PHYTOCHEMICAL AND ANTIMICROBIAL SCREENNING OF THE WHOLE PLANT OF *AESCHYNOMENE SCHIMPERI* Hochst.ex A. Rich.

BY

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FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY, ZARIA

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DECLARATION

I declare that the work in this Thesis entitled "PHYTOCHEMICAL AND ANTIMICROBIAL SCREENNING OF *AESCHYNOMENE SCHIMPERI* Hochst. ex A. Rich" has been carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Project has been previously presented for another degree or diploma at this or any other Institution.

Zakari Salihu ADAMU	_____	_____
Name of student	Signature	Date

CERTIFICATION

This thesis entitled, "PHYTOCHEMICAL AND ANTIMICROBIAL SCREENNING OF *AESCHYNOMENE SCHIMPERI* Hochst. ex A.Rich" by Zakari, Salihu ADAMU meets the regulations governing the award of the degree of Masters of Science in Organic Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Aeschynomene schimperi is a medicinal plant and used as animal food which belongs to the Fabaceae family. The whole plant of *Aeschynomene schimperi* was first extracted exhaustively with petroleum ether (60-80 °C) using a soxhlet extractor. The procedure was sequentially repeated using chloroform, ethyl acetate and methanol. The phytochemical screening revealed the presence of carbohydrates, reducing sugar, glycosides, cardiac glycosides, Triterpenes, flavanoids, steroids, tannins, saponins (hydrolysable), glycosides, alkaloids and combined anthraquinone in the plant. The antimicrobial sensitivity test of the extracts were carried out using nine pathogenic microorganisms; *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa,* Shigella dysenteriae, *Aspergillus nigre, Candida krusei* and *Candida albicans*. *Streptococcus pyogenes, Staphylococcus aureus,* Shigella dysenteriae and *Candida krusei* were sensitive to all the plant extracts while *Proteus mirabilis, Pseudomonas aeruginosa* and *Aspergillus nigre* were resistant to all the plant extracts. *Escherichia coli* and *Candida albicans* were resistant to petroleum ether extract. The petroleum ether extract showed diameters of zones of inhibition of 12-17mm against four microorganisms. Chloroform extract showed diameters of zones of inhibition of 20-27mm against six microorganisms. Ethyl acetate extract showed diameters of zones of inhibition of 20-23mm against the same organisms as in chloroform extract. Methanolic extract showed diameters of zones of inhibition of 17-21mm against the same organisms as in chloroform and ethyl acetate extracts. Chloroform extract had the highest antimicrobial activity of the four crude extracts recording 27mm in diameters of zones of inhibition in Bacteria. Petroleum ether extract recorded the lowest inhibition zone (12mm in diameter). *Candida krusei. Proteus mirabilis, Pseudomonas*

aeruginosa and Aspergillus *nigre* could not respond *at* all to all the extracts. In the petroleum extract, the Minimum Inhibitory Concentration (MIC) was **5 mg/ml**. In the chlororm extract, the MIC was **2.5 mg/ml**, in the ethyl acetate extract, **2.5 mg/ml** was the MIC, while in the methanolic extract, the MIC was **5 mg/ml** for *Candida albicans* and *Candida krusei* and **2.5 mg/ml** for the remaining microorganisms that responded. In the fungi that responded to the test, the MFC was **10 mg/ml**, in the methanolic extract, the MBC was **10 mg/ml** while in the remaining extracts ,there were variations in the MBC of the extracts against the bacteria. Isolation of the compound was from chloroform extract using series of chromatographic processes. By comparison of the spectral data of the isolated compound with those reported in the literature, Stigmast-5-en-3β-ol ($C_{29}H_{50}O$) also known as beta-sitosterol was proposed to be the isolated compound which is one of the possible bioactive constituents of the plant responsible for various pharmacological activities of the plant.

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LIST OF ABBREVIATION

FLO:	Flouconazole, which is an antifungal drug in the market
MBC:	Minimum Bactericidal Concentrations
MFC:	Minimum Fungicidal Concentrations
MIC:	Minimum Inhibitory Concentration
NMR:	Nuclear Magnetic Resonance
^{1}HNMR:	Proton Nuclear Magnetic Resonance
^{13}CNMR:	Carbon-13 Nuclear Magnetic Resonance
SPA:	Sparfloxacin, which is an antibacterial drug in the market
ZB:	Zak-Bal

CHAPTER ONE

1.0 INTRODUCTION

1.1 PHYTOCHEMICALS

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. The term is generally used to refer to those chemicals that may have biological significance, for example antioxidants, but are not established as essential nutrient. Scientists estimate that there may be as many as ten thousand different phytochemicals having the potentials to affect diseases such as cancer, stroke or other ailments (Neuwinger, 2000). Without specific knowledge of their cellular actions or mechanisms, phytochemicals have been considered as drugs for millennia. For example, Hippocrates may have prescribed willow tree leaves to abate fever. Salicin, having anti-inflammatory and pain-relieving properties, was originally extracted from the bark of the white willow tree and later, synthetically produced, became the staple over-the-counter drug aspirin(Brown and Arthur, 2001).Sometimes they can be harmful and sometimes they can be very helpful. There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of cancer, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects (Neuwinger, 2000). Specific phytochemicals, such as fermentable dietary, are allowed limited health claims by the US Food and Drug Administration. An important cancer drug, Taxol (paclitaxel), is a phytochemical initially extracted and purified from the Pacific yew tree(Brown and Arthur, 2001).Some phytochemicals with physiological properties may be elements rather than complex organic molecules. For example, selenium,

which is abundant in many fruits and vegetables, is involved in major metabolic pathways, including thyroid hormone metabolism and immune function. Particularly, it is an essential nutrient and cofactor for the enzymatic synthesis of glutathione, an endogenous antioxidant (Brown and Arthur, 2001).

1.2 SOME USES OF PHYTOCHEMICALS

Phytochemicals exist in various foods and in different concentrations. Broccoli, pumpkin, spinach, squash, yams, and sweet potatoes all contain important carotenoids, an important phytochemical. Flavonoids can be found in many different natural foods, including: berries, soybeans, carrots, tomatoes, cabbage, parsley, and cucumber. Legumes, peas, and beans contain healthy isoflavones. Indole, another phytochemical, is available in cabbage, bokchoy, turnips, brussel's sprouts, kale, and cauliflower. Flaxseeds and walnuts contain Lignans, as well as many other kinds of seeds and nuts. Fatty acids, or lipids, are seen in flaxseeds, hemp oil and hemp seeds, and walnuts (Damery *et al.*, 2011).Eggplant, hemp seeds and oil, peppers, squash, cabbage, cucumber, tomatoes, broccoli, cabbage, and soybeans, contain plant sterols. There are tons of nutrients that are contained by the entire phytochemical family, and these are perfect for any diet. Health problems and diseases can be fought in many ways through phytochemicals, since they readily help your body in several ways. When carcinogens try to enter into the cell walls, phytochemicals help block them. Malignant changes that have already started in the cells by carcinogens are battled. Phytochemicals seem to increase the benefits of the many protective enzymes that are eaten in the diet, by raising enzyme activity. The damage that the body can receive from free radicals is scavenged by phytochemicals that mix with different vitamins to

boost antioxidants (Chantel, 2009). This is done before damage can be caused to the body. Bad cholesterol levels have been shown to be reduced as well. By maintaining a diet that is rich in a variety of vegetables, fruits, and nuts, the benefits of the phytochemicals and vitamins go far in helping one reach maximum health. Knowing the names and benefits of the varied range of existing phytochemicals is not important, but understanding how eating a diet rich in these foods can help your body, is of great importance. One food source that is often overlooked that contains essential phytochemicals is hemp seeds which are abundant in essential fatty acid (Chantel, 2009). There are many phytochemicals and each works differently. These are some possible actions of some of them.

Antioxidant - Most Phytochemicals have antioxidant activities and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Some phytochemicals with antioxidant activities are: allyl sulfides (onions, leeks, and garlic), carotenoids (fruits, carrots), flavanoids (fruits, vegetables), polyphones (tea, grapes) (Dragland, 2003).

Hormonal action - Isoflavones found in soy bean plant, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis (Neuwinger, 2000).

Stimulation of enzymes - Indoles which are found in cabbages, stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals which interfere with enzymes are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries) (Chantel, 2009).

Interference with DNA replication - Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens (Dragland, 2003).

Anti-bacterial effect - The phytochemical, allicin from garlic, has anti-bacterial properties (Brown and Arthur, 2001).

Physical action - Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health (Damery *et al.,* 2011).

1.3 HERBAL MEDICATION

Herbal medicine is also called botanical medicine or phytomedicine which refers to using plants' parts for medicinal purposes (Chantel, 2009). Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing diseases (Izzo and Ernst, 2009). Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes (Abeloff, 2008*).* In the early 19th century, when chemical

analysis first became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plant compounds and, over time, the use of herbal medicines declined in favor of drugs. Almost one fourth of pharmaceutical drugs are derived from botanical sources. Recently, the World Health Organization (2009) estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care (Damery *et al.,* 2011). In Germany, about 600 - 700 plant based medicines are available and are prescribed by some 70% of German physicians. In the past 20 years in the United States, public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicinal use (Abeloff, 2008). Herbal medicine is used to treat many conditions, such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome and cancer, among others. Herbal supplements are best taken under the guidance of a trained health care provider. For example, one study found that 90% of arthritic patients use alternative therapies, such as herbal medicine (Damery *et al.*, 2011).

1.4 AIM AND OBJECTIVES OF THE STUDY

The aim of the research is to screen the plant-*Aeschynomene schimperi* for phytochemical and antimicrobial activities.

The objective of the study is to carry out the structural elucidation of possible isolate(s) from the plant.

1.5 JUSTIFICATION FOR RESEARCH

The frequently mentioned effects of the herbs in the Fabaceae family from the traditional perspective are: dispelling heat, cleaning toxins, resolving swellings; removing water accumulation; opening meridians, vitalizing blood, controlling pain; controlling bleeding; and resolving phlegm accumulation, cough, and lung disorders (Hu, 1980). The bark, leaves, flowers and fruits of *Aeschynomene schimperi* are used in traditional medicine to treat multifactorial diseases like leprosy, gout, acute rheumatic fever, sores, boils, bloody diarrhea, and vaginitis (Joshi, 2000). A number of the herbs in this family have traditional indications in common. Hence there is need for a scientific investigation of the plant for justification (Hu, 1980).

1.6 SCOPE OF THE RESEARCH WORK

This research work would cover the following:

i. Phytochemical screening

ii. Antimicrobial screening

iii. Isolation of active component(s)

iv. Structural elucidation of the active component(s)

1.7 LIMITATION

This research work would be limited to structural elucidation of active component(s) which may also be dependent on the availability of spectroscopic instrument.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 FABACEAE

The **Fabaceae** or **Leguminoseae**, commonly known as the **legume**, **pea** or **bean family**, is a large and economically important family of flowering plants. The group is the third-largest land plant family, behind only the Orchidaceae and Asteraceae, with 730 genera and over 19,400 species (Stevens, 2008).Among the largest genera are Astragalus (over 2,400 species), Acacia (over 950 species), Indigofera (around 700 species), Crotalaria (around 700 species), and Mimosa (around 500 species) (Stevens, 2008).Plants of this family are found throughout the world, growing in many different environments and climates. A good number of them are important agricultural and food plants, including *Glycinemax* (soybean), *Phaseolus* (beans), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Ceratonia siliqua* (carob), and *Glycyrrhiza glabra* (licorice) (Stevens, 2008).

2.2 ETYMOLOGY OF FABACEAE

The name 'Fabaceae' comes from the defunct genus *Faba*, now included in *Vicia*. The term "faba" comes from Latin, and appears to simply mean "bean". Leguminoseae is an older name still considered valid and refers to the fruit of these plants, which are called legumes (Ling, 1995).

2.3 DISTRIBUTION

The Fabaceae have an essentially worldwide distribution, being found everywhere except Antarctica and the high arctic (Stevens, 2008).

2.4 TAXONOMY

Worldwide, there are a total of 790 genera and 17,600 species of the legumes and of these there are 163 genera and 1,252 species that are used as sources of medicinal plants in China. The legumes include trees, shrubs and small herbs. Among the sources of Oriental herbal medicines the Leguminoseae is the fourth largest family in terms of numbers of medicinal genera and species that are used, following the Gramineae (grasses, grains), Compositae (daisies, dandelions), and Orchidacea (orchids). However, in terms of total amount of medicinal materials collected and used the Leguminoseae rates at or near the top (Ling, 1995).

2.5 DESCRIPTION

Fabaceae range in habit from giant trees (like Koompassia excelsa) to small annual herbs, with the majority being herbaceous perennials. Plants have indeterminate inflorescences, which are sometimes reduced to a single flower. The flowers have a short hypanthium and a single carpel with a short gynophore, and after fertilization produce fruits that are legumes (Stevens, 2008).

2.6 AGRICULTURAL & ECONOMICAL IMPORTANCE OF LEGUMES

A large number of legume species are cultivated worldwide as ornamentals (in gardens, as shade trees), living fences and firebreaks, soil binders, green manures, fodder for livestock, forage for honey bees, food for humans, in agro forestry and reforestation (for nitrogen

fixation), pulp for paper production, fuelwoods, timber, sources of chemicals (e.g., dyes, tannins), oils (industrial, food, aromatherapy) and medicines (Graham and Vance, 2003).

2.7 MEDICINAL VALUE OF LEGUME

The ability of the herbs to clear heat and clean toxin may be due, in large part, to antiseptic and antiviral activity of the flavonoids, saponins, and alkaloids generated by this family of plants. The swellings that are resolved may be those that result from internal infections; thus, this quality may also represent anti-infection activity. Anticancer properties have also been noted, especially with the *Sophora* species and the folk remedy trifolium; so swellings that represent tumors might also be affected by appropriate selection and application of the herbs (Liu and Xiao, 1993).The blood-vitalizing activity of the herbs has been attributed to the flavonoid components (Liu and Xiao, 1993).

2.7.1 HIV INHIBITION

A study at the Chinese University of Hong Kong tested a variety of legumes, including French beans, cowpeas, field beans, mung beans, peanuts and kidney beans. They were tested for the ability to inhibit the HIV (human immunodeficiency virus) reverse transcriptase, protease, or integrase, which are enzymes needed for the HIV virus to survive (Liu and Xiao,1993).The results varied across species, but most showed some form of inhibition. In the cowpeas, two different anti-fungal proteins were detected. The alpha protein inhibited the HIV reverse transcriptase, while the beta proteins exhibited high potency in inhibiting HIV protease and integrase. The anti-fungal protein found in peanuts had high potency inhibiting HIV integrase, but with weak inhibiting action against transcriptase and protease. The other legumes had

intermediate-to-low inhibition actions. The study showed that most legumes have the ability to interfere with activities of enzymes that are essential for the replication and survival of HIV (Liu and Xiao, 1993).

2.8 NUTRITIONAL VALUES

Legumes are among the best protein sources in the plant kingdom. The low concentrations of the amino acid, methionine, in legumes may be compensated for simply by eating more of them. Since legumes are relatively cheap compared to meat, eating more legumes may be an alternative to meat for some. According to the protein combining theory, legumes should be combined with another protein source such as a grain in the same meal, to balance out the amino acid levels (Hu, 1980). A variety of protein sources is considered healthy, but they do not have to be consumed at the same meal. In any case, vegetarian cultures often serve legumes along with grains, which are low in the essential amino acid, lysine, creating a more complete protein than either the beans or the grains on their own. Legumes contain relatively low quantities of the essential amino acid methionine, as compared to whole eggs, dairy products, or meat. This means that a smaller proportion of the plant proteins, compared to proteins from eggs or meat, may be used for the synthesis of protein in humans, unless other higher methionine sources are consumed which are complementary in regard to their amino acid profile (Vogel, 2003)

2.9 THE GENUS *AESCHYNOMENE*

Aeschynomene is a genus of flowering plants in the family Fabaceae. They are known commonly as joint vetches. These legumes are most common in warm regions and many species are aquatic (Stevens, 2008).

2.9.1 Examples of Some Species of the Genus

These include: *Aeschynomene abyssinica* (A. Rich.) Vatke, *Aeschynomene aculeata* Schreb., *Aeschynomene acutangula* Welw. ex Baker, *Aeschynomene aegyptiaca* (Pers.) Steud., *Aeschynomene afraspera* J. Léonard *Aeschynomene americana* L., *Aeschynomene angolensis* Rossberg, *Aeschynomene aphylla* Wild, *Aeschynomene arbuscula* Baker f., *Aeschynomene aspera*auct., *Aeschynomene batekensis* Troch. &Koechlin , *Aeschynomene baumii* Harms, *Aeschynomene baumii* Harms var. baumii, *Aeschynomene baumii*var. kassneri (Harms) Verdc., *Aeschynomene bella* Harms, *Aeschynomene benguellensis* Torre, *Aeschynomene bispinosa*Jacq., *Aeschynomene bracteosa* Baker, *Aeschynomene fluminensis*, *Aeschynomene grandiflora*, *Aeschynomene indica*, *Aeschynomene schimperi* Hochst. ex A. Rich. *Aeschynomene uniflora* E. Mey., *Aeschynomene uniflora* E. Mey. var. grandiflora, *Aeschynomene grandiflora* var. grandiflora Verdc., *Aeschynomene upembensis* J.Léonard, *Aeschynomene venulosa* var. grandis Verdc., *Aeschynomene venulosa* Verdc., *Aeschynomene venulosa* Verdc.var. venulosa, *Aeschynomene walteri* Harms, *Aeschynomene wittei* Baker f., *Aeschynomene youngii* Baker f., *Aeschynomene zigzag* De Wild, etc. (Flore and Jean, 1999).

2.9.2 The specie -*Aeschynomene schimperi*

Aeschynomene schimperi is a shrubby aromatic herb found in swampy places, stream banks, lakesides and rock pools often actually standing in water. It is geographically distributed

throughout Africa and in Western Indian Ocean (Neuwinger, 2000). It is a traditional medicine used to treat multifactorial diseases like leprosy, gout, acute rheumatic fever, sores, boils, bloody diarrhea, and vaginitis (Joshi, 2000).

2.9.3 Reported Information on Some Species of the Genus

2.9.3.1 *Aeschynomene grandiflora*

Sesbania grandiflora (S. grandiflora) belonging to the family of Fabaceae is used traditionally for the treatment of broad spectrum diseases in India. S. grandiflora syn. *Aeschynomene grandiflora* commonly known as sesbania and agathi (Tamil) in ayurvedic and indigenous Indian system of medicine belongs to the family Fabaceae. The bark, leaves, gums, flowers and fruits were used to treat multifactorial diseases like leprosy, gout (Joshi, 2000), rheumatism, cancer, liver disorders (Sreelatha *et al.,* 200l), inflammation, ocular diseases (Patil *et al.,* 2011), epilepsy and anemia (Kasture *et al.*,2002). It possesses anti-inflammatory, analgesic, anti-pyretic (Wagh *et al.,* 2009), hypolipidemic (Ramesh *et al*., 2008).Antibacterial (Pimporn *et al*., 2011), free radical scavenging (Gowri and Vansanta, 2010), anti-ulcer (Serti *et al.,* 2001), anti urolithiatic (Doddola *et al.,* 2008), hepatoprotective (Pari and Uma, 2003) and chemo preventive (Laladhas, 2009) activities. The leaves of this plant are the richest source of amino acids, minerals and vitamins, like vitamin A, vitamin C, thiamine, riboflavin and nicotinic acid (Ramesh *et al.,* 2008). It also possesses active biomolecules, and can be used to treat various ailments (Karthiga *et al.,* 2010). The famous ancient text of ayurveda such as Dravyaguna has mentioned the medicinal value of S. grandiflora. All parts of this plant including preparations from the plant parts are used as medicine in south eastern Asia and India (Pimporn *et al*., 2011).

2.9.3.2 *Aeschynomene fluminensis*

Phytochemical investigation of *Aeschynomene fluminensis* leaves and branches (Marlene, 2012). led to isolation of the flavonoid glycosides kaempferol 3,7-di-*O*-α-L-rhamnopyranoside, kaempferol 7-*O*-α-L-rhamnopyranoside, kaempferol 3-*O*-apiofuranosil-7-*O*-rhamnopyranoside, quercitin 3-*O*-α-L-rhamnopyranoside, quercitin 3-*O*-arabinofuranoside, 8-β-D-glucopyranosy l4',5,7-trihydroxyflavanone, the isoflavonoid 4',7-di-hydroxy-isoflavone, the dimer epicatechin-(2β→7, 4β→8)- epicatechin, the polyol 3-*O*-methyl-*chiro*-inositol and two steroids in sitosterol and stigmasterol mixture. These compounds were identified by NMR ^{1}H and ^{13}C and compared with literature data. Anti-inflammatory activity of the crude methanolic extract and its fractions was evaluated (Marlene, 2012).

2.9.3.3 *Aeschynomene indica*

Aeschynomene indica is a swampy medicinal plant used to treat kidney stones and urinary disorders by the Chenchu tribes and the local herbalists. All plant parts; Leaf, Flower and Fruits were screened out for their secondary metabolites in the four selected solvents and observed the presence of alkaloids, flavanoids, phenols, steroids and glycosides. Qualitative analysis of flavonoids (6 identified and 1 unidentified), phenols (19 identified and 5 unidentified),anthocyanidins (3) was carried out. Isochlorogenic acid, coumarin, p-hydroxy benzoic acid as the major phenolic compounds; kaempferol and apigenin as major flavonoids; delphinidin, malvidin and peonidin as the major anthocynadins. Antimicrobial activity of the leaf cold water, hot water, methanol and alcohol extracts are more efficient on the selected four pathogens of gram positive (*Bacillus subtilis, Staphylococcus aureus*) and gram negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria than the control drugs Ampicillin and

Gentamycin with an average inhibitory zone of 20 mm to 41 mm at 10 mg/ml concentration of the drug. Minimum Inhibitory Concentrations were observed between 0.55- 0.75 mg/ml equal to that of the control drugs. Anti-inflammatory activity of the crude methanolic extract and its fractions was evaluated. Aqueous extracts are rich in containing more number of bioactive compounds in all parts (Leaves, Flowers and Fruits). The main constituents are alkaloids, flavonoids, phenols, saponins and tannins. *Aeschynomene indica* was found to contain the flavonoid, reynoutrin, and amino acid potassium aeschynomate (Aruna *et al.*, 2012).

2.9.3.4 *Aeschynomene aspera*

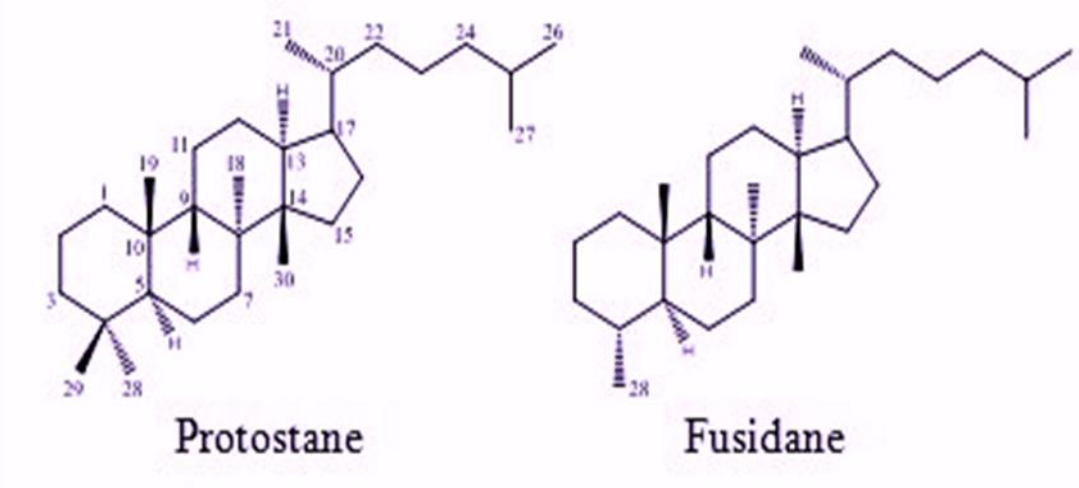
Aeschynomene aspera is a shrub that grows up to 150 centimeter found in the eastern and the southern regions of India. The species of *Aeschynomene* has been traditionally curative agents in colic, jaundice and poisoning (Nadkarni, 2003).The hepatoprotective activity of benzene and alcoholic extracts of root of *Aeschynomene aspera* was investigated in rats for carbon tetrachloride induced hepatotoxicity. LD_{50} values for both extracts determined. The extracts did not produce any mortality even at 5000 mg/kg while LD_{50} of benzene and alcoholic extracts was found to be 100 mg/kg and 200 mg/kg. Hepatotoxicity was induced in rats by intraperitoneal injection of carbon tetrachloride (1 ml/kg /day diluted with olive oil (1:1) for 3 days). Benzene and alcoholic extracts of roots were administered to the experimental rats (100 and 200 mg/kg/d p.o for 3d). The Hepatoprotective effect of these extracts was evaluated by liver function biochemical parameters (total bilirubin, serum protein, alanine amino tranasaminase, aspartate amino transaminase and alkaline phosphatase activities) and histopathological studies of liver. In benzene and alcoholic extracts–treated animals, the toxicity effect of carbon tetrachloride was controlled significantly by restoration of the levels of serum

bilirubin and enzymes as compared to the normal and standard drug silymarin - treated groups. Histology of liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration which further evidence the hepatoprotective activity (Nadkarni, 2003).

2.10 PHYTOCHEMICAL AND PHARMACOLOGICAL IMPORTANCE OF SOME ISOLATED COMPOUNDS FROMTHE FABACEAE (LEGUMES) FAMILY

2.10.1 Protostane and fusidane

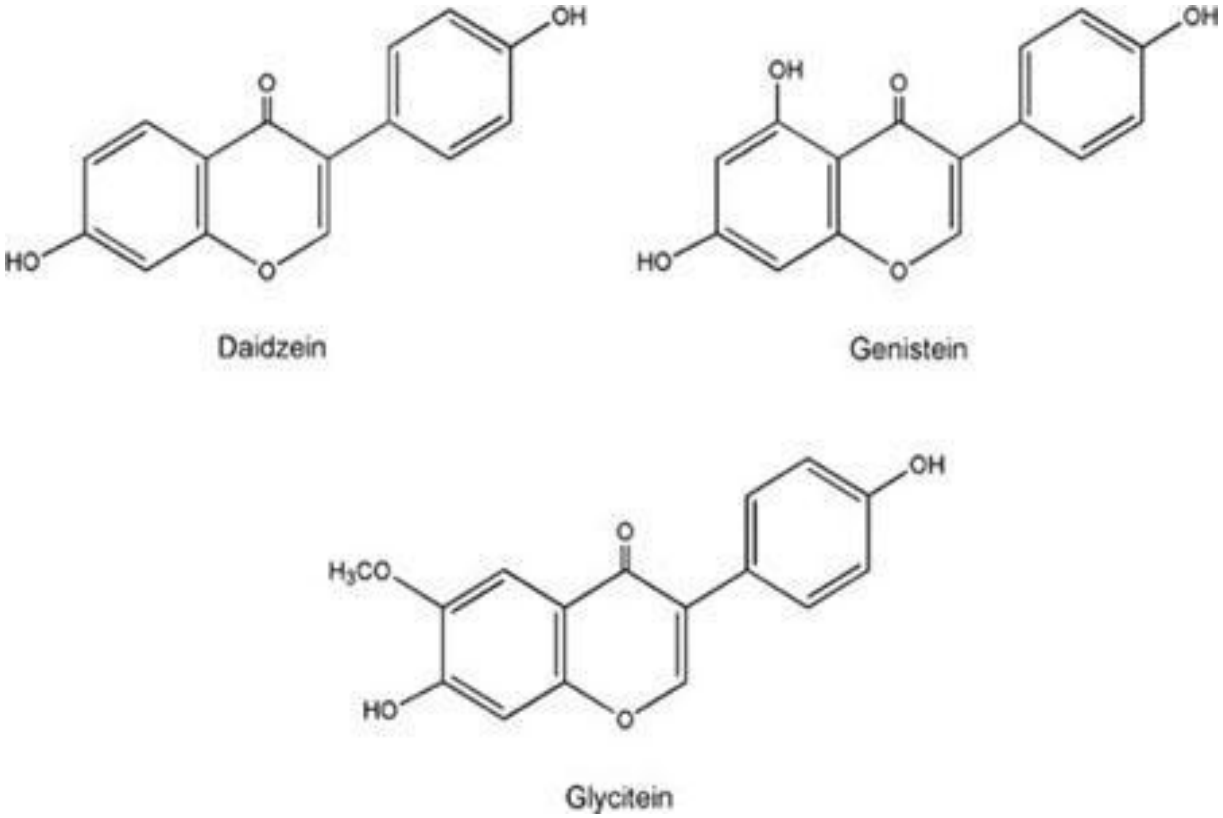
Legumes and the polyphenolic compounds present in the legumes have gained a lot of interest due to their beneficial health implications (Ming *et al.*, 2013). Dietary polyphenolic compounds, especially flavanoids, exert antioxidant properties and are potent inhibitors of Xanthine oxidase (XO) activity. XO is the main contributor of free radicals during exercise but it is also involved in pathogenesis of several diseases such as vascular disorders, cancer and gout. In order to discover new natural, dietary XO inhibitors, some polyphenolic fractions and pure compounds isolated from two legume plant extracts were tested for their effects on XO activity. The fractions isolated from both *Vicia faba* and *Lotus edulis* plant extracts were potent inhibitors of XO. All the pure polyphenolic compounds inhibited XO. These findings indicate that flavanoids' isolates from legume plant extracts (Structure 1) are novel, natural XO inhibitors. Their mode of action is under investigation in order to examine their potential in drug design for diseases related to overwhelming XO action (Ming *et al.,* 2013).



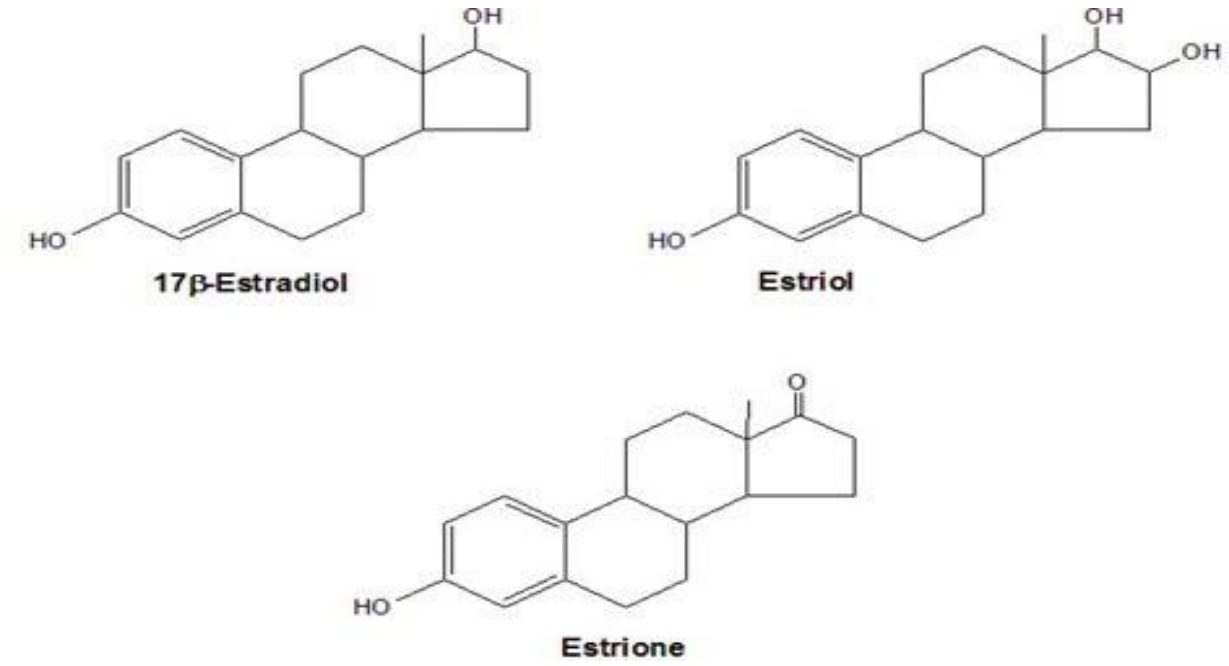
Structure1: Structures of some Flavonoids from Legumes

2.10.2 Isoflavones

Isoflavones are polyphenolic compounds that are capable of exerting estrogen-like effects. For this reason, they are classified as phytoestrogens (Structure 3)-plant-derived compounds with estrogenic activity (Lampe, 2003). Legumes, particularly soybeans, are the richest sources of isoflavones in the human diet. In soybeans, isoflavones are present as glycosides (bound to a sugar molecule). Fermentation or digestion of soybeans or soy products results in the release of the sugar molecule from the isoflavone glycoside, leaving an isoflavone aglycone (Structure 2). Soy isoflavone glycosides are called genistin, daidzin, and glycitin, while the aglycones are called genistein, daidzein, and glycitein (Structure 2) (Lampe, 2003).



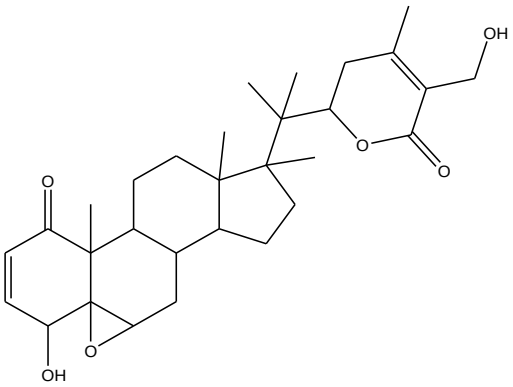
Structure 2: Structures of some Isoflavone aglycone (from soybeans)



Structure 3: Structures of some Phytoestrogens

2.10.3 Steroidal Lactone

Steroidal Lactone (Structure 4) was isolated from *Aeschynomene elongata*, an Ancient plant known for Novel Medicine (Mohammad *et al.*, 2009)

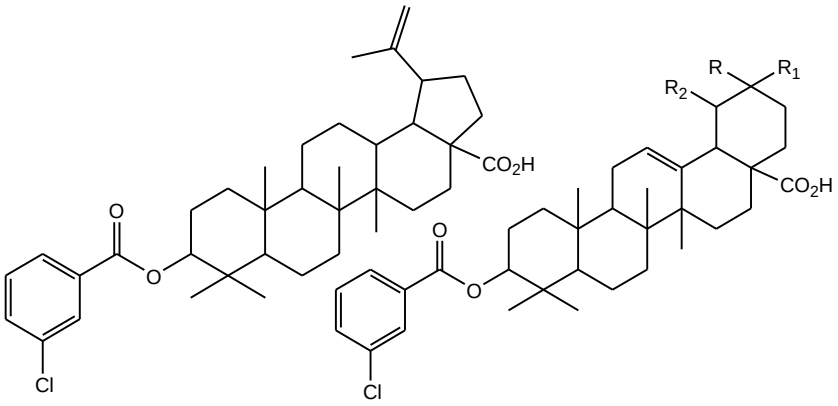


Withaferin A

Structure 4: Structure of Steroidal Lactone (Withaferin A)

2.10.4 Betulinic and Ursolic acids

Betulinic and Ursolic acids (Structure 5) isolated from the aerial parts of Aeschynomene erubescens (Fabaceae) were subjected to different esterification reactions, yielding 12 C-3 position ester derivatives. All compounds were identified using spectroscopic techniques, such as IR, ^{1}H-NMR and MS. The acids and their derivatives were further investigated and were found to possess antioxidant, *Artemia salina* lethality and antimicrobial activity (Brazil, 2012) Betulinic acid is a naturally occurring pentacyclic triterpenoid which has antiretroviral, antimalarial, and anti-inflammatory properties, as well as a more recently discovered potential as an anticancer agent, by inhibition of topoisomerase (Chowdhury *et al.*, 2002). Ursolic acid is a pentacyclic triterpene acid, used in cosmetics that is also capable of inhibiting various cancer cell types by inhibiting the STAT3 (Signal transducer and activator of transcription 3, is a factor which in humans is encoded by the *STAT3*) activation pathway (Shishodia *et al*., 2013.)

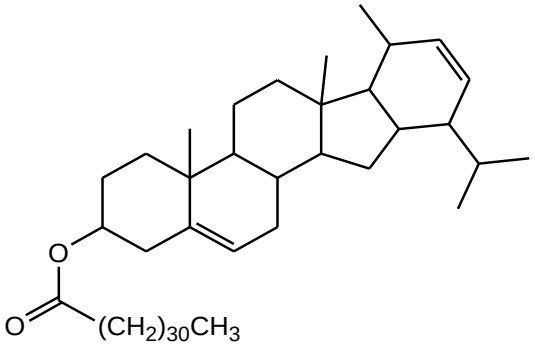


Betulinic acid Ursolic acid

Structure 5: Structures of Betulinic and Ursolic acids

2.10.5 Stigmasterol tritriacontanate

Aeschynomene indica Linn is one of the most important crude drugs in traditional Chinese medicine (TCM), distributing in Southwest, central South, East China, North China. It was used to treat cold, fever, urinary tract infection, dysuria, hematuria, edema, diarrhea, cholecystitis, night blindness, cataracts, eczema, itching and other skin diseases. Isolation and structure elucidation of one new compound, named stigmasterol tritriacontanate (Structure 6), was carried out (Chen *et al*., 2011).



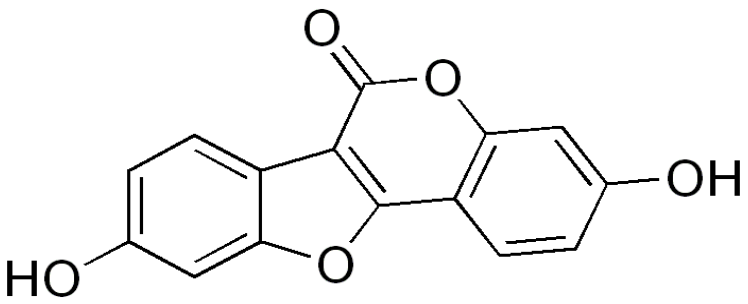
Stigmasterol tritriacontanate

Structure 6: Structure of stigmasterol tritriacontanate

2.10.6 Coumestrol

Coumestrol (Structure 7) is a natural organic compound in the class of phytochemicals known as Coumestans. It has garnered research interest because of its estrogenic activity and its prevalence in some foods, such as soybeans. Coumestrol was first identified by E. M. Bickoff in 1957 (Bickoof *et al*., 1957). It has since been found in a variety of legumes, soybeans, brussels, sprouts, and spinach. Clover and soybeans have the highest concentrations (Amr and Michael,

2007). Coumestrol is a phytoestrogen, mimicking the biological activity of estrogens. The chemical shape of coumestrol orients its two hydroxy groups in the same position as the two hydroxy groups in estradiol, allowing it to inhibit the activity of aromatase and hydroxysteroid dehydrogenase (Blomquist *et al*., 2005). These enzymes are involved in the biosynthesis of steroid hormones, and inhibition of these enzymes results in the modulation of hormone production (Amr and Michael, 2007).

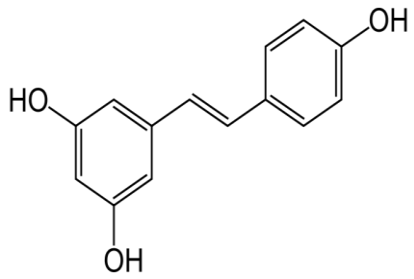


Structure 7: Structure of Coumestrol

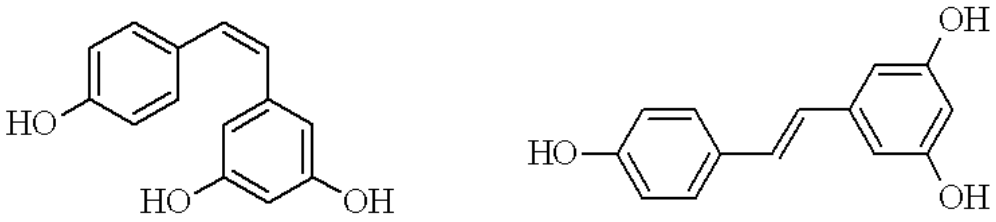
2.10.7 Resveratrol

The anti-inflammatory effects of resveratrols have been demonstrated in several animal model studies. In a rat model of carrageenan-induced paw edema, resveratrols inhibited both acute and chronic phases of the inflammatory process (Gentilli *et al.,* 2001). In an experimental rabbit inflammatory arthritis model, resveratrol showed promise as a potential therapy for arthritis. When administered to rabbits with induced inflammatory arthritis, resveratrol protected cartilage against the progression of inflammatory arthritis (Elmali *et al.,* 2007). Studies show resveratrol inhibits herpes simplex virus (HSV) types 1 and 2 replication by inhibition of an early step in the virus replication cycle (Docherty, *et al.,* 1999). *In vivo* studies in mice found

resveratrol inhibits or reduces HSV replication in the vagina and limits extra vaginal disease (Docherty *et al.,* 2005). The skin of resveratrol-treated animals showed no apparent dermal toxicity, such as erythema, scaling, crusting, lichenification, or excoriation (Docherty *et al*., 2004). Resveratrol (3, 5, 4'-trihydroxystilbene), (Structure 9) is a stilbenoid; a derivate of stilbene. It exists as two geometric isomers: *cis*- (*Z*) and *trans*- (*E*), (Structure 10). The *trans*- and *cis*-resveratrol can be either free or bound to glucose (Mattivi *et al.,* 1995).The *trans*- form can undergo isomerisation to the *cis*- form when exposed to ultraviolet irradiation, a process called photoisomerization (Lamuela-Raventos *et al*., 1995).



Structure 8: Structure of Resveratrol



Structure 9: Structures of Cis and Trans resveratrols**.**

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 MATERIALS AND METHODS

3.2 APPARATUSES USED FOR EXTRACTION

i. Soxhlet extractor

ii. Rotary evaporator

iii. Heating mantle

iv. Steam bath

v. Water bath

vi. Condenser

vii. Silica gel

viii. Developing chamber

ix. Sintered funnel (porosity No. 3)

x. Pre- coated plates for TLC

xi. Ultra violet (UV) Lamp

xii. Incubator

xiii. Autoclave

3.3 SOLVENTS FOR EXTRACTION OF THE PLANT MATERIAL

i. Methanol (64.7^{o}C) by Sigma-Aldrich.

ii. Ethyl acetate (77.1^{o}C) by Sigma-Aldrich

iii. Trichloromethane (61.15^{o}C)by Sigma-Aldrich

iv. Petroleum-ether (60-80^{0}C) by Sigma-Aldrich

3.4 REAGENTS FOR PHYTOCHEMICAL SCREENING.

i. Concentrated sulphuric acid.

ii. Dilute sulphuric acid.

iii. Concentrated Hydrochloric

iv. 10 % Hydrochloric acid

v. Concentrated NaOH

vi. 10 % NaOH

vii. 90 %Ethanol

viii. 5 % Acetone

ix. 5 % H_2S0_4in 5 % ethanol.

x. 28 % Ammonia solution.

xi. Acetic anhydride.

xii. Lead sub-acetate solution.

xiii. Fehling's solution: 7 g copper sulphate and 0.1 ml sulphuric acid were mixed with sufficient distilled water to produce 100 ml: this is Fehling's solution A.

35.2g sodium potassium tartrate and 15.4 g sodium hydroxide were dissolved in 100 ml of distilled water: this is Fehling's solution B. Equal amounts of solutions A and B were mixed together for use.

xiii. Molisch's reagent: solution of 10 % α-Naphthol in alcohol.

xiv. Dragendorff's reagent: 0.85 g of bismuth was dissolved in 10 ml of acetic acid and 40 ml of distilled water was mixed together to give solution A. 8 g of Potassium iodide was

dissolved in 20 ml of distilled water to give solution B. 13.5 ml each of solutions A and B were mixed together in 20 ml of 33 % acetic acid and the volume made up to 100 ml with distilled water to produce Dragendorff's reagent.

xv. Meyer's reagent: 1.36 g of mercuric chloride was added to 60 ml of distilled water. 5 g of potassium iodide was added to 20 ml of distilled water. Both solutions were mixed together and made up to 100 ml.

3.5 EQUIPMENT /MATERIALS USED FOR ANTIMICROBIAL SCREENING

i. Nutrient agar

ii. Sabouraud dextrose agar

iii. Petri -dishes

iv. Autoclave

v. Incubator.

vi. Syringes and needles

vii. Sample bottles

viii. Pipettes.

ix. Distilled water

x. Disinfectant

xi. Test tubes

xii. Dimethyl sulphoxide (DMSO).

xiii. Whatman filter paper

xiv. Cotton wool

xv. Bunsen burner

3.6 MICROORGANISMS USED FOR ANTIMICROBIAL SCREENING:

BACTERIA:

i. *Streptococcus pyogenes.*

ii. *Staphylococcus aureus.*

iii. *Escherichia coli*

iv. *Proteus mirabilis*

v. *Pseudomonas aeruginosa*

vi *Shigella dysenteriae.*

FUNGI:

i. *Aspergillus nigre.*

ii. *Candida krusei.*

iii. *Candida albicans*

3.7 THE PLANT MATERIALS

The whole plant of *Aeschynomene schimperi* was collected from Samaru, Zaria, Kaduna State, Nigeria in October, 2010 and identified by Mallam Musa Galla a taxonomist at the Herbarium, Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria. Accordingly, a voucher specimen number, 1697 was kept there. The plant material was air-dried, pulverized by the use of a wooden mortar and pestle and later stored in a closed container.

3.8 EXTRACTION OF THE PLANT MATERIAL

The air- dried and finely powdered whole plant of *Aeschynomene schimperi* (500 g) was first extracted exhaustively using petroleum-ether (60-80 °C) in a Soxhlet extractor. The procedure was sequentially repeated on the same plant using chloroform, ethyl acetate and methanol in order of increasing polarity (Brain *et al.,* 1989).The extracts were concentrated *in vacuo* using rotary evaporator and allowed to dry under *vacuo*. The weight of the extracts were recorded as shown in Table 4.1.

3.9 PHYTOCHEMICAL SCREENING

Standard procedures (Trease and Evans, 2002) were employed to carry out the phytochemical screening of the extracts to test for the presence or absence of secondary metabolites as described below:

3.9.1 Test for Glycosides

5mlof conc. sulphuric acid was added to each extract (0.2 g) in a test tube and boiled for 15 minutes cooled and neutralized with 20 % KOH and was divided into two portions.

Fehling's solution Test

Fehling's solutions A and B in the ratio of 1:1 was added to the above portions and boiled for few minutes. A brick red precipitate was observed in all the extracts.

Ferric Chloride Test

3 drops of ferric chloride solution was also added to these portions and greenish-black precipitate was observed.

3.9.2 Test for Tannins

About 10 ml of distilled water was added to 0.2 g of the extracts, stirred and then filtered. Few drops of 1 % ferric chloride solution were added to 2 ml of each of the filtrates. The solutions turned greenish black in all the extracts.

3.9.3 Test for Anthraquinones

Free Anthraquinones

Each of the extracts was respectively put in a test tube, treated with 5 ml of chloroform. The content was filtered and 5 ml of 10 % ammonia solution was added to each filtrate, the mixture was shaken. There was no observable reaction in all the test tubes.

Combined Anthraquinones

Each portion (0.2 g) of the extract was boiled with 10 ml of aqueous sulphuric acid and filtered while still hot. The filtrate was shaken with 5 ml of benzene and the benzene layer was separated. To half of its volume, 10 % ammonium hydroxide was added; a pink color was seen in the methanolic extract.

3.9.4 Test for Alkaloids

About 0.2 g each of the extracts was stirred with 5 ml of 1 % aqueous HCl on water bath and then filtered. Each of the filtrates was divided into two and put into two separate test tubes. To the first test tube, few drops of Dragendorff's reagent was added. A blue black color was formed with little precipitation in the extracts of chloroform and petroleum ether. To the second test tube, 1 ml of Meyer's reagent was added; a reddish brown precipitate was also seen in the extracts of chloroform and petroleum ether.

3.9.5 Test for Cardiac Glycoside

Salkowski's test

Each of the extracts (0.2 g) was dissolved in 1 ml of chloroform, 1 ml of conc. H_2SO_4 was carefully added to form a lower layer. Red ring appeared in all the extracts.

3.9.6 Test for Reducing Sugar

A little portion (0.2 g) of each of the crude extracts was dissolved in 2 ml dilute H_2SO_4 and warmed for 3 minutes, 2 ml of 10 % NaOH was added to neutralize the solutions. The solutions were then treated with equal volumes of Fehling's solution A and B. Brick red precipitate was observed in all the extracts.

3.9.7 Test for Flavonoids

Ferric Chloride Test

About 0.2 g of the extract was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drops of 10 % ferric chloride solution were then added. There were yellow colorations in all the extracts.

Shinoda's Test

About 0.2 g of the extract was dissolved in ethanol, warmed and then filtered. Few drops of magnesium solution were then added to the filtrate followed by few drops of concentrated hydrochloric acid. Yellow color was observed.

3.9.8 Test for Steroids/Triterpenoids

Liebermann- Buschard's Test

To 0.2 g of each extract, 2 ml of acetic acid was added; the solution was then cooled in the ice followed by careful addition of conc. H_2SO_4. Red colored rings were observed at the interface.

Salkowski's Test

About 2 ml of chloroform was added to about 0.2 g of each of the extracts, 4 drops of conc. H_2SO_4 were carefully added down the test tube of each, a reddish brown color was seen at the interface.

3.9.9 Test for Saponins

About 0.3 g each of the extracts was shaken vigorously with distilled water in a test tube. Frothing which persisted for sometimes was observed.

3.9.10 Test For Carbohydrates

Molisch's Test

0.2 g each of the extracts was dissolved in 5 ml of distilled water and then heated and allowed to cool to room temperature. Molisch's reagent was added and conc. H_2SO_4 acid was introduced into the mixture with the test tube in an inclined position so that the acid formed a layer beneath the aqueous solution without mixing. The test tube was shaken and the mixture was then allowed to stand for 2 mins. 5 ml of distilled water was added, a reddish brown color was observed.

3.10.0 PREPARATIONS OF THE CULTURE MEDIA, TEST MICROORGANISMS AND CONCENTRATION PLANT EXTRACTS

3.10.1 Preparation of the Culture Media

About 28 g of nutrient agar and Sabouraud dextrose agar were carefully weighed and transferred into two separate, sterilized conical flasks. One liter of distilled water was added to each conical flask and stirred until homogenous mixtures were obtained. These were then autoclaved at 121 °C for 15 minutes and allowed to cool to about 40-50 ^{o}C. About 20 ml was transferred into sterilized petri-dishes and allowed to solidify.

3.10.2 Preparation of Test Organisms

The Microorganisms mentioned earlier were obtained from the Department of Medical Microbiology Laboratory, Ahmadu Bello University Teaching Hospital, Zaria.

3.10.3 Preparation of Concentration of Plant Extracts

Each extract (0.1 g) was dissolved in 10 ml of Dimethyl sulphoxide (DMSO) to obtain a concentration of 10mg/ml. This was the initial concentration used to check for the antimicrobial activities of the plant extracts.

3.11 ANTIMICROBIAL SCREENING

Mueller Hinton and Sabouraud dextrose agar were the media used as the growth media for the microorganisms and the media were prepared according to Sabouraud (1892). Boiled to dissolve and were sterilized at 121 °C for 15minutesthen cooled to 4 0^{o}C, 20 ml of the media was then poured into sterile petri dishes. The plates were covered and allowed to cool and solidify. Filter paper discs of 6mmin diameters were cut and sterilized at 100 °C for 60 minutes.

The discs were then soaked into the solution of the extracts and dried at 45 °C. The media were then seeded with the test microorganisms and the inoculums were spread evenly over the surface of the media by the use of sterile wire loop. The seeded plates were dried at 37 °C for 30 minutes. The discs were then planted on the surface of the seeded media. The media were incubated at 37 °C for 24 hrs for the bacteria, and at 30°Cfor 7 days for the fungi, after which the plates were observed for the zones of inhibition of growth. The zones of inhibition were measured with a transparent ruler and recorded in millimeters (mm).

3.11.1 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (*MIC*) was carried out on the test microorganisms using broth dilution method. Nutrient and sabouraud dextrose broth were prepared (Sabouraud, 1892). 10 ml was dispensed into tests tubes and were sterilized at 121 °C for 15 mins and were allowed to cool. McFarland's turbidity standard scale no. 0.5 was prepared to give turbid solution. Normal saline was prepared and was dispensed into test tubes. The test organisms were then inoculated into the normal saline. To each test organism, separate incubation was made at 37 °C for 6 hrs for the bacteria and at 30 °C for 24 hr for the fungi. After incubation, the broth cultures were diluted with normal saline until the turbidity matched that of the McFarland's scale by visual comparison. At this point the microorganism had a concentration of 1.5x $l0^8$ cfu/ml. Fold serial dilution of the extract was done to give concentrations of 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 mg/ml. The initial concentrations were obtained by dissolving 0.1 g of the extracts in the nutrient broth. 0.1 ml of the standard inoculums of each test organism was then incubated into the different concentrations of the extract in the broth. The nutrient broth was incubated at 37 ^oC for 24 hr. and the Sabouraud dextrose broth at 30 ^oC for 7

days after which the test tubes were observed for turbidity (growth).The lowest concentration of extract in the broth which showed no turbidity was recorded as the Minimum Inhibitory Concentration (MIC).

3.11.2 Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC) of the Crude Extracts

The minimum bactericidal and fungicidal concentrations were carried out to confirm whether the organisms were killed or only their growth was inhibited.

Mueller Hinton and Sabouraud dextrose agar were prepared and sterilized at 121 °C for 15 min, cooled to 45 °C and were poured into Petri dishes, allowed to cool and solidify. The content of the minimum inhibitory concentration in the serial dilution were sub-cultured onto the prepared media. The media were incubated at 37 °C for 24 hr for the bacteria and at 30 °C for 7days for the fungi. The plates were observed for colonies growth. The minimum bactericidal/fungicidal concentrations were obtained from the plates with lowest concentration of the extracts in which there was no growth.

3.12.0 ISOLATION OF PURE COMPONENT

3.12.1 Thin Layer Chromatography (TLC)

This is a separatory technique employed to determine the number of chemical components in the extract and to ascertain the most suitable mobile phases for good resolution. This will greatly assist in taking a decision as to the most suitable solvent system to use in Column Chromatography. Commercially pre- coated TLC plates were used and developed with different ratios of organic solvents taking into cognizance their individual polarities. The resulting chromatograms after air-drying were viewed under visible and ultra- violet (UV) lights of 254

and 366 mm respectively. As revealed by the result of the TLC, the most suitable solvent system with the best resolution to use for the Column chromatography was ethyl acetate and petroleum ether in the ratio of 2:8.

3.12.2 Column Chromatography

Preparation of Column

Silica gel [100 g (60-120 mesh)] for column chromatography was activated in an oven at 100 ^{o}C for 1 hr. It was allowed to cool to room temperature in a desiccator. A slurry was made and carefully packed into the column ensuring that it was properly compacted and uniformly spread.

Loading of the Extract

The chloroform extract (3 g) was dissolved in chloroform and mixed with silica gel to form a dry, finely flowing powder. A non-polar solvent, petroleum-ether *(*60-80 ^{o}C*)* was run through the column under vacuum. The powdered extract was then introduced to the top of the column after draining the solvent to the level of the gel by the aid of sintered glass funnel.

Collection of Fractions

At a flow rate of 40 ml per hr, 30 fractions were collected at 10 ml per fraction with 100 % petroleum ether, then slowly adding up the concentration of the more polar ethyl acetate until 100 % ethyl acetate was achieved and finally washing up with methanol. The fractions collected were spotted using silica TLC and similar fractions (fractions with similar R_f values) were pooled together. Combined fraction "A" showed a single spot as revealed by the UV-light and was further purified using preparative thin layer chromatography. The isolate was allowed to dry which gave a pure substance named Zak-Bal (ZB). Zak-Bal means Zakari Bali. ZB was found to be yellow oil.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 EXTRACTION

The result of the extraction was summarized in Table 4.1 below:

TABLE 4.1 RESULTS OF EXTRACTS IN GRAMS

EXTRACTS	**AMOUNT OBTAINED**
PETROLEUM ETHER EXTRACT (60-80 ^{O}C)	15.00
CHLOROFORM EXTRACT (61.15^{o}C)	12.00
ETHYL ACETATE EXTRACT (77.1^{o}C)	10.20
METHANOL EXTRACT (64.7^{o}C)	8.00

4.2 PHYTOCHEMICAL SCREENING

Phytochemical screening of the crude extracts of petroleum ether, chloroform, ethyl acetate and methanol of the whole plants of *Aeschynomene schimperi* was carried out. As revealed by the results of the screening in Table 4.2, carbohydrates, reducing sugar, glycosides, cardiac glycosides, triterpenes, flavanoids, steroids, tannins and saponins (hydrolysable) were present in all the crude extracts of the whole plants while free anthraquinone was absent in all. glycosides and alkaloids were absent in ethyl acetate and methanolic extracts, combined anthraquinone was present in only the methanolic extract.

TABLE 4.2 PHYTOCHEMICAL SCREENING OF THE CRUDE EXTRACTS OF WHOLE PLANT OF *AESCHYNOMENE SCHIMPERI*

CONSTITUENTS	TEST	PE	CLM	E/A	MeOH
Carbohydrates	Molisch's test	+	+	+	+
Reducing sugar	Fehling's solution (A and B)	+	+	+	+
Glycosides	Fehling's solution (A and B)	+	+	+	+
	$FeCl_3$ Test	+	+	+	+
Free anthraquinones	Benzene, 10%NH_3	-	-	-	-
Combined anthraquinones	Benzene, 10%NH_3	-	-	-	+
Cardiac glycosides	Salkowski's Test	+	+	+	+
Steroids/ Triterpenoids	Liebermann Buchard test	+	+	+	+
	Salkowski's test	+	+	+	+
Flavanoids	Ferric chloride test	+	+	+	+
	Shinoda's test	+	+	+	+
Tannins (hydrolysable)	$FeCI_3$ test	+	+	+	+
Saponins	Frothing test	+	+	+	+
Alkaloids	Meyer's test	+	+	-	-
	Dragendoff's test	+	+	-	-

Key: **+=** present, - =absent, PE =Petroleum ether extract, CLM= chloroform extract, E/A = ethyl acetate extract, MeOH =methanolic extract

4.3 ANTIMICROBIAL TESTS

The antimicrobial sensitivity test of the extracts of petroleum ether, chloroform, ethyl acetate and methanol were carried out using nine pathogenic microorganisms; *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Shigella dysenteriae, Aspergillus nigre, Candida krusei* and *Candida albicans. Streptococcus pyogenes, Staphylococcus aureus, Shigella dysenteriae* and *Candida krusei* were sensitive to all the plant extracts while *Proteus mirabilis, Pseudomonas aeruginosa* and *Aspergillus nigre* were resistant to all the plant extracts. *Escherichia coli* and *Candida albicans* were resistant to petroleum ether extract.

TABLE 4.3 RESULTS OF SENSITIVITY TEST FOR *AESCHYNOMENE SCHIMPERI* EXTRACTS.

TEST ORGANISM	PE	CLM	E/A	MeOH
Streptococcus pyogenes	S	S	S	S
Staphylococcus aureus	S	S	S	S
Escherichia coli	R	S	S	S
Proteus mirabilis	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R
Shigella dysenteriae	S	S	S	S
Candida albicans	R	S	S	S
Candida krusei	S	S	S	S
Aspergillus nigre	R	R	R	R

Key: S= Sensitive, R =Resistant, PE =Petroleum ether extract, CLM= chloroform extract, E/A = ethyl acetate extract, MeOH = methanolic extract.

4.4 ZONES OF INHIBITION OF THE PLANT EXTRACTS AGAINST THE TEST MICROORGANISMS

The crude extracts of the four solvents were tested for zones of inhibition of the test pathogenic microbes. The petroleum ether extract showed diameters of zone of inhibition of 12-17 mm against; *Streptococcus pyogenes, Staphylococcus aureus, Shigella dysenteriae* and *Candida krusei*. Chloroform extract showed diameters of zone of inhibition of 20-27 mm against; *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Candida krusei and Candida albicans.* Ethyl acetate extract showed diameters of zone of inhibition of 20-23 mm against the same organisms as in chloroform extract. Methanolic extract showed diameters of zone of inhibition of 17-21 mm against the same organisms as in chloroform and ethyl acetate extracts. Chloroform extract had the highest antimicrobial activity of the four crude extracts recording 27 mm in diameters of zone of inhibition in Bacteria *(Streptococcus pyogenes and Escherichia coli).* Petroleum ether extract recorded the lowest inhibition zone (12 mm in diameter) in the Fungus, *Candida krusei.* The control drugs (Sparfloxacin and Flouconazole) had varied zones of inhibition against the test microorganisms. The concentration of the control drugs was 2 mg/ml. The results are shown in Table 4.4.

TABLE 4.4 ZONES OF INHIBITION (mm) OF THE EXTRACTS OF THE WHOLE PLANT OF *AESCHYNOMENE SCHIMPERI* WITH CONTROL DRUGS

TEST MIROORGANISMS	**PE**	**CLM**	**E/A**	**MeOH**	**SPA**	**FLO**
Streptococcus pyogenes	17	27	25	20	37	0
Staphylococcus aureus	16	24	23	21	32	0
Escherichia coli	0	27	23	20	41	0
Proteus mirabilis	0	0	0	0	27	0
Pseudomonas aeruginosa	0	0	0	0	24	0
Shigella dysenteriae	17	26	23	20	30	0
Candida albicans	0	22	20	17	0	32
Candida krusei	0	20	20	17	0	30
Aspergillus nigre	0	0	0	0	0	21

Key: 0= No Zone of Inhibition, SPA = Sparfloxacin, FLO = Flouconazole

4.5 MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE EXTRACTS OF THE WHOLE PLANT OF *AESCHYNOMENE SCHIMPERI.*

The Minimum Inhibitory Concentration (MIC) of the extract is the lowest concentration of the extract required to halt the growth of the test microorganisms. The test was carried out to examine the bacteristatic and fungistatic properties of the plant extracts on the test Bacteria and Fungi respectively. *Proteus mirabilis, Pseudomonas aeruginosa* and *Aspergillus nigre* could not respond at all to all the extracts. *Escherichia coli* and *Candida albicans could* not respond to the Petroleum extract. The remaining microorganisms responded to all the plant extracts. In the Petroleum extract, the Minimum Inhibitory Concentration (MIC) is **5 mg/ml** for all the microorganisms that responded. In the chloroform extract, the MIC is **2.5 mg/ml** for all the microbes that responded. **2.5 mg/ml** is the MIC of the ethyl acetate for all the microorganisms that responded where in the methanolic extract, the MIC is **5 mg/ml** for *Candida albicans* and *Candida krusei* and **2.5 mg/ml** for the remaining microorganisms that responded. The results of the test were shown in Table 4.5.

TABLE 4.5 MINIMUM INHIBITORY CONCENTRATION OF THE EXTRACTS AGAINST THE TEST MICROORGANISMS.

TEST MICRO-ORGANISMS	EXTRACTS				
	PE	CLM	E/A	MeOH	
	2.5mg/ml	2.5mg/ml	2.5mg/ml	5mg/ml	2.5mg/ml
Streptococcus pyogenes	*	*	*		*
Staphylococcus aureus	*	*	*		*
Escherichia coli		*	*		*
Proteus mirabilis					
Pseudomonas aeruginosa					
Shigella dysenteriae	*	*	*		*
Candida albicans		*	*	*	
Candida krusei	*	*	*	*	
Aspergillus nigre					

Key:* = MIC

4.6 MINIMUM BACTERICIDAL AND FUNGICIDAL CONCENTRATIONS (MBC/MFC) OF THE EXTRACTS AGAINST THE TEST MICROORGANISMS

The minimum bactericidal concentration (MBC) of the plant extract is the lowest concentration of the extract required to kill the test bacteria while the minimum fungicidal concentration

(MFC) of the extract is the lowest concentration of the extract required to kill the tested fungi. In the fungi that responded to the test, the MFC was **10 mg/ml** for all the extracts. In the methanolic extract, the MBC for all the bacteria that responded was also **10 mg/ml** while in the remaining extracts, there were variations in the MBC of the extracts against the bacteria that were examined as clearly shown by the results in Table 4.6.

TABLE 4.6 MINIMUM BACTERICIDAL AND FUNGICIDAL CONCENTRATIONS (MBC/MFC) OF THE EXTRACTS AGAINST THE MICROORGANISMS

TEST MICRO-ORGANISMS	EXTRACTS					
	PE	CLM		E/A		MeOH
	10mg/ml	10mg/ml	5mg/ml	10mg/ml	5mg/ml	10mg/ml
Streptococcus pyogenes	*		*		*	*
Staphylococcus aureus	*	*		*		*
Eschenchia coci			*	*		*
Proteus mirabilis						
Pseudomonas aenginose						
Shigella dysenteriae	*		*	*		*
Candida albicans		*		*		*
Candida krusei	*	*		*		*
Aspergillus nigre						

Key: * = MBC/MFC

4.7.0 SPECTRAL RESULT

The ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) and ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) were used in the structural elucidation of the isolated compound ZB which was initially obtained as a yellow oil. The chemical shifts (measured in ppm) of the ^{13} C NMR and ^{1}H NMR spectra of the isolated compound ZB were compared with those from the literature and summarized in Table 4.7a and Table 4.7b respectively.

4.7.1 Discussion of the Spectroscopy

From Table 4.7a, the chemical shift of the vinylic carbon of the reported compound was 140.70 ppm while that of the isolated ZB was 135.20 ppm both of which are characteristic chemical shifts of vinylic carbon (C=C) (Silverstein *et al.,* 2005). The range of chemical shifts of 71.80 - 73.70 ppm was for the Methoxy carbon (C-O) which has a characteristic chemical shifts in the range of 60-85 ppm (Berg *et al.,*2006).The exocyclic primary, secondary and tertiary Carbon have ^{13}C NMR chemical shifts in the range of 0-30 ppm, 20-45 ppm and 30-58 ppm respectively (Berg *et al.,* 2006). Carbon in the pentacyclic ring is of the chemical shifts in the range of 23.5-29.5 ppm while Carbon in the hexacyclic ring has chemical shifts in the rage of 24.3-30.3ppm (Silverstein *et al*., 2005). In Table 4.7b, C-5 (vinylic Carbon), C-10 and C-13 were quaternary Carbon. Hydroxyl proton (2.0, d) was on C-3. Methyl protons (0.88, s) and (0.80, d) were on C-18 and C-27 and respectively. Based on the striking similarities between the ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) and the ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) of the isolated compound with reported steroid in Table 4.7a and Table 4.7b respectively and from the positive tests for steroids (Liebermann-Buschard's test) given by ZB, it was assumed to be a compound containing steroidal nucleus and the structure is proposed to be Stigmast-5-en-3β-ol (β-Sitosterol) (Silverstein *et al.,* 2005).

TABLE 4.7a: COMPARATIVE CHEMICAL SHIFTS (ppm) OF ^{13}C NMR OF THE COMPOUND (ZB) WITH THE REPORTED STEROID

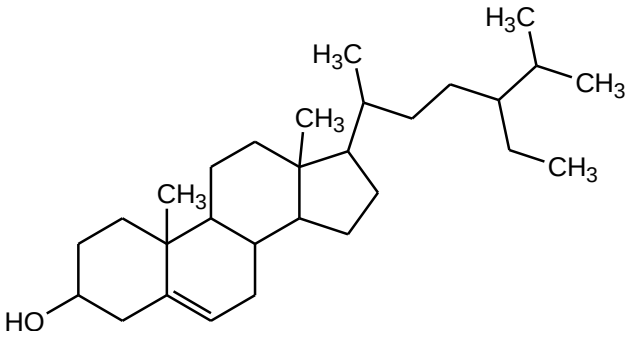
Position of Carbon	β –sitosterol[1]	Recorded (ZB)
1	37.28	**37.31**
2	**31.69**	**31.62**
3	71.82	**73.70**
4	**42.33**	**42.30**
5	140.70	**135.2**
6	121.72	**122.5**
7	**31.69**	**31.62**
8	**31.93**	**31.92**
9	50.17	**50.38**
10	36.52	**36.36**
11	21.10	**22.56**
12	**39.80**	**39.86**
13	**42.33**	**42.34**
14	**56.79**	**56.72**
15	**24.37**	**24.30**
16	**28.25**	**28.24**
17	**56.09**	**56.08**
18	**36.52**	**36.16**
19	21.25	**22.68**
20	33.98	**34.41**
21	**26.14**	**26.16**
22	45.88	**45.31**
23	**23.10**	**23.10**
24	**11.90**	**11.61**
25	28.91	**29.26**
26	18.79	21.00
27	19.80	23.10
28	22.70	23.50
29	21.56	22.16

[1]Silverstein *et al.*, (2005)

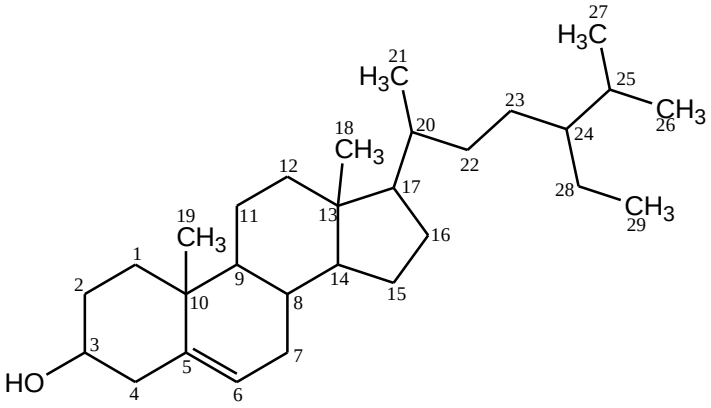
TABLE 4.7b: COMPARATIVE CHEMICAL SHIFTS (ppm) OF ^{1}H NMR OF THE COMPOUND (ZB) WITH THE REPORTED STEROID

Position of Carbon	β –Sitosterol[1]	Recorded (ZB)
1		
2		
3		
4	2.28(d)	2.28(d)
5	-	-
6	5.36(t)	5.36(t)
7	2.03(t)	2.3(t)
8		
9		
10	-	-
11		
12		
13	-	-
14		
15	1.60(t)	1.60(t)
16		
17		
18	0.88(s)	0.88(s)
19		
20	0.68(d)	0.68(d)
21	0.94(d)	0.90(d)
22		
23		
24		
25		
26		
27	0.83 (d)	0.80 (d)
28		
29		
OH(at C-3 position)	2.0(d)	2.0(d)

[1]Arjun, 2010



STRUCTURE 10: Proposed structure of ZB



Stigmast-5-en-3β-ol (β -sitosterol) $C_{29}H_{50}O$

STRUCTURE 11: Proposed Structure of ZB Showing numbering, nomenclature and molecular formula.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The plant *Aeschynomene schimperi* showed the presence of carbohydrates, reducing sugar, glycosides, cardiac glycosides, steroids, triterpenoids, flavanoids, tannins (hydrolysable), saponins, alkaloids and traces of combined anthraquinones. This may account for the broad spectrum activity of the plants against some pathogenic microorganisms tested as testified by the antimicrobial screening of the extracts against the pathogens (Table 4. I).The Isolation of ZB from chloroform extract of the plant was successfully carried out through series of chromatographic processes and based on the available spectral data, Stigmast-5-en-3β-ol (β-Sitosterol) with molecular formula of $C_{29}H_{50}O$ was proposed to be the isolated compound. Further antimicrobial screening could not be carried out as a result of insufficiency of the compound. However, it was variously reported that, β–sitosterol was active against, *Streptococcus pyogenes, Escherichia coli* and *Candida albicans* (Bouic *et al*., 1996). β–sitosterol was isolated from the chloroform extract of *Aeschynomene schimperi* and this is a phytosterol. β-Sitosterol reduces carcinogen-induced cancer of the colon. It shows anti-inflammatory, anti-pyretic, anti-arthritic, anti-ulcer, insulin releasing and estrogenic effects and inhibition of spermatogenesis. But studies have shown that the phytochemical may have other health benefits: easing symptoms of benign prostatic enlargement, reducing risk of cancer and prevention of oxidative damage through its antioxidant activity (Nadkarni, 2007). In traditional medicine the bark, leaves, flowers and fruits are used to treat multifactorial diseases like leprosy, gout, acute rheumatic fever, sores, boils, bloody diarrhea, and vaginitis (Joshi, 2000).

5.2 RECOMMENDATION

There is need for further studies on the toxicity of the plant so as to rule out the possibility of any side effect as a result of using the plant.

REFERENCES

Abeloff, M. **(2008).** *Abeloff's Clinical Oncology,* 4th ed. Philadelphia, PA: Churchill Livingstone, An Imprint of Elsevier, pp. 129-135.

Amr, A. and Michael, B., **(2007)**. *The Anti-Cancer Charm of Flavonoids: A Cup of-Tea Will Do*, 2,2 109–117.

Arjun, P., **(2010).** *International Journal of Pharmaceutical Sciences*, 2, 95-100.

Aruna, C., Chaithra, D., Alekhya, C. and Yasodamma, N., **(2012).** Pharmacognostic studies of aeschynomene indica L. *International Journal of Pharmacy and Pharmaceutical Sciences*, 76-77.

Berg, J. M., John, L., T. and Lubert S., **(2012).** *Biochemistry,* 7th ed. New York, pp. 876-879.

Bickoff, E. M., Booth, A. N., Lyman, R. L., Livingston, A. L., Thompson, C.R. and Deeds, F., **(1957).** Coumestrol, a New Estrogen Isolated from Forage Crops. *Journal of Science*, 126, 3280, 969–970.

Blomquist, C.H., Lima, P.H. and Hotchkiss, J.R., **(2005).** Inhibition of 3a-hydroxysteoid dehydogenase (3a-HSD) activity of human lung microsomes by genistein, daidzein, coumestrol and C18-, C19- and C21 hydroxysteroids and ketosteroids. *Steroids, 70*, 8, 860-865.

Bouic, P.J.D., Etsebeth, S., Liebenberg, R.W., Albrecht, C.F., Pegel, K., V. and Jaarsveld, P.P., **(1996).** *International Journal of Immunopharmacology*, 18, 12, 693-700.

Brain, C. F., Anthony, J. H., Peter, N. G. and Austin, R. T., **(1989).** *Vogel text book of Organic Chemistry*, 5th ed. John Wiley and Sons, Inc., New York, pp.164-165.

Brazil, B.A. **(2012).** Triterpenes and Triterpenoids, *Special article*, 12, 14, 243-249.

Brown, K. M. and Arthur, J. R., (**2001).** Selenium, selenoproteins and human health: *A review Public health nutrition*, 4:2B, 593–598.

Burkill, H.M., **(1995).** *The useful plants of West Tropical Africa*, 2nd ed., 3, Families J–L. Royal Botanic Gardens, Kew, Richmond, United Kingdom, pp. 857 -860.

Chantel, D., **(2009).** *Journal of Health and Fitness*, 7, 44, 231-235.

Chen, J., Tan, X., Lu, W. and Ya, Q., **(2011).** A New Stigmasterol Ester from Aeschynomene indica. *Chinese Herbal Medicines*, 3, 4, 248-250.

Chowdhury, A. R., Mandal, S., Mittra, B., Sharma, S., Mukhopadhyay, S. and Majumder, H.K. **(2002).** Betulinic acid, a potent inhibitor of eukaryotic topoisomerase I: identification of the inhibitory step, the major functional group responsible and development of more potent derivatives, *Public health nutrition*, 8, 182-185.

Damery, S., Gratus, C. and Grieve, R., **(2011).** The use of herbal medicines by people with cancer: A cross-sectional survey, *British Journal of Cancer*, 104, 6, 927-933.

Docherty, J. J., Fu, M. M., Stiffler, B. S., Limperos, R. J., Pokabla, C.M. and DeLucia, A. L., **(1999).** Resveratrol inhibition of herpes simplex virus replication. *Antiviral Res.*, 43, 3, 145–55.

Doddola, S., Pasupulati, H., Koganti, B. and Koganti, V. S., **(2008).** Evaluation of S. grandiflora for anti urolithiatic and antioxidant properties. *Journal of Natural Medicine*, 62, 300-307.

Dragland, S. **(2003).** Several Culinary and Medicinal Herbs are Important Sources of Dietary Antioxidants. *Journal of Nutrition*, 23, 4, 281-290.

Elmali, N., Baysal, O., Harma, A., Esenkaya, I. and Mizrak, B. **(2007).** Effects of resveratrol in inflammatory arthritis. *Inflammation,* 30, 1–2, 1–6.

Flore, M. andJean, L., **(1999).** *Journal of Applied Environmental Microbiology,* 65, 7, 3084-3094.

Gentilli, M., Mazoit, J. X., Bouaziz, H., Fletcher, D., Casper, R. F., Benhamou, D. and Savouret, J. F., **(2001).** Resveratrol decreases hyperalgesia induced by carrageenan in the rat hind paw. *Journal of Life Science,* 68, 11, 1317–21.

Gowri, S.S. and Vansanta, K., **(2010).** Free radical scavenging and antioxidant activity of leaves from agathi (S. grandiflora) (L.) Pers. *AmeEura Journal of Science Resources*, 5, 2, 114 -119.

Graham, P. H. and Vance, C. P., (**2003**). Legumes: importance and constraints to greater use. *Journal of Plant Physiology*, 131, 872-877.

Hostettmann, K., Hostettmann, M. and Morston, A., **(1998).** *Preparative Column Chromatography: Applications in Natural Product Isolation*, 2nd ed. Heildelberg, Berlin, pp. 40-42.

Hu, S., **(1980).** *An Enumeration of Chinese Materia Medica*. Chinese University Press, Hong Kong. 83, 31, pp. 345-354.

Izzo, A.A. and Ernst, E., **(2009).** Interactions between herbal medicines and prescribed drugs: An updated systematic review. *Drugs.* 69, 13, 332-338.

Joshi S., **(2000).** Grandiflora. Leguminoceae: *Text book of medicinal plants.* New Delhi: Oxford Publishing, pp. 130-132.

Karthiga, K., Kumaravel, M., Keerthana, R., Rukkumani, R. and Raviteja, V., (**2010).** Protective role of S. grandiflora on oxidative stress status during alcohol and PUFA induced hepatotoxicity. *Journal of Pharmaceutical Res*, 3, 12, 2959-2963.

Kasture, V. S., Deshmukh, V. K. and Chopde, C. T., **(2002).** Anxiolytic and anticonvulsive activity of S. grandiflora leaves in experimental animals. *Phytother Res*; 165, 455-460.

Kuiper, G. G., Lemmen, J. G., Carlsson, B., Corton, J. C., Safe, S. H., van der Saag P. T., van der Burg, B. and Gustafsson, J. A., **(1998).** Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*, 139 10, 4252–4263.

Laladhas, K. P., **(2009).** A novel protein fraction from S. grandiflora shows potential anticancer and chemoprotective efficacy in vitro and in vivo. *Journal of Cell Mol Med,* 613, 200-207.

Lampe, J. W., **(2003).** Isoflavonoid and lignan phytoestrogens as dietary biomarkers. *Journal of Nutrition,* 133, 3, 966-969.

Lamuela-Raventos, R. M., Romero-Perez, A. I., Waterhouse, A. L. and de la Torre-Boronat, M. C., **(1995).** Direct HPLC Analysis of cis- and trans-Resveratrol and Piceid Isomers in Spanish Red Vitisvinifera Wines. *Journal of Agricultural and Food Chemistry,* 43, 2, 281–283.

Leopold, J., **(2006).** Vacuum Dry Column Chromatography. *Journal of Organic Chemistry*, 47, 4593-4594.

Ling, Y., **(1995).** *A New Compendium of Materia Medica.* Science Press, Beijing. pp.1001-1006

Liu, C. and Xiao P., **(1993).** *An Introduction to Chinese Materia Medica.* Beijing Medical University Press, Beijing, pp. 1780-1783.

Marlene, C., **(2012).** Phytochemical study and evaluation of anti-inflammatory activity of Aeschynomene fluminensis vell. (Fabaceae).*Quím. Nova*, 35, 11, 2241-2244.

Mattivi, F., Reniero, F. and Korhammer, S., **(1995).** Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. *Journal of Agric Food Chem, 43* 7, 1820–1823.

Ming Z., Tanja, G., Jordan, G., Jin-Ao, D. and Chun-Tao, C., **(2013).** Protostane and Fusidane Triterpenes*: A Mini-Review*, 184, 4054-4080.

Mohammad, H. M., Elisabeth, M., Mercedes, B., Rosa, M. C. and Javier, P., **(2009).** Steroidal Lactones from Aeschynomene elongata, an Ancient Plant for Novel Medicine. *Journal of Food Chem,* 14, 7, 2373-2393.

Nadkarni, K. M., **(2003).** *Indian Materia Medica,* Popular prakashan (P) Ltd. Mumbai, pp. 52-54.

Neuwinger, H. D., **(2000).** *African traditional medicine: a dictionary of plant use and Application,* Medpharm Scientific, Stuttgart, Germany, 5, pp. 589-592.

Pari L. and Uma, A., **(2003).** Protective effect of S. grandiflora against erythromycin estolate induced hepatotoxicity. *Journal of Medicinal Food*, 58, 439-443.

Patil, R. B., Nanjwade, B. K. and Manv, F. V. **(2011).** Evaluation of anti-inflammatory and anti arthritic effect of S. grandiflora bark and fruit of Terminalia chebula in rats. *International Journal of Pharmacol Bio Sci*, 5, 1, 37-46.

Pimporn, A., Srikanjana, K. and Siriporn, O., **(2011).** Antibacterial activities of S. grandiflora extracts, *Drug Discovery*, 5, 12-17.

Ramesh, T., Mahesh, R., Sureka, C. and Begum, V. H., **(2008).** Cardio protective effects of S. grandiflora in cigarette smoke exposed rats. *Journal of Cardiovas Pharmacol*, 52, 338-343.

Sabouraud, R., **(1892).** *Analytical Dermatology*, Syphil. 3, 1061, pp. 321-327.

Serti, J. A., Wieze, G., Woisky, R. G. and Carvalho, J. C., **(2001).** Anti-ulcer activity of the ethanol extract of S. grandiflora. *Brazil Journal of Pharmaceutical Sciences*, 37, 107-111.

Shishodia, S., Majumdar, S., Banerjee, S. and Aggarwal, B. B., (**2003).** Ursolic acid inhibits nuclear factor-kappa B activation induced by carcinogenic agents through suppression of Ikappa Balpha kinase and p. 65 phosphorylation: correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1, *Cancer Res.*, 63, 15, 75–83.

Silva, G., Lee, I. and Kinghorn, A., **(1998).** *Special problem with the extraction of plants.* Human press New Jersey, 48, pp. 1-27.

Silverstein, R. M., Webster, F. X. and Kiemle, D. J., **(2005).** *Spectrometric Identification of Organic Compounds*, 7th ed., John Wiley and Sons, New York, pp. 234-239.

Sofowora, E., **(1993).** *Medicinal Plants and Traditional Medicine in Africa*, 1st ed. John Wiley and Sons, New York, pp. 1-23.

Sreelatha, S., Padma, P. R. and Umasankari, E., **(2011).** Evaluation of anticancer activity of ethanol extract of S. grandiflora (AgatiSesban) against Ehrlich ascites carcinoma in Swiss albino mice. *Journal of Ethnopharmacol*, 134, 984-987.

Stevens, P. F., **(2008).** Angiosperm Phylogeny Website Version 9, June, 2008, Mobot.org.

Trease, C. J. and Evans, W., **(2002).** *A text book of* Pharmacognosy, 5th ed. Elsevier Ltd. Edinburghpp, pp. 20 -23.

Vogel, S., **(2003).** *Prime Mover–A Natural History of Muscle*. W. W. Norton & Company, Inc., USA: p. 301.

Wagh, V. D., Wagh, K. V., Tandale, Y. N. and Salve, S. A., **(2009).** Phytochemical, pharmacological and phytopharmaceutics aspects of S. grandiflora (Hadga): *A review Journal of Pharmacy Res*, 2, 5, 889-892.