PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF *Spermacoce verticillata* (Rubiaceae) *G.F.W. Meyer*

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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL STUDIES OF

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G.F.W. Meyer

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BEING

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AUGUST, 2016

DECLARATION

I declare that the work in this dissertation titled **"*Phytochemical screening and antimicrobial studies of Spermacoce verticillata"*** was performed by me in the Department of Pharmaceutical Chemistry of Ahmadu Bello University Zaria under the supervision of prof. U. U Pateh and Prof. M.I Sule.

The information obtained from literature has been duly acknowledged in the work and list of references provided. No part of this thesis was previously presented for the award of another degree and / or diploma at this or any other institution.

…… **……..**

HALIMATU SAIDU **DATE**

CERTIFICATION

This dissertation titled: "***Phytochemical screening and antimicrobial studies of Spermacoce verticillata"*** by HALIMATU SAIDU meets the regulations governing the award of Master of Science Degree at Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENT

All praises and gratitude are due to Allah, the Creator, the cherisher and Sustainer of the world. The best of salutation of peace and blessing be upon His chosen prophet Muhammad (SAW), the members of his household and all those who follow him on the path of righteousness.

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DEDICATION

To my beloved late Father Alhaji Muhammadu Saidu. May his gentle soul rest in peace.

ABSTRACT

The plant *Spermacoce verticillata* is used in ethnomedicine for the treatment of itches, diarrhoea, sores and other ailments. The hexane soluble fraction of acetone extract of the whole plant *Spermacoce verticillata* was subjected to phytochemical and antimicrobial screening. The phytochemical techniques employed were dry column vacuum chromatography and preparative thin layer chromatography. The antimicrobial activity was studied using agar diffusion, and broth dilution methods. The result of preliminary phytochemical screening carried out on the crude acetone extract revealed the presence of carbohydrates, Flavonoids, Anthraquinones, terpernoids, Tannis and Steroids; Tannins flavonoids, and Steroids/terpenes were also found to be present in the hexane fraction. Extensive chromatographic separation of the hexane soluble fraction using silica gel, dry column chromatography, followed by preparative thin layer chromatography led to the isolation of stigmasterol. The structure of this compound was established by spectral analysis including 1D and 2D NMR. The result suggest that the hexane fraction of *Spermacoce verticillata* plant possess phytochemical constituents that may be useful in the management of microbial diseases.

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LIST OF ABBREVIATION

WHO = world health organisation

SVE = spermacoce vertiscillata extract

TLC = thin layer chromatography

PTLC = preparative thin layer chromatography

NMR = Nuclear magnetic resonance

MIC = minimum inhibitory concentration

MBC = minimum bactericidal concentration

MFC = minimum fungicidal concentration

HF= Hexane fraction

AE= Acetone extract

ND = Not determined

IUCN= International union for conservation of nature and natural resources

CHAPTER ONE

INTRODUCTION

1.0 General Introduction

The use of plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available (Peach, 1995). However, the systemic study of plants for detecting antimicrobial activity is of comparatively recent origin. These investigations have been triggered by the emergence and spread of antibiotic resistant microorganisms causing the effective life-span of existing antibiotics limited. Hence the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of new anti-infective agents (Peach, 1995).

It is evident, that even though scientific advances have been made in our quest to understand the physiology of the body, biotechnology and the treatment of diseases, natural products remain a crucial, cheap and uncontroversial component of the comprehensive health care strategy for the future (Patwadhan, 2005). Natural products such as plant extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drugs discoveries because of the unmatched availability of chemical diversity (Cosa *et al.*, 2006).

In the last decades, various studies have demonstrated that plants serve as reservoirs for innumerable microorganisms known as endophytes; these microorganisms live in the host plant at least for one period of their life cycle, without causing apparent harm to them (Petrini, 1991; Bacon and White, 2000). Even with the existence of uncountable epiphytic and soil microorganism, diverse studies have shown the potential of endophytes as a promising source of natural products for the discovery of a variety of different classes of bioactive molecules to be

applied in medicine, industry and Agriculture (Schulz *et al.,* 2002; Joseph and Priya, 2011). Investigations have shown that the search for microorganisms of biotechnological interest based in the ethno botanical pharmacology represents an alternative to discover new microorganisms and bioactive molecules (Li *et al*., 2005, Qin *et al;* 2011). Based on these principles, the bio prospection of microorganisms isolated from new ecological niches constitutes an important strategy for obtaining more efficient and less toxic antibiotics, which may ultimately control pathogenic bacteria that are resistant to the diverse antibiotics commonly used in current time (Appelbaum and Jacobs, 2005).

The use of the plant to treat diseases continue to play a key role in health care. According to the World Health Organization (WHO), 80% of the developing world's rural population depends on traditional medicines for its primary healthcare needs (Bannerman *et al;* 1993). In some African countries such as Ghana, Mali, Nigeria and Zambia, the first line treatment for 60% of the children with high fever resulting from malaria is the use of herbal medicines at home (WHO, 2003). In these societies, the tradition of collecting, processing, and applying plants and plant-based medications have been handed down from generation to generation. Traditional Medicine, with medicinal plants as their most important components are sold in market places and prescribed by traditional healers in their homes (VonMaydell, 1996).

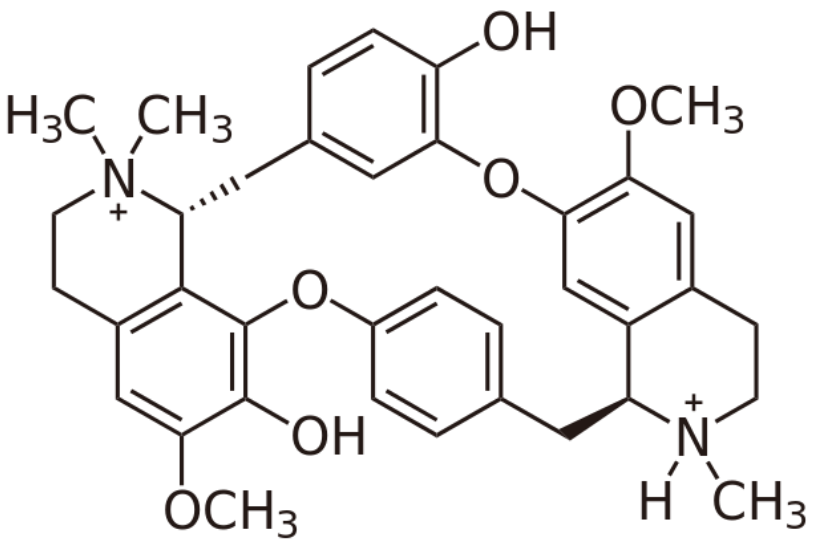
The development of resistance to most of the available antimicrobial agents and the high costs of treatment consequent upon this resistance has necessitated a search for new, safe, efficient and affective agents for the management of infection (Okwu and Uchenna, 2009). These research for new effective agents against infectious diseases and other diseases such as cancers, diabetes, cardio-vascular, neurological, respiratory disorders, among others; has led to increased interest in

existing information about the remedies of these diseases from natural sources, principally the plants (Karou *et al*.,2007;Ouattara *et al*., 2007). Because of this strong dependence on plants as medicines, ethno pharmacological studies have been conducted to determine their safety and efficacy and on the other hand to find out new active principles from plants (Zongo *et al*., 2010; Ouattara *et al*., 2011).

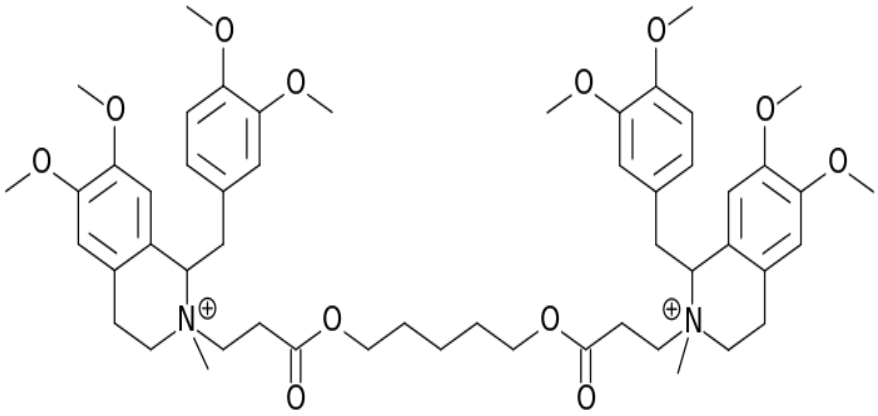
Herbal medicine is now globally accepted as a valid alternative system of therapy in the form of pharmaceuticals, functional foods, a trend recognised and advocated by the World Health Organisation (WHO, 2003). Various studies around the world, especially in Europe, have been initiated to develop scientific evidence-based rational herbal therapies. Though ancient medical treatises have remained largely undocumented as the knowledge of their use are mainly being passed down from generation by word of mouth. New plants sources of medicine are also being discovered (WHO, 2003).

Herbal medicine can be valuable to modern medicine in many ways. Plants in the tropics are often used as direct source of drugs, e.g. the alkaloid tubercurarine (I), is used as muscle relaxant in surgery, and till date chemists are unable to produce it synthetically, although its analogue atracurium besylate (II) was synthesed (WHO, 2003).

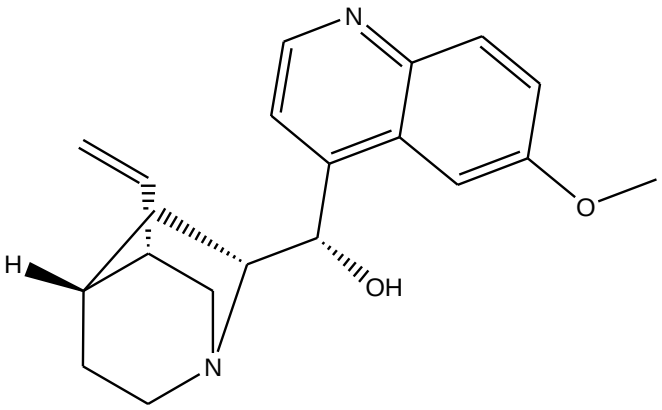
In medicine, trees of the genus Cinchona are of great interest because of their alkaloids, the most familiar being quinine, (III) the first effective agent in treating malaria (WHO, 2003).



I

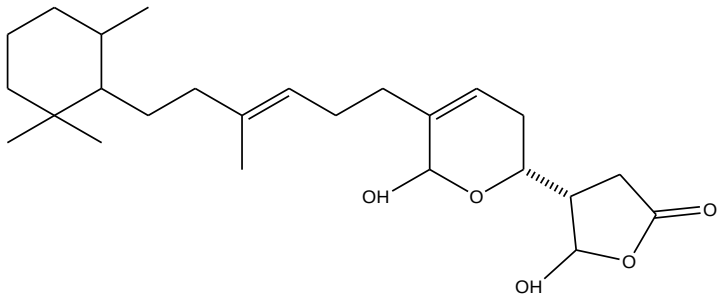


II



III

The search for biologically active molecules from marine organisms is a relatively new but rapidly expanding branch of natural products chemistry. It was estimated that about 3000 novel natural products were isolated in the last 30 years alone. These were isolated from sponges, corals, and sea-dwelling microorganisms. Example of bioactive compounds isolated from marine source is manoalide (IV) from a sponge, *Litffariella variabilis.* It is a 25-carbon marine natural product with anti-inflammatory activity which acts by selectively inhibiting cyclooxygenase **(COX)-**enzyme (DeRosa *et al*., 1998).



IV

Plant kingdom has been haphazardly investigated; some families have been relatively well studied while others were almost completely overlooked. In general, of the estimated 250,000 higher plant species discovered, only about 6% have been screened for biologic activity and a reported 15% have been evaluated phytochemically (Fabricant and Farnsworth, 2001). A major challenge to herbal medicine and development of drugs from herbal medicine is the rapid rate of extinction among the global flora; the world genetic flora is rapidly diminishing. Tropical rain forest plants, which are found exclusively in developing countries, are being destroyed at the rate that many scientists believe to be unjustifiable from both economic and ecological perspectives (Principe, 1991). According to International Union for Conservation of Nature and Natural Resources (IUCN) and the World Wide Fund for nature (WWF), 60,000 higher plant species could become extinct or near extinct by the middle of this century if the present trend continues. The main causes of deforestation and subsequent extinction of plant species are: outgrowth of population and rural poverty shifting cultivation, agricultural conservation, fuel wood gathering and impact of large development projects (Allen and Bernes, 1985).

Higher plants have been described as chemical factories that are capable of synthesizing unlimited numbers of highly complex and unusual chemical substances whose structure could escape the imagination of synthetic chemist forever; considering that many of these unique gene sources may be lost forever through extinction and that plants have a great potential for producing new drugs of great benefit to mankind, some action need to be taken to reverse the current apathy (Farnsworth, 1988).

The validation of the folkloric claims of these medicinal plants will provide scientific basis for the conservation of tropical medicinal resources, the deployment of the beneficial ones as phytomedicine in the primary health care and the development of potential bioactive constituents.

These could provide novel lead compounds or precursors in drug development, and utilization of isolated compounds as investigative, evaluative and other research tools in drug development and testing processes.

The approved and regulated chemical armamentarium available today is failing to provide effective health care for even one-third of human population (Henry, 2000). In addition, a number of significant global disease, including cancer, malaria, tuberculosis and certain viral, fungal and bacterial infections are showing significant pattern of drug resistance to the known therapeutic agents, and the time and cost of developing a new drug from initial discovery to final approval are both too long and too expensive (Cordell *et al*., 2001), because of the affirmation reasons it is obtained that drug discovery is necessary in many diseases such as cancer, heart diseases, lung diseases, HJV-infection, alzheimers diseases, arthritis and resistant fungal, bacterial and viral infections. Others are malaria, infantile diarrhea and tuberculosis (Cordell *et al.,* 2001).

1.1 Bacterial infections

Infectious disease is one of the leading causes of death world-wide and accounts for approximately 50% of death in tropical countries (Iwu *et al.,* 1999). Long before the discovery of the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles was well accepted. Since antiquity, humans have used plants to treat common infectious diseases, and some of these traditional medicines are still included as part of the habitual treatment of various maladies. For example, the use of bearberry *Arctostaphylos uvaursi* and cranberry juice *Vaccinium macrocarpori* to treat urinary tract infection is reported in different manuals of phytotherapy, while species such *as* lemon balm *Melissa officinalis*\ garlic *Allium sativum,* and tee tree *Melalenca alternifolia* are described as broad-spectrum antimi-

crobial agents (Mendonca-Filho, 2006). That being said, it has generally been the essential oils of these plants rather than their extracts that have had the greatest use in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal, and biliary systems, as well as on the skin. In the case of *Melaleuca alternifolia*, for example, the use of the essential oil (tee tree oil) is a common therapeutic tool to treat acne and other infectious troubles of the skin (Mendonca-Filho, 2006). Antimicrobial resistance is one of the biggest challenges facing global public health. Although antimicrobial drugs have saved many lives and eased the suffering of many millions, poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and bad governance in developing countries have tremendously limited the benefits of these drugs in controlling infectious diseases. The development of resistance to the pathogens has worsened the situation, often with very limited resources to investigate and provide reliable susceptibility data on which rational treatments can be based as well as the means to optimize the use of antimicrobial agents (Mendonca-Filho, 2006).The emergence of multidrug-resistant isolates in tuberculosis, acute respiratory tract infections, and diarrhea, often referred to as the diseases of poverty, has had its greatest toll in developing countries. The epidemic of HIV/AIDS, with over 30 million cases in developing countries, has greatly enlarged the population of immune compromised patients. The disease has left these patients at great risk of numerous infections and even greater risk of acquiring highly resistant organisms during long periods of hospitalization (Mendonca-Filho, 2006).

Antibiotic resistance can occur via three general mechanisms: prevention of interaction of the drug with target, efflux of the antibiotic from the cell, and direct destruction or modification of the compound. The emergence of multidrug resistance in human and animal pathogenic bacteria as well as undesirable side-effects of certain antibiotics has triggered immense interest in the

search for new antimicrobial drugs of plant origin (Ahmad and Beg, 2001). Many plants are used in treating bacterial related diseases. Only a small fraction of these plants has been investigated (Cart and Rogers, 1987). Several compounds were isolated and have demonstrated good antimicrobial activity, and these include: Asiaticode, an anti-microbial compound isolated from *Centellaasiallca* (used traditionally in skin diseases and leprosy), has been studied in normal as well as delayed-type wound healing. The results indicated significant wound healing in both models. Another compound, cryptolepine, isolated from *Crytolepis sanguinolenta* which was found to be active against *Campylobacter* species, has been used traditionally in Guinea Bissau in the treatment of hepatitis and in Ghana for the treatment of urinary and upper respiratory tract infections and malaria (Angeh, 2006).

The development of organic chemistry took place along with the study of plants, mainly in the 19^{th} century, when the first studies on plants were scientifically recorded. This ended up in the isolation of some active compounds from plants (Wheelwring, 1974). It is the vegetal kingdom that has contributed in a more meaningful way, to the supply of useful substances for the treatment of human diseases (Galhiane, 2006).

1.2 Statement of Research Problem.

Infectious diseases are the number one cause of death world-wide, and in tropical countries it accounts for approximately 50% of death (Iwu*et al*., 1999). Apart from resistance, some antibiotics have serious undesirable side effects which limit their applications. Therefore, there is a serious need to develop new antimicrobial agents that are very effective with minimal side effects. Higher plants represent a potential source of novel antibiotic, prototypes (Maureer-Grims *et al.,* 1996).

1.3 Justification of the Study.

Spermacoce verticillata has been used traditionally in the management of microbial infections. Literature search in scientific data base showed that no much phytochemical work was reported on the plant.

Many plant extracts from other species of the family have been tested and found to have antimicrobial activities. This makes it necessary to search for drugs that may be more potent. This will go a long way to proposing a template or lead that can be used for designing other drugs.

Aims of the study:

The aim of the research is to carry out phytochemical studies on *Spermacoce verticillata,* and evaluate its anti-microbial activities.

1.4 Objectives of the Study.

To carry out preliminary phytochemical screening of *S. verticillata* using standard procedures.

To isolate some of the biologically active compounds from the plants

To elucidate the structure of the isolated compounds using spectroscopic analysis

To validate the anti-microbial activities of the plant

1.5 Statement of Research Hypothesis.

The whole plant of *Spermacoce verticillata* contains phytochemical constituents with antimicrobial activity.

CHAPTER TWO

Literature Review:

2.1 *Spermacoce verticillata*

Spermacoce verticillata grows on moist soil, both acid and alkaline of all textures derived from nearly all types of rocks.

2.1.1 Morphology of *Spermacoce verticillata*:

Spermacoce verticillata is a fine-stemmed scrambling shrub that may reach a few meters of lateral extension and 1.2 m in height as a free-standing plant. The square stems are herbaceous to semi-woody in their first year, becoming woody and more rounded in the following year. The brown stems reach a maximum diameter of about 8 mm.

2.1.2 Geographical distribution

Spermacoce verticillata appears to be a native of the New York and possibly Africa, but the original range is uncertain. It grows as a native or naturalized species from Florida through the West Indians, and Texas through Central and South America to Argentina, and throughout the moist portions of Tropical Africa and Madagascar (Burkill, 2000).

The species grows in areas that receive from about 750 to 3000 mm of annual rainfall from near sea level to 600 m or more of elevation in Puerto Rico. It grows on sand and caliche in Paris and opening in Texas. The plant is a shrub, it seedling grow slowly at first but begin rapid growth after about 6 months. *S. verticillata* shrubs appears to live at least 4 years and probably much longer in Pueto Rico (Jones,1975).

2.1.3 Classification of the plant *Spermacoce verticillata.*

Taxonomic Name: *Spermacoce verticillata* L.

Kingdom: Plantae

Family: Rubiaceae

Genus: *Spermacoce*

Species: *verticillata*

Common/Local Names:

English: Shrubby false Button weed, Borrerie verticillee

Hausa: Karya Garma.

2.1.4 Ethno medicinal uses:

The plant is used in traditional medicine for wide variety of remedies, most frequently for skin conditions. In Africa, the leaf extracts are used to treat leprous conditions, ulcers, and gonorrhoeal sores (Burkill, 2000). A lotion is prepared to relieve skin itches (Liogier, 1990). Other preparations are used internally to treat diarrhoea, as a diuretic in the treatment of schistosomiasis, and as an abortive. An essential oil extracted from the leaves has been shown to inhibit *Escherichia coli* and *Staphylococcus aureus* (Burkill, 2000).

The plant is used in many remedies in the management of diverse diseases including abdominal irritation, abortion, abscesses, anaemia, arthritis, acarida's, asthenia, baby growth delay, chancre, chicken pox, conjunctivitis, constipation, cough, dermatitis, diabetes, diarrhoea, dizziness, dysentery, dysmenorrhoea, eczema, epilepsy, evil eye, fever general weakness, gonorrhoea, headache, hepatitis B, itchy rashes, infant umbilicals, pains internal inflammation, jaundice, kidney diseases, leprous macular, lumbago, lymphadenitis, malaria, mental disorders, mycoses, obesity, oedema, ovarian cyst, paralysis and nerve diseases, pinworm, poisoning, snake bites,

splenomegaly, sterility, syphilis, tape worm, urinary retention, vomit and wounds. Rubiaceae may be considered as a major component of sub-Saharan folk medicine (Simplice *et al,*. 2011).

2.1.5 Pharmacological activities of *Spermacoce verticillata* and some genera of the family

Literature survey has shown that some species possess antimicrobial, anti-diarrheal, antitumor, antioxidant, and anti immflammatory activity.

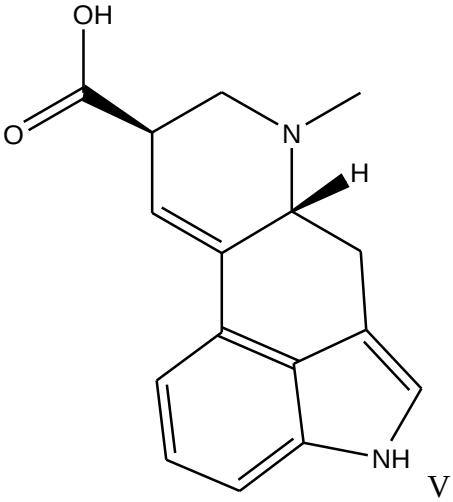
Spermacoce verticillata is a member of rubiaceae family. It is a large family of 630 genera and about 13000 species found worldwide, especially in tropical and warm regions. These plants are not only ornamental but they are also used in African folk medicine to treat several diseases. The family is among plants of wide usage in traditional medicine that are continuously screened in laboratory for pharmacological properties. According to their wide distribution, these plants are used in all parts of the world as ornamental foods and remedies. The most economically important member of the family are the two species of shrub *Coffea canephora* (also known as coffea robusta) and *Coffea arabica*, used in the production of coffee. *Gardenia jasminoides* is widely grown garden plant and flower in frost free climates worldwide. Several other species from the genus are also seen in horticulture. The genus *Ixora* also contain plants seen cultivated in warmer climate gardens. In medicine trees of the genus *cinchona* are of great interest because of malaria.(Simplice *et al.*, 2011).

Rubiaceae is a family of flowering plants, variously called the madder family, bedstraw family or coffee family. The family takes its name from the Madder genus Rubia. Other plants such as Gardenia, Cinchona, Gambier, Ixora., Naucleaceae and The ligonaceae have been included in the family, Now-a-days there are about 630 genera and more than 13000 species in the family; making the Rubiaceae one of the six largest angiosperm families including Asteraceae, Orchidaceae, Fabaceae, Poaceae and Euphorbiaceae in terms of number of genera and species.

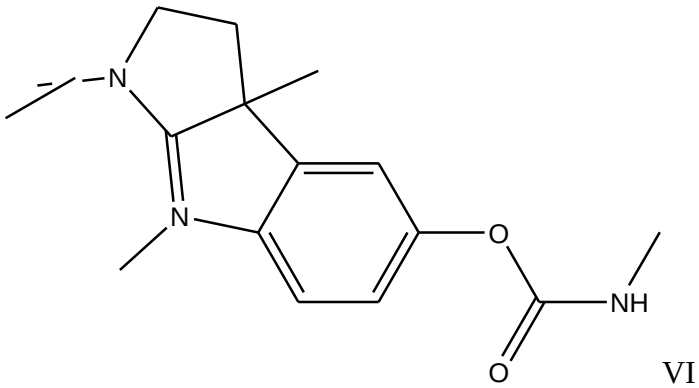
Rubiaceae species are concentrated in warmer and tropical climates around the world (Dalziel, 1957). A wide variety of growth forms are present in the Rubiaceae. Shrubs are most common, but members of the family can also be trees, lianas or herbs. Species are mainly woody, less than 20% of the genera are herbaceous. A large number grow in sub Saharan Africa. The most represented are Anthosperrneae, Morindeae, Spermacoceae, Cinchoneae, Naucleae, Coffeae, Gardenieae and Pavetteae, (Dalziel, 1957).

Chemistry of Rubiaceae

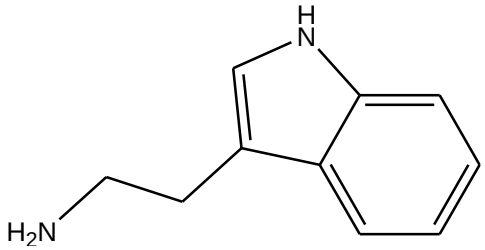
Various natural products occur in Rubiaceae plants extensive phytochemical investigation has been realised regarding the natural occurrence of terpenoids, anthraquinones and indole alkaloids in the family. The occurrence of alkaloids seems to be a rule in this family, although Leal and Elisabetsky (1996) demonstrated the absence of alkaloids in psychotriacartha_genesis. The alkaloids of Rubiaceae are indolique alkaloids. They may occur in tetracyclic or Penta cyclic rings. Lysergic acid (V) is an isoprenoidindole alkaloids, Physotigmine (VI), Tryptamine (VII) are Non- isoprenoidindole alkaloids Leal and Elisabetsky (1996).



V



VI



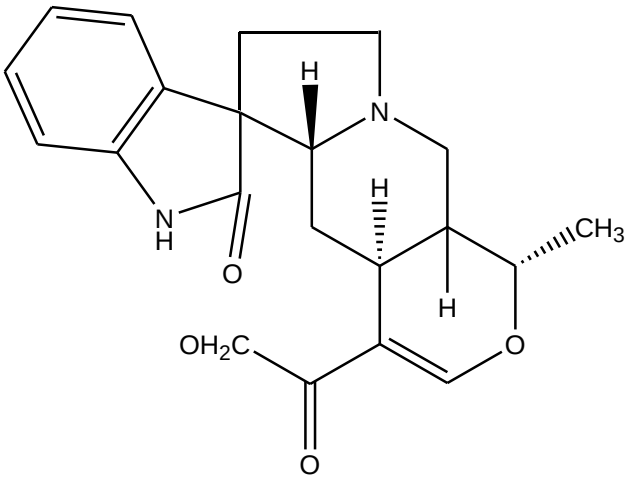
VII

The occurrence of alkaloids in some Rubiaceae is well documented. The leaves of M. Inermis contain tetracyclic and pentacyclic oxy-indole and indole alkaloids including uncarine D (VIII), rhynchophylline (IX), rotundifoline (X). The proportion of these compounds is variable and depends on the growing location and the season of harvest. A number of monoterpene indole alkaloids including nauclefine 1 and 2 have been isolated from nauclea species. The main alkaloids of N.pobeguine were identified as strictosamide, (XI) 5(s)-5-carboxystritosidine (XII), 19-o-methylangustoline (XIII), 3-o-b-D-fusosyl-quinovic acid (XIV), 3-keto quinovic acid (XV) N. latifolia contains duerise phytochemicals such as alkanoids, flovonoids, steroids and glycosides. (Toure *et al.*, 1996).

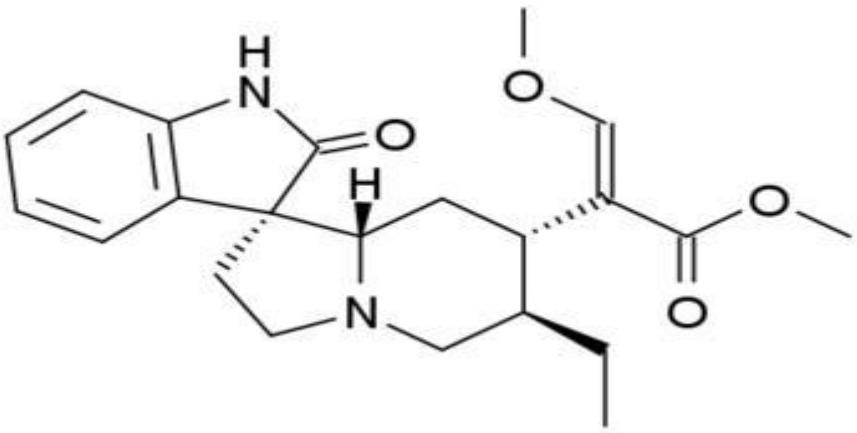
Naucleafoline, nauclecline (XVI) and naufoline (XVII) were isolated from the leaves. Naucleidial and epin-naucleidal have been isolated from an antiviral preparation produced by nesting *Nauclea latifolia* fruits (Morah, 1994).

Furthermore, five monoterpene indole alkanols naucleamids Naucleamide H_{16}=a (XVIII), Naucleamide H_{16}=b (XIX), Naucleamide C (XX), Naucleamide D (XXI), were found to occur in the bark and wood of the plant. Naucleamide E (XXII) was the unique monoterpene indole

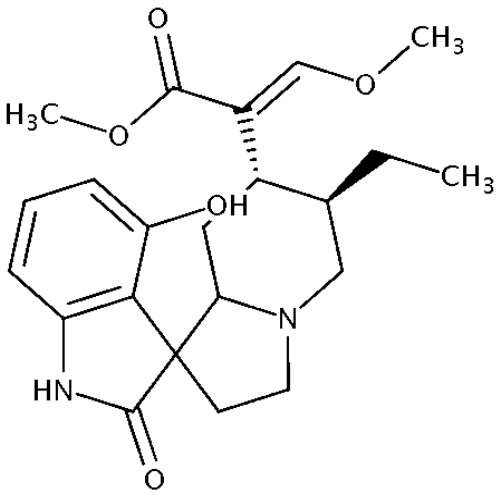
alkaloids pocessing a pentacyclic ring system with an amino acetal bridge. (shigermori *et al.*, 2003). Furthermore, a new indole alkaloids 9-meitroxy-3-epi a-yohim bine (XXIII) was isolated as a minor component. (Takayama*etal*., 2004).



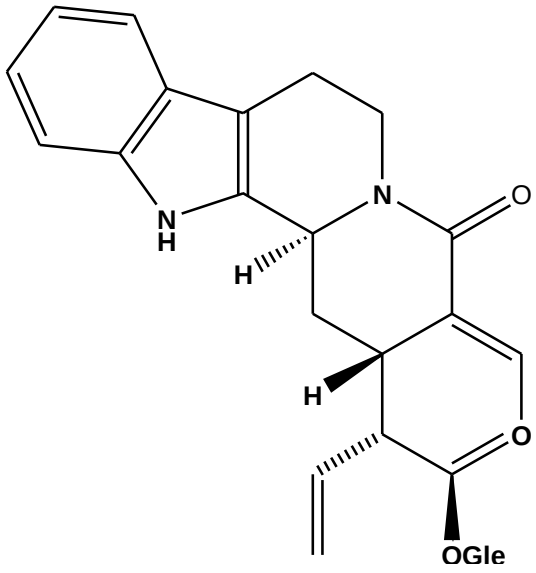
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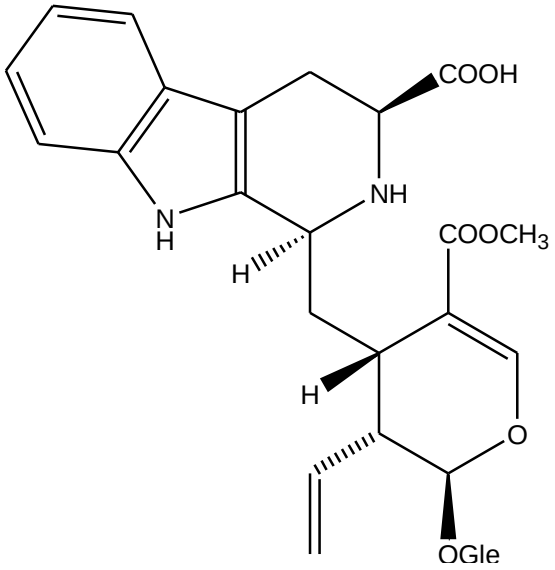
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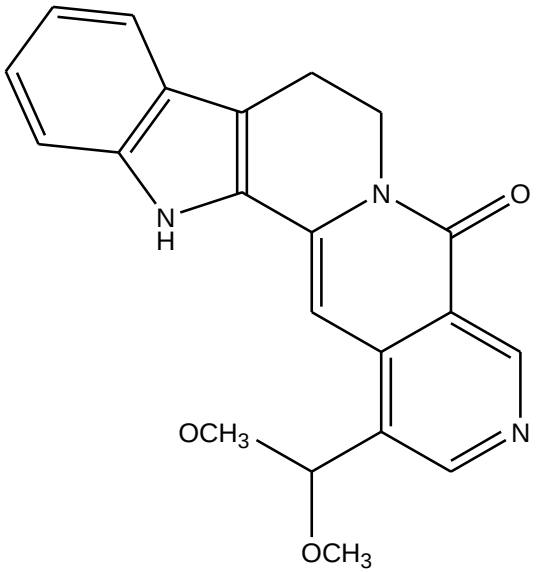
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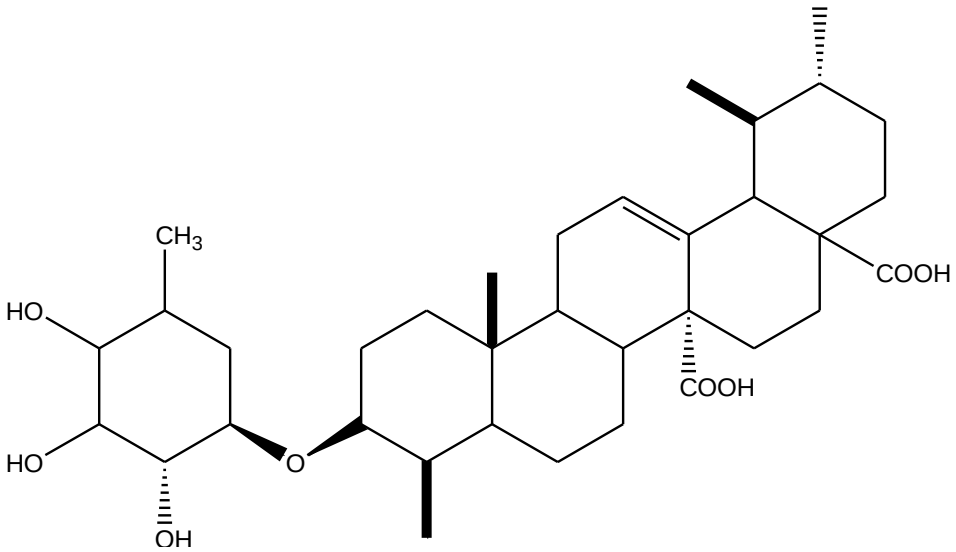
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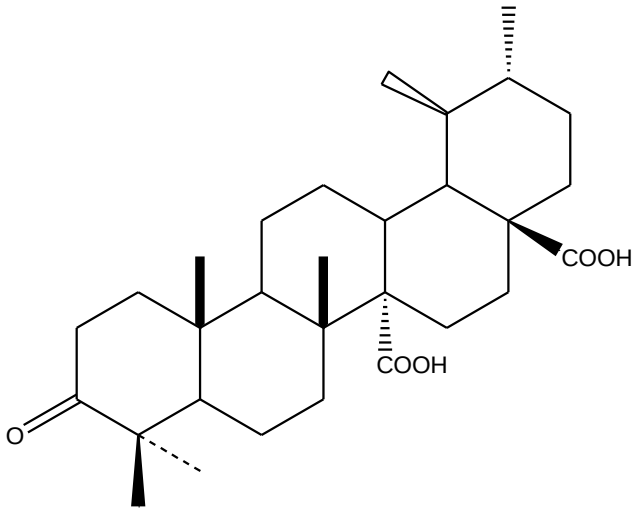
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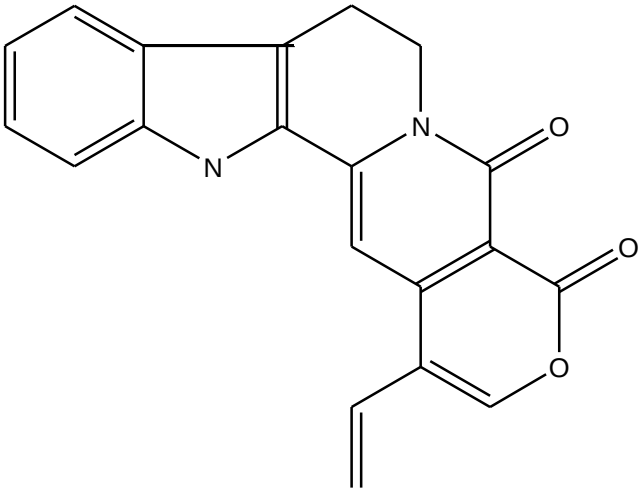
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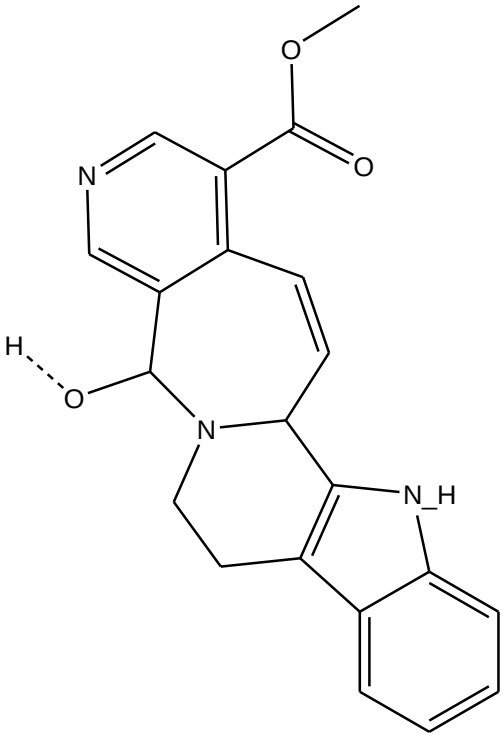
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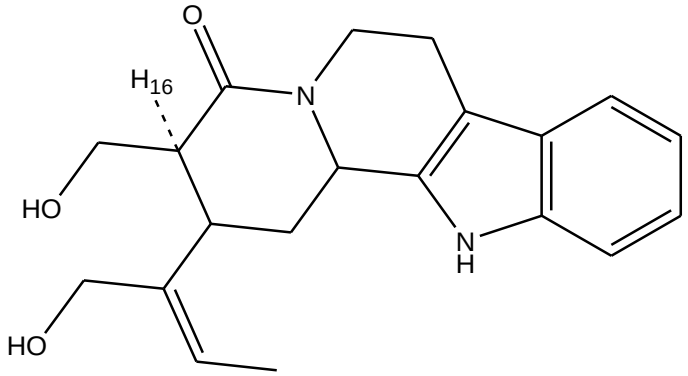
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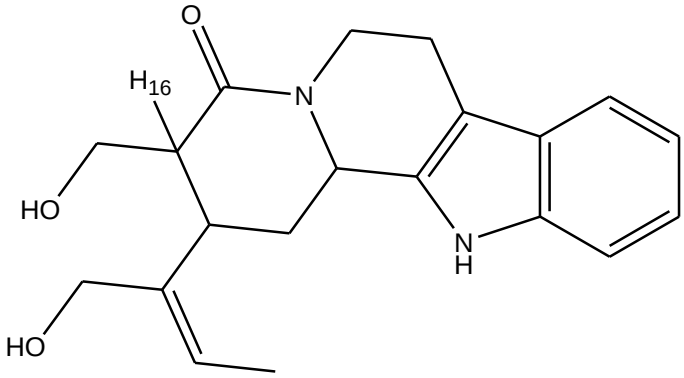
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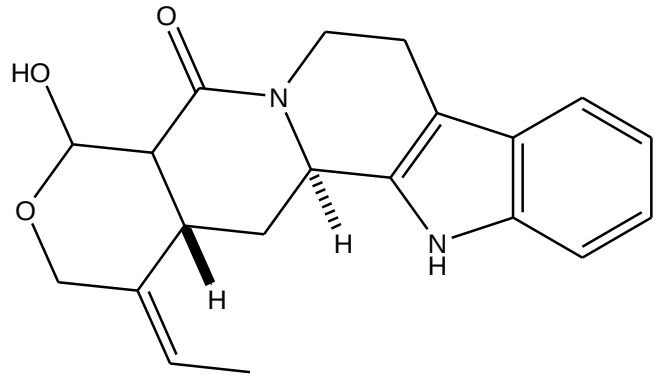
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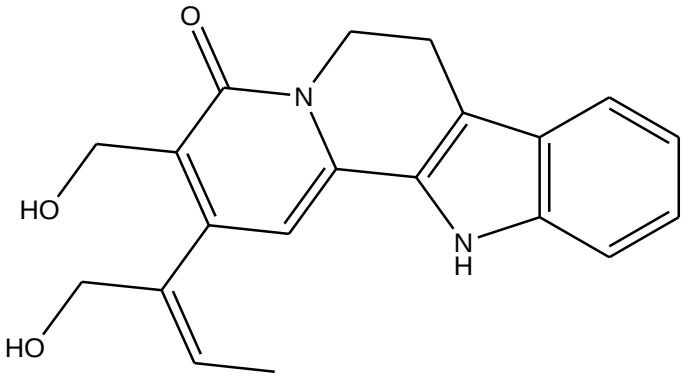
XVIII



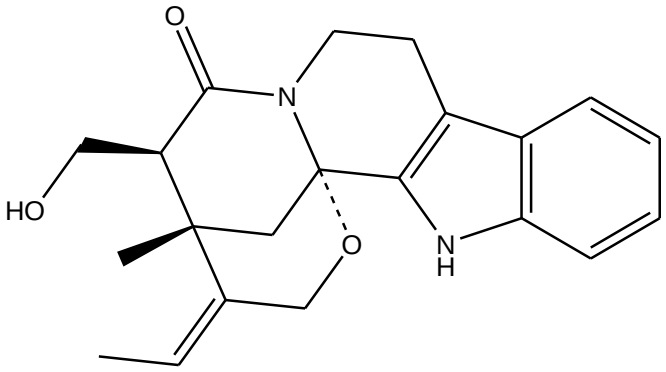
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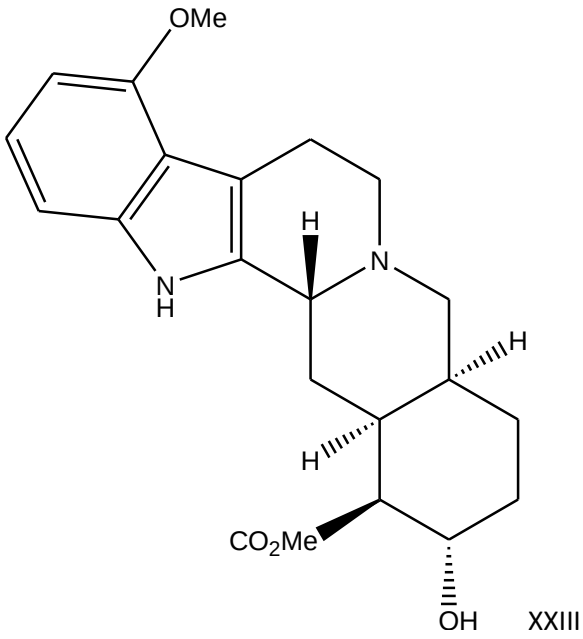
XX



XXI



XXII



XXIII

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 Solvents/Reagents

Solvents used were of analytical grade. Silica gel for column chromatography was of mesh size 60-120µm. The thin layer chromatography (TLC) plates used were aluminium plate pre-coated with silica gel.Pf $_{254}$, 0.2mm; preparative TLC 20x20cm, 0.25

3.2 METHOD

3.2.1 Collection, identification and preparation of plant materials:

The plant was collected from Turunku village, Igabi L.G.A Kaduna State, Nigeria in the month of June, 2012 and was authenticated at the herbarium unit, Biological Sciences Department, Ahmadu Bello University Zaria-Nigeria, where a voucher specimen (No.672) was deposited. The whole plant was air-dried and made into powder using clean mortar and pestle and subsequently referred to as powdered plant material, and stored for use.

3.2.2 Extraction:

The powdered plant material (1400g) was extracted with acetone using soxhlet apparatus. The solvent was removed in-vacuum to yield a residue (60 g) referred to as *Spermacoce verticillata* acetone extract (SVAE).

3. 2. 3 Partitioning (Liquid- Liquid partitioning)

The acetone extract (45g) of the plant was suspended in distilled water and the water soluble fraction was partitioned with hexane, chloroform, ethyl acetate and N- butanol to yield hexane, chloroform, ethyl acetate and N-butanol soluble-fractions were obtained, respectively.

3.3 Preliminary phytochemical Screening

Portion of the acetone extract was subjected to preliminary phytochemical screening for the presence of chemical constituents including Flavonoids, Steroid, alkaloids, terpenes, etc.

3.3.1 Test for Sterols/terpenes

a. *Liebermann-Buchard test:*

Concentrated acetic acid (1ml) was added to 1ml chloroform and cooled to 0^0C then one drop of concentrated sulphuric acid was added to the cooled mixture followed by the extract. The solution was observed for blue, green, red and orange that changes with time (Silva *et al.,* 1998)

b. *Salkowski test:*

A little quantity of extract was dissolve in 1 ml of chloroform and to it 1 ml of concentrated sulphuric acid was added down the test tube to form two phases. It was then observed for the formation of red or yellow coloration at the interphase (Silva *et al*., 1998).

3.3.2 Test for Flavonoids

a. *Shinoda test*:

To an alcoholic solution of the extract three pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid and observed for orange, pink or red to purple colour to indicate the presence of flavonoids (Sofowora, 1993).

b. *Sulphuric acid test:*

*Th*e sample was dissolved in concentrated sulphuric acid and the colour change was observed (Silva *et al.,* 1998)

c. *Ferric chloride test*

Extract was boiled with water and filtered. To 2 ml of filtrate, two drops of freshly prepared ferric chloride solution was added; green, blue or violet colouration indicate the presence of phenolic hydroxyl group (Cannel, 2000).

d. *Sodium hydroxide test*

2 ml solution of extract was dissolve in 10% aqueous sodium hydroxide solution and filtered to give yellow colour, a change in colour from yellow to colourless on addition of dilute HCl indicates the presence of flavonoids (Cannel, 2000).

3.3.3 Test for alkaloids

Crude extract (0.5g) was stirred with 5 ml of 1 % aqueous hydrochloric acid on a water bath and filtered. Three ml of the filtrate was divided into three. To the first portion few drops of freshly prepared Dragenoff reagent was added and observed for formation of orange to brownish precipitate. To the second potion 1 drop of Mayer reagent was added and observed for formation of white to yellowish or cream colour precipitate. To the third portion 1 drop of Wagner reagent was added and was observed for a brown or reddish or reddish-brown precipitate (Silva *et al*,. 1998).

3.3.4 Test for tannins

A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride was added to the filtrate, formation of a blue- black, or green precipitate was taken as evidence for the presence of tannins (Trease and Evans, 1996).

3.3.5 Test for anthraquinones

a. *Free anthraquinones*

The extract was shaken with 10 ml of benzene, the content was filtered, and 5 ml of 10 % ammonia solution was added to the filtrate, the mixture was shaken. Presence of a pink, red or violet colour in the ammoniacal layer (Lower phase) indicates the presence of free anthraquinone (Cannel, 2000).

b. combined anthraquinones.

The extract was boiled with 10 ml of aqueous sulphuric acid and filtered hot. The filtrate was shaken with 5 ml benzene layer was separated and half its own volume, 10 % NH_4OH was added. A pink, red or violet colouration in the ammonia phase (lower phase) indicates the presence of combined anthraquinone or anthraquinone derivatives (Cannel, 2000)

3.3.6 Test for saponins.

0.2g of the extract was shaken with water in a test tube. Frothing which persisted for 15 minutes indicates the presence of saponins (Silva *et al.*1998).

3.4 Chromatographic procedures:

3.4.1 Thin layer Chromatography:

Thin layer chromatography was carried out on TLC aluminium sheet precoated with silica gel 60 PF_{254} layer thickness of 0.2 mm.

Technique: One way ascending

Spotting and development: Spots were applied manually using narrow capillary tube; plates were dried using air blower and developed at room temperature using a Shandon chromatotank.

Solvent system; various solvent systems were used depending on the material, which include:

- Hexane (100%)
- Hexane: Chloroform (2:1)
- Hexane: Chloroform (1:1)
- Hexane: Ethyl acetate (10:1)
- Hexane: Ethyl acetate (8:2)
- Hexane: Ethyl acetate (7:3)

Detection: Spots on TLC plates were visualised under UV light (254 and 366 mm) and sprayed with 10% sulphuric acid, followed by heating at 110^0C for 5-10 min.

3.4.2 Column chromatography

The following column conditions were employed in running the column chromatography.

i. *Technique*- Gradient elution

ii. *Column*- Glass column with sintered disc at the bottom of various dimensions.

iii. *Stationary phase*- silica gel, 60-120 mesh size.

iv. *Column packing*- Wet slurry method.

v. *Sample loading/application*- The sample was applied using dry load method (Cannell, 1998), the sample was dissolved in small amount of Hexane, and it was then mixed with a small quantity of silica gel, dried, tritured and then loaded on top of the column.

vi. *Solvent for elution*- Various solvents were used. Elution was carried out using one or mixture of the following solvents:

Hexane

Chloroform

Ethyl acetate

3.4.3 Gel filtration chromatography

Gel filtration was performed using sephadex LH-20 (sigma)

Packing method: The sephadex gel was suspended in methanol and allowed to swell for 24 hrs. prior to use. It was then poured into the column and allowed to settle tightly.

Sample application: The sample was dissolved in small volume of the eluent and applied on top of the column.

Eluting Solvent: Methanol.

3.5 CHROMATOGRAPHIC SEPARATION

3.5.1 Column chromatography

The hexane fraction was subjected to thin layer chromatography so as to isolate phytochemical compounds present in the extract. The Stationary phase was silica gel (60-120 mesh size), gradient elution technique was employed and the eluents were analysed using TLC. Eluents having the same TLC profile were merged together. Eluents having one single sport after been

subjected to TLC using at least 2 solvents systems were considered as a single compound. While eluents having more than one sport when subjected to TLC was subjected to further gel filtration using Sephadex LH-20.

Column Chromatography of Hexane fraction

Hexane fraction 5g was chromatographed over silica gel packed column of dimension 75 by 3.5 cm, the column was eluted continuously using Hexane, then Hexane: Chloroform mixture, Chloroform 100% then Chloroform: ethyl acetate mixture as solvent system. One hundred and ten fractions (30ml) each were collected. The fractions were pooled together based on their TLC profiles which gave five major fractions and the column was finally washed with methanol. Fraction five which showed two major spots and two minor spots was subjected to gel filtration using sephadex LH-20 and eluted with methanol. Twelve fraction were collected. Fraction 5-8 (X) showed one major and one minor spot were further purified. Fraction X was subjected PTLC using chloroform: ethyl acetate 4:1 as the solvent system. The major spot was scraped; the adsorbent was thoroughly washed with chloroform. The solution was concentrated to afford pink amorphous compound coded S1. It was then subjected to spectroscopic analysis to elucidate its chemical structure.

3.5.3 Preparative thin layer chromatography (PTLC)

Fraction X was subjected to PTLC using $CHCl_3$: EA (4:1) as solvent system. It was carried out using fluka silica gel precoated glass plated 20x20 cm with layer thickness of 0.25 mm. A thin line about 1.5 cm from the bottom of the plate was drawn with a pencil. The sample to be separated was dissolved in minimum amount of chloroform to give an approximate concentration

of 20 mg/ml. it was then applied uniformly along the thin line using capillary tube. The plate was allowed to dry after which it was developed using hexane: Ethyl acetate (8:2) as solvent system. The developed plate was air dried in a fume cupboard and the position of the band of interest was marked with pencil and scraped off on a foil paper. The scrapped sorbent was size reduced using pestle and mortar, transferred to a sintered glass funnel and washed repeatedly with chloroform and the solution obtained was evaporated to give the compound coded S1 (Gibbons and Gray, 1998). The structure of S1 was subjected to spectroscopic analysis to elucidate its structure.

3.5.4 Spectroscopic Analysis;

a. Proton and carbon-13 NMR Spectra

NMR spectra were obtained on a Bruker AVANCE (400 MHz for ^{1}H and 125 MHz) spectrometer, using the residual peaks as internal standard. Chemical shift values were reported in parts per million (ppm) relative to appropriate internal solvent standard. The NMR solvent used for these measurements is deutrated methanol. Isolated compound was subjected to spectral analysis for structural elucidation of the isolated compounds using ultraviolet (UV) spectroscopy, Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and Mass spectroscopy (GC-MS). (Silverstein *et al*; 1991).

3.5.5 Melting point:

The melting point of the isolated compound was determined using the Gallenkhamp melting point apparatus.

3.6 Antimicrobial screening

The antimicrobial activities of *S.verticillata* crude extract was determined using some pathogenic microbes obtained from the Department of Medical Microbiology A B U Teaching Hospital, Zaria were: *Staphylococcus aureus, Salmonella typhii, Eschericia coli, (ATCC11775), Candida albican, Streptococcus feacalis, Bacillus subtilis, Campylobacter Sp., Pseudomanas aeruginosa, Shigella dysenteria and Candida krusei.*

Stock concentration was prepared by dissolving 0.2 g of the extract was weighed and dissolved in 10 mls of DMSO to obtain a concentration of 20 mg/ml, this was the initial concentration of the extract used to check the antimicrobial activities from the crude of the plant. (Souza *et al.,* 2012).

Muller Hinton agar was the medium used as the growth medium for the microbe and was prepared according to the manufacturer's instructions, sterilized at 121^{o}C for 15mins into sterile petri dishes and was allowed to cool and solidify. (Souza *et al.*, 2012).

Diffusion method was the method used for screening the extract. The sterilized medium was seeded with 0.1 ml of the standard inoculum of the test microbe. The inoculum was spread evenly over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6mm in diameters a well was cut at the centre of each inoculated medium. (Souza *et al.,* 2012).

0.1ml of the solution of the extract of the concentration 20mg/ml was thus introduced into each well on the medium and the medium was incubated at 37^{0}C for 24hrs, after which it was checked

for the zone of inhibition of growth, the zone was measured with a transparent ruler and the result recorded in millimetre. (Souza *et al.,* 2012).

3.6.1 Test organisms

Clinical isolates were obtained from the Department of Medical Microbiology ABUTH Zaria including: *Staphylococcus aureus, Salmonella typhii, Eschericia coli,(ATCC11775),Candida albican, Streptococcus feacalis, Bacullus subtilis, Campylobacter Sp., Pseudomanas aeriginisa, Shigella dysenteria and Candida krusei.* All the organism were obtained from the Department of medical Microbiology Ahmady Bello Universuty Teaching Hospital Zaria.

3.6.2 Susceptibility Test

The antimicrobial activities of crude plant acetone extract and its hexane fraction of the plant was determined using stock concentration of 5mg/ml. The microorganisms were maintained on agar slant. The inoculant were prepared by inoculating the test organisms in nutrient broth and incubating them for 24hrs at 37^{o}C for bacteria, while for fungi sabraud dextrose broth was used and was incubated for 48hrs. After incubation, the broth cultures were diluted to 1:1000 formed gram positive bacteria and 1:5000 for the gram negative bacteria. One millilitre of the diluted cultures were inoculated into a sterile molten nutrient agar at 45^{o}C and poured into sterile petri dish. Similarly 1ml of the diluted fungal suspension was poured into sterile saboraud dextrose agar plates and the excess sucked up with Pasteur pipette. These were swirled gently and allowed to solidify. Wells were bored on the solidified oculated nutrient agar plates using cork borer number 4. The wells were filled respectively with equal volume of 0.1ml of each extract and spafloxacin standard (5mg/disc) were placed on the agar plate. One hour was allowed for the

extract to diffuse into the agar after which the plates were incubated overnight at 37^0C and 25^0C for fungi and bacteria respectively. At the of incubation period, diameter of inhibition zone was measured and recorded. The extract, fractions and standard antibiotic, were tested in duplicate and minimum zone of inhibition were calculated for each extract and standard antibiotics. (BSAC, 2010).

3.6.3 Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration of the extract was carried out using the broth dilution method.(Sidney *et al.,* 1978; Volk Kobia *et al., 2001*).

Mueller Hinton broth was prepared, 10mls was dispensed into test tubes and the test tubes sterilized at 121^oC for 15mins, the broth was allowed to cool.MC- far-lands turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10mls was dispensed into sterile test tube and the test microbe was inoculated and incubated at 37^oC for 6hrs.Dilution of the test microbe was made in the normal saline until the turbidity marched that of the Mc- farlands scale by visual comparison at this point the test microbe has a concentration of about $1.5x10^8$c/hr. Two- fold serial dilution of the extract in the sterile broth was made to obtain the concentration of 20mg/ml. 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml. Having obtained the different concentrations of the extract in the sterile broth, 0.1ml of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at 37^oC of 24hrs after which the broth was observed for turbidity (growth), the lowest concentration of the extract in the broth, which shows no turbidity was recorded as the MIC.

3.6.4 Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC)

MBC/MFC were carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121^{o}C for 15mins, poured into sterile petri dishes and was allowed to cool. The content of the MIC tubes and the following tubes in the serial dilution was then sub cultured onto the prepared medium, incubation was made at 37^{o}C for 24hrs after which each plate of the medium was observed for colony growth, the MBC/MFC were the plates with lowest concentration of the extract without colony growth.

CHAPTER FOUR

4.0RESULTS

4.1.0 Preliminary phytochemical Screening

The results of preliminary phytochemical screening of the hexane and crude acetone extracts revealed the presence of flavonoids steroids alkaloids Tannins and terpenes (table 4.1).

Table 4.1 phytochemical constituents of *Spermacoce verticillata*

TEST	ACETONE EXTRACT(AE)	HEXANE FRACTION(HF)
Alkaloids	+	-
Carbohydrates	+	-
Flavonoids	+	-
Anthraquinones	+	-
Saponins	-	-
Steroids/triterpenes	+	+
Tannins	+	-
Glycosides	ND	ND

Key + = present and - = not present ND= Not Determine

4.1.2 Results of thin layer chromatography

Thin layer chromatography of the crude acetone extract using Hexane: ethylacetate (10:1) as solvent system yielded 2 distinct spots with several zones/tailing showing different characteristic colours when sprayed with 10% sulphuric acid spraying reagent followed by heating (Table 4.2)

Table4.2 TLC of crude acetone extract using Hexane: Ethyl acetate (10:1)

Spot	R_f Value	Colour in 10%H_2SO_4 acid
1	**0.3**	**Pink**
2	**0.54**	**Green**

Thin layer chromatography of the crude acetone extract using Hexane: Chloroform (8:2) as solvent system yielded 2 distinct spots (Table 4.3)

Table4.3 TLC of crude acetone extract using Hexane: Chloroform (8:2)

Spot	R_fValue	Colour in 10%H_2SO_4
1	**0.36**	**Pink-violet**
2	**0.62**	**Green**

4.2: Results of chromatographic separation

Table 4.4 Eluate obtained from column chromatography of hexane fraction.

Fraction	Eluting solvent	Solvent system	No. of spots
1	Hexane 100%	Hexane:chloroform 8:2	2
2	Hexane:chloroform 95:5%	Hexane:chloroform 8:2	4
3	Hexane:chloroform 90:10%	Hexane:chloroform 8:2	4
4	Hexane:chloroform 85:15%	Hexane:chloroform 8:2	5
5	Hexane:chloroform 80:20%	Hexane:chloroform 8:2	5
6	Hexane:chloroform 75:25%	Hexane:chloroform 8:2	5
7	Hexane:chloroform 70:30%	Hexane:chloroform 8:2	5
8	Hexane:chloroform 65:35%	Hexane:chloroform 8:2	6
9	Hexane:chloroform 60:40%	Hexane:chloroform 8:2	7
10	Hexane:chloroform 55:45%	Hexane:chloroform 8:2	7
11	Hexane:chloroform 50:50%	Hexane:chloroform 8:2	7
12	Hexane:chloroform 30:70%	Hexane:chloroform 8:2	9
13	Hexane:chloroform 20:80%	Hexane:chloroform 8:2	4
14	Hexane:chloroform 10:90%	Hexane:chloroform 8:2	3
15	Ethyl acetate 100%	Hexane:chloroform 8:2	Mixed spots
16	Methanol 100%	Hexane:chloroform 8:2	Stained plate

4.2.1 Isolation of compound S1

Fractions X were pooled together based on their TLC profiles. It was subjected to gel filtration using sephadex in methanol and further purified using PTLC which yielded a single homogeneous spot coded S1 (21.2mg). it was subjected to chemical and spectroscopic analysis to elucidate its chemical structure.

4.2.2 Thin layer chromatographic analysis of S1

PTLC analysis of S1 using hexane ethyl acetate (8:2) revealed single spot with $R_f$value of 0.48

TLC profile of S1

Hexane: ethyl acetate (8:2)



4.2.3 Solubility of S1

S1 was found to be soluble in chloroform.

4.3 Spectroscopy

4.3.1 Results of NMR

^{1}H NMR (ohm ppm, 600 MHz, DMSO-6).

4.3.1.1 Proton magnetic resonance (^{1}H-NMR) of S1 (fig.1)

S1 revealed three regions typical of a steroids nucleus, between 0.5- 2.5ppm, representing over lapping methyl, methylene and methine protons, an oxymethine proton signal around 3.5ppm and olefinic protons at 5.10 & 5.38ppm.

4.3.1.2 Carbon-13-NMR (^{13}C- NMR) (Fig 2)

The carbon NMR spectrum of SI revealed overlapping methyl methylene and methine signals between 11.8ppm - 56.8ppm, an oxymythine carbon signal at 71.9ppm and four olefinic signals respond at 121.7ppm - 140.7ppm, these values are typical of stigmasterol.

The ^{1}H and 13C NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlations. The above spectral data supported the presence of sterol skeleton having a hydroxyl group at C-3 position with two double bonds at C-5/C-6 and C-20/C-21 with six methyl groups. Thus, the structure of S1 was assigned as the known compound stigmasterol. The physical and spectral data are consistent to the reported literature values (Patch *et al.,* 2009) of stigmasterol.

Table 4.5 ^{1}H and ^{13}C NMR Data of S1 compared with those reported in the literature (Pateh *et al., 2009*)

Position	^{13}C (Reference)	^{1}H(Reference)	^{1}H (SI)	^{13}C (S_1)
1	37.3			37.26
2	31.6			31.67
3	71.8	3.52	3.62	71.81
4	42.3			43.31
5	140.8			140.76
6	121.7	5.357	5.31	121.71
7	31.9			31.90
8	31.9			31.90
9	51.2			50.16
10	36.5			36.51
11	21.1			21.21
12	39.7			39.68
13	42.3			42.22
14	56.9			56.87
15	24.4			24.26
16	28.4			28.92
17	56.1			55.96
18	11	0.699s	0.68	12.05
19	21.2	0.01s	1.02d	21.08
20	40.5			40.49
21	21.2	1.02d	1.01s	23.07
22	138.3			138.31
23	129.3			129.28
24	51.2			51.24
25	31.9			29.15
26	21.2	0.795d	0.795d	18.98
27	19	0.846d	0.82	19.40
28	25.5			25.40
29	12.1	0.804d	0.845t	12.25

Proton ^{1}HNMR

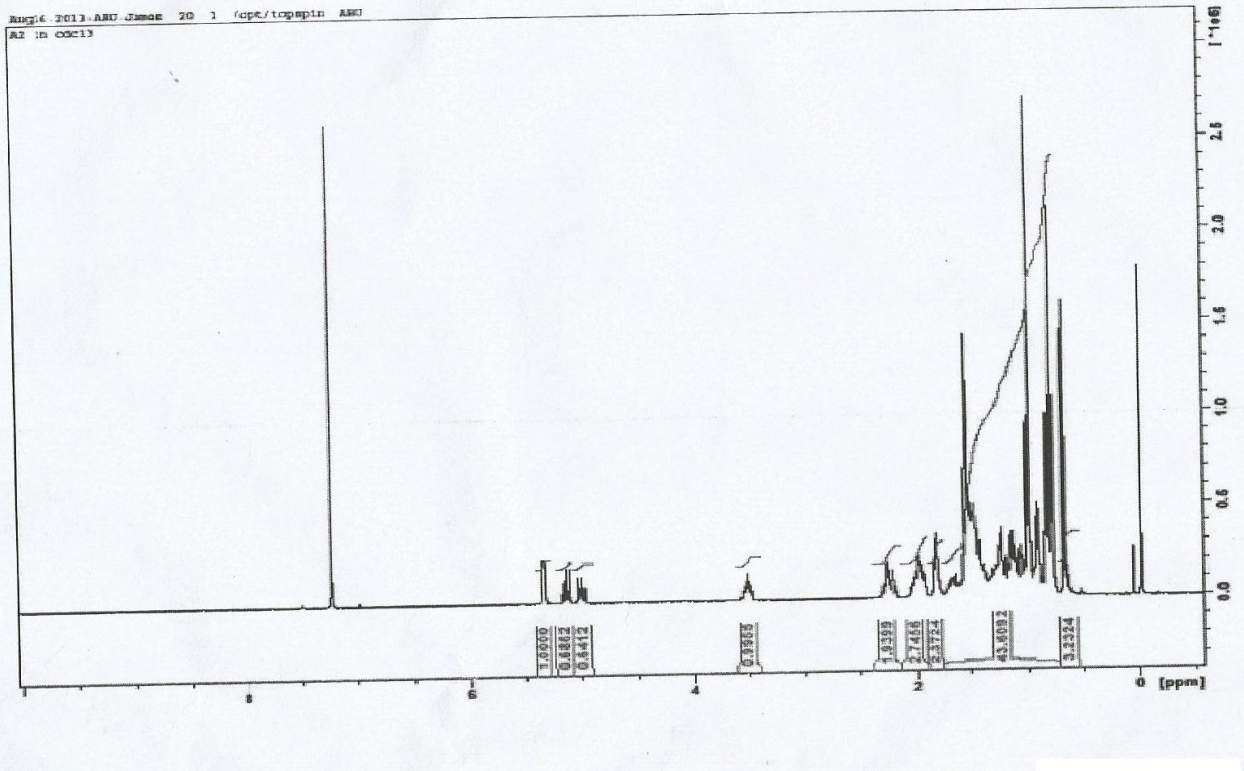


Figure 1 ^{1}H NMR spectrum of S1

Fig 4.1:

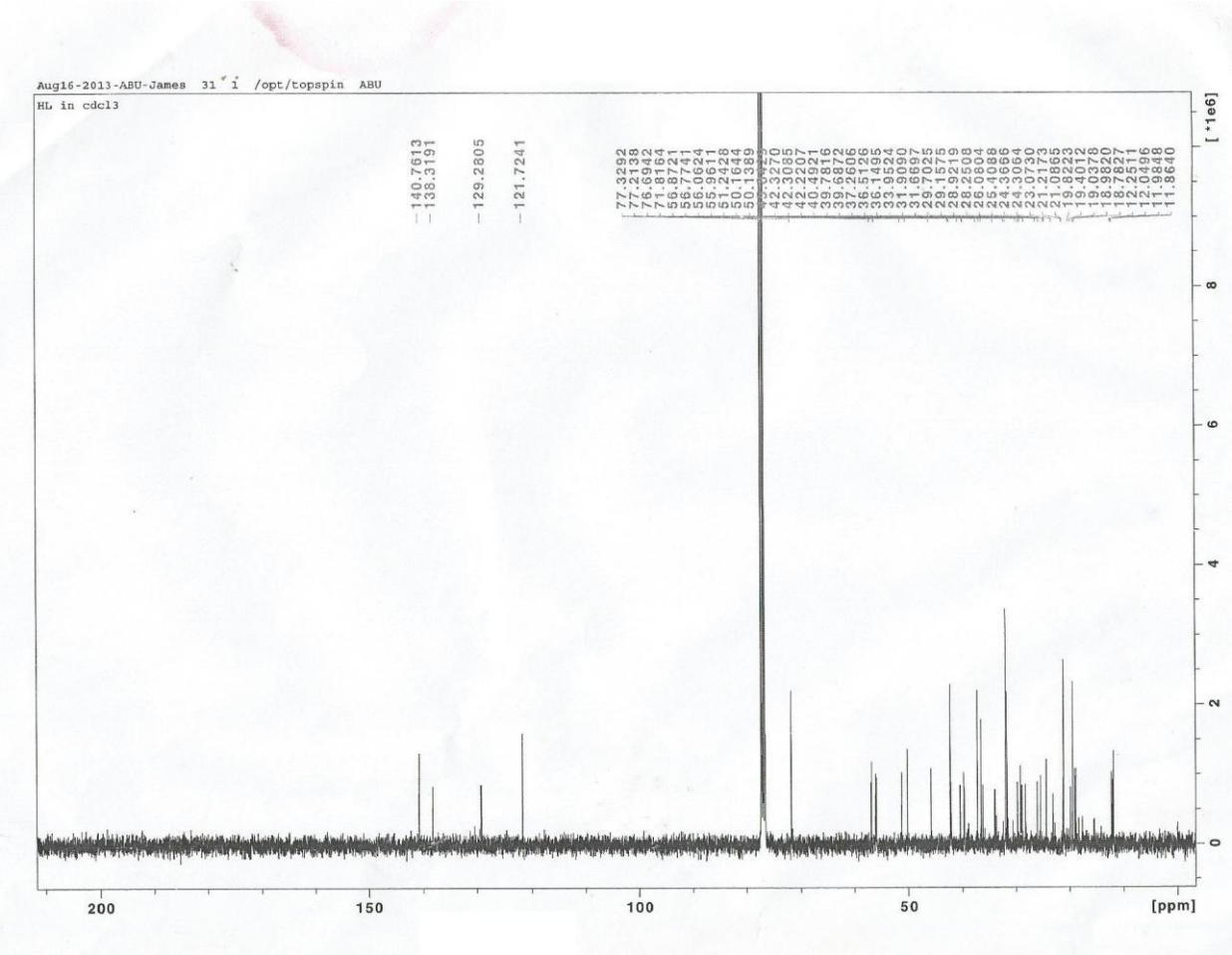

Aug16-2013-ABU-James 31 1 /opt/topspin ABU
HL in cdcl3
140.7613
138.3191
129.2805
121.7241
77.3292
77.2138
76.6942
71.8164
56.8721
56.7741
56.0624
55.9911
55.9218
51.2644
50.1389
42.3270
42.3085
42.2207
40.4021
39.7816
39.6872
37.2906
36.1445
33.9524
31.9090
31.6697
29.7025
29.1575
28.9219
28.2508
26.0004
25.4088
24.3655
24.1730
23.0730
21.2173
21.0865
19.8823
19.4012
19.0378
18.9820
18.7827
12.2511
12.0496
11.8648
11.8640
[*1e6]
200
150
100
50
[ppm]

Fig 4.2

4.3 Result of Antimicrobial activities

The antimicrobial activities of the acetone extract is summarized in (table 4.6) the ecetone extract showed activity against all the test organisms except *Pseudomonas aeruginosa, Salmonella typhi and Candida krusei.* Sparfloxacin, a standard antibiotic at concentration of 5mg/ml showed activity against all organisms with the exception of *Campylobacter sp*. and *candida albicans* and *krusei* which are *fungal*. The crude acetone extract was found to have MIC at 5.00mg/ml and MBC at l0mg/ml.

Table 4.6 Susceptibility Test of the crude extract of *S.vertiscillata* against the test organisms

Test organism	Extract (mg/ml)	Sparfloxacin (mg/ml)	Fluconazole(mg/ml)
S. aureus	S	S	R
S.feacalis	S	S	R
B. subtilis	S	S	R
E. coci	S	S	R
Campylobacter sp	S	R	R
P. aeruginosa	R	S	R
S.dysenteriae	S	S	R
S. typhi	R	S	R
C.albicans	S	R	S
C. krusei	R	R	S

KEY S= Sensitive R= Resistanc

Table 4.7 Zone Of Inhibition of the Extract against the test Microorganism (mm)

Test organism	Extract (mg/ml)	Sparfloxacin (mg/ml)	Fluconazole (mg/ml)
S.aureus	22	38	0
S. feacalis	25	35	0
B. subtilis	30	42	0
E. coci	20	35	0
Campylobacter sp	21	0	0
P. aeruginosa	0	37	0
S. dysenteriae	27	36	0
S. typhi	20	32	0
C.albicans	20	0	35
C. krusei	0	0	37

Table 4.8 Minimum Inhibition Concentration (MIC) of the Extract against the test Organisms

Test organisms	20g/ml	10g/ml	5g/ml	2.5g/ml	1.25g/ml
S. aureus	-	-	ox	+	++
S. feacalis	-	-	ox	+	++
B. subtilis	-	-	-	Ox	+
E. coli	-	-	Ox	+	++
Campylobacter sp	-	-	Ox	+	++
P. aeruginosa	ND	ND	ND	ND	ND
S. dysenteriae	-	-	-	Ox	+
S. typhi	-	-	Ox	+	++
C. albicans	-	-	Ox	+	++
C. krusei	-	-	ox	+	++

Table 4.9 Minimum Bactericidal/Fungicidal Conc. (MBC) Of The Extract Against The Microbes

Test organism	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
S. aureus	Ox	+	++	+++	+++
S.feacacis	-	Ox	+	++	+++
B.subtilis	-	Ox	+	++	+++
E.coci	Ox	+	++	+++	+++
Campylobacter sp	Ox	+	++	+++	+++
P.aeruginosa					
S.dysenteriae	_	Ox	+	++	+++
S. typhii	Ox	+	++	+++	+++
C. albicans	Ox	+	++	+++	+++
C. krrusei	-	-	-	-	-

KEY - = No colony growth, ox = MBc/MFc, + = scanty colonies growth, ++ = moderate colonies growth, +++ = heavy colonies growth.

CHAPTER FIVE

5.0 DISCUSSION

5.1 PHYTOCHEMICAL STUDIES

The result of preliminary phytochemical screening carried out on the crude acetone extract revealed the presence of alkaloids, carbohydrates, flavonoids, steroids/triterpenes, tannins and anthraquinones. These phytochemical constituents were reported to possess biological activities. Tannins is a major constituent of canberry juice, it has for long been used to treat bacterial infection of the bladder (Avorn*el al.*, 1994). While some flavonoids have anti-tumour, anti-bacterial/or anti-fungal property, some are used in domestic veterinary medicine, particularly in the form of ointment for its anti-hepatoxic properties (Trease and Evans 1996). Steroids and triterpenes are classified as active anti- microbial compounds. (Rojas *et. al.*,1992).

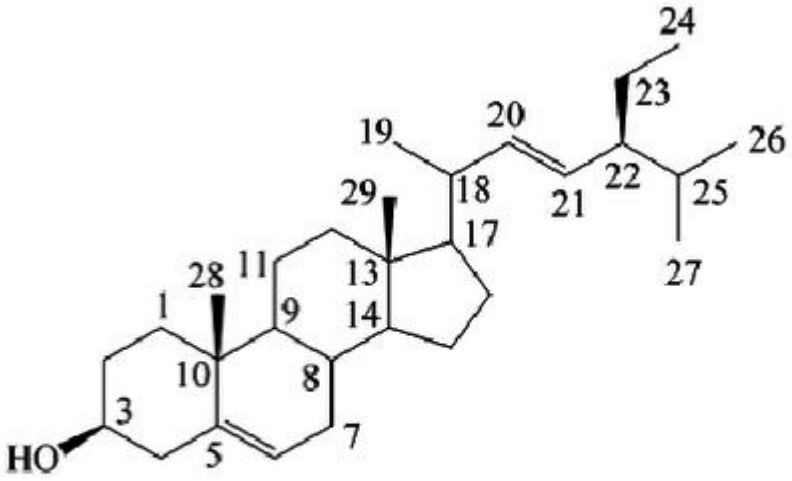
Elucidation of S1

Column chromatography of hexane fraction using silica gel filtration and preparative TLC led to the isolation of S1. The identity of this compound was confirmed by ^{1}HNMR, and ^{13}CNMR. The ^{1}HNMR spectra of SI showed signals between 0.5 - 2.5 ppm representing the overlapping methyl, methylene, and methine protons, and oxymethine proton signal at 3.5 ppm and olefinic proton at 5.38 ppm (Pateh Iet al., 2009).

The ^{13}CNMR spectrum experiments clearly indicated the olefinic carbons at 140.76 (C-5), l21.71(C-6), l38.31(C-22), l29.28(C-23) and hydroxylated carbon around 71.8 (C-3). Further confirming the presence of stigmasterol nucleus in the molecule (Pateh *et al., 2009).*

The comparison of the spectral data with available literature (Pateh *et al.,* 2009) established the structure of S1 as Stigmasterol. (fig. 6)

PROPOSED STRUCTURE



Stigmasterol (fig.6)

5.2 ANTIMICROBIAL ACTIVITY

The crude extracts of the *S.V* exhibited a broad spectrum anti- bacterial activity on several microbial pathogens including reference strains and clinical isolates, the reference strains such as *S. aureus, E. coli, E. feacalis, P. aeruginosa* are often used. The extracts were tested using the agar diffusion assay and the broth micro dilution assay.

Acetone extract showed antimicrobial activity against all the organisms' tested with the exception of *Pseudomonas aeruginosa, Salmonella typhi* and *Candida krusei.* It also showed resistance against all organisms except *Candida albicans* and *Candida krusei.* This showed that the plant is slightly an anti-fungal.

It is important to note that the strong activity of crude acetone extract against *Bacillus subtilis,* S.*feacalis* and *S.dysenteriae* indicates that the plant can be used as a good source of antimicrobial agent, most especially for the treatment of diarrhoea, which is caused by *S.dysenteriae.*

5.3 SUMMARY

Preliminary phytochemical screening of the acetone extract of *Spermacoce verticillata* revealed the presence of flavonoids, steroids/triterpenes, tannins, anthraquinones, alkaloids and carbohydrates.

Extensive phytochemical investigation led to the isolation of stigmast -5, 22-dien -3β-ol.

Biological studies on the acetone extract showed that the extract exhibited antimicrobial activity against *Staphylococus aureus, Streptococus feacalis, and Bacillus subtilis, E. coli, Campylobacter sp., Shigella dysenteriae, Salmonella typhii* and *Candida albicans.* The extract showed resistance to *Candida krusei* because it has slightly or nor antifungal activity.

5.4 CONCLUSION

Based on the findings in this work, it can be concluded that the use of *Spermacoce vertiscillata* in the treatment of skin and diarrhoea related to bacterial infections has scientific basis. To the best of our search, this is the first time these activities are being reported. The antimicrobial activity observed may be linked to stigmasterol isolated and other steroids/triterpenes present in the extract.

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