bark.....	34
Table 4.2:	Macroscopical Features of Powered*S. araliaceae* Stem Bark.....	35
Table 4.3:	Ash Values, Moisture Content and Extractive Values.....	37
Table 4.4:	Thin Layer Chromatogram of the Hexane Extract of the Stem Bark of *S. araliacea* using Specific Spraying Reagents.....	40
Table 4.5:	Fractions Collected from the Column Chromatography of the Hexane Extract of the Stem Bark of *S. araliacea*.....	42
Table 4.6:	Proton NMR of Compound UF and Hentriacotane.....	47
Table 4.7:	The Effects of the Hexane Extract of the Stem Bark of *S. araliacea* on Formalin Induced Paw Oedema in Rats.....50	

LIST OF PLATES

Title Page

Plate I: *Steganotaeniaaraliacea*Tree……..... 12

Plate II:TLC profile of the Hexane Extract *S. araliacea*Stem Bark..... 39

Plate III**:** TLC profile of Compound UF..... 44

LIST OF APPENDICIES

Title Page

APPENDIX A: Determination of Ash Value of powdered Stem Bark of *S.* araliacea……. 67

APPENDIX B: Determination of Acid insoluble Ash of powdered Stem bark of *S. araliacea*. 68

APPENDIX C: Determination of water soluble Ash of powdered Stem Bark of *S. araliacea*. 69

APPENDIX D: Determination of Water-Extractive value of powdered Stem Bark of *S. araliacea*…….. 70

APPENDIX E: Determination of Alcohol – Soluble Extractive value of powdered Stem Bark of *S. araliacea*..... 71

APPENDIX F: Determination of Acute toxicity of the Hexane extract of *S. araliacea* Stem barkusing Lorke's Method…… 72

APPENDIX G: Procedure for the preparation of spray reagents used for TLC..... 73

APPENDIX H: TLC Profile of the Hexane Extract of Stem Bark of *S. araliaceae* developed in Hexane-Ethylacetate (10:1) sprayed with Liebermann Burchard reagent and viewed under day light (A) and sprayed with Aluminium Chloride viewed under long UV light (B)…… 74

LIST OF ABBREVIATIONS

CC	Column chromatography
°C	Degree Celsius
Fig.	Figure
g/gm	Gram
Hz	Hertz
mg	Milligram
µg	Microgram
µL	Microliter
MHz	Mega Hertz
Na	Sodium
Ca	Calcium
K	Potassium
NMR	Nuclear Magnetic Resonance
ppm	Parts Per Million
R_f	Retention factor
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography
UV	Ultra Violet
v/v	Volume by Volume
w/w	Weight by Weight
α	Alpha

β	Beta
δ	Delta
C	Carbon
^{1}H - NMR	Proton Nuclear Magnetic Resonance
i.p	Intra-peritoneal route of administration
SAHE	Hexane extract of stem bark of *S. araliacea.*
SEM	Standard error of the mean
iNOS	inducible nitric oxide synthase
COX	cyclooxygenases
LOX	lipoxygenase
MMP	matrix metalloproteinase
cpDNA	chloroplast DNA
RPS16	Ribosomal protein S16
MAbs	Monoclonal Antibodies
FTIR	Fourier-Transform Infra-Red spectroscopy
MS	Mass Spectroscopy

CHAPTER ONE

1.0INTRODUCTION

Medicinal preparations derived from natural sources, especially from plants, have been in widespread use since time immemorial. Ancient texts of India and China contain exhaustive depictions of the use of a variety of plant-derived medications (Samuelsson, 2004). In fact, plants remain the main source of medicines for a large proportion of the world's population, particularly in the developing world, despite the advent of the pharmaceutical chemistry during the early twentieth century, which brought with it the ability to synthesize an enormous variety of medicinal drug molecules and allowed the treatment of previously incurable and/or life-threatening diseases(Ahmad *et al.*, 2006).

Over the years, however, synthetic drugs have been plagued by unwanted side-effects, toxicity, and inefficiency, among other problems. In addition, the search for new drugs against a variety of illnesses through chemical synthesis and other modern approaches has not been encouraging. These factors, as well as the emergence of new infectious diseases, the proliferation of disorders such as cancer, and growing multidrug resistance in pathogenic microorganisms, have prompted renewed interest in the discovery of potential drug molecules from medicinal plants. (Ahmad *et al.*, 2006)

1.1 Definition and Scope of Pharmacognosy

The term pharmacognosy was first used between 1811 and 1815, and originally referred to "materia medica". The knowledge of drug materials or pharmacology. It is derived from two words , pharmakon (a drug) and gignosko (to acquire a knowledge of) (Evans, 2002). Later on, pharmacognosy became restricted to that branch of pharmacy investigating "medicinal

substances from the plant, animal and minerals in their natural, crude, or unprepared state, or in the form of such primary derivatives as oils, waxes, gums, and resins" (Hocking, 1997). Although this latter definition may have been appropriate for the descriptive and microscopical applications of pharmacognosy which were developed from the 19th century until the middle of 20th century (Pratt and Youngken 1956; Wallis 1967), it became necessary for the subject to be redefined as it subsequently broadened in scope to deal with the chemical components of crude drugs. For example, pharmacognosy was stated to be "an applied science that deals with the biologic, biochemical, and economic features of natural drugs and their constituents" (Tyler*et al.*, 1998).

In further attempt to update the scope of this field in amanner consistent with scientific activities ongoing at the beginning of the 21st century, pharmacognosy has been recently defined as "a molecular science that explores naturally occurring structure-activity relationships with a drug potential" (Bruhn and Bohlin, 1997). As practiced today, pharmacognosy involves the broad study of natural products from various sources including plants, bacteria, fungi, and marine organisms (Balunas and Kinghorn, 2005). Pharmacognosy includes both the study of botanical dietary supplements, including herbal remedies as well as the search for single compound drug leads that may proceed through further development into Food and Drug Administration-approved medicines (Tyler, 1999; Cardellina II, 2002).

1.2 Taxonomy of Medicinal Plants

Medicinal plants have diverse botanical characteristics. They belong to various plant families which, frequently, comprise characteristic similar active ingredients (as a result of similarities in the biosynthetic pathways). As an example, the plant-family Labiateae (Lamiaceae) comprise a

large number of essential oil-containing species (lavender, thyme, rosemary, sage, etc) whereas other plant families, like the Solanaceae are characterized by the occurrence of several alkaloid-containing species (Belladona, thorn apple, tobacco, etc.) (Bradley and Michael, 2008)

Phylogenetic systems classify medicinal plants according to their purported evolutionary relationships or heredity characteristics, although remarkably even to date, phylogenic systems are to a large extent based on the former artificial system of Linneaus. More refined morphological systems take into consideration the morphological traits of plants and evaluate them according to the principles of evolution and inheritance (Bradley and Michael, 2008).In recent decades, more and more attention has been paid to the use of plant-derived chemical information giving rise to chemo-taxonomy or phytochemical plant systems. Similar to other domains of plant systematic, the recent trends to use cytological and molecular biological traits are expected to bring about changes in the already established phylogenetic systems (Bradley and Michael, 2008).

1.3 Chemistry and Biological Activity of Plant Extracts

Amedicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis (WHO, 2011). Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically activeThese non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals (‘phyto-‘ from Greek - phyto meaning ‘plant’) or phytoconstituents and are responsible for protecting the

plant against microbial infections or infestations by pests (Abo *et al.*, 1991; Liu, 2004; Nweze *et al.*, 2004; Doughari *et al.*, 2009).

The study of natural products on the other hand is called phytochemistry. Phytochemicals such as flavonoids, steroids, triterpenes, alkaloids etc have been isolated and characterized from fruits such as grapes (*Vitis vinifera*) and apples (*Malus domestica)*, vegetables such as broccoli (*Brassica oleracea*) and onion (*Allium cepa*), spices such as turmeric (*Curcuma longa*), as well as many other sources (Doughari and Obidah, 2008; Doughari *et al.*, 2009).Different plant parts and components (roots, leaves, stem barks, flowers or their combinations, essential oils) have been employed as extracts in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin (Rıos and Recio, 2005; Adekunle and Adekunle, 2009).

1.4 Extraction, Isolation and Identification of Bioactive Constituents from Plants

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials followed by further separation and characterization. The basic operation include stepssuch as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to ensure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional uses (Fabricant and Farnsworth, 2001), then it is required toprepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional ‘herbal’ drug.

The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compounds from natural sources. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, the extraction with hexane is used to remove chlorophyll (Cosa*et al.*, 2006).

Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as thin layer chromatography, column chromatography using silica gel (60-120 mesh) and sephadex LH-20 , flash chromatography, andhigh performance liquid chromatography should be used to obtain pure compounds. The pure compounds are then used for the determination of structure andbiological activity. Besides that, non-chromatographic techniques such as immunoassay, which use Monoclonal Antibodies(MAbs), phytochemical screening assay, Fourier-Transform Infra-Red spectroscopy (FTIR), Mass Spectroscopy (MS) and Nuclear Magnetic Resonance Spectroscopy (NMR) can also be used to facilitate the identification of the bioactive compounds (Sasidharan *et al*, 2011).

1.5 Statement of the Research Problem

Inflammation is the response of living tissues to injury and it involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Perianayagam *et al.*,2006). Due to its implication in virtually all human and animal diseases, inflammation has become the focus of global scientific research, more so, since the currently used synthetic anti-inflammatory agents both steroidal and non-steroidal are prone to evoking serious adverse reactions (Dharmasiri *et al.,* 2003; Park *et al.,* 2004).

1.6 Justification of the Study

Plants constitute potentially important avenue leading to novel therapeutic agents for inflammation which may not only ameliorate the inflammation but also may be safe, relatively inexpensive, highly tolerated and convenient for use. Due to the reported presence of phytochemical constituents like the polyacetylenes, steroids, terpenes, flavonoids in plants from the Apiaceae family that possess anti-inflammatory activity (Christensen and Brandt, 2006;Watson and Dallwitz, 1992), there is need to carryout studies on *Steganoteania araliacea* as a potential source of anti-inflammatory agents because of its ethnobotanical use like the use of the bark in the treatment of asthma by the Zigula and Sukuma people of South Africa (Watt and Breyer-Brandwijk, 1962) and rheumatism in East Africa (Hedberg *et al*, 1983)

1.7 Hypothesis

The hexane extract of the stem bark of *Steganotaenia araliacea*has phytochemical constituents with anti-inflammatory activity.

1.8 General Aim

To carryout phytochemical and anti-inflammatory studies on the stem bark of *Steganotaenia araliacea*.

1.9Specific Aims and Scope of the Study

- **Specific aim I**: To evaluate some pharmacognostic properties of the stem bark of *Steganotaenia araliacea*.
- **Specific aim II:**To carry out phytochemical studies on the hexane extract of the stem bark.
- **Specific aim III:**To evaluate the anti-inflammatory activity of the hexane extract of the stem barkin experimental rat model.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Taxonomic Classification of *Steganotaenia araliacea* Hoschts

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Apiales

Family: Apiaceae

Sub-family: Saniculoideae

Genus: *Steganotaenia*

Species: *Steganotaenia araliaceae*Hoschts

The *Apiaceae* family is closely related to the Araliaceae. The family Apiaceae has traditionally been divided into the subfamilies Hydrocotyloideae, Saniculoideae and Apioideae as described by Drude in 1898 (Plunkett *et al.,* 2004). Cladistic analyses indicate that this taxonomy is not quite satisfactory. An investigation on the morphology of a few representatives of Apiaceae and Araliaceae indicated that the two families are not monophylethic (Judd *et al.,* 1994). According to molecular data, Apioideae and Saniculoideae are monophyletic, while Hydrocotyloideae is polyphyletic: Hydrocotyleis on the same clade as Araliaceae, while Bowlesiais on the same clade

as Apioideae and Saniculoideae (Plunkett *et al.,* 2004). Molecular studies with the aim towards a new classification within Apioideae have revealed some lineages which have been recognized at tribal and sub-tribal levels, among a large "rest group" of unresolved genera (Downie et al., 2000).The Apiaceae family has about 2,850 species and genera of about 420 (Watson and Dallwitz, 1992). Examples of important species of the Apiaceae family are;

- Medicinal plants, cordiments and spices: fennel (*Foenuculum vulgare*), coriander (*Coriandum sativum*), caraway (*Cacum carvi*), dill (*Anethum graveolens*), parseley (*Petroselenum crispum*), Anise (*Pimpinella anisum*), asafoetida (*Ferula asafoetida*) etc.
- Vegetables: carrot (*Daucus carota*), parnsnip (*Pastinaca sativa*) and oeleny (*Apium graveolens*)

2.2 Botanical Description of the Family Apiaceae

The plants in the family areherbs (mostly), or shrubs (some), or 'arborescent', or trees (few); bearing essential oils, or without essential oils; resinous, or not resinous. They appear occasionally as switch-plants or 'normal' plants; occasionally with the principal photosynthesizing function transferred to stems (e.g., *Platysace compressa*), or phyllodineous (e.g. *Lilaeopsis*). Leaves are well developed (usually), or much reduced (sometimes, in switch forms). The Plants may be succulent (occasionally, e.g. *Crithmum*), or non-succulent; autotrophic. (Watson and Dallwitz, 1992)

They are annual, or biennial, or perennial; with a basal aggregation of leaves, or without conspicuous aggregations of leaves. They may be helophytic, or mesophytic, or xerophytic (e.g., *Eryngium*). The leaves are small to large; alternate, or alternate and opposite (the upper sometimes more or less opposite); 'herbaceous' (usually), or leathery (occasionally), or fleshy

(rarely); petiolate, or perfoliate (e.g., the upper leaves of *Bupleurum rotundifolium*); more or less sheathing. The stomata present on the leaves are of the anomocytic, or paracytic type. The trichomes present are mostly glandular (including unicellular, dendroid and stellate forms)(Watson and Dallwitz, 1992).

The flowers aggregated in 'inflorescences' which may appear in umbels (nearly always) or in heads. The perianth of the flowers arewith distinct calyx and corolla (usually, but the calyx usually very reduced). Theandroecium is exclusively of fertile stamens and microsporogenesis is stimultaneouswhile thegynoecium is two carpelled and syncarpous. The fruits are non-fleshy(schizocarp),the integument sometimes united with the pericarp while the seeds areendospermic and oily with well differentiated embryo (often small). Germination is phanerocotylar (Watson and Dallwitz, 1992).

2.3 Reported Phytochemical Constituents of the Apiaceae Family

Phytochemical constituents reported in the family include sugars which weretransported as sucrose (*Bupleurum*) and inulin was absent (umbelliferose recorded). Polyacetylenes were reported (falcarinone). Alkaloids were reported in some members (poisonous umbellifers usually toxic via polyacetylenes). Anthraquinones were also detected (*Bupleurum*, *Heracleum*); polyacetate derived. Saponins/sapogenins were reported to be present or absent in some members. The Flavonols reported in the family were kaempferol, or kaempferol and quercetin (mostly both)(Watson and Dallwitz, 1992).

2.4Description of Subfamily Saniculoideae

Apiaceae subfamily Saniculoidae, as treated by Drude (1898) and Wolff (1913), comprises two tribes (Saniculeae and Lagoecieae), nine genera and approximately 330 species (Calvino and

Downie, 2007). The plants are mostly herbaceous, with often spiny or bristly simple leaves. Their flowers are arranged primarily in simple (rarely compound) umbels or heads that are surrounded by showy bracts. Their fruits comprise an exocarp covered in scales, bristles, or prickles (or rarely are glabrous or tuberculate), a mesocarp with calcium-oxalate crystals scattered throughout, and a parenchymatous endocarp. The most common base chromosome numbers of the subfamily are x = 7 and x = 8 (Calvino and Downie, 2007).

2.5Botanical Description of the Genus *Steganotaenia*

The genus is made up of two species *Steganotaenia commiphoroides*Thulin and*Steganotaenia araliacea* Hochst. Both species are found in Africa. *S. araliacea* (Plate 1) (Synonyms: *Peucedanum araliaceum* Benth. & Hook f.; *P. fraxinifolium* Hiern) is the most common in West Africa. It is commonly called carrot tree. In Northern Nigeria it is called *Hanno* in Hausa (Agunu, 1997). It is a small, softwooded tree up to 12m. The barkis yellowishgreen or grey, rather waxy and peeling off in papery strips or rectangles (Plate II). The leavesare simply pinnate having 3-5 pairs of serrate leaflets with a terminal one (Plate III). It produces tiny white flowers in compound umbels during the dry season. The fruits are small, flat and 2-winged (Hutchinson and Dalziel, 1958; Irvine, 1961).



Plate I: ***Steganotaenia araliacea*****tree**(Source: Botanical garden Department of Pharmacognosy and Drug Development Ahmadu Bello University, Zaria)

2.5.1 Ethnobotanical uses of*S. araliacea.*

The root is used to treat: snakebites in India (Selvanayahgam *et al.*,1994); menstrual problems, abdominal pains, malaria and snakebite in Tanzania (Chhabra *et al.,* 1993); bilharzias, sore throat and swellings caused by allergies in East Africa. It is used in multicomponent prescriptions to treat heart palpitations, severe abdominal pains and gonorrhoea (Hedberg *et al.,* 1983). The bark is used to treat asthma by the Zigula and Sukuma people of South Africa (Watt and Breyer-Brandwijk, 1962); leukaemia and malaria in Tanzania (Chhabra *et al.*,1993; Gessler *et al.*, 1995); rheumatism by rubbing the ash into scarifications. In East Africa, the decoction is mixed with milk to treat dysentery and flatulence (Hedberg *et al.,* 1983), treatment of HIV, oedema and gastric reflux in Tanzania(Omolo *et al.,* 2014). The water extract of the leaf is used to treat: gonorrhoea, sore eyes and sore throat in Zimbabwe (Gelfand*et al.,* 1985); convulsions in Gambia (Irvine, 1961).

The whole plant is also reported to cause abortion in goats (Watt and Breyer-Brandwijk, 1962). The central core of the root, when wrapped around the penis is claimed to increase the size of the latter (Buchanan, 1975).The stem bark is reported to be used for diueresis by people of Zaria in Nigeria (Agunu, 1997)

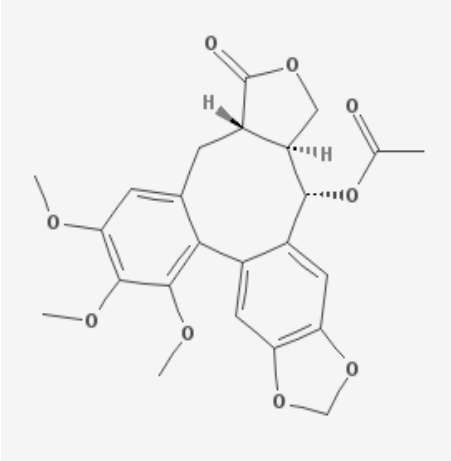
2.5.2 Phytochemistry and biological activity of*S. araliacea.*

The ethanol extract and chromatographic fractions of the bark have been shown to possess antiviral (Beuscher *et al.*, 1994) and cytotoxic properties (Taafrout *et al.,* 1983a). The leaves and bark were also reported to have molluscicidal activity (Kupchan *et al*., 1973; Kloos *et al.,* 1987). The n-hexane extract of the stem bark reportedly demonstrated insect repellent/ antifeedant properties (Abubakar *et al.,* 2001). The 70% ethanol extract of the fresh root bark

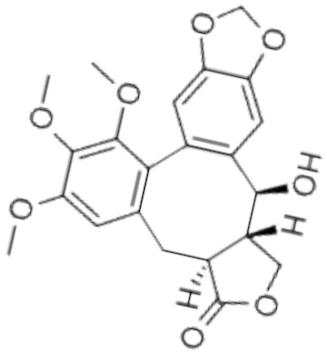
was however inactive in antibacterial tests against *B. subtilis*and *E. coli* at 100g/mL. It was also inactive in antiviral (against rhinovirus Type 2), antifungal (*Penicillium crustosum*), and anti-yeast (*Saccharomyces cerevisiae*) test systems (Taniguchi *et al.,* 1978). The aqueous, ethanolic and methanolic extracts have been shown to possess diuretic activity (Agunu *et al*., 2003).

Several lignans have been isolated from the stem bark and entire plant. These include: araliangine (Taafrout *et al.,* 1983a), neoisostegane (Hicks and Sneden, 1983; Taafrout *et al.,* 1983b; 1984b), prestegane A (Taafrout *et al.,* 1983c), prestegane B**(III)** (Taafrout *et al.,* 1984a), steganacin**(I),** steganangin**(IV)** (Kupchan *et al.,*1973), steganol**(II)** (Wickramaratne *et al.,* 1993), steganolide A (Taafrout *et al.,* 1986), steganolides B, steganolide C (Robin *et al.,*1986), steganone (Hughes and Raphael, 1976) and 10-demethoxystegane (Meragelman *et al.,* 2001). Also, triterpenoid glycosides (saponins) have been isolated from the leaves. These include: glycosides of barrigenol R1**(V)**(now known as barringtogenol C) and steganogenin with glucose, galactose and rhamnose in their sugar portions (Lavaud *et al.,* 1992). Protocatechuic acid has been isolated from 50% ethanol extract of the bark (Alemika *et al.,* 2004). Flavonoids apigenin-4'-glucoside and sophoraflavone B were isolated from the methanolic extract of the stem bark(Omolo *et al.,* 2014).

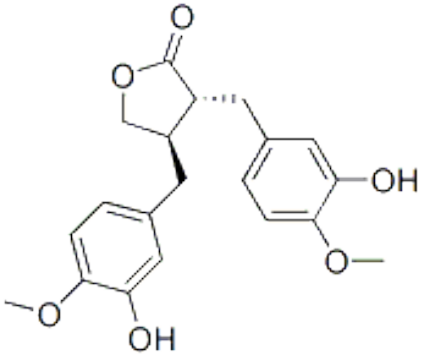
The reported major components of hydrodistilled essential oils from the highly aromatic leaves are limonene**(VI)**, β-phellandrene**(VIII),**α-pinene**(VII)**, sabinene**(IX),** β-cryophyllene and cryptone (Orwa *et al.,* 2009).

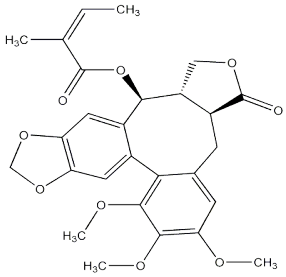


Steganacin (I)



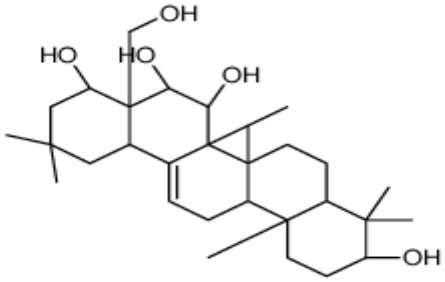
Steganol (11)

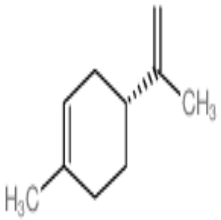




Steganangin (IV)

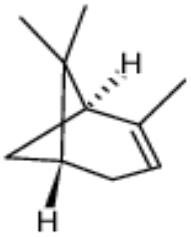
Prestegane B (III)



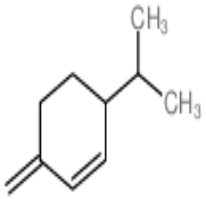


Limonene (VI)

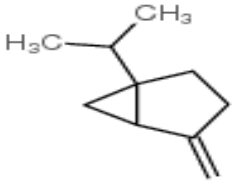
Barrigenol(V)



α-pinene (VII)



β-phellandrene (VIII)



Sabinene (IX)

2.6Inflammation

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness,swollen joint that is warm to touch, joint pain, its stiffness and loss of joint function. Inflammation is either acute or chronic. Under specific circumstance, it could turn into a chronic state and subsequently become a causative factor in the pathogenesis. Inflammation is a self-defense reaction in its first phase, hence regarded as the main therapeutic target and often, the best choice to treat the disease and alleviate the symptoms (Shailasree *et al*., 2012).

2.6.1 Acute inflammation

Acute inflammation may be an initial response of the body to harmful stimuli. An increased movement of plasma and leukocytes, especially granulocytes from the blood into the injured tissues is observed. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injured tissue (Shailasree *et al*., 2012).

2.6.2 Chronic inflammation

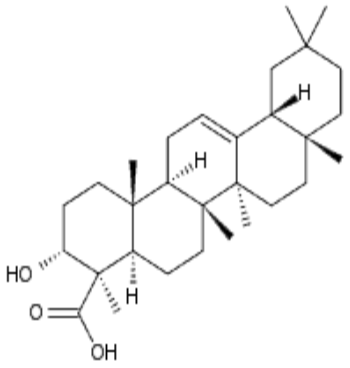
Prolonged inflammation also known as chronic inflammation is when the inflammatory response is out of proportion resulting in damage to the body. The different types of allergies and many autoimmune diseases viz, asthma, rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus are a few examples of chronic inflammations(Shailasree *et al*., 2012).

2.7Plant-derived Compounds with Anti-inflammatory Activity

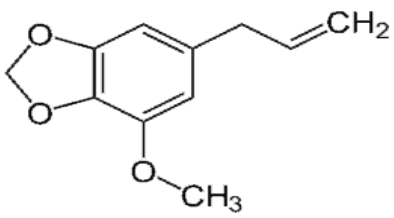
Chemical compounds from plants have been screened for their capacity to modulate the expression of pro-inflammatory signals thereby assessing their capacity as anti-inflammatory agents.Polyphenols, flavonoids, terpenes, quinines, catechins, alkaloids and antioxidants are phytochemical compounds targeted for anti-inflammatory activity. Potent anti-inflammatory plant compounds include guggulsterone[4,17(20)-pregnadiene-3,16-dione], a plant sterol from *Commiphora mukul*, boswellic acid**(X)**, a pentacyclic triterpenic acid and its derivatives viz., acetyl-β-boswellic acid, 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid (Safayhi *et al*., 1995; Takada *et al*., 2006), curcumin from turmeric, resveratrol from red grape seeds, genistein from soy, quercetin**(XIV)** (onions), silymarin (artichoke), withanolides (Ashwagandba), tea polyphenols, ranberries and peanuts. Table 2.1 shows some isolated bioactive compounds from plants that have been reported to possess anti-inflammatory activity.The mechanism of anti-inflammatory activity for these bioactives were identified to be by inhibition of NF-kB proteins activation and down-regulating the expression of inflammatory marker enzymes viz, inducible nitric oxide synthase (iNOS), cyclooxygenases (COX) -1, -2; 5-lipoxygenase (LOX) and matrix metalloproteinase (MMP)-9 (Khan*et al.*, 2007).

Table 2.1: Some Isolated Compounds from Plants with Anti-inflammatory Activity

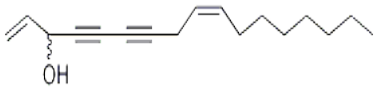
S/N	Plant	Family	Isolated compound	Reference
1	*Cyperus rotundus*	Ciperaceae	β – sitosterol**(XIX)**	Gupta *et al.,* 1980
2	*Bryophyllum pinnatum*	Crassulaceae	β – sitosterol**(XIX)**	Hema *et al.,* 1987
3	*Boswella serrata*	Bursersceae	Boswellic acid**(X)**	Singh *et al.,* 1986
4	*Embelia ribes*	Myrsiticaceae	Embellin**(XV)**	Thompson, 1971
5	*Rosmarinus officinnalis*	Labiatae	Rosmarinic acid**(XVII)**	Harbone, 1989
6	*Myristica fragrans*	Myristacaceae	Myristicin**(XI)**	Ozaki *et al.,* 1989
7	*Panax ginseng*	Araliaceae	Vanillic acid**(XVIII)**	Van sumere., 1989
9	*Erica australis*	Ericaceae	Protocatechuic acid**(XIII)**	Van sumere., 1989
10	*Cassia alata*	Fabaceae	Kaempferol	Palanichamy and Nagarajan, 1990
11	*Physalis minima*	Solanaceae	Quercetin**(XIV)**	Sethuraman and Sulochana, 1988
12	*Solanum lacintatum*	Solanaceae	Solasodine**(XVI)**	Roddick, 1986
13	*Foenuculum vulgare*	Apiaceae	Falcarinol**(XII)**	Christensen and Brandt, 2006
14	*Oldenlandia diffusa*	Rubiaceae	Hentriacontane	Kim *et al.,* 2011



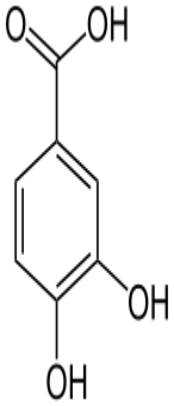
Boswellic acid(X)



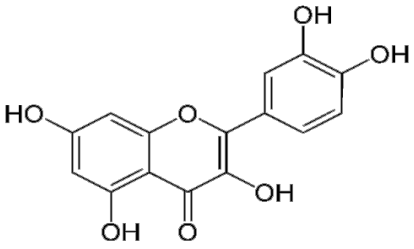
Myristicin(XI)



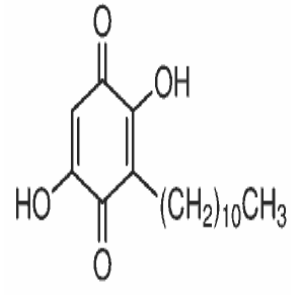
Falcarinol (XII)



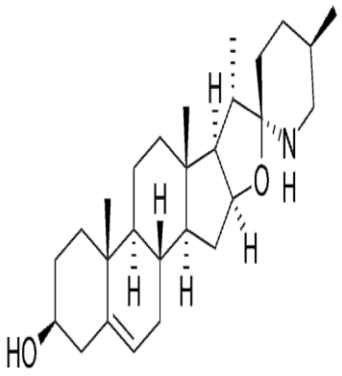
Protocatechuic acid(XIII)



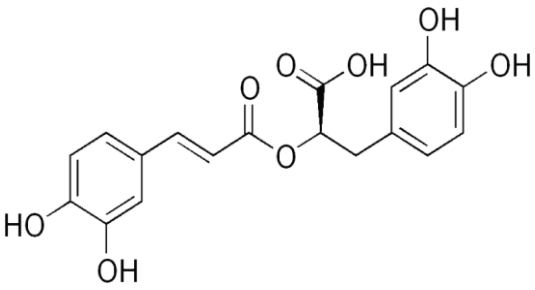
Quercetin(XIV)



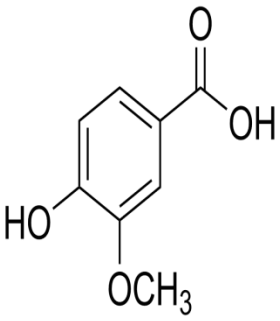
Embellin (XV)



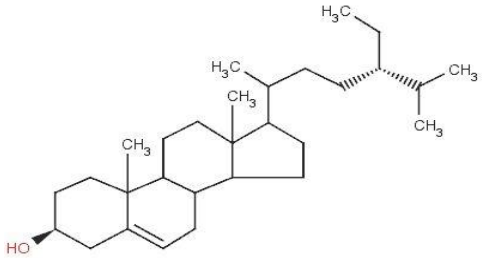
Solasodine(XVI)



Rosmarinic acid (XVII)



Vanillic acid (XVIII)



β-sitosterol (XIX)

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 List of Chemicals and Reagents

All solvents and reagents used in this work were of reagent grade. All references to water indicate distilled water.

3.1.1 List of chemicals

Ethanol (Qualikems fine chem., Nandesari, India)

Methanol (Sigma Aldrich St. Louis, MO, USA)

Ethyl acetate (Qualikems fine chem., Nandesari, India)

Chloroform (Qualikems fine chem., Nandesari, India)

Acetone (Qualikems fine chem., Nandesari, India)

N-hexane (Qualikems fine chem., Nandesari, India)

Formalin (Qualikems fine chem., Nandesari, India)

Ketoprofen (May and Baker, Nigeria)

3.1.2List of reagents for chromatographic studies

All reagents used for chromatographic studies were freshly prepared (see Appendix VII)

3.1.2.1Universal detecting reagents

10% Sulphuric Acid in Methanol

P-Anishaldehyde/concentrated Sulphuric acid

3.1.2.2 Specific detecting reagents

* Dragendorff reagent for alkaloids and other nitrogen-containing compounds.
* Bontrager reagent for anthracene derivatives
* Aluminium chloride solution for flavonoids
* Liebermann-Burchard reagent: Acetic anhydride - sulphuric acid for 5-3-sterols (cholesterol and esters), steroids and triterpene glycosides

3.2 List of Materials and Equipments

3.2.1Chromatographic materials

Silica gel was used for both column and thin layer chromatographic (TLC) techniques. The types used are:

• Silica gel (60-120 mesh) for column chromatography (Merck, Germany)

• Pre-coated thin layer chromatography (TLC) aluminium plates (Silica gel 60 F_{254}) for TLC analysis (Merck, Germany).

3.2.2 Materials for anti-inflammatory studies

- Digital vernier caliper
- 1 ml syringe

3.2.3 Source of laboratory animals used

Male Wister Rats (100-184g) from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

3.3 Plant Collection, Preparation and Identification

Leaves and stem bark of *S. araliaceae*were collected from Auchan Town of Ikara Local Government Area of Kaduna State, Nigeria in May, 2014. The plant was identified and authenticated at the Herbarium Unit by Malam Namadi Sunusi of Department of Biological Sciences, Ahmadu Bello University, Zaria. A reference sample (Voucher number, 3871) has been deposited in the Herbarium.

The stem bark collected was air dried at room temperature for seven days after which the dried bark was pulverized using pestleand mortar. The pulverized bark was then further air dried until completely dried.

3.4 Pharmacognostic Evaluation of Stem Bark

3.4.1Macroscopical evaluation of whole and powdered stem bark

An initial macroscopical study was carried out on the whole and powedered stem bark using mainly organoleptic methods. This is based on thecolour, surface characteristics, texture and fracture characteristics; odour and taste (WHO, 2011).

3.4.1.1Colour

The untreated plant material was examined under diffuse daylight. The colour of the sample was then compared with that of a reference sample reported in the literature (WHO, 2011).

3.4.1.2 Surface characteristics, texture and fracture characteristics

The untreated plant material was examined using magnifying lens (6x to 10x). The plant material was then touched to determine if it is soft or hard, bend and rupture to obtain information on brittleness and the appearance of the fractured plane whether if it is fibrous, smooth, rough, granular etc (WHO, 2011).

3.4.1.3 Odour

The air over the plant material in a beaker was inhaled slowly and repeatedly. First, the strength of the odour (none, weak, distinct, strong) and the odour sensation (aromatic, fruity, musty, mouldy, etc) was determined(WHO. 2011).

3.4.2Physico-chemical evaluation of powdered stem bark of *S. araliacea*

3.4.2.1 Determination of total ash

Four gram of the ground air-dried plant material was weighed in a previously ignited and tarred crucible. The material was spread in an even layer and was ignited gradually increasing the heat to 500˚C until it turned white, indicating the absence of carbon. It was then cooled in a desicator and weighed. The total ash content was calculated as percentage of air-dried plant material. The procedure was repeated two more times and the average was taken. The result was then expressed as mean ± SEM(WHO, 2011).

3.4.2.2Determination of acid-insoluble ash

To the crucible containing the total ash, 25ml of hydrochloric acid was added and covered with a watch-glass and boiled for 5minutes. The watch-glass was rinsed with hot water and the liquid added to the crucible. The insoluble matter was then collected on an ashless filter-paper and

washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter was then transferred into the original crucible. It was then dried on a hot-plate and ignited to a constant weight. The residue was then cooled in a desicator and then weighed without delay. The acid-insoluble ash content was calculated as percentage of the air-dried plant material. The procedure was repeated two more times and the average was taken. The result was then expressed as mean ± SEM (WHO, 2011).

3.4.2.3 Determination of water-soluble ash

To another crucible containing the total ash, 25ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a crucible. It was then washed with hot water and ignited in a crucible at 400˚C for 15 minutes. The weight in milligram(mg) was then subtracted from the weight of the total ash. The water-soluble ash content was then calculated as percentage of the air-dried plant material. The procedure was then repeated two more times and the average was taken. The result was then expressed as mean ± SEM.(WHO, 2011)

3.4.2.4Determination of Moisture Content by Loss on Drying Method

Five grams of the prepared air-dried plant material was weighed in a previously dried and tarred flat weighing bottle. The sample was then dried in an oven at 105˚C. It was dried until two consecutive weighings were obtained. The loss in weight of the air-dried plant material was thenobtained by subtracting the new weight from the initial weight. Apercentage weight loss was calculated with reference to the original weight of the sample. The procedure was repeated two more times and the average was taken.The result was then expressed as mean ± SEM (WHO, 2011).

3.4.2.5 Determination of alcohol-soluble extractive value

The powdered air-dried plant material (5 g) was weighed and macerated with 100 ml of 90% ethanol in a stoppered flask for 24 hours shaking frequently during the first six hours. It was then filtered and 25 ml of the filterate was evaporated to dryness in a tared shallow dish. It was then dried to constant weight at 110^0C. The percentage of alcohol – soluble extractive with reference to the air dried drug was then calculated (Brain and Turner, 1975). This procedure was repeated twice and the average recorded. The result was then expressed as mean ± SEM.

3.4.2.6 Determination of water-soluble extractive value

The powdered air-dried plant material (5 g) was weighed and macerated with 100 ml of 0.25% chlolroform water (1: 400) in a stoppered flask for 24 hours shaking frequently during the first six hours. It was filtered and 25 ml of the filterate was evaporated to dryness in a tared shallow dish. It was dried to constant weight at 110^0C. The percentage of water – soluble extractive with reference to the air dried plant material was calculated (Brain and Turner, 1975).This procedure was repeated twice and the average recorded. The result was then expressed as mean ± SEM.

3.4.2.7 Determination of petroleum ether-soluble extractive value

The powdered air-dried plant material(5 g) was weighed and a thimble pack was prepared. The plant material in the pack was then extracted with solvent petroleum ether (40 – 60˚C) in a continuous extraction (Soxhlet) apparatus for 6 h. 25ml of the extract was then evaporated and dried at 105°C to a constant weight. The percentage of petroleum ether-soluble extractive with reference to the air-dried plant material was then calculated(Brain and Turner, 1975). This procedure was repeated twice and the average recorded. The result was then expressed as mean ± SEM

3.5Extraction of Plant Material

The pulverized plant material (1Kg) was extracted by maceration using n-hexane (2.5L) for seven days. The extract was then collected and filtered. The solvent from the filtrate was then evaporated at room temperature until completely dried.

3.6Phytochemical Studies on the Hexane Extract of the Stem Bark of *S. araliacea*

3.6.1 Thin Layer Chromatographic (TLC) Profiling

The extract was dissolved in n-hexane and applied on pre-coated silica gel TLC plates as a spot with the aid of capillary tube about 0.5 cm above the bottom edge and 0.5 cm away from the sides. The spot was allowed to dry and the plate placed in a chromatank containing the mobile phase that has been prepared in the tank at least 30 minutes earlier. The mobile phase was allowed to run along the TLC plate in an ascending manner due to capillary action, carrying with it the components of the extract or the mixture. When the mobile phase reached the desired distance, the plate was removed, the solvent front marked and the plate dried. The separated compounds were located by observing the chromatogram under ultra-violet light (254 and 366nm) for fluorescence. This was followed by spraying with 10% H_2SO_4 in methanol and heating at 110°C for 5 minutes. This method was used for all TLC analysis (Ghani, 1990). The solvent systems;hexane 100%, hexane- ethylacetate in ratios 10:1,9:1, 8:2 and 7:3 were used.

Different chromatograms were developed and sprayed using reagents both universal (10% sulphuric acidin methanol) and specific (ferric chloride for phenolic compounds, Drangedorff for alkaloids,Liberman Burchard reagent for steroids and terpenoids; aluminium chloride for flavonoids etc) to check for the presence or absence of various secondary metabolites. The solventhexane- ethylacetate (10:1) gave an optimum resolution and was thus, used for further analysis.

3.6.2 Column chromatography

The extract was subjected to column chromatography using Silica gel (60-120 mesh, Qualikems fine chem., Nandesari, India) as stationary phase and ran by gradient elution technique where n-hexane and ethyl acetate were employed as mobile phase. The silica gel 100g was packed in a column (3.5cm x 40cm) with hexane using wet method. The column was allowed to stabilize before the extract (2g) adsorbed on some amount of the silica gel was packed on top of the prepared column and a cotton wool placed on the packed column. Elution began with hexane (100%) and then followed by gradual introduction of ethyl acetate (5%, 10%, 15% etc) until ethyl acetate (100%) was used. The flow rate of the column was 20 drops/min.20 ml aliquots were collected and analyzed using TLC. Similar fractions were pooled together for further purification.

3.6.2.1 Purification of isolated compound

The column fractions with a similar single spot indicating the isolation of a compound were pooled together dried at room temperature and further purified on a smaller column using 10g of silica gel (60-120 mesh) eluted with hexane and ethyl acetate as mobile phase just as in 3.6.2. 5ml aliquots were collected and monitored using TLC. Fractions with single spots on TLC were pooled together and solvents evaporated at room temperature. The isolated compound was collected, weighed, designated as compound UF and stored in dessicator for further analysis.

3.6.3 Identificationof isolated compound UF

3.6.3.1 Physico-chemical Evaluation of Compound UF

The melting point of the pure compound was determined using Electrothermal Melting Point apparatus. About 2 mg of the sample was transferred into a capillary tube and inserted into the

melting point apparatus. With the aid of a thermometer, the uncorrected melting point was recorded.

3.6.3.2 Nuclear magnetic resonance (NMR) spectroscopic analysis

The Proton NMR (1H NMR) spectra of compound UF was determined at the Chemistry Department, School of Physics and Chemistry, University of Kwazulu Natal, South Africa. Chemical shifts were reported in δ (ppm) and coupling constants in Hz.The analysis was conducted on Bruker AVANCE 600Hz spectrometer, using TMS (trimethylsilane) as internal standard. .NMR solvent used was deutrated chloroform ($CDCl_3$).

3.7Acute Toxicity Testing

3.7.1Determination of Median Lethal dose (LD_{50})

LD_{50} determination of the hexane extract of the stem bark of *S. araliacea* was conducted using the method of Lorke's (1983) by the intraperitoneal (i.p.) route in rats. The method was carried out in two phases. In the initial phase, 3 groups of three rats each were treated with the hexane extract of the stem bark suspended in Tween80 at doses of 10, 100 and 1000 mg/kg body weight i.p and observed for signs of toxicity and death over 24 hours. In the second phase, 4 groups each containing one ratwas injected with 50, 100, 200 and 400mg/kg doses of the extractsuspended in Tween 80 i.p based on the result in phase 1. The LD_{50} value was determined by calculating the square root of the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1and 1/1).

3.8Anti-inflammatory Studies

The anti-inflammatory activity of the hexane extract of the stem bark of *S. araliacea* was evaluated using the formalininduced paw oedama in male rats (Turner, 1965) as modified by

(Abage and Fageyinbo, 2012). Twenty five (25) rats (100-184g) were divided into 5 groups of five animals each. Group 1 served as negative control (Tween80) and group 5 as positive control (Ketoprofen 2mg/kg IP). Groups 2-4 were treated with graded doses in 35, 70 and 140mg/mg of the extract suspended in Tween80 based on LD_{50} of the extract intraperitoneally respectively. Acute inflammation was induced by sub-plantal injection of 0.1ml of 2% formalin into the right hind paw. Treatments were administered 30minutes before induction of oedema. Paw thickness were measured using a vennier caliper 30minutes before and at 1, 2, 3, 4 and 5 hours after induction of oedema. The paw odema thickness was determined as the difference between the paw thickness at 0 hour and the various time intervals after the induction of the oedema. The percentage (%) inhibition of the paw oedema was calculated using the formular below

$$\%\ inhibition\ = \frac{To\ -\ Tt}{To} \times 100$$

Where Tt is the paw oedema thickness of rats given the test extract at a corresponding time and To is the paw oedema thickness of the rats in the negative control group at the same time.

Experimental groups were compared with the vehicle and reference drug control groups for statistical significance using student t test at $p < 0.05$

3.9 Statistical Analysis

Student t-test and one way analysis of variance (ANOVA) were used to compute the data were applicable. The results were expressed in mean ± SEM

CHAPTER FOUR

4.0RESULT

4.1 Pharmacognostic Evaluationof*S. araliacea* Stem Bark.

4.1.1 Macroscopical evaluation of *S. araliacea*stem bark.

4.1.1.1 Whole stem bark

The stem bark was observed to be yellowish green and flat; short fractured and waxy. It possesses an aromatic odour. The summary of these parameters are shown in the Table 4.1

4.1.1.2 Powdered stem bark

The summary of the parameters evaluated for the powdered stem bark are shown in Table 4.2.

Table 4.1: Macroscopical Features of *S. araliaceae*Whole Stem Bark

Evaluation	Features

Shape	Flat
Colour	Yellowish green
Surface	Waxy and short fractured
Texture	Smooth
Odour	Aromatic

Table 4.2: Macroscopical Features of Powdered*S. araliaceae*Stem Bark

Evaluation	Features
Colour	Brown
Odour	Aromatic
Taste	Sweet bitter

4.1.2 Physico-chemical evaluation of the powdered *S. araliacea*stem bark

4.1.2.1Ash values

The percentages of total-ash, acid-insoluble ash and water-soluble ash values were observed to be 10.67±0.30%, 4.00±0.50% and 1.25±0.14% respectively as in Table 4.3.

4.1.2.2Moisture content

The percentage of the moisture content by loss on drying was found to be 8.00±0.76% as in Table 4.3.

4.1.2.3 Extractive values

The percentages of the water-soluble extractives, alcohol-soluble extractives and petroleum ether-soluble extractive values were determined as 2.87±0.18, 6.67±0.22 and 1.27±0.18 respectively as in Table 4.3.

Table 4.3: Ash Values, Moisture Content and Extractives Values

Physico-Chemical Paramater	**Inference/Values (%w/w) ±SEM***
Total ash	10.67±0.30
Acid-insoluble ash	4.00±0.50
Water-soluble ash	1.25±0.14
Moisture content	8.00±0.76
Water-soluble extractive	2.87±0.18
Alcohol-soluble extractive	6.67±0.22
Petroleum ether-soluble extractive	1.27±0.18

*Mean of 3 determinations

4.2Extraction Yield of Powdered*S. araliacea* Stem Bark with Hexane

The hexane extract was dried under room temperature to give a yellowish green gummy mass after maceration with 2.5L of n-hexane for seven days.

Weight of stem bark powder = 1kg

Weight of extract = 13g

Percentage yield = $\frac{13 \times 100}{1000}$ = 1.3%w/w

4.3Phytochemistry of the Hexane Extract of the Stem Bark

4.3.1Thin-layer chromatographic profile

The TLC of the hexane extract of *S. araliaceae* stem bark were observed to give good separation using hexane-ethylacetate (10:1) as the solvent system and sprayed with 10% sulphuric acid in methanol as the detecting reagent (Plate IV)..The thin-layer chromatographic profile with specific reagent showed the presence of steroids/terpenoids (Table 4.3).



Plate II: TLC Profile of the Hexane Extract of *S.araliaceae* Stem bark developed in Hexane-Ethylacetate (10:1) sprayed with 10% Sulphuric acid in Methanol andviewed under day light with R_f values 0.15(A), 0.30(B), 0.58(C). 0.77(D), 0.88(E) and 0.95(F). .

Table 4.4:Thin-Layer Chromatogram of the Hexane Extract of *S. araliacea* using Specific Reagents

Constituents	Spraying Reagents	Observation	Inference
Flavonoids	Aluminium chloride	Fluorescence of spots under long UV range with colours, pink and blue	-
Steroids and terpenes	Libermann-Burchard reagent	Reddish and violet coloured spots	+
Alkaloids	Dragendroff reagent	No coloured spots	-
Anthracene derivatives	Bontragers reagent	No coloured spots	-

Key: (+) indicate presence while (-) indicates absence

4.3.2 Result of the column chromatography of the hexane extract of the stem bark

The column chromatography gave 100 fractions of 20ml aliquots which were allowed to concentrate by drying under room temperature and screened using TLC. Fractions with similar TLC profiles were pooled together. A total of 13 combined fractions (coded: CF1-CF13) were obtained as in Table 4.4.

Table 4.5: Fractions Collected from Column Chromatography of the Hexane Extract of Stem Bark of *S. araliacea*

Name of Combined Fractions	Eluting Solvent	Collections
CF1	100% (hexane)	1-10
CF2	95:5 (hexane-ethylacetate)	11-16
CF3	95:5 (hexane-ethylacetate)	17-20
CF4	90:10 (hexane-ethylacetate)	21-25
CF5	90:10 (hexane-ethylacetate)	26-30
CF6	85:15 (hexane-ethylacetate)	31-36
CF7	85:15 (hexane-ethylacetate)	37-40
CF8	80:20 (hexane-ethylacetate)	41-50
CF9	70:30 (hexane-ethylacetate)	51-60
CF10	60:40 (hexane-ethylacetate)	61-70
CF11	50:50 (hexane-ethylacetate)	71-80
CF12	40:60 (hexane-ethylacetate)	81-90
CF13	30:70 (hexane-ethylacetate)	91-100

4.3.3Isolation of compound

Combined fraction CF3 (Collection 17-20) on dying gave a white waxy solid which was then eluted and purified on a smaller column using 10 g of silica gel 60 – 120 mesh size. The compound gave one brown spot on TLC with an R_f value of 0.9 using 100% hexane as solvent system, R_f value of 0.92 using hexane-ethyl acetate (10:1) as the solvent system and 10% sulphuric acid in methanol as the spraying reagent(Plate V). From the TLC profile of the isolated compound minor impurities were still present. The isolated compound was then coded as Compound UF.



(A) (B)

Plate III: The TLC Profile of Compound UF Isolated from the Hexane Extract of *S. araliaceae* developed in

- **(A) Hexane 100% sprayed with 10% Sulphuric acid in Methanol viewed under day light.R_f value 0.90.**
- **(B) Hexane-Ethyl acetae ratio (10:1) sprayed with 10% Sulphuric acid in Methanol viewed under day light. $R_f$value 0.92.**

4.4Identification of Compound UF

4.4.1 Physical properties of compound UF

Weight: 50mg

Physical appearance: a white waxy solid

Melting point: 66-68˚C

Solubility: soluble in hexane, ethyl acetate and chloroform.

4.4.2 Proton NMR of compound UF

The characteristics signals of ^{1}H NMR spectrum of compound UF as shown in Fig: 4.1 are broad singlet at chemical shift 1.27 indicating a long chain of methylene groupsand a triplet at chemical shift 0.89 indicating terminal methyl groups.

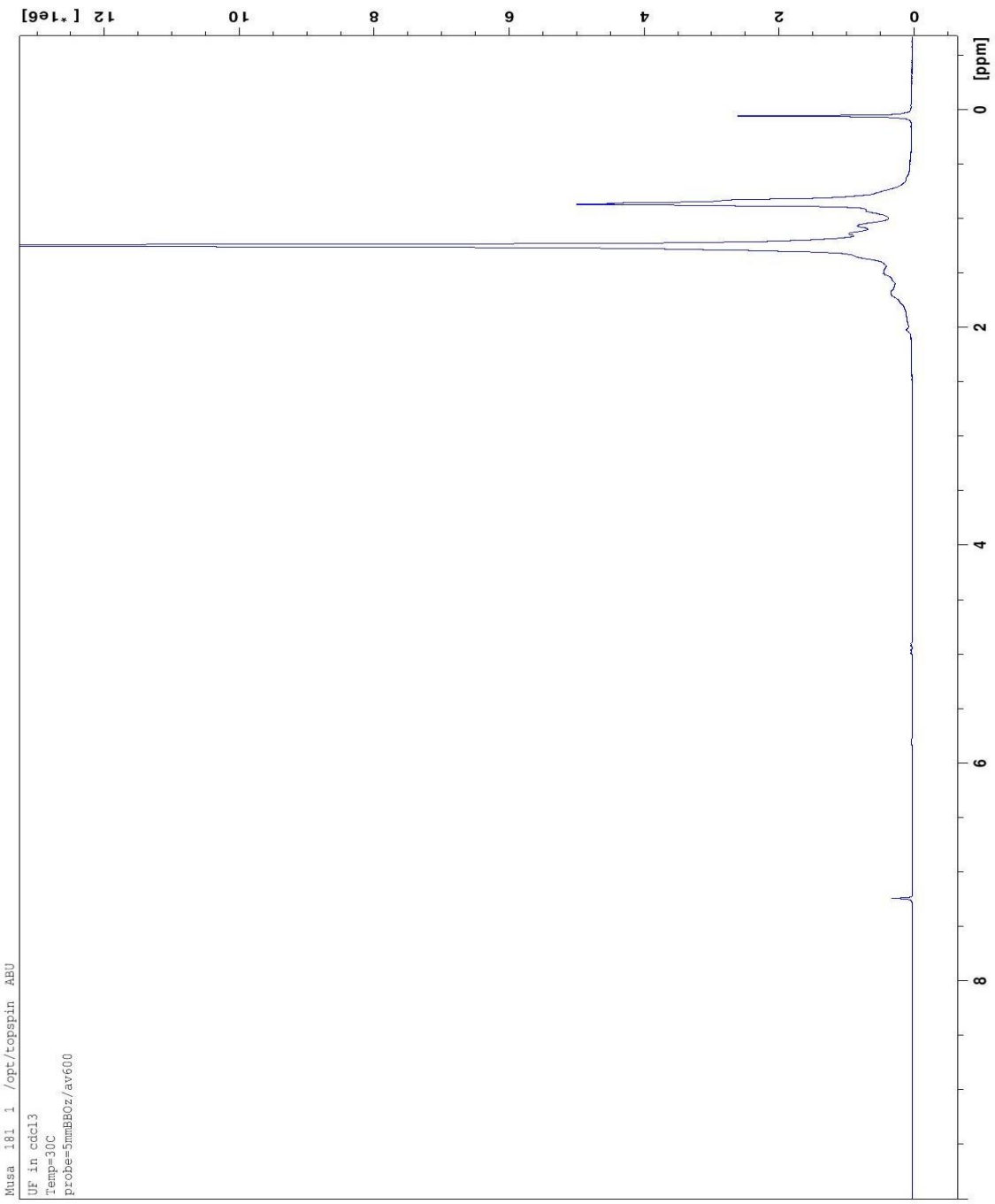


Fig 4.1: Proton NMR Spectra of Compound UF in $CDCl_3$

Table 4.6: Proton NMR of Compound UF and Hentriacontane

δ H ppm UF	δ H ppm*	δ H ppm**
0.89(t)	0.89(t)	0.89 (t)
1.27 (s)	1.27 (s)	1.27 (s)

*Haque *et al* 2008, **Licea*et al* 2012.

4.4.3 Proposed Structure of compound UF

The proposed structure of compound UF is Hentriacontane $C_{31}H_{64}$

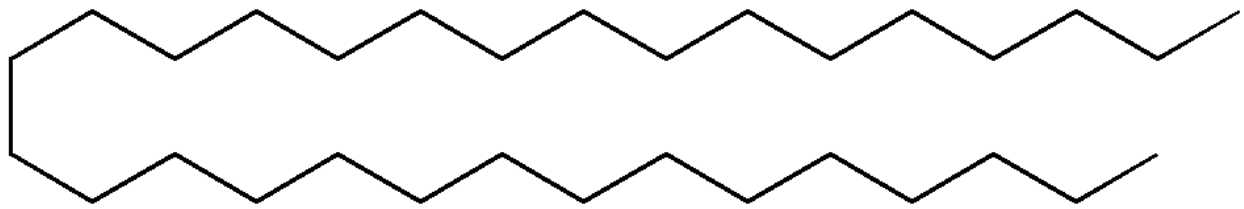


Fig 4.2: Proposed Structure of CompoundUF($C_{31}H_{64}$)Isolated from the Hexane Extract of the Stem Bark of S. araliacea.

4.5Acute toxicity testing (Median Lethal Dose LD_{50})

The acute toxicity studiesof the hexane extract of the stem bark of *S. araliaceae*using the Lorke method (1983) showed the death of all the three rats that received 1000mg/kg of the extract, one death among the rats that received 100 mg/kg but no death among the rats that received 10mg/kg after 24 hours in the first phase of the study. In the second phase of four rats in each group they received 50, 100, 200 and 400mg/kg of extract. After 24 hours the only death recorded was the rat that received 400mg/kg of the extract. The median lethal dose (LD_{50}) of the extract was calculated to be 282.84mg/kg.

4.6Anti-inflammatory Studies

The effect of the hexane extract of the stem bark of *S. araliacea* on formalin induced paw oedema in rats at different time intervals is shown on Table 4.7.

Table 4.7:The Effects of the Hexane Extract of the Stem Bark of *S. araliacea* on Formalin Induced Paw Oedema in Rats.

Treatment mg/kg (i.p)	MeanPaw Oedema Thickness (mm) ±SEM and Percentage inhibition				
	1hr	**2hrs**	**3hrs**	**4hrs**	**5hrs**
Tween80	0.83±0.09	1.29±0.11	1.15±0.07	0.55±0.09	0.40±0.09
SAHE (35)	0.63±0.10 (24%)	0.76±0.03* (41%)	1.12±0.21 (26%)	0.62±0.11	0.51±0.16
SAHE (70)	0.58±0.05* (30%)	0.75±0.04* (42%)	0.71±0.15* (38%)	0.55±0.04	0.40±0.08
SAHE (140)	0.57±0.13 (31%)	0.64±0.09* (50%)	0.48±0.07* (58%)	0.49±0.09	0.31±0.04
Ketoprofen (2)	0.49±0.07* (40%)	0.47±0.09* (64%)	0.47±0.05* (59%)	0.50±0.07	0.35±0.05

Values are ±SEM (n=5).* Statistical significant at P< 0.05. SAHE – Hexane extract of stem bark of *S. araliacea.*

i.p – Intra-peritoneal route of administration.

CHAPTER FIVE

5.0 DISCUSSION

In the pharmacognostic evaluation ofthe stem bark of *S. araliacea*,the macroscopical examination of the whole stem (Table 4.1)was in agreement with that reported by Orwa *et al*(2009) while the powdered stem bark (Table 4.2) shows was as reported by Abubakar (1997). The macroscopical examination of medicinal plants is the first step in the evaluation of herbal medicines for identity and quality as described by the WHO (2011) Quality Control of Hebal Materials.

The physico-chemical evaluation of the prepared powdered stem barkof *S. araliacea* show that the total ash as 10.67% which wasthe total material remaining after incineration of the bark. This includes both 'physiological ash' which is derivedfrom the plant tissue itself, and 'non-physiological ash', which isthe residue of the extraneous matter adhering to the plant surface. It is usually made up of chlorides, carbonates, silicates and silica (African Pharmacopea, 1985). The acid-insoluble ash was determined to be 4% which is part of total ash that measures the amountof silica present, especially as sand and siliceous earth. A high acid-insoluble ash value usually indicates faulty collection or aldulteration of the plant material.Some reported official acid insoluble ash value are; Capsicum not more than 0.15% w/w, Euphorbia whole herb 3.0% w/w, cannabis leaf 5.0%w/w, belladonna 3.0% w/w, clove 0.75% w/w (African Pharmacopiea, 1985). Water-solubleash is the water soluble portion of the total ash and was determined to be 1.25%.The value obtained for some official drugs are: *Zingiber officinale* 1.7% w/w, *C. jamus* 4.23% w/w (Musa, 2004).

The moisture content of the powdered stem bark was determined by the loss on drying method to be 8.00%. The percentage of active chemical constituents incrude drugs is mentioned on air-dried basis. Therefore, the loss ondrying of plant materials should be determined and the watercontent should also be controlled. This is especially important formaterials that absorb moisture easily or deteriorate quickly in thepresence of water. High percentage may activate enzymes that can catalyse the breakdown of medicinally active chemical compounds. In addition, also susceptibility to microorganisms and insect pest can be encouraged by high moisture content (Musa, 2004).

The extracts obtained by exhausting plant materials with specific solvents are indicative of approximate measures of their chemical constituents extracted with those solvents from a specific amount of air-dried plant material. The water-soluble extractive value, alcohol-soluble extractive value and petroleum ether-soluble value for the powdered stem bark of *S. araliaceae* were determined to be 2.87%, 6.67% and 1.27% respectively.From the results above petroleum ether been a non-polar solvent was expected to extract only non-polar constituents and from the value (1.27%) indicates that the concentration of non-polar constituents in the bark is low. Also, the water used was expected to extract highly polar constituents, again the value obtained (2.86%) indicated a low concentration of this constituents. However, the alcohol extractive value (6.67%) suggests notable extraction of combination of polar and non-polar constituents by the alcohol. The above indicates that alcohol is the best solvent for the extraction of the plant material. The values of the physico-chemical evaluation of the powdered crude drug determined above can serve as reference in determining the quality and purity of the powdered stem bark of *S. araliacea.*

The Thin layer chromatographic (TLC) profiling of the hexane extract of the stem bark using hexane-ethylacetate (10:1) as solvent system and sprayed with 10% sulphuric acid in methanol revealed that the solvent system gave a clear separation on the TLC of some components of the extract(Plate IV). In Table 4.1 where the Chromatograms were sprayed with specific reagents, the extract was shown to contain steroids/terpenoids which the extract has been previously been reported to contain (Abubakar *et al.,* 2001) are important phytochemicals that may be responsible for some of the biological activity of the extract.

The column chromatography of 2g of the hexane extract on silica gel yielded 100 fractions which were pooled together into 13 combined fractions (CF1-CF13) based on their TLC profiles (Table 4.4). Compound UF was isolated from CF3 (50mg).

Compound UF (TLC profile on Plate V) was a white waxy solid soluble in hexane, ethylacetate and chloroform with m.p 66 - 68˚C.The ^{1}H-NMR spectrum, which showed a triple at δ 0.89 for the terminal methyl protonsand a huge unsplit singlet at δ 1.27 formethylene protons of long alkyl chain. The high intensity the signalat δ 1.27 suggested the presence of largenumber of methylene protons. It may thereforebe concluded that compound UF is a long chain hydrocarbon.With the m.p.64 - 68˚C and its ^{1}H-NMR spectrum, compound UF iscloser to Hentriacontane ($C_{31}H_{64}$), a hydrocarbon that hasbeen reported to be isolated and characterized by Haque *et al* (2008) and Licea*et al* (2012) from other plant species. Hentriacontane is an n-alkane and has been reported to be isolated from several plants including*Scabiosa comosa*(Dargaeva *et al.,* 1976),*Syzygium jambos*(Tessmann *et al.,*2002), *Terminalia arjuna* (Ahmad *et al.,* 1982 ; Haque *et al.,*2008), *Gymnosperma glutinosum* (Licea*et al.,*2012) and so forth. It is reported for the first time in *Steganotaenia araliaceae.*The carbon chains in plant alkanes are usually odd-

numbered, between twenty-seven and thirty-three carbon atoms in length and are made by the plants by decarboxylation of even-numbered fatty acids (Baker, 1982).

The acute toxicity testing of hexane extract of the stem bark of was carried out determining the median lethal dose (LD_{50}) which is the dose of the extract that will kill 50% of the test animals using the method described by Lorke (1983). The $LD_{50}$of the extract was determined to be 282.84mg/kg using the i.p route of administration in rats and the extract was found to be moderately toxic using the Hodge and Sterner scale (1956).

The most widely used primary test to screen new anti-inflammatory agents' measures the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent (Winter *et al.,* 1962). The formalin induced paw oedema in rats was used to evaluate the anti-inflammatory activity of the hexane extract.The peak of the inflammation was at the 2^{nd} hour after the induction of the inflammation as shown in Table 4.7. At that hour there was a dose dependent statistically significant ($p<0.05$)inhibition of the paw oedema in the rats (Table 4.7). The standard drug used as the positive control was Ketoprofen (2mg/mg) which is a non-steriodal anti-inflammatory drug (NSAIDS) caused 64% inhibition of paw oedema and the extract at the dose of 35 mg/kg showed 41% inhibition, 70 mg/kg 42% and 140 mg/kg 50% inhibition (Table 4.7).

Activity of formalin to cause acute inflammation is biphasic, consisting of an early neurogenic component followed by a two phase later tissue-mediated response. In the first phase of the inflammation, there is a release of histamine, serotonin and kinin, while the second phase is related to the release of prostaglandins (Gupta *et al.,* 2006; Turner *et al.,* 1971). The peak activity of the extract was at the second phase (2^{nd} hour after induction of inflammation) which is related

to the release of prostaglandins just as that of the standard drug Ketoprofen which inhibits the cycloxygenase (COX) enzyme preventing the release of prostaglandins (Williams *et al.,* 1999) and as such the extract can be proposed to act via this process.

Hentriacontane the compound isolated from the hexane extract of the stem bark of has been reported to possess anti-inflammatory activity because it was shown to ameliorate the expression of inflammatory mediators (TNF-α, IL-6, PGE (2), COX-2 and iNOS) (Kim *et al*., 2011). Also the TLC profiling of the extract showed the presence of steroids/terpenoids. The steroids/terpenoids have been reported to possess anti-inflammatory effects and the effects have been attributed to various mechanisms including inhibition oflipoxygenase and cycloxygenase activities (Andrikopoulos *et al.,* 2003).

CHAPTER SIX

6.0 SUMMARY CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

In this study the hexane extract of the stem bark of *S. araliacea* (family: Apiaceae) was evaluated for its anti-inflammatory activity and with a view to isolate bioactive compounds from it. The morphological features of the stem bark were described as yellowish-green in colour, flat, smooth in texture,waxy and short fractured with aromatic odour, while the powdered stem barkwas described as brown in colour with an aromatic odour and a sweet bitter taste. The physico-chemical properties of the bark were determined as total ash value 10.67%, acid-insoluble ash value of 4.00%, water-soluble ash value 1.25%, moisture content 8.00%, water-soluble extractive value 2.87%, alcohol-soluble extractive value 6.67% and petroleum ether-soluble extractive value 1.27%. The TLC profiling of the hexane extract of the bark showed the presence of steroids/terpenoids. The column chromatography of the hexane extract of the stem bark lead to isolation of compound UFwhich was identified using the ^{1}H-NMR spectroscopy and its physical properties. Compound UF was identified as Hentriacotane based on the available spectroscopic data and physical properties which agreed well with those reported in the literature. The acute toxicity studies of the hexane extract revealed LD_{50} of 282.84mg/kg suggesting the extract is moderately toxic. The effect of the hexane extract on formalin induced paw oedema in rats revealed a statistically significant ($p<0.05$) dose dependant inhibition of the paw oedema when compared to the reference. 35,70 and 140mg/kg of the extract showed 41%, 42% and 50% inhibition at the peak of the paw oedema. This indicates a dose dependent anti-inflammatory activity of the extract.

6.2 Conclusions

At the end of the study the following conclusions were made:

- Some pharmacognostic characters of the stem bark of *S. araliacea* were determined which can be used as reference in determining the identity, quality and purity of the stem bark.
- The phytochemical studies of the hexane extract of the stem bark of *S. araliacea*showed the presence of steroids/terpenoids by TLC profiling of the extract. Hentriacontane a known compound was isolated from the extract by column chromatography and identified by comparing its spectroscopic data and physical properties to reported data.
- The extract also showed a significant dose dependant anti-inflammatory activity on formalin induced paw oedema in rats.

6.3 Recommendations

- It is recommended that a bio-assay guided isolation of the hexane extract of the stem bark of *S. araliacea* should be carried out so as to isolate the other anti-inflammatory constituents in the extract.
- Chronic toxicity studies should be carried out on the extract as to ascertain its safety.
- Other *in-vivo* and *in-vitro* models of testing anti-inflammatory activity should be used to test the extract so as to ascertain its mechanism of anti-inflammatory activity.

REFERENCES

Abubakar, M.S. (1997): Pharmacognostic and investigation of anti-insect activity of the stem bark of *Steganotaenia araliaceae*Hochsts (F. Umbelliferae): Unpublished M.Sc. Thesis A.B.U., Zaria, Nigeria.

Abubakar, M.S., Abdurahman, E.M., Nock I.H., Haruna , A.K. and Garba M. (2001); The evaluation of pest control properties of *Steganotaenia araliaceae ; Journal of Herbs Spices and Medicinal Plants*; 8(1), 51-58.

Abo, K.A., Ogunleye, V.O., Ashidi, J.S. (1991): Antimicrobial poteintial of *Spondias mombin*, *Croton zambesicus* and *Zygotritonia crocea*.*Journal of Pharmacological Research*. 5(13):494-497.

Adekunle, A.S., Adekunle, O.C. (2009): Preliminary assessment of antimicrobial properties of aqueous extract of plants against infectious diseases. *Biology and Medicine*. 1(3):20-24.

African Pharmacopiea (1986): General Method of Analysis. Vol. 11, 1st edition Scientific, Technical and Research Commision (STRC) Organization of African Unity (O.A.U) pp 121 – 208.

Abage, E.O., Fageyinbo, M.S. (2012): Evaluating Anti-inflammatory activity of aqueous root extract of *Strophathus hispidus* D.C (Apocynaceae): *International Journal of Applied Research in Natural Products*, 4(4): 7-14.

Agunu A. (1997): Microscopical and pharmacological studies of the sterm bark of *Steganotaenia araliacea* Hochst (Apiaceae): Unpublished M.Sc. Thesis A.B.U., Zaria, Nigeria.

Agunu, A., Abdurahman, E.M., Andrew,G. O., Muhammed, Z. (2005): Diuretic activity of the stem-bark extracts of *Steganotaenia araliacea* hochst (Apiaceae). *Journal of Ethnopharmacology* 96: 471-475.

Ahmad,I., Ajil, F. and Owais, M. (2006) *Modern Phytomedcine*. Wiley-VCH GmbH & CO.KGaA. Weiheim. Pp 1

Ahmad, M.U. Mullah, K.B., Saha, K.C. (1982): Hentriacontane, Arachidic stearate and myristyl oleate from the fruits of *Terminalia arjuna* Bedd. *Journal of Bangladesh Academy of Sciences,* 6(1&2): 61-65.

Andrikopoulos N.K, Kaloria AC., Assimopolou N.A. (2003): Biological activity of some naturally occurring resins, gums and pigments against in-vitro LDLoxidation, *Phytotherapy Research.*7:501-507.

Alemika, T.E, Onawumi, G.O. and Olugbade T.A., (2004). Protocatechuic and saponin mixture from*Steganotaenia araliceaa* stem bark. *Nigerian Journal of pharmaceutical Research* 3(1): 9-15.

Bakers, E.A. (1982): Chemistry and morphology of plant epicuticular waxes pp. 139-165. In "The plant cuticle" edited by Cutler, D.F., Alvin, K.L and Price C.E. Academic press London. ISBN 0-12-19920-3.

Balunas, M.J., Kinghorn, A.D. (2005): Drug discovery from medicinal plants:*Life Sciences*, 78: 431-441

Barbosa-Filho, J.M., Piuvezam, M.R., Moura, M.D., Silva, M.S., Lima, K.V.B., Leitãoda-Cunha,EV., Fechine, I.M., Takemura, O.S. (2006): Anti-inflammatory activities of alkaloids - a twenty-century review:*Revista Brasileira de Ciências Farmacêuticas.* 16:109-22

Beuscher, N., Bodinet, C., Neumann-Haefelin, D., Marston, A., Hostettmann, K. (1994): Antiviral activity of African medicinal plants; *Journal of Ethnopharmacology*.42(2), 101-9.

Brain, K.R and Turner, T.D. (1975): Practical evaluation of phytopharmaceuticals. Wright – Scientechnica, Bristol.1st Ed. 144.

Bradley, C.B., Micheal, J.B. (2008): Phytomedicine 101: plant taxonomy for preclinical and clinical medicinal plant researchers. *Journal of the Society for Intergrative Oncology*, 6(04): 150-157

Bruhn, G., Bohlin, L. (1997):Molecular Pharmacognosy: an explanatory model. *Drug Discovery Today*, 2:243-246

Buchanan, D.J. (1975): Penile burns due to application of roots.*Medical Journal of Zambia* 9, 42-4.

Calviño, C.I., Downie, S.R.(2007): Circumscription and phylogeny of Apiaceae subfamily Saniculoideae based on chloroplast DNA sequences, *Molecular Phylogenetics.and Evolution*, doi:10.1016/j.ympev.2007.01.002.

Cardellina II, J.H. (2002): Challenges and oppurtunities confronting the botanical dietry supplementindustry: *Journal of Natural Products*, 65 (7): 1073-1084.

Christensen, L.P. and Brandt, K. (2006) Bioactive polyacetylenes in food plants of the Apiceace family: Occurrence, bioactivity and analysis. *Journal of Pharmaceutical and Biomedical analysis* 41: 683-693.

Chhabra, S. C.; Mahunnah, R.L.A. and Mshiu, E.N. (1993): Plants used in traditional medicine in Eastern Tanzania. VI. Angiosperms (Sapotaceae to Zingiberaceae) *Journal of Ethnopharmacology*. 39(2), 83-103.

Cosa, P., Vlietinck, A.J., Berghe, D.V., Maes, L. (2006). Anti-infective potential of natural products: How todevelop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology*, 106: 290–302

Dargaeva, T.D., Brutko, L.I. (1976): Hentriacontane from *Scabiosa comosa*. *Chemistry of Natural of Compounds.*12: 471.

Dharmasiri, J.R., Jayakody, A.C., Galhena, G., Liyanage, S.S.P., Ratnasooriya, W.D. (2003): Anti-inflammatory andanalgesic activities of mature fresh leaves of *Vitex negundo*.*Journal ofEthnopharmacology*, 87: 199-206.

Doughari, J.H., Human, I.S., Bennade, S., Ndakidemi, P.A. (2009): Phytochemicals as chemotherapeuticagents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal Plants Research*. 3(11): 839-848.

Doughari, J.H. and Obidah, J.S. (2008). Antibacterial potentils of stem bark extracts of *Leptadenia lancifoli*against some pathogenic bacteria.*Pharmacologyonline* 3: 172-180.

Downie, S.R., Katz-Downie, and D.S., Watson, M.F. (2000): A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA rpl16 and rpoC1 intron sequences: Towards a supra-generic classification of subfamily Apioidae.*American Journal of Botany*. 87: 273–292.

Evans, W.C. (2002). *Trease and Evans Pharmacognosy*, 15th edition, W.B Saunders Company Ltd. PP 135-137.

Fabricant, D.S., Farnsworth, N.R. (2001): The value of plantsused in traditional medicine for drug discovery. *Environmental Health Perspectives*, 109: 69–75.

Gelfand, S.M.; Drummond, R.B. and Ndemera, B. (1985): The traditional medical practitioner in Zimbabwe; Mambo Press, Zimbabwe; 2nd Reprint 1993; pp. 271, 335.

Gessler, M.C.; Mysuya, D.E.; Nkunya, M.H.H.; Mwasumbi, L.B.; Schar, A.; Heinrich, M. and Tanenr, M. (1995): Traditional healers in Tanzania: the treatment of malaria with plant remedies; *Journal of Ethnopharmacology*. 48(3), 131-44.

Ghani, A. (1990): Introduction to Pharmacognosy, Ahmadu Bello University Press Ltd, Zaria-Nigeria: 187- 197.

Gupta, M., Mazumder, U.K., Gomathi. P., Thamilselvan,V.(2006):Anti-inflammatory evaluation of leaves of *Plumeria acuminate*:*BMC Complementary and alternative medicine*, 36(6): 1472-6882.

Gupta, M.B., Nath, R., Srivastava, N., Shanker, K., and Bhargava, K.P. (1980): Anti-inflammatory and antipyretic activities of β-Sitosterol. *Planta Medica*. 39, 157–163

Harbone, J.B. (1989): Phytochemical Methods. 2nd ed. Chapman and Hall, London.Pp 32

Haque, M.Z., Assaki, M.A., Ali, M.U., Ali, M.Y.,and Al-Maruf, M.A. (2008): Investigations on *Terminalia arjuna* fruits: Part 1- isolation of compounds from petroleum ether ether fractions: *Bangladesh Journal of Science and Industrial Research*, 43(1): 123-130.

Hedberg, I.; Hedberg, O.; Madati, P.J.; Mshigeni, K.E.; Mshiu, E.N.and Samuelsson, G. (1983): Inventory of plants used in traditional medicine in Tanzania Part III. Plants of the families Papillionaceae Vitaceae; *Journal of Ethnopharmacology*. 9(2/3), 237-60.

Hema, D.M., Tidjani, E., Bassene, J.L., and Pousset, L. (1987):African medicinal plants. XXIV. Study of the antiinflammatory action of *Bryophyllum pinnatum* (Crassulaceae).*Plant Med. Phytotheraphy.* 20, 231–235.

Hicks, RP. And Sneden, A.T. (1983): Neoisostegane, a new bisbenzocyclooctadiene lignan lactone from *Steganotaenia araliacea* Hochst; *Tetrahedron Letters*. 24(29), 2987-90.

Hodge, H.C., J.H. Sterner. (1956). Combined tabulation of toxicity classes. In: Spector, W.S., ed. *Handbook of toxicology*, Philadelphia, W.B. Saunders Company, Vol. 1

Hockings, G.M. (1997): *A Dictionary of Natural Products*. Plexus Publishing Inc. Med Ford. Pp65

Hughes, L.R.; Raphael, R.A.(1976): Synthesis of the antileukemic lignan precursor (+)-Steganone; *Tetrahedron Letter*.18, 1543-6.

Hutchinson, J. and Dalziel, J.M. (1958): Flora of West Tropical Africa vol. I part2; Published by Crown Agents for Oversea Governments and Administrations, London. 2nd Ed. (Revised by R. W. J. Keay) pp. 694-7; 751-6.

Irvine, F.R. (1961): Woody plants of Ghana; Oxford University Press, London. Pp. 508-11, 575-6.

Judd, W.S., Sanders, R.W.,Donoghue, M.J. (1994):Angiosperm family pairs: preliminary phylogenetic analyses.*Harvard papers in Botany*, no. 5, January 1994.

Khan, H., Khan, M.A., Hussan, I. (2007): Enzyme inhibition activities of the extracts from

rhizomes of *Gloriosa superba* Linn. (Colchicaceae):*Journal of Enzyme Inhibition and Medicinal Chemistry*. 22:722-25.

Kim, S.J., Chung, W.S., Kim, S.S., Ko, S. G., and Um, J.Y. (2011), Antiinflammatory Effect of *Oldenlandia diffusa* and its Constituent, Hentriacontane, through Suppression of Caspase-1 Activation in Mouse Peritoneal Macrophages. *Phytotherapy Research*., 25: 1537–1546. doi: 10.1002/ptr.3443

Kloos, H., Thiongo, F.W., Ouma, J.H., Butterworth, A.E. (1987): Preliminary evaluation of some wild and cultivated plants for snail control in Machakos district, Kenya.; Journal of Tropical Medicine and Hygiene. 90(4), 197-204.

Kupchan, S.M.; Britton. R.W.; Ziegler, M.F.; Gilmore, C.J.; Restivo, R.J. and Bryan, R.F.(1973): Steganacin and steganangin, novel antileukaemic lignan lactones from *Steganotaenia araliacea;Journal of American Chemical Society*. 95, 1335.

Licea, R.Q., Casttilo, R.M., Florres, R.G., Laatsch, H., Star, M.J.V., Martinez, H.H., Guerra, P.T., and Padilla, C.R. (2012): Bioassy-Guided isolation and identification of cytotoxic compounds from *Gymnosperma glutinosum* leaves: *Molecules*, 17: 11229-11241

Lavaud, C.; Massiot, G.; Le Men-Oliver, L.; Viari, A.; Vigny, P. and Delaude, C. (1992): Saponins from *Steganotaenia araliacea*; *Phytochemistry* 31(9), 3177-81.

Lewis, D.A. (1989): Anti-inflammatory drugs from plant and marine sources. Birkhause Verlag, Basel. Wiley and Sons. Chichester, England.

Liu, R.H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *Journal of Nutrition*. 134(12 Suppl):3479S-3485S.

Lorke D. (1983): A new approach to toxicity. *Archieves of Toxicology*.54: 275-287.

Meragelman K.R., McKee T.C. and Boyd M.R. (2001); 10-Demethoxystegane, a new lignan from*Steganotaenia araliacea*; *Journal of Natural Products*. 64, 1480-1482.

Musa, K.Y. (2004). Phytochemical and Biological studies of *Dyschoriste perrottetii* Nees Family; Acanthaceae.Unpublished PhD Thesis, Ahmadu Bello University, Zaria.

Nweze, E.L., Okafor, J.L., Njoku, O. (2004) :Antimicrobial Activityies of Methanolic extractsof *Trume guineesis* (Scchumn and Thorn) and *Morinda lucinda* used in NigerianHerbal Medicinal practice. *Journal of Biological Research and Biotechnology*. 2(1): 34-46.

Omolo, J.J., Maharaj, V., Naidoo, D., Malebo,H.M., Mtullu, S.,Lyaruu, H.V.M., and deKoning, C. B. (2014): Flavonoids of Steganotaenia araliacea. *American Journal of Research* Communication, 2(8): 52-6

Orwa C, Mutua A, indt R, Jamnadass R, and Simons A. (2009)*Steganotaenia araliacea* hochst

(Apiaceae) Agroforestree Database: a tree reference and selection guide version 4.0 (http://www.worldagrofrestry.org/af/treedb/). Retrieved 24th September, 2014.

Ozaki, Y., Soekeni, S., Rosina, Y., and Suganda, A. (199):Anti-inflammatory effect of mace, aril of *Myristica fragans* Houtt, and its active principles. *Journal of Pharmacology*. 49, 155–164.

Palanichamy, S., and Nagarajan, S. (1990): Anti-inflammatory activity *Cassia alata* leaf extract and kaempferol-3-O-sophoroside. *Fitoterapia* LXI, 44–47.

Park, J.H., Son, K.H, Kim, S.W., Chang, H.W., Bae, K., Kang, S.S., and Kim, H.P. (2004): Anti-inflammatory activity of *Synurusdeltoids*.*Phytotheraphy Research*. 18: 930-933.

Perianayagam, J.B., Sharma, S.K., and Pillai, K.K. (2006): Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. *Journal of Ethnopharmacology* 104: 410-414.

Plunkett, G.M., Chandler, G.T. and Kowry II,P.P., Pinney, S.M., and Sprenkle, T.S.(2004): Recent advances in understanding Apiales and a revised classification.*South African Journal of Botany* 70: 371–381.

Pratt, R., and Younken, H.W. (1956): *Phharmacognosy. The Study of Natural Crude Substances andCertain Allied Products.*2nd edn. J.B.Lipprincott Company, Philadelphia

Rìos, J.L., Recio, M.C. (2005). Medicinal plants and antimicrobial activity.*Journal of Ethnorpharmacology*. 00: 80-84

Robin, J.P.; Davoust, D. and Taafrout, M. (1986): Steganolides B and C, new lignin analogs of episteganacine; *Tetrahedron Letter*. 27(25), 2871-4.

Roddick, J.G. (1986): Steroidal alkaloids of the Solanaceae. In:*Solanaceae Biology and Systematics*. D´Arcy, W.G., Ed. Columbia University Press, New York. pp. 201–222.

Safayhi, H., Sailer, E.R., Ammon, H.P. (1995): Mechanism of 5-lipoxygenase inhibition by acetyl-11-keto-beta-boswellic acid:*Molecular Pharmacology*. 47:1212-16.

Samuelsson, G. (2004):*Drugs of Natural Origin:a Textbook of Pharmacognosy*, 5th Swedish Pharmaceutical Press, Stockholm.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M, and Yoga Latha, L. (2011): Extarction, isolation and characterization of bioactive substances from plant extracts: *The Journal of Traditional, Complementary and Alternative Medicines*, 8(1): 1-10.

Selvanayahgam, Z.E.; Gnanevendhan, S.G.; Balakrishna, K and Rao, R.B. (1994): Antisnake

venom botanicals from ethnomedicine; *Journal of Herbs, Spices, and Medicinal Plants;* 2(4), 45-100.

Sethuraman, V., Sulochana, N. (1988): The antiinflammatory activity of *Physalis minima*. *Fitoterapia*LIX, 335–336.

Shailasree S., Ruma K., Kin K.R., Niranjana S.R., and Prakash H.S. (2012): Potential Anti-inflammatory Bioactives from Medicinal Plants of Western Ghats, India:*Pharmacognosy Communications* volume 2/issue2/Apr-Jun 2012. 1-12

Singh, G.B., Atal, C.K.,and Zahn, M. (1986): Pharmacology of an extract of salai guggal from *Boswellia serrata*, a new steroidal antiinflammatory.*Agente Actions* 18, 407–412

Taafrout, M.; Rouessac, F.; and Robin, J.P. (1983a): Araliangine, a new bisbenzocyclooctadienolactone lignan from *Steganotaenia araliacea* Hochst; *Tetrahedron Letter*. 24(2), 197-200.

Taafrout, M.; Rouessac, F.; and Robin, J.P. (1983b): Neoisostegane, a new bisbenzocyclooctadienolactonic lignin from *Steganotaenia araliacea*Hochst.; *Tetrahedron Letter.*24(29), 2983-6.

Taafrout, M.; Rouessac, F.; and Robin, J.P. (1983c): Prestegane A, from *Steganotaenia araliacea* Hochst: the first natural dibenzylbutanolide lignan with a meta-phenol. A short synthesis of cis and trans (DL) arctigenin. *Tetrahedron Letter*.24(31), 3237-8.

Taafrout, M.; Rouessac, F.; and Robin, J.P. and Davoust, D. (1984a): Isolation of prestegane B from*Steganotaenia araliacea* Hochst, primary lignan bis-(meta-hydroxy-benzyl)-butanolide of plant origin –Isolation and synthesis in one step of dimethylmetaphenol.; *Tetrahedron Letter*. 25(37), 4127-8.

Taafrout, M.; Rouessac, F.; and Robin, J.P.; Hicks, R.P.; Shillady, D.D.andSneden, A.T. (1984b): Neoisostegane, a new bisbenzocyclooctadiene lignan lactone from*Steganotaenia araliacea* Hochst.; *Journal of Natural Products*. 47(4), 600-6.

Taafrout, M.; Landais, Y.; Robin, J.P. and Davoust, D. (1986): Isolation, stereochemistryand synthesis of steganolide A, a new bisbenzocyclooctadienolactone lignan of *Steganotaenia araliacea*; *Tetrahedron Letter*. 27(16), 1781-4.

Takada, Y., Ichikawa, HBadmaev, V., and Aggarwal, B.B. (2006): Acetyl-11-ketobetaboswellic acid potentiates apoptosis, inhibits invasion, and abolishes osteoclastogenesis by suppressing NF-k B and NF-k B-regulated gene expression. *JournalofImmunology*. 176:3127-40.

Taniguchi, M., Chapya, A, Kubo, I., Nakanishi, K. (1978): Screening of East African plants for

antimicrobial activity I: *Chemical and Pharmaceuticl Bulletin*. 26, 2910-3

Tessmann, D.J., andDianese, J.C. (2002): Hentriacontane: a leaf hydrocarbon from *Syzygium jambos*with stimulatory effects on the germinationof urediniospores of *Puccinia psidii*.*Fitopatologia Brasileira* 27:538-542.

Thompson, R.H. (1971):*Naturally Occurring Quinones*. 2nd ed. Academic Press, London. Pp.22

Turner, R. (1965): Screening Methods in Pharmacology,Anti-inflammatoryagent, Academic Press New York, London, 13: 158.

Turner, R., Crunkhon, P., and Meacock, S.(1971): Mediators of the inflammation induced in the rat paw by carrageenan.Screening Methods in Pharmacology. Anti-inflammatory Agent, Academic Press, New York, London, *British Journal of Pharmacology*, 42: 392 402.

Tyler, V.E. (1999): Phytomedicine: back tothe future. *Journal of NaturalProducts*, 62 (2): 1589-1592

Tyler, V.E., Brady, and L.R., Robbers, J.E. (1998): *Pharmacognosy*. 9th edn. Lea & Febiger, Philadelphia.

Van Sumere, C.F. (1989):Phenols and phenolic acids. In:*Methods inPlant Biochemistry*.Vol. 1.Plant Phenolics.Harbone,J.B., Ed. Academic Press, London. pp. 29–74.

Wallis, T.E. (1967): *Textbook of Pharmacoggnosy*. 5th edn J &A Churchhill Ltd. London

Watson, L., and Dallwtz, M.J. (1992): The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version: 19th August 2014. http://delta-intkey.com

Watt, J.M. and Breyer-Brandwijk, M.G. (1962): The medicinal and poisonous plants of Southern and Eastern Africa; 2nd Ed., E&S Livingstone Ltd., London.

WHO. (2011): Quality control methods for Herbal materials.WHO library cataloguing-in-publication data. PP 11-33.

Williams, C.S., Mann, M., and DuBois, R.N. (1999):The role of cyclo-oxygenases in inflammation, cancer, and development. *Oncogene,18*:7908-7916.

Winter, C.A., Risley, E.A., Nuss, W. (1962): Carrageenan-induced oedema in hind paw of rats as an assay for anti-inflammatory drugs. *Proceedings of Society of Experimental Biology and Medicine*, 111: 544-547

Wickramaratne, D.B.M.; Pengsusarp, T.; Mar, W.; Chai, H.B.; Chagwedera, T.E.; Beecher,

C.W.W.; Farnsworth, N.R.; Kinghorn, A.D.; Pezzuto, J.M. and Cordell, G.A.(1993): Novel antimitotic dibenzocyclo-octadiene lignan constituents of the stem bark of *Steganotaenia araliacea;Journal of Natural Products*. 56(12), 2083-90

’.

APPENDIX A

Determination of Ash Value of powdered Stem Bark of *S. araliacea*

Weight of plant material = 4g

Description	1	2	3
Constant weight of crucible (g)	38.30	39.30	38.76
Weight of crucible and Ash (g)	38.74	39.7239.20	
Weight Weight of Ash (g)	0.44	0.42	0.44
Ash Value (%)	11.00	10.50 11.00	
Average mean (%)		10.67±0.30	

Sample calculation

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

$$\text{Ash Value} = \frac{0.44 \times 100}{4\ g} = 11.00\ \%w/w$$

APPENDIX B

Determination of Acid insoluble Ash of powdered Stem bark of *S. araliacea*

Weight of plant material = 4g

Description	1	2	3
Constant weight of crucible (g)	38.30	39.30	38.76
Weight of crucible and Acid insoluble ash (g)	38.46	39.49	38.74
Weight of Acid insoluble ash (g)	0.18	0.19	0.20
Acid Insoluble Ash Value (%)	4.50	4.75	5.00
Average mean (%)		4.83	

Sample calculation

$$\text{Acid Insoluble Ash(AIA) value} = \frac{\text{weight of AIA}}{\text{Initial weight of drug}} \times 100$$

$$\text{AIA Value} = \frac{0.18 \times 100}{4\ g} = 4.00\ \%w/w$$

APPENDIX C

Determination of water soluble Ash of powdered Stem Bark of *S. araliacea*

Description	1	2	3
Constant weight of crucible (g)	38.30	39.30	38.76
Weight of crucible and Ash (g)	38.74	39.72	39.20
Weight of Ash (g)	0.44	0.42	0.44
Weight of water Insoluble ash (g)	0.39	0.36	0.40
Weight of water soluble ash (g)	0.05 0.06	0.04	
Water soluble Ash Value (%)	1.25	1.50	1.00
Average mean (%)		1.25	

Sample calculation

$$\text{Water soluble Ash(WSA) value} = \frac{\text{Wt of Ash - Wt of Water Insoluble Ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{AIA Value} = \frac{(0.42 - 0.36) \times 100}{4g} = 1.50\ \%\text{w/w}$$

APPENDIX D

Determination of Water-Extractive value of powdered Stem Bark of *S. araliacea*

5 g of the powdered leaves was used in 100 ml of 90% ethanol

Description	1	2	3
Constant weight of crucible (g) 124.00	127.00	125.5	
Weight of crucible and content after heating (g)	124.13	127.16	125.64
Alcohol extractive content (g)	0.13	0.16	0.14
Alcohol extractive Value (%)	2.60	3.20 2.80	
Average mean (%)		2.87	

Alcohol extractive val = Wt of cruc. & content after heat (g) - Constant wt. of cruc. (g) X 100

Initial weight of Drug.

APPENDIX E

Determination of Alcohol – Soluble Extractive valueof powdered Stem Bark of *S. araliacea*

5 g of the powdered Stem Bark was used in 100 ml of 0.25% chlolroform water.

Description	1	2	3
Constant weight of crucible (g)	124.00	126.00	124.50
Weight of crucible and content after heating (g)	124.35	126.27	124
water extractive Value (%)	6.25	6.75	7.00
Average mean (%)		6.67	

Water extractive val= Wt of cruc.& content after heat (g) - Const wt. of cruc. (g) X 5 X 100

Initial weight of Drug.

APPENDIX F

Determination of Acute toxicity of the Hexane extract of *S. araliacea* Stem barkusing Lorke's Method

Phase I

Group	Dose $mgKg^{-1}$	No. Of death/ No. Of Animal
1	10	0/3
2	100	1/3
3	1000	3/3

Phase II

Group	Dose $mgKg^{-1}$	No. Of death/ No. Of Animal
1	50	0/1
2	1000/1	
3	200	0/1
4	400	1/1

$LD_{50} = \sqrt{\text{Minimum toxic dose x Maximum tolerated dose}}$

$= \sqrt{200 \times 400}$

$= \sqrt{80{,}000}$

$= 282.84 \text{ mg } Kg^{-1}$

APPENDIX G

Procedure for the preparation of spray reagents used for TLC

1. **Dragendorff reagent** for alkaloids and other nitrogen-containing compounds. Solution a: 0.85 g Bismuth(III) nitrate was dissolved in 10 ml glacial acetic acid and 40 ml water.

Solution b: 8 g potassium iodide was dissolved in 20 ml water.

Stock solution: Equal parts of a and b was mixed. Spray solution: 1 ml stock solution was mixed with 2 ml glacial acetic acid and 10 ml water before use (Stahl, 1988).

2. **Liebermann-Burchard reagent:** This reagent was prepared fresh. 5 ml Acetic anhydride is placed in an ice bath, to this is added 5 ml concentrated sulphuric acid. The mixture is added to 50 ml ice cold absolute ethanol. After spraying, it was heated for 10 min at 110°C. The compounds produce spots visible under ultraviolet light (360 nm) Characteristic of sterols (cholesterol and esters), steroids and triterpene glycosides (Stahl, 1988).

3. **Sulphuric acid (general visualisation reagent)**: 10% sulphuric acid in ethanol was prepared. The chromatogram was sprayed with this reagent and allowed to dry for 10 minutes in the air and heated to 110°C for maximal visualisation of the spots (Stahl, 1988).

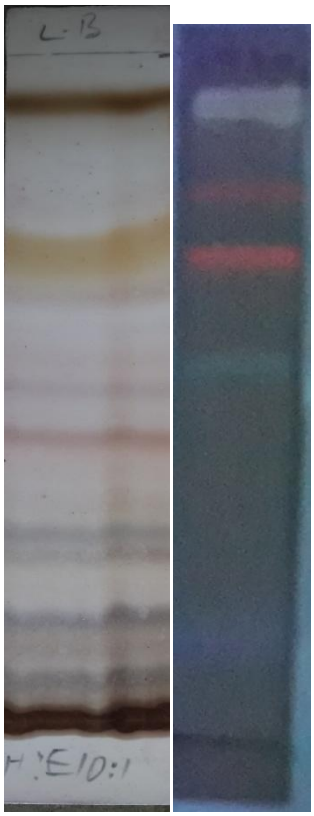
4. **Aluminium chloride for flavonoids:** 1% ethanolic solution of aluminium chloride was prepared and sprayed on the chromatogram. A yellow fluorescence in long-wave UV light is indicative of a flavonoid (Stahl, 1988).

5. Anisaldehyde - sulfuric acid for sugars, steroids, terpenes.

A solution of 0.5 ml anisaldehyde in 50 ml glacial acetic acid and 1 ml 97% sulfuric acid was prepared freshly before use. After-treatment, it was heated to 100-105°C until maximal visualisation of the spots were seen (Stahl, 1988).

APPENDIX H

TLC Profile of the Hexane Extract of Stem Bark of *S.araliaceae* developed in Hexane-Ethylacetate (10:1) sprayed with Liebermann Burchard reagent and viewed under day light (A)and sprayed with Aluminium Chloride viewed under long UV light (B).



(A) (B)