PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES ON THE LEAF OF *Senna singueana* (Delile) Lock(FABACEAE)

BY

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BY

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JUNE, 2018

DECLARATION

I declare that the work in this dissertation entitled "Phytochemical and Antimicrobial Studies on the Leaf of *Senna singueana* (Delile) Lock(Fabaceae)"has been carried out by me in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Sarah Ene, ODOH _____ _____

Name of student Signature Date

CERTIFICATION

This dissertation entitled "Phytochemical and Antimicrobial Studies on the Leaf of *Senna singueana* (Delile) Lock(Fabaceae)" by Sarah Ene ODOH meets the regulations governing the award of the degree of Master of Science in Pharmacognosy of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to God almighty for giving me the strength and ability to carry out this work. I alsodedicate this research to my late beloved grandmother Enuwa Cecilia ODOH for her ceaseless and countless prayers to God almighty on my behave throughout her life time. I also dedicate this work to my two lovely daughters Olohikondu and Enenu-Ondugbe ODUMA whom God blessed me with in the course of this program.

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May God bless them all.

ABSTRACT

Senna singueana (Dell) Fabacea is a shrub widely used traditionally to treat numerous disease conditions such as sexually transmitted diseases (STD), intestinal complications as a laxative, antimalaria,antimicrobialand antioxidant effects. The pharmacognostic standardization of the leaf of the plant was assessed to determine its organoleptic features, microscopical, and chemo-microscopical characters as well as physico-chemical parameters, phytochemical and anti-microbial activity. The study was aimed at evaluating the pharmacognostic, phytochemical constituents and the anti-microbial activity of leaf extracts. The investigations started with the qualitative microscopic and macroscopic evaluation of the leaf material and establishment of its quality parameters, including physicochemical and phytochemical evaluation. The powdered leaf was then extracted successively using soxhlet apparatus in n-hexane, ethylacetate and methanol to obtain a brownish hexane Extract (HE), brownish ethylacetate extract (EE) and brownish gummy methanol extract (ME). The extracts were subjected to phytochemical analysis and then, evaluated for their antibacterial and antifungal activity. The hexane extract was subjected to column chromatography with the aim of isolating a bioactive phytoconstituent. The leaf was found to be elliptical with entire margins with an obtuse apex, its 2.52 cm long and 1.65 cm wide, it has smooth texturewith a characteristic taste and distinct odour. Microscopically the leaf has numerous paracytic stomata on the lower surface. It has a lot of unicellular trichomes.Chemo-microscopical analysis revealed the presence of aleurone grains, starch grains, tannins and cutins surrounded by layers of cellulose and lignified walls. Physico-chemical parameters such as moisture content (6.03%), total ash content (7.33%), water soluble ash content (4.5%), and acid insoluble ash content (1.5%). Phytochemical screening revealed the presence of many therapeutically important classes

of phytoconstituents such as alkaloids, flavonoids, phenolic, sterols, triterpenoids, saponins and carbohydrates with varied presence in the three different extracts which was further confirmed by the use of specific spray reagents on thin layer chromatography (TLC). EE gave the highest zone of inhibition for *E.coli*(27mm) while ME gave 21mm and HE 17 mm at the same (10 mg/mL) onthe tested Gram positive organisms. EE also gave the highest zone of inhibition of 22mm followed by ME(20mm) and the hexane extract (18 mm) for *Staphylococcus aureus*. From the hexane extract, column and thin layer chromatography led to the isolation and identification of a triterpenoid. The structure of this compound couldn't be determined as the quantity was so minute. The study would therefore serve as a useful tool for the standardization of the leaves of *Senna singueana* ensuring and validating its identity, purity and quality. Also validates its use in treatment of skin infections.

TABLE OF CONTENTS

Declaration iii

Certification iv

Dedication v

Acknowledgements vi

Abstract vii

Table Of Contents ix

CHAPTER I 1

1.0 INTRODUCTION 1

1.1 Statement of Research Problem **6**

1.2 Justification of Research **7**

1.3 Hypothesis **7**

1.4 Aims of Study **7**

1.5 Specific Objectives **8**

CHAPTER II 9

2.0 LITERATURE REVIEW **9**

2.1 Description of Fabaceae Family **9**

2.2 Characteristics of Members of Fabaceae Family **9**

2.3 Taxonomy: **10**

2.5 Botanical Description of the Genus Senna. **12**

2.6 Senna Systematics and Taxonomy 12

2.7 Some Species of Senna 13

2.8 Ethnomedicinal Uses of Some Senna Species 13

2.9 Some Phytochemicals Reported From Some Species of Senna 14

2.10 Antimicrobial Activity of *Senna* Species. 17

2.11 Botanical Description of *Senna Singueana* 17

2.12 Origin, Ecology and Distribution of *S. Singueana* 22

2.13 Management 22

2.14 Ethnomedicinal Uses / Biological Activities of *S. Singueana* 22

CHAPTER III 27

3.0 MATERIALS AND METHODS 27

3.1 Materials, Chemicals, Equipment, Solvents, Reagents/Solution 27

3.2 List of Reagents and Solvents 27

3.3 List of Equipment 27

3.4 Plant Collection, Identification and Preparation 28

3.5 Pharmacognostic Evaluation of the Leaves of *Senna Singueana* 28

3.5.1 Macroscopic Examination 28

3.5.2 Microscopical Examination on the Leaves of *S.singueana* 29

3.5.3Chemo-Microscopic Studies on the Leaves of *S. singueana* 29

3.6 Determination of Physicochemical Constants of the Powdered Leaves of *S.Singueana* 31

3.6.1 Moisture Content 31

3.6.2 Ash Values 32

3.6.3 Solvent Extractive Values 33

3.7 Phytochemical Analysis of *S. singueana* Leaves 34

3.7.1 Extraction of *S. Singueana* Leaves 34

3.7.2 Preliminary Phytochemical Screening 36

3.8 Thin Layer Chromatographic Analysis. 39

3.9 Column Chromatography 40

3.9.1 Preparative Thin Layer Chromatography 40

3.10 Antimicrobial Activity of the Leaves Extract of *Senna singueana* 41

3.10.1 Collections of Clinical Isolates. 41

3.10.2 Preparation of Stock Solution 42

3.10.3 Preparation of Culture Media and Standard Turbid Solution 42

3.10.4 Screening of the Extracts for Antimicrobial Activity 43

3.10.5 Minimum Inhibition Concentration of Extracts 43

3.10.6 Minimum Bactericidal and Fungicidal Concentrations (MBC/ MFC) 44

3.10.7 Synergistic Activity of Extracts with Standard Drugs 44

3.11 Statistical Analysis 45

CHAPTER IV 46

4.0 RESULTS 46

4.1 Plant Collection and Identification **46**

4.2 Macroscopical Evaluation of *Senna Singueana* Leaf **46**

4.3 Microscopical Features of *Senna Singueana* Leaves **47**

4.4 Chemomicroscopical Studies of *S. Singueana* Powdered Leaves **50**

4.5 Physical Constants of *Senna Singueana* Leaves **51**

4.6 Extraction of *Senna Singueana* Leaves **52**

4.8 Thin Layer Chromatoghraphic Profile of *S. Singueana* Leaves Crude Extracts **54**

4.9 Column Chromatography **70**

4.9.1 Preparative TLC of SS5 Column Fractions 71

4.9.2 Isolation of the Compound 72

4.10 Antimicrobial Studies of *S. Singuena* Crude Extracts **73**

4.10.1 Antimicrobial Activities and Zones of Inhibitions of Extracts. 73

4.10.2 Synergistic Activity of Senna Extracts with Drugs 77

4.10.5 Minimum Inhibitory Concentration (MIC) of Extracts against the Microbes 79

4.10.6 Minimum Bactericidal/Fungicidal Concentration of Senna Singueana Extract Against the Microbes (MBC/MFC) 81

CHAPTER V 82

5.0 DISCUSSION 82

CHAPTER VI 91

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS 91

6.1 Summary **91**

6.2 Conclusion **93**

6.3 Recommendations **93**

REFERENCES **95**

APPENDIX: **103**

LIST OF FIGURE

Figure **Title** **Page**

Fig. 2.0 Structures of some Phytochemicals isolated from *Sennasingueana* 15

Fig. 3.1: Flowchart of Soxhlet Extraction of *S.singueana* Leaves..... 35

LIST OF TABLES

Table	Title	Page
Table 1.1:	Some Common Plants with Antimicrobial and Therapeutic Properties	2
Table 1.2:	Some Important Phytochemicals Present in Medicinal Plants	6
Table 4.1	Organoleptic Evaluationof *Senna singueana* leaf	46
Table 4.2	Chemomicroscopical Studies of *S.singueana* Powdered Leaves	50
Table 4.3	Physical Constants of *S. singueana* Leaves	51
Table 4.4	Weight, Percentage yields and pH of *S. sinqueana* Extracts	52
Table 4.5	Preliminary Phytochemical Screenings of S. *singueana* Leaves Crude Extracts	53
Table 4.6	Summary of TLC profile of Extracts Using General Spray Reagent P-anisaldehydes	57
Table 4.7	Summary of Column Chromatography N-hexane Extract	70
Table 4.8	Zone of Inhibition of *S. sinqueana* ExtractsAgainst Bacterial Isolates	74
Table 4.9	Zone of Inhibition of *S. sinqueana* Extracts Against Fungi Isolate	75
Table 4.10	Zone of inhibition of synergistic effect of Senna extracts and against Ciprofloxacin against bacteria isolate	76
Table 4.11	Zone of inhibition of synergistic effect of Senna extracts and against Fluconazole against Fungi isolate	77
Table 4.12	MIC of *S. singueana* extracts against the microbes	79
Table 4.13	Minimum bactericidal/fungicidal concentration of *S.singueana*extract against the microbes (MBC/MFC)	80

LIST OF PLATES

Plate	Title	Page
Plate I.	Picture of *S. singueana* Shrub in it Natural Habitat	19
Plate II a:	Picture of *S. singueana* Leaves and Flowers	20
Plate II b.	Picture of *S. singueana* Pods Containing Seeds	21
Plate III:	Photomicrograph of the Transverse section of *S. singueana*Leaf through the Midrib (mag.X100)	47
Plate IV:	Upper Epidermis Showing Numerous Trichomes	48
Plate V:	Photomicrograph of the Lower Epidermis of *S. singueana*Leaf showing some features (mag.x 100)	49
Plate VI:	Chromatogram of Hexane extract developed in Hexane:Ethyl acetate 19:1 revealed 8 spots. Colours are Purple and Dark Blue	54
Plate VII:	Chromatogram of Ethylacetate Extract developed in Hexane:Ethylacetate 7:3 revealed 6Spots. Colours are Green and Dark Blue	55
Plate VIII:	Chromatogram of Methanol Extract developed in chloroform: methanol 4:2. Colours are purple, Dark Blue and Yellow	56
Plate IX:	Chromatogram of Hexane Extract developed in Hexane: EthylAcetate 19:1 sprayed with Liebermann-Buchard Reagent revealed 7 Spots. Colours are Green, Dark Blue and Purple	59
Plate X:	Chromatogram of ethylacetate Extract developed in hexane: ethylacetate 7:3 sprayed with Liebermann-Buchard reagent revealed 3 spots. Colours are Green and Purple	60
Plate XI:	Chromatogram of Methanol Extract developed in Chloroform: Methanol 8:2 sprayed with Liebermann-Buchard Reagent revealed 4 spots.Colours are Green and Purple	61
Plate XII:	Chromatogram of hexane Extract developed in Hexane: ethylacetate 19:1 sprayed with ferric chloride Reagent revealed 3 spots. Colours are Light Green	62
Plate XIII:	Chromatogram of Ethylacetate Extract developed in Hexane: ethylacetate 7:3 Sprayed with Ferric Chloride Reagent revealed 4Spots. Colours are Blue-Black	63

Plate XIV: Chromatogram of Methanol Extract developed in Chloroform: Methanol 8:2 Sprayed with Ferric Chloride Reagent revealed 3 Spots. Colours are Blue-Black 64

Plate XV: Chromatogram of Hexane Extract developed in Hexane: ethylacetate 19:1 Sprayed with Bontrager Reagent revealed 2 Spots. Colour is Orange 65

Plate XVI: Chromatogram of Ethylacetate Extract developed in Hexane: Ethylacetate 7:3 sprayed with Bontrager Reagent revealed 4 spots. ColourisGreen 66

Plate XVII: Chromatogram of Methanol Extract Chloroform: Methanol 8:2 sprayed with Bontrager Reagent Revealed 3 Spots. Colour is Yellow 67

Plate XVIII: Chromatogram of Ethyl acetate Extract Hexane: Ethyl acetate 7:3Sprayed with Aluminium Chloride Sprayed and viewed under UV (254 nM) revealed 5 Yellow Florescence Spots. 68

Plate XIX: Chromatogram of Methanol Extract Chloroform: Methanol 8:1Sprayed with Aluminium chloride sprayed and viewed under UV (254 nM) revealed 8 Yellow Florescence Spots. 69

Plate XX: TLC of Hexane Crude Extract compared to Pooled Fraction 11-18(SS5) using 100% Hexane as Solvent System. H = Hexane Extract, F= Pooled Fraction 11-18 71

Plate XXI: TLC of Compound SA using Hexane 100% as Solvent system yielding a Single Purple Color Spotwith Rf value of 0.56 72

ACRONYMS AND ABBREVIATIONS

B.H. P:	British Herbal Pharmacopoeia
Fig:	Figure
FAA:	Formalin Acetic Acid Alcohol
g:	Gram
G.A.A:	Glacial Acetic Acid
Hcl:	Hydrochloric Acid
H_2SO_4:	Sulphuric Acid
LD_{50}:	Median Lethal Dose
kg:	Kilogram
ml:	Milliliter
mm:	Millimeter
mg:	Milligram
UV:	Ultraviolet Light
Vol.:	Volume
w/w:	Weight per Weight
WHO:	World Health Organization
%:	Percentage

CHAPTER I

1.0 INTRODUCTION

1.1 Traditional Medicines

Countries in Africa, Asia and Latin America use traditional medicine to meet some of their primary health care needs. The World Health Organization (WHO, 2003) defined Traditional Medicine as the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. Many rural communities in the aforementioned countries have great faith in traditional medicine, particularly in the explicable aspects as they believed that it is the wisdom of their fore – fathers which also recognized the social, cultural and religious background which orthodox medicines seems to neglect. Traditional medicine is the oldest, most tried and tested form of medicine and it is as old as man himself (WHO, 2003). Plants have formed the bases of traditional medicine system.

1.2 Medicinal Plants

The World health organization (2008) defines medicinal plants as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs. The medicinal qualities of plant is its ability to produce a host of bioactive molecules (secondary metabolites) which can be used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivores. Table 1.1 shows some common medicinal plants and their uses especially in microbial related ailments. The therapeutic activity of herbs is because of various constituents present in them. The

therapeutic efficacy of medicinal plants also depends on the quality and quantity of chemical constituents which may vary depending on various factors, among which is the geographical localities which shows quantitative variation in their chemical constituents *(*Joshi *et al.*, 2004).

It is important to stress the relevance of medicinal plants to the majority of Nigerian population and the world at large especially those living in rural areas that mostly have limited access to orthodox medicine. In such communities, the rising cost of imported medications and other raw materials for medicines remain a challenge.

Table 1.1 Some Common Plants with Antimicrobial and Therapeutic Properties

Specie	**Common name**	**Traditional uses**	**Family**
Eugenia caryophyllata	Clove	antiseptic and analgesic effects, anticoagulant and anti-inflammatory effect, expectorant and antiemetic	Myrtaceae
Cinnamomum cassia	Cinnamon, chinese cassia	As antimicrobial, dysmenorrhea, diarrhea and other GI disorders.	Lauraceae
Myristica fragrans	Nutmeg	Used in higher doses for their aphrodisiac and psychoactive properties.	Myristicaceae.
Origanum vulgare	Wintersweet, Mediterranean oregano	As antimicrobial, antispasmodic, antidiabetic, and antioxidant	Lamiaceae
Allium cepa	Onion	used as an antimicrobial, cardiovascular-supportive, anticancer/antioxidant	Liliacea
Abrus precatorius	Indian licorice	Has been used as an antimicrobial, treatment of fever, cough and cold, aphrodisiac.	Fabacea

(Joy *et al*. 1998)

1.3 Pharmacognosy

As practised today, pharmacognosy includes the extensive study of natural products from plants, bacteria, fungi and marine organisms, botanical dietary supplements as well as herbal remedies (Cardellina, 2002). It is estimated that 90% of the crude drugs are originated from plant sources while the remaining are from other two sources (Joy *et al*. 1998).Pharmacognosy has a natural link with other scientific fields such as Pharmacology, Analytical Chemistry, Microbiology, Plant Tissue Culture, Biotechnology and Genetic Engineering etc. andencapsulates all of these fields into a distinct interdisciplinary science (Balunas and Kinghorn, 2005). Pharmacognosy has also a very vital link with pharmaceutics and various traditional systems of medicines which help the pharmacognosists to dispense, formulate and manufacture drugs of natural origin in the best accepted allopathic form. The knowledge of chemotaxonomy, extraction, purification, plant tissue culture etc helps in the complete understanding of pharmacognosy along with coming up of better technologies for collection and preparation of crude drugs (Jarald and Jarald, 2007).

Pharmacognosy, nowadays, focuses on finding novel and unique molecules and revealing unknown targets by studying such molecules in nature. It is now well understood that pharmacognosy is one of several scientific disciplines that have strategic position in connecting biology with chemistry and even medicine. New and improved strategies regarding the selection of organism, bioassays techniques, isolation procedures, and structure elucidation are constantly developed based on the advancements in pharmacognosy (Claeson and Bohlin, 1997). Pharmacognosy provides basis for the study of secondary metabolites (natural product molecules) which are beneficial for their ecological, medicinal, gustatory or other functional properties. The natural species which are the basis for medicinally important compounds are of

the origin of biological kingdoms, particularly marine invertebrates, plants, fungi, and bacteria(Gupta *et al.,* 2010)

Plants have been considered as potential source of medicines for curing various ailments and disorders since the dawn of civilization (Table 1.1) and led to the establishment of the conventional knowledge of plants all around the sphere. Initially these medicines were utilized in the form of crude drugs, poultices, teas, tinctures, powders, and other herbal formulations. The particular plants to be used and the methods of application for a specific ailment were passed down by our fore-fathers orally (Balick and Cox, 1997 and Samuelsson, 2004).

Medicinal plants are plants containing potentially active ingredients used to cure disease or relieve pains (Okigbo *et al.,* 2008). Plants play a therapeutic and restorative role in protecting human beings from the adverse effects of diseases and other complications, thus considered to have a beneficial role in healthcare system. That is one of the reasons why majority of population of developing countries still rely on herbal medicines (Fellows, 1991).

Significant increase in medicinal plants usage has been recorded continuously both for traditional users and pharmaceutical industry. Medicinal plants provide opportunities for biological screening: methods useful for the industry and trends in the pharmacological investigations of natural products (Ozturk and Ozturk, 2008). Plants are the natural and most easy accessible source of therapeutically active biological principles, thus there is a need to screen out plants for development of new drugs. For this purpose plants have been assayed widely but still large number of them has not arrived to the conventional health care system (Esimone *et al.,* 2003; Bhattarai *et al*., 2006).

1.4 Phytochemistry

Phytochemistry is the study of phytochemicals, which are chemicals derived from plants. Specifically, phytochemistry describes the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insects attacks and plants diseases. They also exhibit a number of protective functions for human consumers (Samuelsson, 2004)

Phytochemicalsare defined as organic compounds and other chemicals synthesized by plants through metabolic processes aided by sunlight, involving CO_2, H_2O vapour and chlorophyll. Generally, phytochemicals are characterized by specific functions they perform in plants and animals. Classical phytochemical methodologies enabled the discovery of a vast array of bioactive secondary metabolites from various source materials including terrestrial plants, terrestrial micro-organisms, marine organisms, and terrestrial vertebrates and invertebrates (Tyler *et al.*, 1988). Metabolites are naturally-occurring organic compounds synthesized by plants through metabolic activities in plants, aided by enzymes. About 150 phytochemicals have been studied in detail (Mamta *et al*., 2013).

In wide-ranging dietary, phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, bacteria, herbs and spices. Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds (Mamta *et al*., 2013). Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues and levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions.

Table 1.2: Some Important Phytochemicals Present in Medicinal Plants

Classes	Main group of compound	Mechanisms of action
Antibacterial / antifungal	Terpenoids, alkaloids and phenolics	Inhibitors of microoganisms / reduces risk of fungal infections'
Detoxifying agents	Indoles, aromatic isothiocyanates, flavones, coumarines, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Antioxidants	Polyphenolics,flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation

Source**:** Mamta *et al*., 2013

1.5 Statement of Research Problem

Antibiotic resistance is a worldwide problem. Infectious diseases are major causes of morbidity and mortality in the developing world and accounts for about 50% of all deaths (El- Mahmood, 2009). Each year in the United States, at least 2 million people acquire serious infections with bacteria that are resistance to one or more antibiotics designed to treat those infections. At least 23,000 people die each year in the United States as a direct result of these antibiotics- resistance infections (Ahmad *et al*., 2009).

Microbial infections are considered a major threat to human health because of unavailability of vaccines or limited chemotherapy (Ofokansi *et al*., 2013). Also due to antibiotics overuse and misuse, there is an increase in resistant strains which do not respond to conventional antibiotics.

1.6 Justification of research

Traditional plant medicines and its ethno pharmacological uses have paved way for current orthodox medicines through the process of isolation of its active metabolites (Evans, 2009)

Despite the claimed ethno medicinal uses of the leaves of *S. singueana* in the treatment of various ailments including conjunctivitis, cough, wounds in leprosy mostly caused by pathogenic microorganism, there are no scientific evidence to either support or debunk these traditional claims; no available data on standardizationfor quality control and no active constituents present in *S. singueana* leaves have been isolated and characterized.

1.7 Hypothesis.

The plant *Senna singueana* contains chemical compounds which are responsible for its antimicrobial activity in traditional medicines.

1.8 Aims of study

The main aim of the present research is to analysed the phytochemical constituents and evaluate the antimicrobial properties of the extracts.

1.9 Specific Objectives

The specific objectives of the study are to:

I. Provide some pharmacognostic features of the leaves of *S. Singueana*.

II. Evaluate the phytochemicals present in the leaves of *S. Singueana.*

III. Evaluate the antimicrobial activity of the leaves of *S. singueana* extracts.

CHAPTER II

2.0 LITERATURE REVIEW

2.1 Description of FabaceaeFamily

The fabaceae family comprises one of the largest families of flowering plants numbering some 700 genera and 20,000 species. It is the third largest family of flowering plants. It is surpassed in size only by the orchid family (Orchidaceae) with about 1000 genera, 28,000 species and the sunflower family (Asteraceae) with about 32,913 acceptable species name in 1,911 genera (Christenhusz and Byng, 2016).

They are mostly herbs but include also shrubs and trees found in both temperate and tropical areas. The leaves are stipulate, nearly always alternate, and range from pinnately or palmately compound to simple.

The five largest of the genera are Astragalus (over 3,000 species), Acacia (over 1000 species), indigofera (around 700 species), Crotaleria (around 700 species) and Mimosa(around 500 species), which constitute about a quarter of all legume species. Fabaceae are economically and culturally important plants due to their extraordinary diversity and abundance.They include wide variety of edible vegetables which can also be used in horticulture and agriculture, as a food, oil and fats (Christenhusz and Byng, 2016).

2.2 Characteristics of Members of Fabaceae Family

Members of the Fabacea formerly referred to as the legume family are generally characterized by simple to compound leaves and the production of fruits known as legumes or pods, which splits open as it dries, releasing the seeds. Leguminous fruits come in an enormous variety of shapes

and sizes, including indehiscent pods that do not split open. The leaves are usually arranged spirally with stipules present that are sometimes large and leaf-like or developed into spines (Lewis *et al.,* 2005). Flowers are usually regular or irregular bisexual with a single superior carpel (hypogynous to perigynous). The principal unifying feature of the family is the fruits known as 'legume' .which are typically one-chambered pods with parietal placentation along the adaxial suture (Armstrong, 2006).

Molecular and morphological evidence supports the fact that the Fabaceae is a single monophyletic family (Lewis *et a*l., 2005).

2.3 Taxonomy

The Fabaceae are placed in the order Fabales according to most taxonomic systems, including theAPG111 SYSTEMand had been traditionally divided into three subfamilies, the Caesalpinioideae, Mimosoideae, and Papilionoideae. As Lewis and colleagues (2005) pointed out that, while there has been some disagreement as to whether Fabaceae should be treated as one family (composed of three subfamilies) or three, there is a growing body of evidence from morphology and molecules to support the legumes being one monophyletic family. This view has been reinforced not only by the degree of interrelatedness of taxonomic groups within the legumes compared to that between legumes and its relatives, but also some molecular phylogenetic studies (Kajita *et al.,* 2001; Wojciechowski, 2003; Wojciechowski *et al.,* 2004) showed strong support for a monophyletic family that is more closely related to Polygalaceae, Surianaceae, and Quillajaceae, which together form the order Fabales (Angiosperm Phylogeny Group, APG, 2009).

A recent study conducted by the Legumes Phylogeny Working Group (LPWG) (2017), divided the family Fabaceae into six subfamilies:

- **Cercidodeae** : 12 genera and 335 species. Mainly tropical *e.g cersis*
- **Detariodae** : 84 genera and 760 species. Mainly tropical. *e.g tamarindus*
- **Deparquetiodeae**: 1 genus and 1 species. West and Central Africa.e.g deparquetia
- **Dailiodaea:** 17 genera and 85 species. Widespread throughout the tropics. *Dalium*
- **Caecalpinodeae** : 148 genera and ~4400 species, *e.g senna, acacia* Includes the former subfamily Mimosoideae.
- **Fabiodeae (Papilionoideae)**: 503 genera and ~14,000 species. Astragalus. *Pisum*

The recognition of these subfamilies is based on some characteristics particularly of the flower, including size, symmetry, aestivation of petals, sepals (united or free), stamen number and heteromorphy, pollen (single or polyyads), but also presence of a pleurogram, embryo radical shape, leaf complexity and presence of root nodules (Lewis *et al.*,2005). Differences in these characteristics led to the view that the Mimosoideae and Papilionoideae are unique and distinct lineages in the family which arose independently within a paraphyletic "basal" caesalpinioid assemblage. The *Dimorphandra* group of tribe Caesalpinioideae and papilionoid tribe Swartzieae were considered likely transitional groups between them, respectively (Polhill, 1994).

2.4 Distribution and Habitat of members of Fabaceae family

Legumes are particularly diverse in tropical forest with seasonally dry aspect and temperate shrub lands. Ranging in habit from large trees to annual herbs, the family is cosmopolitan in distribution and well represented throughout temperate and tropical regions of the world (Rundel, 1989). They are usually absent in Antarctica and the high Arctic. The preference of fabaceae from semi-arid to arid habitat is related to a nitrogen-demanding metabolism, which is

thought to be an adaptation to climatically variable habitat whereby leaves can be produce economically and opportunistically (McKey, 1994).

2.5 Botanical Description of the genus Senna.

Senna, a large genus of flowering plants in the family Fabacea- Caesalpinioideae includes herbs, shrubs and trees native throughout the tropics. The number of species is estimated to be from 260– 350*(*Randel and Barlow 1998, Marazzi *et al.*, 2006).

The leaves are pinnate with opposite paired leaflets. The inflorescences are racemes at the ends of branches or emerging from the leaf axils, flowers are yellow in colour, has five sepals and five usually yellow petals. There are ten straight stamens. The stamens may be different sizes, and some are staminoids. Fruit is a legume pod containing several seeds (Christenhusz and Byng, 2016).

2.6 Senna Systematics and Taxonomy

The genus Sennahas had a complex taxonomic history (Singh, 2001). What is now known as Senna was included by Linnaeus in his concept of Cassia in *Species Plantarum*.Marazzi*et al.*,(2006) stated that in 1754, Philip MillersegregatedSennafrom Cassia in the fourth edition of The Gardeners Dictionary. Until 1982, many authors, following Linnaeus, did not recognize Sennaand Chamaecrista, but included them in a broadly circumscribed*Cassiasensu lato*. Phylogenetic analyses of DNA have shown that Chamaecrista, Cassia, and Sennaare all monophyletic, but the relationships between these three genera have not been resolved (Marazzi*et al.*, 2006). They are therefore shown in phylogenetic trees as a tritomy.

2.7 Some species of Senna

Senna comprises the following species according to ILDIS (2017), USDA, (2017)Missouri Botanical Garden(2013).

Senna acanthoclada, Senna acclinis, Senna aciphylla, Senna aculeata (*Senna acunae, Senna acuparata, Senna acuruensis, Senna acuta, Senna acutisepala, Senna affinisSenna alata* (syn.candlebush, Christmas candle, empress candleplant, candlestick tree, ringworm bush, seven-golden-candles), *Senna alcaparra, Senna occidentalis, Senna alata, Senna fistula, Senna obstusifolia, Senna alexandrina*(syn.Alexandrian senna, Indian senna, Tinnevelly senna, true senna), *Senna andrieuxii, Senna angulata, Senna angustifolia, Senna bosseri, Senna bracteosa, Senna brongniartii* (Gaudich.), *Senna burkartiana, Senna cajamarcae, Senna cana, Senna candolleana, Senna capensis, Senna cardiosperma, Senna caudata*(syn.Costa Rica, Panama), *Senna singueana* (Delile), *Senna skinneri* (Benth.), *Senna smithiana* (Britton & Killip), *Senna socotrana(serrato), Senna tuhovalyana, Senna uncata, Senna undulata, Senna uniflora, Senna unijuga, Senna urmenetae, Senna vargasii, Senna velutina, Senna venusta,* and *Senna versicolo.*

2.8 Ethnomedicinal uses of Some Senna species

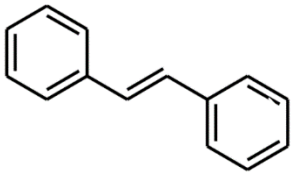
Senna species are widely used traditionally to treat a number of disease conditions such as ringworm, jaundice and some forms of intestinal complications e.g *Senna didymobotya*(Singh *et al*., 2003).Others are laxative effects (both leaves and pods of Alexandra senna are used as laxative), antimicrobial effect and antioxidant effects e.g.*Senna occidentalis* (Odeja *et.al.*,2014), antimalarial effects e.g.*Senna alata* (Kayembe *et.al.*, 2010).

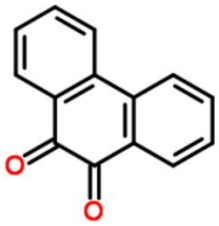
2.9 Some Phytochemicals Reported from some Species of Senna

The genus Senna is known to be a rich source of anthraquinones,and flavonoids (Singh and Tiwari, 1982;Anthony *et al.*, 2013). Senna contains abundant of glycosides such as anthraquinone known as senna glycosides or sennosides. Column chromatographic separation of the root extract of *Senna didymobotrya*(Fig 2.1) affordeda stilbene derivative (1) and a phenylanthraquinone (2) (Alemayehu *et al.*,2015).

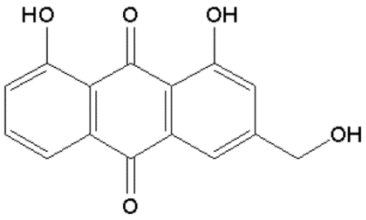
Alkaloids, flavonoids, resins, antraquinones, saponins, tannins, and phenols have been identified in the leaves (fresh and dried) and seeds extracts of *S. alata* (Archana *et al.*, 2012). Phytochemical investigation of *Cassia alata* Linn (Fig 2.1) led to the isolation of six known anthraquinones: aloe-emodin (3), emodin [4], ω-hydroxyemodin (5), lunatin [6], physcion (7), and ziganein (8), six flavonoids: apigenin (9), 7,4′-dihydroxy-5-methoxyflavone [10], diosmetin (11), kaempferol (12), luteolin [13], and trans-dihydrokaempferol (14) as well as one stilbene,trans-resveratrol (15)(Promgool *et al.*,2014).

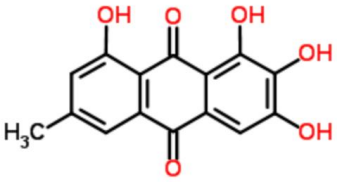
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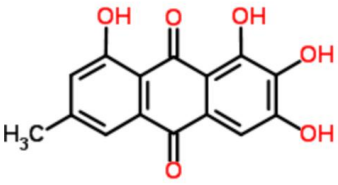


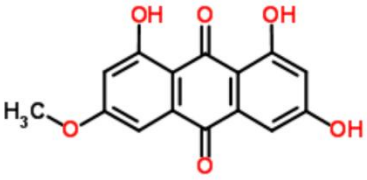
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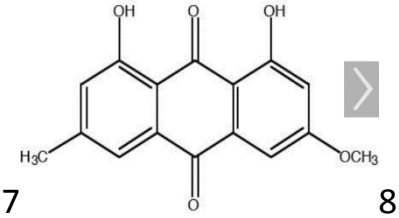




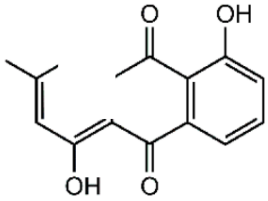
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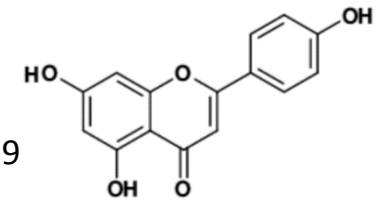


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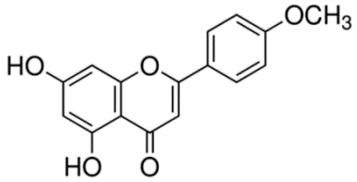
Fig. 2.1: Chemical Structures of some Phytochemicals Isolated from *Senna singueana*

Source*:* (Alemayehu *et al.*,2015; Promgool *et al.*,2014).

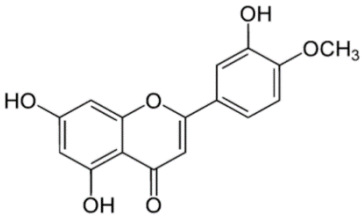
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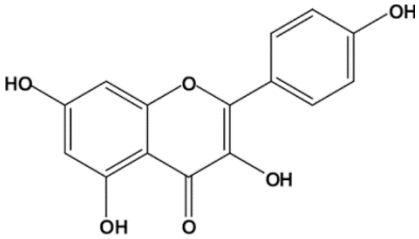
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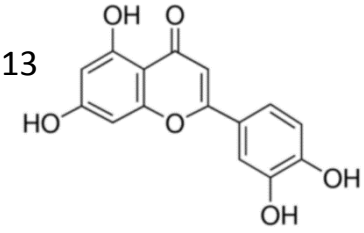
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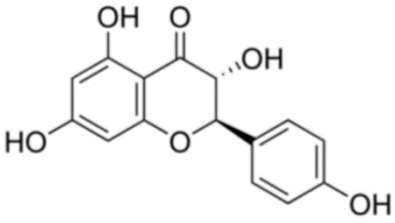
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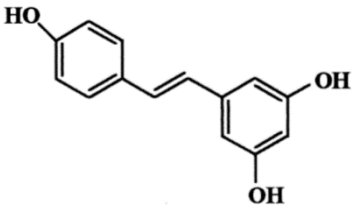


Figure 2.1 Contd:

Source*:* (Alemayehu *et al.*,2015; Promgool *et al.*,2014).

In *Senna fistula*, evaluation of phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, protein and amino acids, saponins, and triterpenoids revealed the presence of most of constituents in polar extracts (ethanol, methanol, and aqueous) compared with nonpolar extracts (petroleum ether and chloroform)(Panda *et. al*., 2012).

2.10 Antimicrobial Activity of *Senna*Species

Senna alata leaves extract showed both antibacterial and antifungal activities (Owoyale *et al*., 2005; Ogunjobi and Abiala, 2013). The tested organisms include: *Escherichia coli, Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Mucor, Rhizopus, Aspergillus niger, Candida albicans and Saccharomyces*. The methanol extract was the most active of the three crude extracts. Other extracts used were petroleum ether and ethanol. Highest activity of the methanolic extract was displayed against *Pseudomonas aeruginosa*(17mm) *and Staphylococcus aureus* (12mm). However, methanolic crude extract has a narrow spectrum of activity not being able to inhibit successfully *Candida albicans* and *Saccharomyce*s (Owoyale *et al*., 2005). Antimicrobial properties of the leaves extracts of *Senna obstusifolia* were investigated against clinical and laboratory isolates of both bacteria and fungi using the disc diffusion method. Acetone extract demonstrated the highest activity followed by dichloromethane, methane and hexane extracts (Doughari *et al*., 2008).

2.11 Botanical Description of *Senna singueana*

Senna singueana plant (Plate I) is a shrub or small tree, scaly fissured and thick bark, with deciduous and open crown. branchlets glabrous to densely pubescent, bark reddish, becoming grey-brown and rough with age(Burkil, 1995). It has a compound leaf (plate II), with 4-10 pairs

of oval leaflets, 2.5-5 cm long, with a conspicuous gland between each pair of leaflets, rounded at apex, glabrous or nearly (Endo and Naoki, 1980, Khelet and Hansen, 2004).

Flowers are yellow (Plate II) to deep yellow in colour, fragrant, in racemes to 15 cm, flower stalks 2-4 cm, with conspicuous glands and often aggregated towards branchlet-ends and often produced when the plant is without leaves; (Bein *et al.*, 1996; Khelet and Hansen, 2004). Pods linear, straight or somewhat twisted, torulose, slightly compressed, 5-26 cm long, indehiscent, with stiff and rather hard valves, glabrous to pubescent, rounded to abruptly acute and often apiculate at apex; yellowish when ripe. Seeds dull brown, almost circular, flattened, 5-6 mm in diameter, with a small areole 2-2.5 x 1-1.5 mm on each face (Neuwinger, 2000; Adzu *et al.*, 2003).

The tree grows up to 15 metres tall and brings forth its flowers before the onset of rains (one of its unique characteristics).

The leaflets are not particularly variable in size or shape, but range from completely glabrous to pubescent. It bears the name '*Cassia singueana* Delil*e* var. subgeabsens' (Brenan, 1967 and Kokwaro, 1993). This is presumably a corruption of 'subglabrescens' (Ken*et al*., 2014).

English names includescrambled eggs, sticky pods, wild cassia and winter cassia..Locallyit is called 'Rumfu' in Hausa, "Rumbu'among the Kanuri speaking people in Northern Nigeria. Shadarat al bashime in Arabic (Niger) (Burkil, 1995).



Plate I: Picture of *Senna singueana* Shrub in it Natural Habitat



Plate II a. Picture of *S.singueana* Leaves and flowers



Plate II b: Picture of *S.singueana*podContaining Seeds

2.12 Origin, Ecology and Distribution of *S. sisngueana*

Senna singueana originated from mainland tropical Africa and is probably only absent from Gabon, Djibouti and Somalia (Burkil, 1995).*Senna singueana* occurs in thickets, woodland, savanna and dry evergreen forest, often on termite mounds, from sea-level up to 2250 m altitude. It is found in areas with an annual rainfall of 500–1000 mm (Neuwinger, 2000).*S. singueana* is a common medicinal plant which is widely distributed in the wild in Africa including Nigeria, Namibia, Zambia, Malawi, cote d'ivoire, south to Congo and Mozambique (Ken *et al.,* 2014).

2.13 Management

Senna singueana is propagated by seedlings, including wildlings, and is quick growing. Dry seed stored in an airtight container remains viable for at least 3 years. Seed germinates in about 9 days with an average germination rate of 78%.If a tree with a straight stem is required, protection against browsing animals is needed.

2.14 Ethnomedicinal uses / Biological activities of *S. singueana*

Leaves: The leaves are eating as vegetable in Tanzania and Malawi. Bananas are wrapped in the leaves to speed ripening. The leaves have been shown to have anthelmintic, antiviral and antibacterial properties; it also contains tannins and are astringent. An infusion of the leaves is used as a remedy for venereal disease, malaria, convulsions, epilepsy, coughs, intestinal worms, constipation, heartburn and stomach-ache (Bein*et al.*, 1996). A hot water infusion of the leaves is drunk and the warm leaves are applied as a compress to treat fever. The leaves, either as a decoction or infusion, or as a dried powder, are applied to wounds caused by leprosy and syphilis. An infusion of the leaves is applied as eye drops to cure conjunctivitis (Owoyale *et al*., 2005).

Stem bark: the stem bark is used as dye for textiles in Europe and Zambia and for tanning hides in large parts of East Africa.Extracts of the stem bark are taken to cure stomach complaints. Like the leaves, the stem bark is used to treat skin disorders and malaria (Ken*et al.,* 2014).

Roots: Extracts of the root bark have shown significant analgesic, antipyretic, anthelmintic and antiplasmodial activity. A decoction of the roots is used to treat wounds and as a remedy for diarrhoea, convulsions, dementia and Sexually Transmitted Diseases. The roots are used to treat venereal diseases, stomach complaints and as a purgative. The roots are also used to cure impotence caused by diabetes. The ash of burnt roots is eaten mixed with porridge to cure abdominal pain. Phytochemical screening of the root extract revealed the presence of phenols, saponins, tannins and some traces of anthraquinones (Hemen and Lalita, 2012).

Fruits: The fruits are used in Sudan for tanning skin (Ken*et al.,* 2014).

Various works has been done on the plant which supported the medicinal uses its parts in different parts of the world. These include;

1. (Hibeng *et al.,*2015), evaluated *Senna singueana* leaf extract as an alternative or adjuvant therapy for malaria. The extracts from the root bark of this plant exhibited antiplasmodial activity; however, the leaves are the more sustainable resource. Hence, *S. singueana* leaf is a potential alternative or adjuvant therapy for malaria, as 70% aqueous ethanol extract of *S. singueana* leaf was safe to mice and possessed some parasite suppression effect. Co administration of the extract with Chloroquine appeared to boost the overall antimalaria effect, indicating that the combination may have a net health benefit if used as an adjuvant therapy.
2. Ibrahim and Islam (2014), studied the anti-diabetic effects of the acetone fraction of *Senna singueanna* stem bark in a type 2 diabetes rat model, the result suggest that orally

administered the acetone fraction could ameliorate most of the type 2 diabetes-induced abnormalities in a type 2 diabetic model of rats.

3. Antinocieptive activity of the root extracts of *Senna singueana* was carried out by Hellen *et al.*(2012), using both tail flicks and hot plates methods, although, they documented that the root extract of *S. singueana* (100mg/Kg) showed no significant antinocieptive activity in both methods, but 200mg/Kg of the extract showed remarkable antinocieptive activity in both methods.
4. Missa*et al*. (2015) reported the phytochemical screening, total phenolics content and antioxidants activity of *S.singueana* leaves and seeds. They reported that the extract have reducing power on DPPH radical scavenging abilities. Ethyl acetate and methanol of the leaves have the highest total reducing power whereas, the methanol and ethyl acetate extracts of the seeds part have more potent free radical scavenging activity than all the other extracts using DPPH free radical scavenge capacity assay. They also reported the presence of alkaloids, coumarins, flavonoids, sterols, saponins and tannins. Quantitative analysis of the two parts of *Sennasingueana* for phenolic flavonoids and tannins compounds revealed that the total phenolic content ranged from 122.75 to 376.1 and 286.94 to 1990 mg/g of dry weight of leaves and seeds extracts respectively which expressed as gallic acid equivalent.
5. Nutritional Composition and Fatty Acids Analysis of *Senna singueana*Leaves and Seeds as studied by Missa*et al*.(2015) which showed the proximate analysis and fatty acid composition of the seeds and leaves using Association of Analytical Chemist (AOAC) (1990)methods. Theyreported that the seeds contain crude protein (14.88%), crude fiber (15.85%), moisture (4.07%), total ash (7.67%), total carbohydrate (55.68%), total sugar

(0.078%) and total oil (1.85%). While the results of proximate analysis of theleaves showed that the crude protein (11.38%), crude fiber (1.36%), moisture (2.92%), total ash (6.93%), total carbohydrate (74.09%), total sugar (0.08%) and total oil percentage (3.32%). The fixed oils extracted from seeds and leaves were evaluated for chemical composition. While the GC-MS analysis showed the presence of various saturated and unsaturated fatty acids such as stearic acid (5.330%), oleic acid methyl ester (1.986%), Behenic acid methyl ester (3.06%) and palmatic acid methyl ester (1.515%) of *Senna singueana* leaves and the fatty acid found in the seeds part were eicosanoic acid, methyl acid (4.17%), hexadecanoic acid, methyl ester (1.515%), teteracosanoic acid, methyl ester (3.64%) and 16 - octadecenoic acid, methyl ester(26.01%).

Also leaves and seeds indicate the presence of calcium, magnesium, iron, zinc, magnesium and copper at different concentrations (Missa *et al.*, 2015).

6. In an *in vivo* assessment of the antimalarial activity of *Senna singueana*by Ior*et al.*, (2015), 50% aqueous ethanol*e*xtractof *S. singueana* barks suppressive and prophylactic anti-plasmodial activities against chloroquine sensitive strain of *Plasmodium berghei berghei* in mice showed that 400-600 mg/kg has chemosuppressive effects(72.7% to 90.5%). The pathological effects associated with malaria infections (pyrexia and weight loss or poor weight gain)also showed a promising results. They also report the presence of carbohydrates, alkaloids, tanins, flavonoids, cardiac glycosides, saponins and steroids in the plant, with an oral median lethal dose of both extracts was greater than 5000mg/kg body weight.

Although *Senna singueana* has numerous medicinal uses, research into its pharmacology has been scarce and restricted to the root bark (Adzu *et al.*, 2003). Because of their many medicinal

uses, research into the properties of the leaves is warranted. The leaves are a more sustainable source of medicine than root or stem bark (Kew, 1988).The morphological variation in the species in connection to its uses and variation in phytochemistry needs further research(Adzu *et al.*, 2003).

CHAPTER III

3.0 MATERIALS AND METHODS

3.1 Materials, Chemicals, Equipment, Solvents, Reagents/Solution

3.2 List of reagents and solvents

Acetic acid (Avondale Laboratory, England)**,** Aluminum chloride**,** Anisaldehyde (Sigma-Aldrich, St. Louis, MO, USA)**,** Chloral hydrate (BDH Laboratory Chemicals Division, POOLE. England)**,** Chloroform**,** Dragendorff Reagent, Picric acid reagent, Mayer's reagent, Ethyl acetate (JHD, AR; Lobal Chem, India)**,** Ferric chloride**,** Glycerol (BDH Laboratory Chemicals Division, POOLE. England),Hexane (JHD, AR; Lobal Chem, India), Hydrochloric acid (BDH Laboratory Chemicals Division, Poole. England)**,** acetic anhydride, chloroform, suiphuric acid **,** Methanol (JHD, AR; Lobal Chem, India)**,** Methylene blue**,** Phloroglucinol,Sudan Red Solution**,** Tetraoxosulphate (VI) acid (Sigma-Aldrich, St. Louis, MO, USA)**,** TLC silica gel 60 F_{254} pre-coated plates (Merk-Germany)**,** Zinc chloride (Tianji Trade Limited Company, China). Culture media, DMSO, Ciprofloxacin (Maxter Bio-Genics, India).

3.3 List of Equipment and Apparatus

Ash less filter paper, Compound microscope (Fisher Scientific, UK), Dessicator, Disposable syringes, Glass Slides and Cover slips, KERN EW Electronic Balanced, Laboratory glass wares (Funnel, Conical flasks, Beakers, Measuring cylinder), Mechanical shaker (Stuart Scientific Flask Shaker, Great Britain), Metallic cages and feeding bottles for rats, Microtome (C 740527, Cambridge Instrument Company Ltd, London and Cambridge, England), Oven, Photographic camera, Plant press (localyl made), Slide dryer (Hospital and Lab. Supply Ltd, London, UK), Stage Micrometer and Ocular Lens (Graticules Ltd, Ton bridge, Kent. England), TLC tanks (Uni

kit® TLCChromatank®, Shandon, Germany), UV lamp, Water bath (HHS, Mc Donald Scientific International).

3.4 Plant Collection, Identification and Preparation

The fresh plant parts (the leaves, fruits and flower) were collected by a local plant collector (Mallam Ibrahim) and was identified as *Senna singueana* by a taxonomist (Mallam Namani Sanusi) at the herbarium section of the Department of Botany,Ahmadu Bello University, Zaria Nigeria, in August, 2016 with the voucher specimen number 6863. Sufficient quantity of the leaves were collected and dried at room temperature. It was powdered and stored at room temperature in a close container for further uses.

3.5 Pharmacognostic Evaluation of the Leaves of *Senna singueana*

Experimental Design; Pharmacognostic standards of the leaves of *S. singueana* were studied by examining the organoleptic, microscopicaland chemomicroscopical characteristic in details to establish some pharmacopoeial standards and characters for it identification.

3.5.1 Organoleptic Evaluation

Macroscopic observations conducted on the leaves of the plant include: colour, odour, taste, texture, shape and fracture (Brain and Turner, 1975).

(a) Colour: The colour of the dried leaves was determined under diffused daylight and result was recorded.

(b) Odour: Powdered leaves was placed on the palm of the left hand and air was blown using the right hand odour were perceivedusing nose.

(c) Taste: The dried and powdered leaves was placed on the palm and tasted using the tongue.Taste was noted and result was recorded.

(d) Surface texture: The texture of the dried sample of the leaves was determined by feeling of its outer and inner surfaces using the thumb and first finger.

(e)**Size.** A graduated ruler in centimetres/millimetres was used for the measurement of both the length and width of 25 pieces of theleaves.

3.5.2 Microscopical examination on the Leaves of *S.singueana*

(a) Surface Preparation and AnatomicalSection

Anatomical section of the leaf sample was examined under the microscope and features were described by using the terms according to Dutta, (2003) and Evans, (2002).

The transverse section across the midrib of the fresh leaf of *S. singueana*was prepared using a blade.Leaf epidermises (upper and lower) were peeled. The prepared sections were cleared using 70% chloral hydrate solution and boiled on a water-bath for thirty minutes to remove obscuring materials. The cleared sample was mounted on a microscope slide, using dilute glycerol to moisten and enhance it view under the microscope. Features were observed under the microscope and appropriate images of the diagnostic features were taken and documented (WHO, 2011).

3.5.3 Chemo-microscopic Studies on the Leaves of *S.singueana*

This was carried out on the powdered leaves of the plant. Small amount of the finely ground powdered leaves was cleared in a test-tube containing 70% chloral hydrate solution. The cleared sample was mounted on a microscope slide, using dilute glycerol. Using various detecting reagents, the presence of some cell inclusions and cell wall materials were detected in accordance with Brain and Turner, (1975) and Evans,(2002).

(a) *Test for Cellulose:*Two drops of iodinated zinc chloride were added to the cleared sample on a glass slide.This was allowed to stand for 2 minutes. One drop of sulphuric acid was added. The cover- slip was placed and observed.The present or absence of blue colour will indicates the presence or absence of cellulose in the cell walls.

(b) *Test for Lignins:*Two drops of phloroglucinol was added to the cleared sample and allowed to stand until almost dry. A drop of hydrochloric acid was added and cover slip was placed.This was observed under the microscope. The present or absent of red colour on the anatomical section will indicates the presence or absence of lignin.

(c) *Test for Suberin:* Two drops of Sudan red was added to the cleared powdered sample on a slide. The cover slip was placed and this was gently heated over hot water bath for 2 minutes. The slide was then observed under the microscope.The present or absent of orange red colour will indicates the presence or absence of suberin in the cell wall.

*(d) Test for Gum and Mucilage:*A drop of ruthenium red was added to the cleared sample on a glass slide. The cover slip was placed and observed under the microscope. The present or absent of pink colour will indicates the presence or absence of gums and mucilage**.**

(e) *Test for Starch grains:*Two drops of N/50 iodine solution was added to the cleared sample and cover slip was placed. This was observed under the microscope.The present or absent of blue-black or reddish-blue coloration on some grains will indicate the presence or absence of starch.

(f) *Aleurone grains:* A few drops of millon's reagent were added to the powdered leaf sample. Cover slipwas placed and observed under the microscope. The present or absent of yellowish brown to brown colour will indicates the presence or absence of aleurone grains.

(g) *Test for Calcium oxalates:* To the cleared sample on a glass slide, cover slip was placed and this was observed under the microscope. It was removed and two drops of hydrochloric acid was added and then observed under the microscope.The present or absent of the dissolution of shining crystals on the anatomical sections of the leaves will indicates the presence or absence of calcium oxalates.

(h) *Test for Calcium carbonates:* concentrated hydrochloric acid was added to the powdered sample on the glass slide. A cover slip was placed and observed under the microscope.The present or absent of effervescences on the slide showed the presence of calcium carbonate.

(i) *Test for Tannins:* A single drop of ferric chloride was added to the cleared sample on a glass slide. The cover slip was placed and this was observed under the microscope. The present or absent of greenish black colour on some cells of the anatomical sections of the leaves will indicates the presence or absence of tannins

3.6 Determination of Physicochemical Constants of the Powdered Leaves of *S.singueana*

The quantitative physical standard (solvent extractive values, ash values, moisture content) were determined as described in the British Pharmacopoeia (2012).

3.6.1 Moisture Content

The loss on drying method was adopted for this procedure. The powdered leaves of the plant (3g) were weighed in a crucible. It was then heated for 1hour in an oven maintained at 105^0C cooled in a desiccator and re-weighed. The procedure was repeated until no further loss in weight was obtained. The moisture content was determined in percentage as:

$$\text{Moisture Content (\%)} = \frac{Weight of Water Lost}{Original Weight of Sample} \times 100$$

3.6.2 Ash Values

(i) Total Ash

The total ash of the powdered leaves of the *S. singueana* was determined by weighing 2 g of the powdered sample in a crucible. This was heated gently at 450^0C. Heating was done until all the carbon was removed. The total ash value was calculated in percentage as:

Total Ash Value (%) = $\frac{Weight\ of Residual\ \ Ash}{Weight\ of\ Sample}\ x\ 100$

(ii) Acid Insoluble Ash

Dilute HCl (25 mL) was added to the ash obtained in (i) above. This was boiled for 5 minutes and the insoluble matter was collected on ashless filter paper. The beaker containing the acid and crucible were washed in hot water and the washings were passed through the filter. The washing was continued until the residue was free of acid. The residue and filter were dried gently in an oven and ignited in a tarred crucible. It was allowed to cool and weighed. The acid insoluble ash was calculated in percentage as:

Acid Insoluble Ash (%) = $\frac{Weight\ of Residual\ \ Ash}{Initial\ \ Weight\ of\ Sample}\ x\ 100$

(iii) Water Soluble Ash

The water soluble ash value of the powdered leaves of the plant was determined separately. The ash obtained following method (i) above was used. The procedure used to obtain the water soluble ash was the same as in (ii) above except that water was used instead of dilute HCl. The water soluble ash was determined in percentage as:

Water ash (%) = $\frac{Weight\ of Initial\ as\ \ h - Weight\ of Residual As\ \ h}{Initial Weig\ \ ht of Sample} \times 100$

3.6.3 Solvent Extractive Values

(i) Alcohol Soluble Extractive Value

Powdered drug (5 g) was macerated with 100 mL of alcohol in a stoppered flask, shaking was done with the aid of a mechanical shaker during first 6 hrs and allowed to stand for 18 hr. It was filtered after 24 hr. The filtrate (20 mL)was evaporated in a tarred evaporating dish at 105℃ in an oven and weighed. Alcohol soluble extractive value was calculated (in percentage) with reference to the initial weights of the extract, five determinations were recorded and the average determined.

Alcohol Extractive Value (%) = $\frac{Weight\ of\ residure \times 5}{Weight\ of\ Sample} \times 100$

(ii)Water Soluble Extractive Value

The dried powdered leaves (5g) was weighed into a 250 mL glass stoppered conical flask and 100 mL of Chloroform-water (few drops of chloroform in water inorder to preserve it from fungi growth) was added to macerate the powder for 6 hours with frequent shaking by using mechanical shaker and was allowed to stand for 18 hours. It was then filtered rapidly and 25 mL of filtrate was transferred into a previously dried and weighed evaporating dish and evaporated to dryness on a hot water bath. This was further dried in the oven at 105^0C for 6 hours, cooled in a desiccator for 30 minutes and then weighed without delay. The percentage water extractive value was calculated using the following formula:

$$\%\ Water\ Extractive\ Value = \frac{Weight\ of\ Extract\ in\ 20ml\ X\ 5}{Original\ Weight\ of\ Powder}\ X\ 100$$

3.7 Extraction and Phytochemical Analysis of *S. singueana* leaves

3.7.1 Extraction of *S. singueana* leaves

Extraction of the plant material was done using the method described by (Kokate*et al.*, 2003). The pulverized plant sample (500 g) was extracted with n-hexane, ethyl acetate and methanol successively in a soxhlet apparatus (Fig. 3.1). The extracts were concentrated to dryness using the water bath. The extacts were stored in desiccator for subsequent use. The percentage yield of each extract was calculated using the formula:

Percentage yield of extracts $= \frac{Weight\ of\ total\ extract}{Weight\ of\ powdered\ \ material} \ x\ 100$

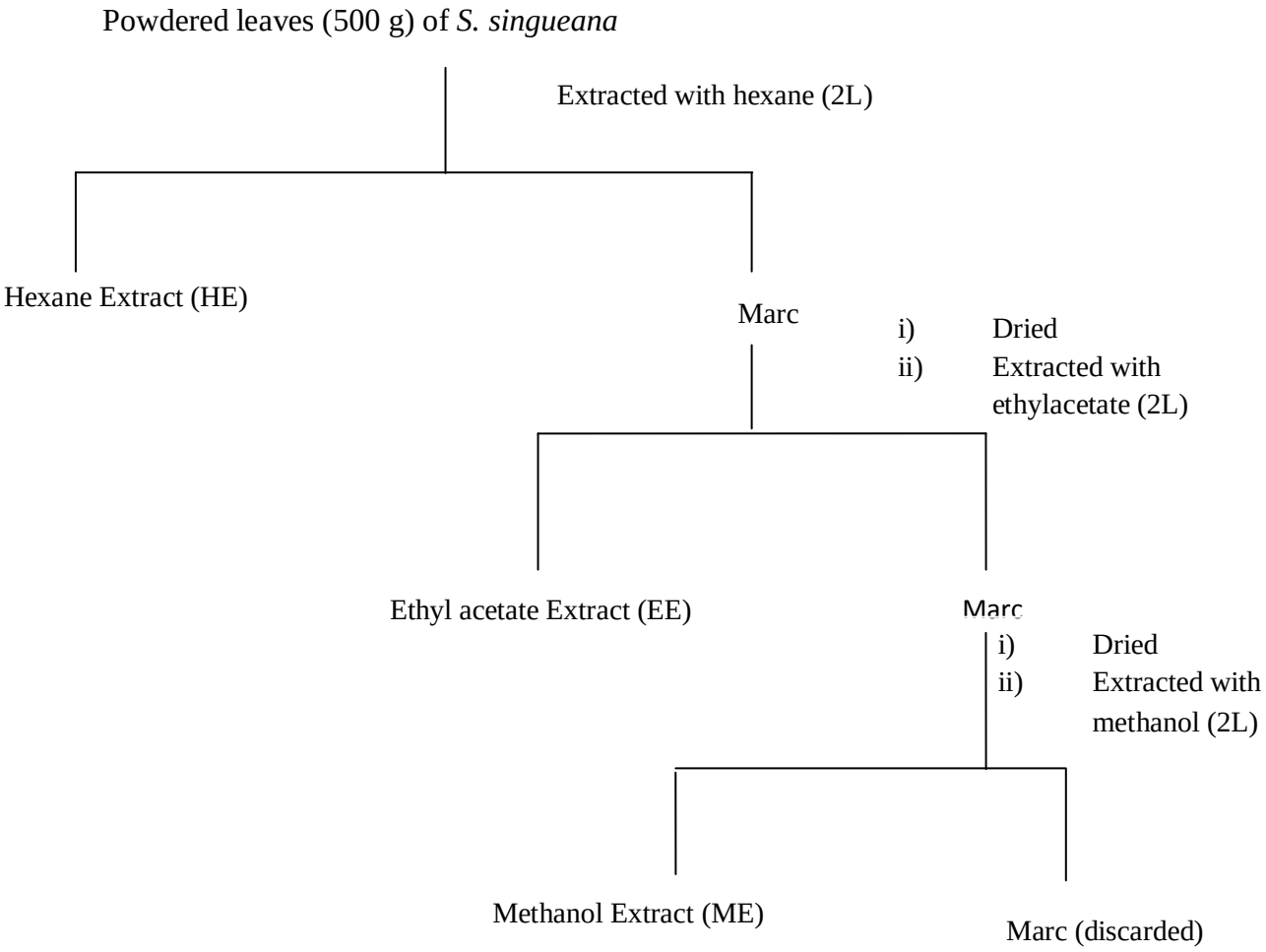


Fig. 3.1: Flowchart of Soxhlet Extraction of *S. singueana*Leaves

3.7.2 Preliminary Phytochemical Screening

Preliminary phytochemical tests werecarried out on the hexane extract (HE), ethyl acetate extract (EE) and methanol extract (ME) according to standard procedures as follows:

(i) Test for carbohydrates (Molisch's test)

To the small portion of the three extracts (HE, EE and ME) in three separate test tube, 3 drops of Molisch's reagent was added individually, thenfollowed by addition of concentrated sulphuric acid. The formation of a reddish coloured ring at the interface indicates the presence of carbohydrates (Evans, 2002).

(ii) Test for saponins (Frothing test)

To the small portion of the extracts (HE, EE and ME) in three separate test tubes, 10 mL of distilled water was added and then shaken continuously for 30 seconds. The solutionswere allowed to stand for 30minutes; the formation of a persistent froth for 15 minutes indicates the presence of saponins (Evans, 2002).

(ii) Test for flavonoids

a. Shinoda test: The extract was dissolved in 2 mL of methanol and pieces of metallic magnesium chips were added. This was followed by additions of few drops of concentrated hydrochloric acid.The formation of a pink, orange, or red to purple coloration indicates the presence of flavonoid (Evans, 2002).
b. Sodium hydroxide test:The hexane extract (0.5 g) was dissolved in water and filtered; 2 mL of 10% aqueous sodium hydroxide solution was then added. The solution was observed for the presence of yellow colour, a change in colour from yellow to colourless on addition of dilute hydrochloric acid was used as an indication for the presence of

flavonoids. The procedure above was repeated for ethyl acetate and methanol extracts (Evans, 2002).

(iv) Test for tannins

a. Lead sub-acetate test: To the small portion of the extract 4 drops of lead acetate solution was added. The formation of a cream coloured precipitate indicates the presence of tannins (Evans, 2002).

b. Ferric Chloride Test: To the second portion of each of the extracts in a test tube, 1 mL of ferric chloride solution was added. The green or dark green precipitate indicates the presence of condensed tannins (made up of catechins and epicatechins) while a blue or blue – black precipitate indicates the presence of hydrolysable tannins.

(v) Test for steriods/ triterpenes

a. Salkowski test: The hexane extract(0.5 g) was dissolved in 2 mL of chloroform and 1 mL of concetrated sulphuric acid was added carefully from the side of the test tube to form a lower layer. A reddish brown coloration at the interface indicated the presence of steroidal ring. The procedure above was repeated for ethyl acetate and methanol extracts (Sofowora, 2008).

b. Lieberman-Burchard test: Two mL of acetic anhydride was added to 0.5 g of the hexane extract dissolved in 2 mL of chloroform. 1 mL of sulphuric acid was then added gently by the side of the test tube to form the lower layer. The formation of reddish brown or violet brown ring at the junction of the two liquids and a greenish or bluish colouration in the upper layer indicates the presence of sterols and/ or triterpenes. The procedure above was repeated for ethyl acetate and methanol extracts (Evans, 2002).

(vi). Test for Alkaloids

a. Dragendoff's Test: The extract (0.2g) was dissolved in 2 mL of 1% aqueous hydrochloric acid with continuous stirring on a water bath. The mixture was filtered and few drops of Dragendoff's reagent was added,formation of rose red precipitate indicates the presence of alkaloids (Evans, 2002).
b. Mayer's test: The acidic solution of the extract(2 mL) in a test tube, few drops of Mayer's reagent were added, the formation of cream precipitate indicates the presence of alkaloids (Evans, 2002).

(vii*). Test for Anthraquinones*

a. Bontrager's test:The extract (0.5 g) was dissolved in 5 mL chloroform. It wasshaken and filtered. an equal volume of 10% ammonia solution was added to the filtrate with continuous shaking. The formation of bright pink colour in the aqueous upper layer indicates the presence of anthraquinone (Evans, 2002).

b. Modified Bontrager's test:The hexane extract 500 mg was boiledwith 2 mL of dilute sulphuric acid and filtered when hot. The filtrate after cooling at room temperature was shaken with 5 mLchloroform. Thechloroform layer was separated and to half of its volume, 10% ammonium hydroxide was added. The formation of pink, red or violet coloration in the ammonia phase (lower phase) is an indication for the presence of combined anthraquinones or anthraquinone derivatives. The procedure above was repeated for ethyl acetate and methanol extracts.

(viii)*Test for Cardiac Glycosides (Keller-Kiliani Test)*

A small portion of the extract was dissolved in 1mL glacial acetic acid containing traces of ferric chloride solution. The solution was then transferred into a dry test tube to which an equal volume of sulphuric acid was added. The formation of brown ring at the interface indicates the presence of a deoxy sugar. (Evans, 2002).

3.8 Thin Layer Chromatographic Analysis.

The crude extracts of hexane, ethyl acetae and methanol were subjected to thin layer chromatography. In this procedure, precoated TLC plates were used to carryout the thin layer chromatography by one way ascending technique. Capillary tubes were used to manually apply spots on the TLC plate and the chromatogram was developed in an air tight chromatographic tank at room temperature employing the solvent systems listed below. The spots were visualized by the use of spray reagent (*p*- anisaldehyde) followed by heating in an oven at 105^{o}C for about 3 minutes. The various TLC solvents system and adsorbent used include:

i. Adsorbent: silica gel G thickness 0.25mm activated at 105^{o}C for 1hr
ii. Solvent system. Hexane 100, Hexane: ethyl acetate (9:1, 3:2, 7:3), chloroform: methanol 4:1

More chromatograms from best solvent systems that resolved the extracts were developed for HE,Hexan 100 percent, 19:1 Hexane:Ethyl acetate, , for EE, Hexane:Ethyl acetate7:3, and for ME 4:1 chloroform: methanol. Specific reagents were used on the chromatograms. Specific reagents used include**:**Aluminium chloride for flavonoids, Ferric chloride for phenolic compounds, Dragendorff reagent for alkaloids, Bontrager's reagent for anthraquinones,

Liebermann - Buchard sprayfor steroids and triterpenes and UV light at 254 and 366nm photographs of the plates were taken immediately after they were developed.

3.9 Column Chromatography

The n- hexane extract (HE) was subjected to column chromatography. A 75 cm by 3.5 cm glass column and a stationary phase silica gel of 60-120 mesh size were used. Wet packing and dry sample loading was used for silica gel column chromatography. Gradient elution was used starting with 100% n-hexane solvent, and gradually the polarity was increased by successive addition of solution of hexane:ethylacetate (19:1) . Small fractions of the eluate were collected sequentially in labelled beakers. A total of 100 collections of 20ml each for the first 50 collections and 50 ml for the last 50 collections were made and the composition of each fraction was analysed by TLC. The collection was pooled together on the basis of their TLC profiles to obtain 16pooled fractions coded SS1 – SS16.

3.9.1 Preparative Thin Layer Chromatography

Based on the results of the TLC of the pooled fractions from column chromatography,pooled fractions 11 – 18 coded (SS5) was purified by Preparative TLC using solvent system, hexane: ethyl acetate 19: 1. A blade was used to scrap off the silica containing the compound and this was placed on a filter paper and wash with hexane solvent. The filtrate was analysed using TLC plate for an appearance of a single spot.Spot was visualized using p anisaldehydes for general and Libermam buchard for specific spray.

3.10 Antimicrobial Activity of the Leaves Extract of *Senna singueana*

3.10.1 Collections of clinical isolates.

Twelve (12) clinical isolates were collected from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH)Shika - Zaria. The experiment was carried out using agar well diffusion method as described by Azoro(2002) and Mobasher (2005).The clinical isolates used include:

1. *Campylobacter jejuni*-Gram negative bacteria
2. *Candida krusei*- Fungi
3. *Candida tropicalis*- Fungi
4. *Candidiaalbican*- Fungi
5. *Escherichia coli*– Gram negative bacteria
6. *Neisseria gonorrhoea* – Gram negative bacteria
7. *Proteus mirabilis*– Gram negative bacteria
8. *Pseudomonasaeruginosa*– Gram negative bacteria
9. *Salmonella typhi*– Gram negative bacteria
10. *Shigella dysentery*– Gram negative bacteria
11. *Staphylococcus aureus*– Gram positive bacteria
12. *Streptococcus feacalis*– Gram positive bacteria

3.10.2 Preparation of Stock Solution

The hexane, ethylacetate, and methanol extracts (0.1 g) each in separate sample bottles of wereweighed and dissolved in 10 mLof dimethyl sulfoxide (DMSO) to obtain a concentration of 10mg/L. This was the initial concentration of the extract used to determine the antimicrobial activities from the plant. Two - fold serial dilution of the extracts were made to obtain the concentrations of 5mg/mL, 1.25mg/mL and 0.63mg/mL. The initial concentration was obtained by dissolving 0.1g of the extract in 10 ml of the sterile broth. Ciprofloxacin and fluconazole were used as the positive control drugs for bacteria andfungirespectively (Azoro, 2002).

3.10.3 Preparation of Culture Media and Standard turbid solution

The culture media for determination of antibacterial and antifungal activities were prepared according to the manufacturer's instructions;

a. Using the Mueller Hinton agar: The agar (36g) was weighed and dissolved in 1000 mL of distilled water, gently heated over the flame to dissolve and sterilized at 121^{o}C for 15minutes.The prepared medium was then poured into sterilized petri dishes and was allowed to cool and solidify. Mueller Hinton agar was used as the growth medium for the microbes.
b. Subourand dextrose agar**:** The agar (28g) was weighed and dissolved in 1000mL of distilled water, gently heated over the flame to dissolve and was sterilized at 121^{o}C for 15 minutes.

The Standard Turbid Solution: Mc– Farland 0.5 Barium sulphate Turbidity Standard: This was prepared by dissolving 0.5 mL of 0.004 M BaCl in 99.5 mL of 0.3 N sulphuric acid using normal saline containingthe isolates to give turbid solution.

3.10.4 Screening of the Extracts for Antimicrobial Activity

The agar diffusion method was used for screening of the extracts for antimicrobial activities.

The inocula were poured and spread evenly over the surface of the sterilized solid medium by the use of a sterile swab. Using the standard cork – borer (6mm in diameter), wells were boredat the centre of each inoculated medium. The solutions of the extracts (0.1 mL) wereintroduced into each well on the inoculated medium. Incubation was made at 37^{o}C for 24 h. The plates were then observed for zones of inhibitions of growth. The zones were measured using a transparent ruler and the result recorded in millimetres (Azoro, 2002; Mobasher, 2005).

3.10.5 Minimum Inhibition Concentration of Extracts

The Broth Dilution method was used to determine the minimum inhibition concentration of the extracts (EUCAST, 2000).Mueller Hinton broth was preparedand was dispensed into 5 test tubes (double strength of 10 mL to the first test tube and then single strenght 5 mL each to the rest of the test tubes) and sterilized at 121^{o}C for 15min, the broth was then allowed to cool.Normal saline (10 mL) was prepared and dispensed into a separate sterile test tube and the test microbes were inoculated and incubated for 6 hrs at 37^{o}C**.** Dilution of the test microbes was done in the normal saline until the turbidity marched that of the Mc – Farland's scale by way of visual comparison. At this point, the test microbes have a concentration of about 1.5 x 10^{8}CFU/ mL.The initial concentration of extracts was obtained by dissolving 0.1g of the extract in 10 mls of the sterile broth Two – fold serial dilution of the extracts in the broth were made to obtain the concentrations of 10 mg/mL, 5mg/mL, 2.5mg/mL, 1.25mg/mL and 0.63mg/mL..Having obtained the different concentrations of the extracts in the sterile broth,0.1ml of the microbial suspension was then inoculated into the different concentrations of the extracts**.** Incubations were made at 37^{o}C for 24 hours.The test tubes of the broth were then observed for turbidity(growth) and the

lowest concentration of the extract in the broth which shows no turbidity was recorded as the minimum inhibition concentration of extract (EUCAST, 2000).

3.10.6 Minimum Bactericidal and Fungicidal Concentrations (MBC/ MFC)

This was done to show whether the test microbes were killed or their growth was inhibited. The Mueller Hinton agar was prepared, sterilized at 121^{o}C for 15minutes, poured into sterile petri dishes and was allowed to cool and solidify.The content of the test tubes from the MIC that shows no growth in the serial dilution was then sub – cultured onto the prepared medium.This was incubated at 37 ^{o}C for 24 hours. The plate with the lowest concentration of extract without colony growth was the Minimum bactericidal / Fungicidal concentrations (MBC/MFC) of the extracts (EUCAST, 2000).

3.10.7 SynergisticActivity of Extracts with Standard Drugs

The *in - vitro* evaluation of antimicrobial activity of combination of ciprofloxacin, fluconazole with *Sennasingueana* extracts against the microbes (bacteria and fungi) was determined. Each of the extracts (0.1g)was weighed and the standard drugs ciprofloxacin and fluconazole (1mg) dissolved in 10mL dimethyl sulfoxide (DMSO) to obtain a concentration of 10mg/mL of extracts and 0.1mg/mL of drugs.Mueller Hinton agar was the medium used as growth medium for the bacteria and sabourand dextrose agar was used for the fungi.The media (Mueller Hinton and Saboraud dextrose agar) were both prepared according to the manufacturer's instructions. Sterilized at 121^{o}C for 15 min, pouredinto sterile petri dishes and was allowed to cool and solidify.The extracts were screened using agar diffusion methods.The sterilized media were inoculated with the test microbes, Mueller Hinton agar with the bacteria and Saboraud dextrose agar with the fungi.The inoculum was spread evenly over the surface of the media using a sterile swab (EUCAST, 2000).

Using the standard cork borer (6mm), a well was bored at the center of each inoculated media. Solution of the extract plus the drug (0.1mL) was introduced into the well on the inoculated medium.Incubation of the inoculated medium was made at 37^{o}C for 24 hours after which the plates were observed for the zone of inhibition of growth. The zones were measured with the aid of a transparent ruler and results recorded in millimetres(EUCAST, 2000).

3.11 Statistical Analysis

The data generated werestatistically analyzedand expressed as mean ± standard error of mean (SEM) for all values (Chatterjee, 2015).

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CHAPTER IV

4.0 RESULTS

4.1 Plant Collection and Identification

The plant parts (leaves, flower and fruit) were collected from the wide in Zaria and identified as *Sennasingueana* (Fabaceae), by Mal. NamaniSanusi and a specimen voucher number (6863) was given and deposited in the Herbarium unit of at the Department of Botany Ahmadu Bello University, Zaria in August, 2016.

4.2 Organoleptic Evaluation of *Senna singueana* leaf

The morphological charactersof*Senna singueana* leaves are summarized below

Table 4.1Organoleptic features of *Senna singueana* leaf

S/No	Morphological Characters	Observations
1	Colour	Green
2	Odour	Characteristics
3	Taste	Distinct
4	Texture	Smooth
5	Shape	Elliptical
6	Apex	Obtuse
7	Venation	Compound
8	Size	2.52cm by1.65cm

4.3 Microscopical Features of *Senna Singueana* Leaves

The transverse (TS) section of the leaves through the midrib (plate III) shows covering trichomes, spongy parenchyma, palisade cells, lower and upper epidermis, vascular bundles consisting xylem and phloem tissues, parenchyma cells.The lower epidermis (plate IV) shows Paracytic type of stomata as the stoma is surrounded by two subsidiary cells parallel to the longitudinal axis of the pores and the guard cells.

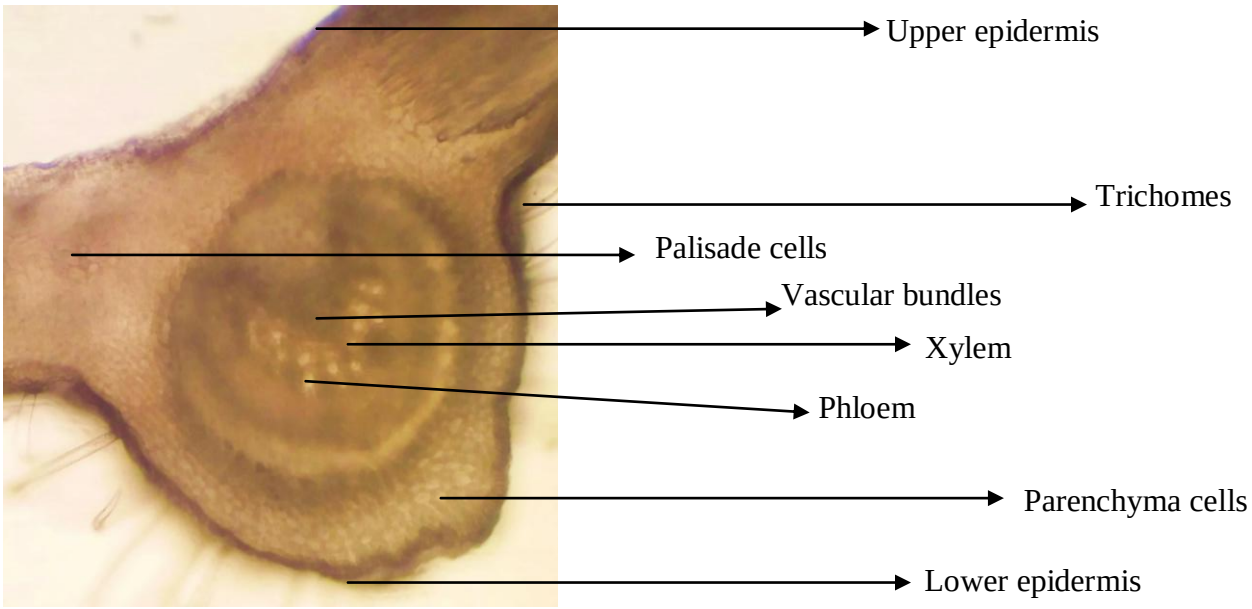
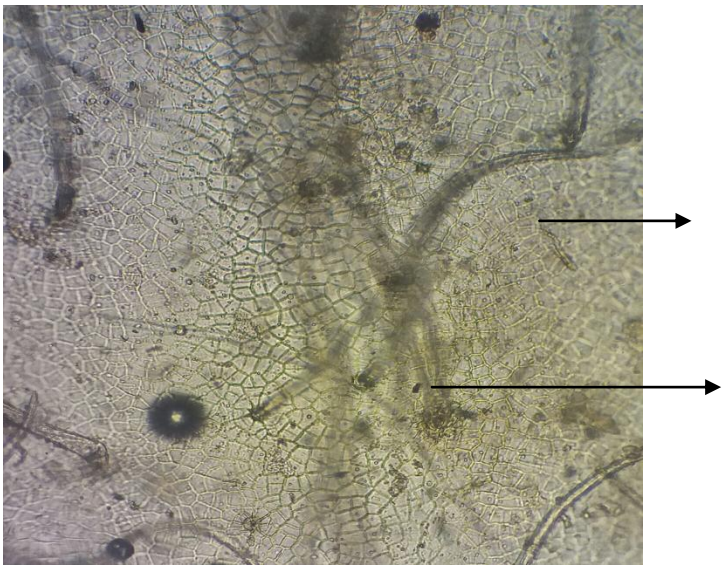


Plate III: Photomicrograph of the transverse section of *S. singueana* leaf through the midrib (Magnification 10 x 10)



(Mag. X 200)

Plate IV: Photomicrograph of Upper Epidermis of *Senna singueana* leaves.

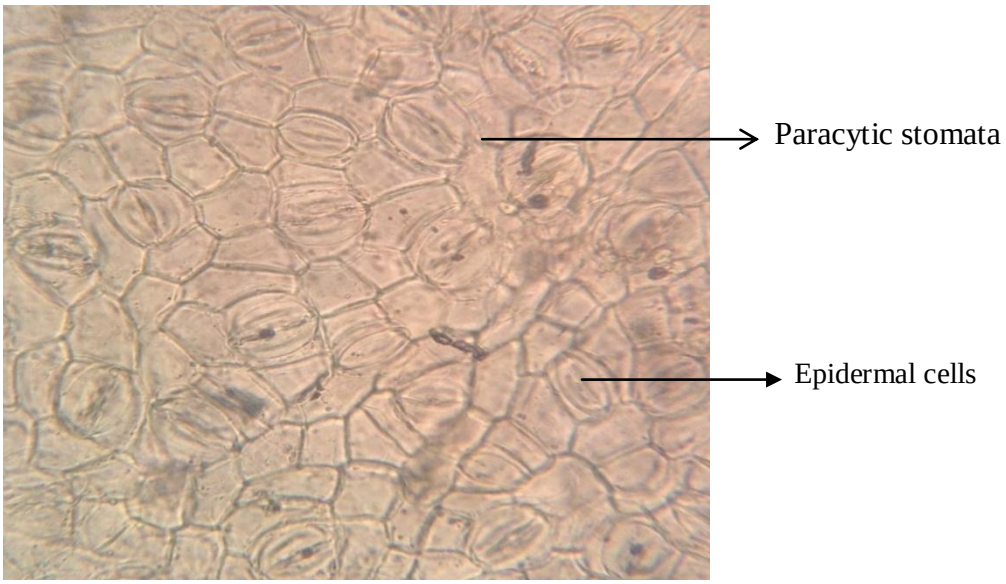


Plate IV: Photomicrograph of the lower Epidermis of *S. singueana* leaf showing some features(Mag.x 100)

4.4 Chemomicroscopical Studies of *S.singueana* Powdered Leaves

Chemomicropical examination of the powdered leaves revealed the presence of ergastic cell contents and cell wall materials as presented in table 4.2.

Table 4.2: **Chemomicroscopical Studies of *S.sngueana* Powdered Leaves**

Constituents	**Detecting agent**	**Inference**
Starch	N/50 iodine	Present
Lignin	Phloroglucinol	Present
Tannin	5 % $FeCl_3$	Present
Mucilage	Ruthenium red	Present
Calcium carbonate	HCl	Absent
Calcium oxalate	HCl	Present
Cellulose	Chlor zinc iodine	Present
Suberin	Sudan red	Present
Aleurone grains	Iodine in ethanol	Present

4.5 Physical Constants of *Senna singueana* Leaves

The physical constants (Table 4.3)of the *Senna singueana* leaves showed that the average moisture content of the powdered leaves is 6.03%, percentage total ash value is 7.33%, and acid insoluble ash value is 1.50%, Water soluble ash value 4.50% while the alcohol and water extractives values are36.66% and 31.33% respectively.

Table 4.3: Physical Constants of *S. singueana* Leaves

Parameters	**Values(%W/W)***
Moisture content	6.03± 0.87
Total ash value	7.33±1.30
Acid insoluble ash value	1.50± 0.02
Water soluble ash value	4.50± 0.00
Alcohol extractive value	36.67± 0.88
Water extractive value	31.33±0.88

* Mean of 5 counts± SEM

4.6 Extraction of *Senna Singueana* Leaves

The extraction of the powder leaves of*S. singueana*(500g) gave the percentage yield of the extracts as presented in table 4.4.

Table 4.4 Weight, percentage yields and pH of *S. singueana* extracts

Extract	Weight(g)	% yield (W/W)	pH
n- hexane	13.06	2.61	4.56
Ethyl acetate	44.22	8.84	4.60
Methanol	85.90	17.18	4.76

4.7 Preliminary Phytochemical Screening

Preliminary phytochemical screening of *S. singueana* leaves crude extracts shows that the leaf extracts contain some phytochemical constituents. Results are summarized in Table 4.5.

Table 4.5 Preliminary Phytochemical Screenings of S. *Singueana* Leaves Crude Extracts

Tests	n-hexane	Ethyl acetate	Methanol
Alkaloids			
Mayer	Absent	Present	Present
Dragendorff's	Absent	Present	Present
Carbohydrates			
Molish's	Absent	Absent	Present
Fehling	Absent	Absent	Present
Saponin			
Frothing's	Absent	Absent	Present
Tannins			
Ferric chloride	Absent	Absent	Present
Lead sub acetate	Absent	Absent	Present
Anthraquinones			
Bondragers	Absent	Absent	Present
Modified bondragers	Absent	Absent	Absent
Cardiac glycosides			
Keller-kilani	Present	Present	Present
Flavonoids			
Shinoda	Absent	Present	Present
NaOH	Absent	Present	Present
Triterpenes/ steroids			
Salkowski	Present	Present	Present
Lieberman-burchard	Present	Present	Present
Proteins			
Xanthoproteic	Absent	Absent	Present

4.8 Thin Layer Chromatoghraphic Profile of *S. singueana* Leaves Extracts

The Plates (VI– VIII) show the TLC separations profiles of the leaves extracts of *S.singueana* using p – anisaldehydes general dectecting agent. The hexane extract was developed in hexane:ethyl acetate 19:1 andit revealed 8 spots. The ethyl acetate extract was developed in hexane: ethylacetate 7:3 and it revealed 6 spots.The methanol extract was developed in chloroform: methanol 4:1and it revealed 6 spots.

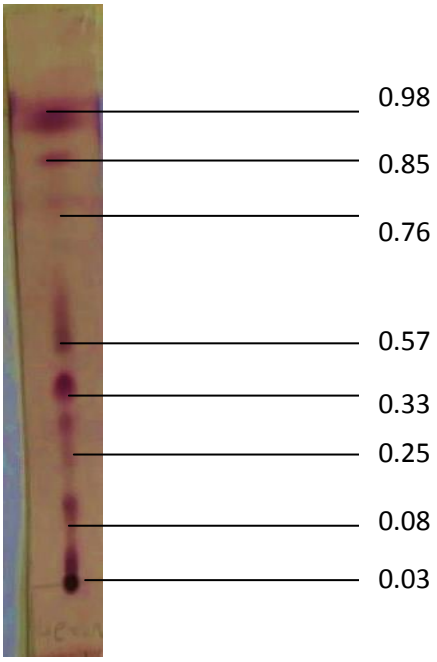


Plate VI:Chromatogram of hexane extract developed in solvent system hexane: ethylacetate 19:1Colours are purple and dark blue

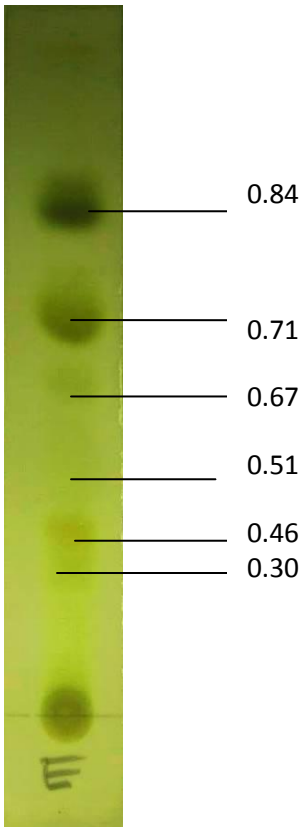


Plate VII: Chromatogram of ethyl acetate extract developed in solvent system hexane: ethyl acatae 7:3, colours are dark blue and green

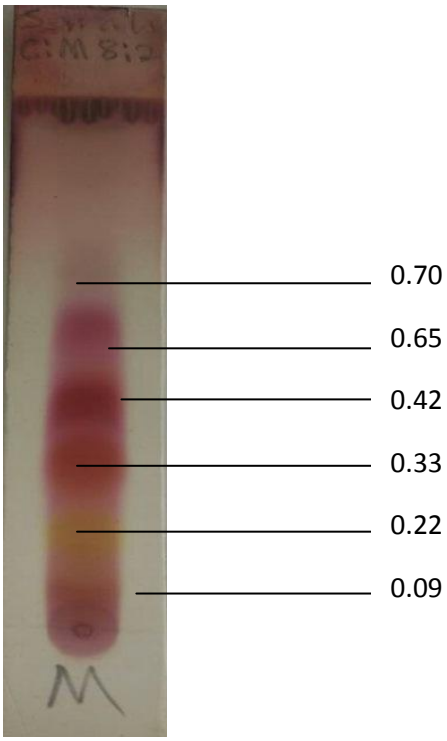


Plate VIII: Chromatogram of methanol extract, developed in solvent system chloroform: methanol 4:1.Colours are purple and yellow

Table 4.6: Summary of TLC profile of Extracts

a. Using general spray reagent (p-anisaldehydes)

Extracts	Solvent System	Colour after spray	No. of Spots	R_f Values
n-hexane	Hexane:Ethyl acetate, (19:1)	purple	8	0.98, 0.85, 0.76, 0.57, 0.33, 0.25, 0.08, 0.03,
Ethylacetate	Hexane:Ethyl acetate, (7:3)	Dark blue, green	6	0.30 (dark-green), 0.46, 0.51, 0.67,(dark-blue) 0.71,(dark-blue) 0.84(dark-blue)
Methanol	Chloroform:Methanol (4:1)	Purple. yellow	6	0.7, 0.65, 0.42, 0.33, 0.22, 0.09

b. Plates (IX– XIX) show the chromatogram of *S. singueana* extracts sprayed with various specific detecting agents

Hexane extract:Sprayed with Liebermann-Buchard spray, (Plate IX) revealed 7 spots. Coloursare green, dark blue and purple (Positive for triterpenes/steroids).

The hexane extract when sprayed with ferric chloride reagent (Plate XII), revealed 3 spots. Colours arelight Green (positive for phenolic compounds)

Ethyl acetate extract: When sprayed with Liebermann-Buchard spray, (Plate X) revealed 3 spots. Coloursare green and purple. (Positive for triterpenes/steroids)

When sprayed with ferric chloride reagent. (Plate XIII) revealed 4 spots. Colours are blue-Black (positive for phenolic compounds)

When sprayed with aluminium chloride sprayed and viewed under 354nmUV.(Plate XVIII)revealed 5 yellow florescence spots (positive for flavonoids).

Methanol extract: when sprayed with ferric chloride reagent. (Plate XIV) revealed 3 spots. Colours are blue-Black (positive for phenolic compounds).

Whensprayed with Liebermann-Buchard spray. (Plate XI) revealed 5 spots. Colours aregreen and purple(Positive for triterpenes/steroids).

When sprayed with aluminium chloride spray and viewed under UV 354nm (Plate XIX)revealed 5 yellow florescence spots (positive for flavonoids).

When Sprayed with 5% potassium hydroxide solution (Plate XVII) revealed 3 spots. Colour is yellow (positive for anthraquinones).

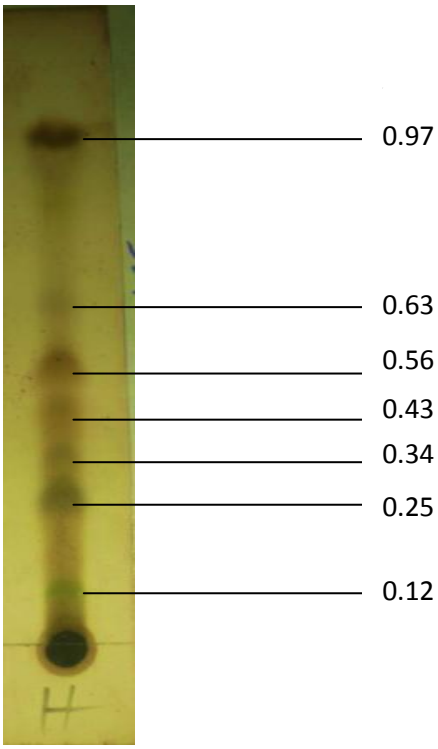


Plate IX: Chromatogram of hexane extract developed in hexane: ethylacetate 19:1 sprayed with Lieberman Buchard's reagent.

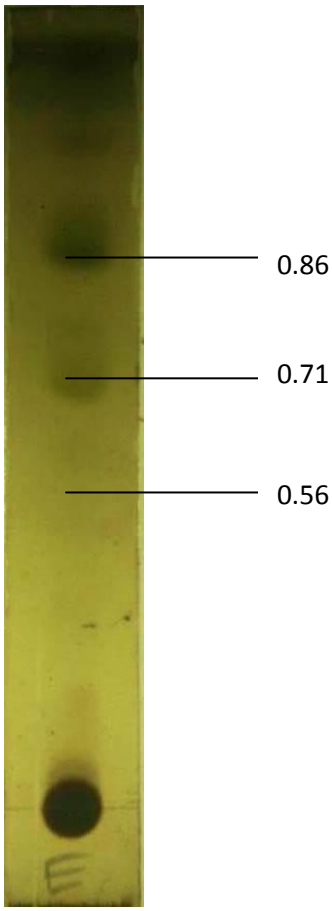


Plate X: Chromatogram of Ethylacetate extract developed in hexane: ethylacetate 7:3
Liebermann-Buchard's Reagent

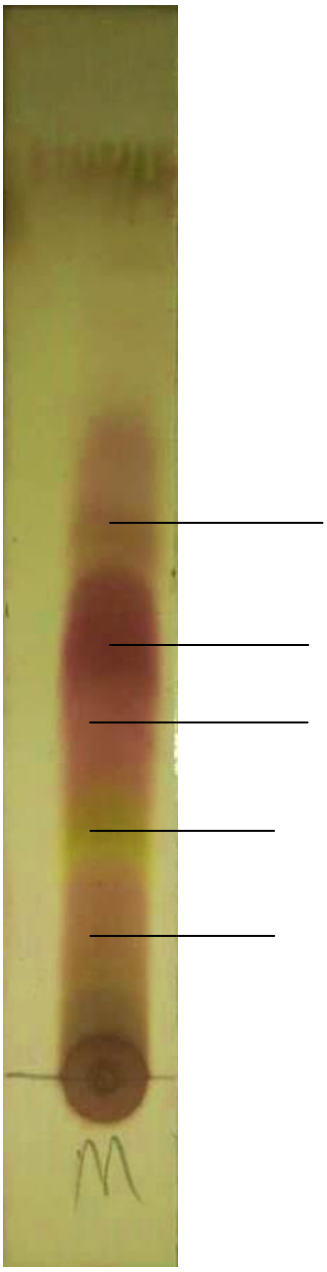


Plate XI: Chromatogram of Methanol extract developed in Chloroform:Methanol 4:1 sprayedwith Liebermann-Buchard's reagent.



Plate XII: Chromatogram of hexane extract developed in hexane: ethylacetate 19:1 sprayed with ferric chloride's reagent

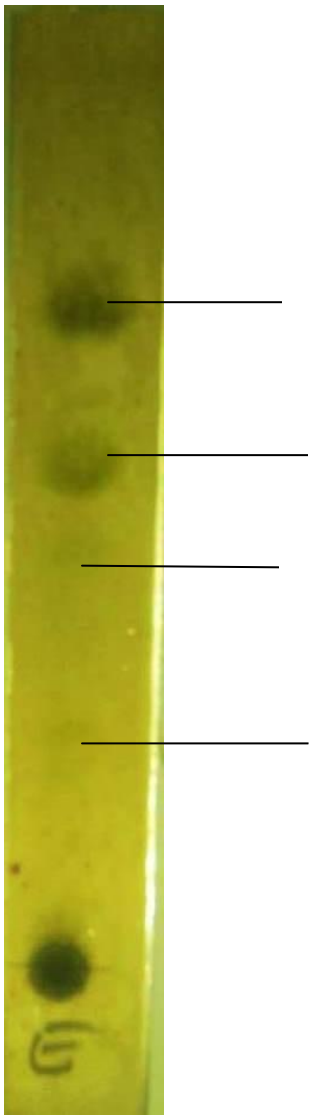


Plate XIII: Chromatogram of ethylacetate extract developed in hexane: ethylacetate 7:3 sprayed with ferric chloride's reagent.

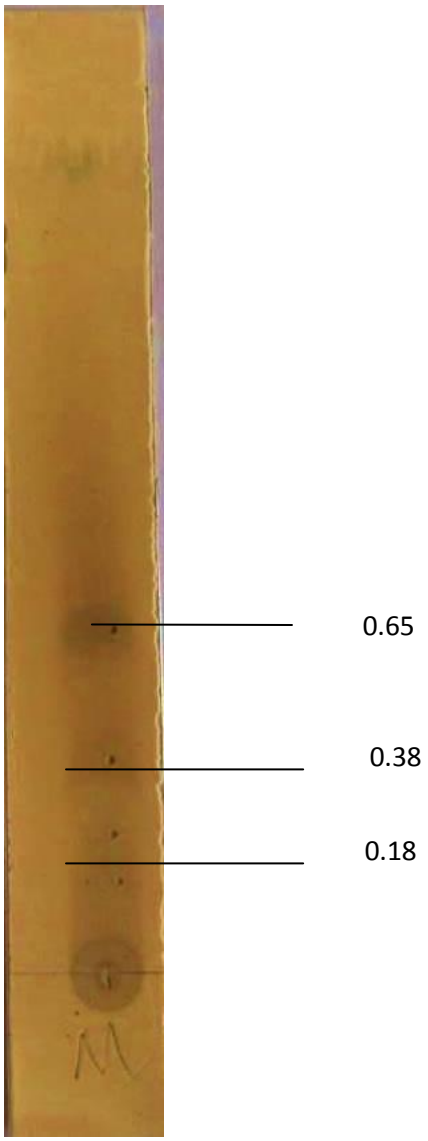


Plate XIV: Chromatogram of methanol extract developed in chloroform: methanol 4:1sprayed with ferric chloride's reagent.



Plate XV: Chromatogram of hexane extract developed in hexane: ethylacetate 19:1 sprayed with 5% potassium hydroxide.

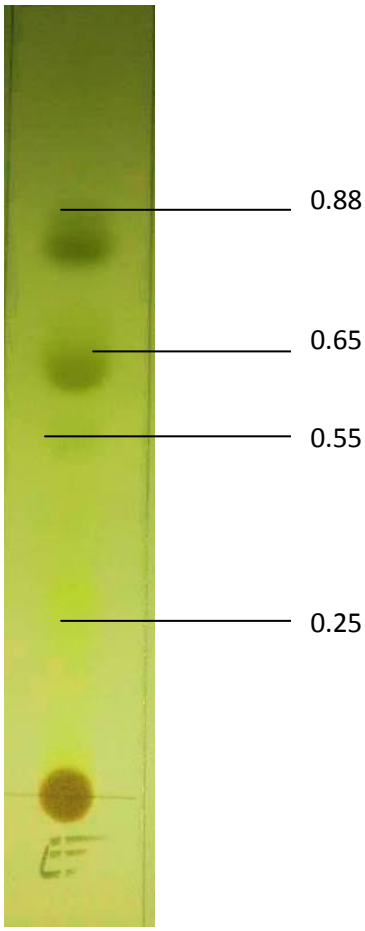


Plate XVI:Chromatogram of ethyl acetate extract developed in hexane: ethylacetate 7:3 sprayed with 5% potassium hydroxide. Colour was dark blue and green

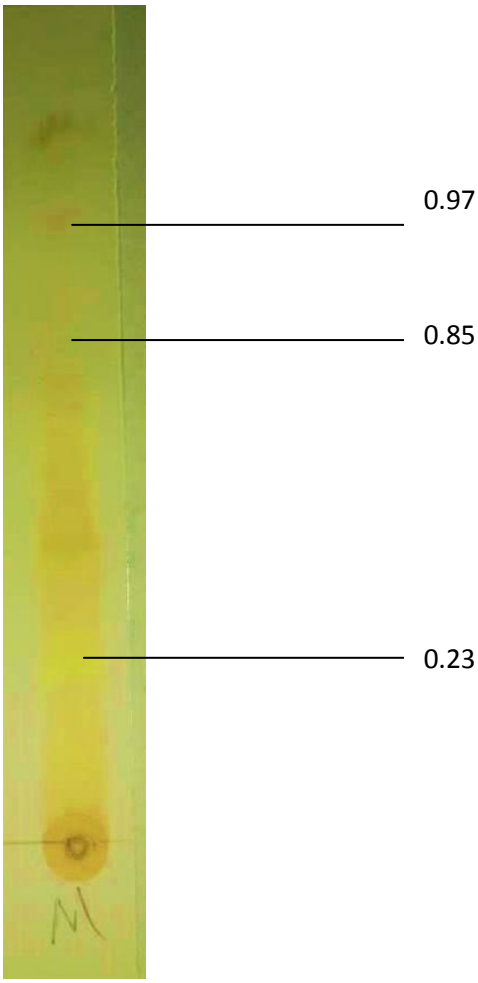


Plate XVII:Chromatogram of methanol extract sprayed with 5% Potassium hydroxide reagent revealed 3 spots.

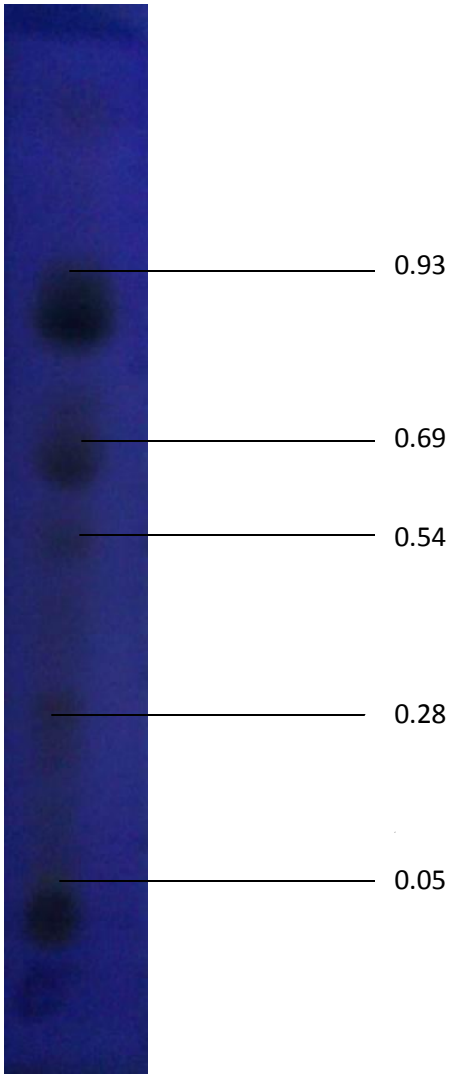


Plate XVIII: Chromatogram of ethyl acetate extract developed Hexane: ethyl acetate 7:3sprayed with aluminium chloride reagent viewed under UV (254 nM).

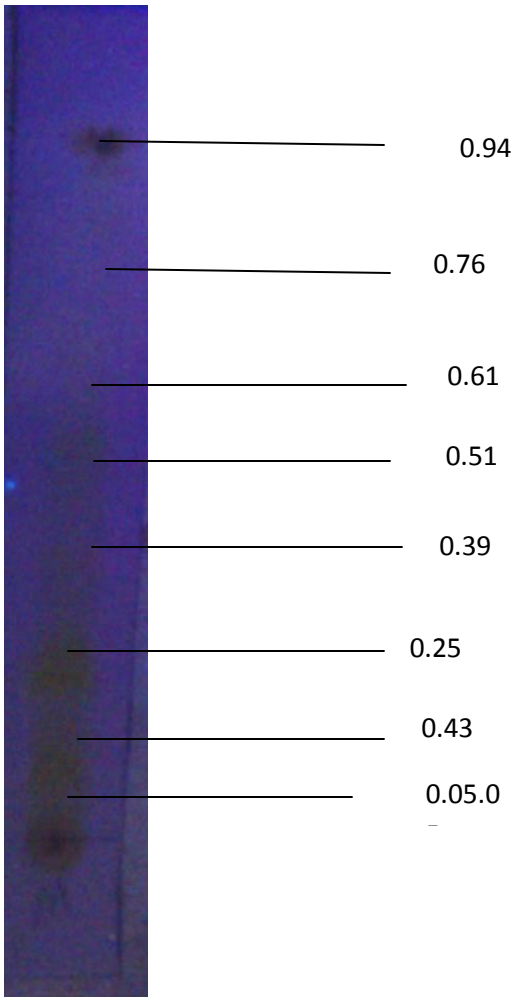


Plate XIX: Chromatogram of methanol extract Chloroformsprayed with aluminium chloride reagent viewed under UV(254 nM.)it revealed 8 yellow fluorescence spots.

4.9 Column Chromatography

The n- hexane extract of *S. Singueana*.100 fractions were collected. Fractions of like spots were pooled together and further analysed using TLC.

Table 4.7 Summary of Column Chromatography of N-hexane Crude Extract

Number of pooled column fractions and codes	Weighed(g)	No of spots	Solvent system for TLC
1-2 (SS1)	0.07	2	Hexane, 100%
3(SS2)	0.04	1	Hexane, 100%
4-5(SS3)	0.22	2	Hexane, 100%
6-9(SS3)	0.01	3	Hexane, 100%
10(SS4)	0.01	3	Hexane, 100%
11-18(SS5)	- 0.01	1	Hexane, 100%
19-25(SS6)	0.02	2	Hexane, 100%
26-31(SS7)	0.01	1	Hexane, 100%
32-42(SS8)	0.0	0	Hexane, 100%
43-45(SS9)	0.02	3	Hexane, 100%
46-51(SS10)	0.0	0	Hexane, 100%
52-65(SS11)	0.01	1	Hexane, 100%
66-82(SS12)	0.05	2	Hexane :Ethyl acetate; 19:1
83-87(SS13)	0.52	5	Hexane :Ethyl acetate; 19:1
88-90(SS14)	0.47	5	Hexane :Ethyl acetate; 19:1
91-100(SS16)	0.28	3	Hexane :Ethyl acetate; 19:1

SS = *Senna singueana*

4.9.1 Preparative TLC of SS5 Column Fraction

The column fraction 11-18 (SS5) was purified using Preparative Thin Layer Chromatography (PTLC) as shown inplate XX.

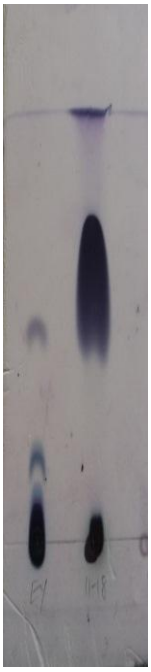


Plate XX: TLC of hexane extract compared to pooled fraction 11-18(SS5) using hexane: ethyl acetate 19: 1 as solvent system and using p- anisaldehyde as the spray (H = Hexane extract, F= pooled fraction 11-18).

4.9.2 Isolation of the compound SA

The compound was isolated using Preparative TLC (PTLC) and a single purple coloured spotwith R_f value 0.56 was obtained as showed Plate XXI

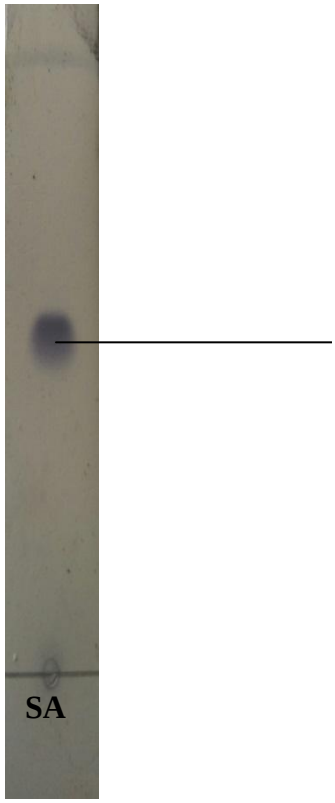


Plate XXI: TLC of compound SA using 100% hexane as solvent system

4.10 Antimicrobial Studies of *S. Singuena* Extracts

4.10.1Antimicrobial activities and zones of inhibitions of extracts.

The extracts at 10 mg/mL concentration (n-hexane, ethylacetate and methanol) demonstrated antimicrobial activities against the test bacteria and fungi isolates used. Zones of inhibition of hexane extract ranged from 17 – 19mm;methanol extract ranged from 20 – 22 mm while ethylacetate extract ranged from 21 – 27 mm. Ciprofloxacin and Fluconazoleat 10µg/mL concentration showed zones of inhibitions which ranged from 32-41mm and 32-35mm respectively.

Ethylacetate extract demonstrated the highest activity with 27mm zone diameter of inhibition. This was followed by methanol extract with 22mm zone diameter of inhibition. The hexane extract with the least activity showed 17mm zone diameter of inhibition (Tables 4.8 and 4.9)

Table 4.8 Zone of inhibition (mm) of*Senna singueana* extract (10mg/mL) against Bacteria Isolates

	n-Hexane	Ethyl acetate	Methanol	Cipro. (10µg/mL)
Staphylococcus aureus	18	22	20	32
Streptococcus feacalis	19	25	21	37
Escherichia coli	17	27	21	38
C. jejuni	0	0	0	0
Nesseiria gonorrheae	0	0	0	0
Salmonella typhi	18	21	20	41
Shigella dysentery	0	0	0	40
Pseudomonas aeruginosa	0	0	0	32
Proteus mirabilis	0	0	0	0

4.9: Zone of inhibition (mm) of *S. singueana* extracts (10mg/mL) against fungi isolates

	hexane	Ethyl acetate	Methanol	Fluconazole (10µg/mL)
Cadida albican	18	23	20	32
Cadidakrusei	0	0	0	37
Cadidatropicalis	17	27	22	34

4.10.2 Synergistic Activity of*S. Singueana*Extracts with Drugs

The extracts combined with the drugs (ciprofloxacin, fluconazole) shows an increased in activity also with ethyl acetate extract showing the highest synergistic activity. This is shown in Tables 4.10 and 4.11

Table 4.10 Zone of inhibition (mm) of synergistic effect of *S. singueana* extract (10mg/mL) and Ciprofloxacin (0.1mg/mL) against Bacterial Isolates

	Cipro	HE+ cipro	EE + Cipro	ME+ Cipro
Staphylococcus aureus	32	27	37	30
Streptococcus feacalis	37	23	27	27
Escherichia coli	38	24	42	31
C. jejuni	0	20	27	21
Nesseiria gonorrheae	0	0	0	0
Salmonella typhi	41	30	47	37
Shigella dysentery	40	28	43	32
Pseudomonas. aeruginosa	32	20	27	27
Proteus mirabilis	0	20	23	21

Key: HE= hexane, EE = ethyl acetate, ME =methanol, Cipro = ciprofloxacin

Table 4.11 Zone of inhibition (mm) of synergistic effect of *S. singueana*extracts(10mg/mL)and fluconazole (1mg/mL)against fungi Isolates

	HE+flu	EE+flu	ME+flu
Candida albican	26	27	22
Candida krusei	0	0	0
Candida tropicalis	23	30	25

Key: HE= hexane, EE = ethyl acetate, ME =methanol, flu = fluconazole

4.10.5Minimum inhibitory concentration (MIC) of extracts against the microbes

Minimum inhibitory concentration (MIC) of hexane extract (table 4.12) was at 5mg/mL against all test pathogens, ethylacetate extract was at 2.5mg/mL for all pathogens except *E. coli* which was at 1.25mg/mL and MIC for methanol extract was 2.5mg/mL for all test pathogens. Ethyl acetate extract exhibited better activity at MIC against*E.coli* at 1.25mg/mL followed by methanol extract at MIC of 2.5mg/mL and lastly by hexane extract at MIC value of 5mg/mL.

Table 4.12 MIC of*S. singueana* extracts against the microbes

Test organisms	Hexane (mg/mL)					Ethyl acetate (mg/mL)					Methanol (mg/mL)				
	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63	10	5	2.5	1.25	0.63
Staphylococcus aureus	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
Streptococcus feacalis	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
Escherichia coli	-	*	+	++	+++	-	-	-	*	+	-	-	*	+	++
Salmonellatyphi	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
Candida tropicalis	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
Candida albican	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++

KEY : - No turbidity(no growth), * mic, + (light growth), ++ (moderate growth), +++(heavy growth)

4.10.6 Minimum bactericidal/fungicidal concentration of *S.singueana*extract against the microbes (MBC/MFC)

This test was carried out to determine whether the test microbes were killed or their growths were only inhibited. Ethyl acetate extract showsMinimum BactericidalConcentration at lowest concentration of 2.5 mg/mL. The hexane and methanol extracts of *S. singueana*werebactericidal at 10 mg/mL as shown in Table 4.13.

Table 4.13 Minimum bactericidal/fungicidal concentration of*S. singueana* extractsagainst the microbes (MBC/MFC)

Microorganisms	**n-hexane (mg/mL)**					**Ethylacetate (mg/mL)**				**Methanol (mg/mL)**					
	10	**5**	**2.5**	**1.25**	**0.63**	**10**	**5**	**2.5**	**1.25**	**0.63**	**10**	**5**	**2.5**	**1.25**	**0.63**
Staphylococcus aureus	*	+	++	+++	+++	*	+	++	+++	+++	*	+	++	+++	+++
Streptococcusfeacalis	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++
Escherichia coli	*	+	++	+++	+++	-	-	*	+	++	*	+	++	+++	+++
Salmonellatyphi	*	+	++	+++	+++	*	+	++	+++	+++	*	+	++	+++	+++
Candida tropicalis	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++
Candida albican	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++

Key > * = MBC/MFC, = No colony growth+ = scanty growth, ++ moderate growth, +++ heavy growt

CHAPTER V

5.0 DISCUSSION

Interest in herbal medicines has increased worldwide, therefore, there is a need for proper identification of the plant materials to ensure some level of standards for such products. Proper identification can be achieved through pharmacognostic and phytochemical studies.

The need to recognize and describe plants has always been especially important because of their use for food and medicinal purposes (Bowles, 2004). Plant classification is constantly changing. Shifts in species alignments and groupings are made as new evidence comes to light hence a need for voucher herbarium specimen for future reference.

Macroscopical evaluation provides the simplest and quickest means to establish the identity and purity of a drug (Chanda, 2013). To ensure reproducible quality of herbal products, there is need for proper control of starting material.The World Health Organization, (WHO, 1998), states that macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and degree of purity of such materials and should be carried out before any tests are undertaken.

Transverse sectionof the lamina consists of the epidermis, numerous simple, uniseriate trichomes. Trichomes occur in a multitude of forms and sizes and play a role in plant defense, especially with regard to phytophagous insects. Trichomes may also complement the chemical defense of a plant by possessing glands which exude terpenes, phenolics, alkaloids or other substances which are olfactory or gustatory repellents. Vascular bundles were also prominent, well differentiated and consisted of xylem and phloem cells. Vascular bundles contain xylem and phloem. Xylem conducts water and

mineral salts from the roots to the leaves. Phloem tissues translocate manufactured food, soluble and organic products of photosynthesis from the leaves to the growing regions/ rest of the plants. S. *singueana* leaf also showed the presence of abundant paracytic type stomata as indicated by their unique features of two subsidiary cells at the opposite pole surrounding the stomata and numerous epidermal cells (Plate V). These are features similar to results obtained from the findings on *Senna siamea* (Lam.) leaf by Esievo *et al.,* (2016).

The leaf powder indicated the presence of cell inclusions and ergastic substances such as calcium oxalate crystals, lignin, tannins, and mucilage, cellulose, suberin and aleurone grains. These materials are either food storage products or waste products of metabolism.Calcium oxalate crysyals are considerable importance as a diagnostic tool in the identification and evaluation of vegetable drugs. They are used in detection of adulteration of crude drugs.

The moisture content of the leaf powder was found to be 6.03 %. This value falls within the limits for water content (6 -14%) for vegetable drugs (Abere and Onwukaeme, 2012). High water content promotes the growth of microorganisms leading to degradation and spoilage. Ash values are also criteria to detect the purity and quality of crude drugs as they determine the level of inorganic compostion and other impurities present in the drug (Gupta and Sharman, 2012). The total ashes, acid- insoluble ash, water - soluble ash, are 5.00%, 1.50% and 4.50% respectively. These values suggest minimal contamination in the leaf sample. Extractives values are other criteria used to judge the purity of crude drug and to determine adulteration. These values estimate the extractable constituents in a giving amount of plants material when extracted with a giving solvent. The compositions of these constituents depend on the nature of the drug and the solvent used for the extraction. The alcohol soluble and water soluble extractive values of *S.*

singueana leaf were 36.66% and 31.33% respectively. The alcohol solvent yield more extractableconstituents than the aqueous medium. Hence it can be said that there are more non polar constituents in the leaf of *S. Singueana.*

Extraction is the separation of medicinally active portion of plants (animal) tissues using selective solvents known as menstruum through standard procedures. Water is a universal solvent, it's cheap and non-toxichence, its use primarily as solvent by traditional healers but alcohol is still given preference in terms of choice of solvent when it comes to medicinal plant researches because it dissolves alkaloids, alkaloidal salts and glycosides and it does not allow growth of mould and bacteria(Gupta *et al.*, 2010). The choice of solvent in a research involving plants depend on so many factors among which include the diversity of different phytochemicals to be extracted and also what the extract is intended to be used for. The result showed that methanol had the highest yield followed by ethyl acetate and n hexane. Highly polar solvent have the ability to extract more of the phytochemical constituents,and it is also an indication that most of the constituents present in the leaf are more soluble in methanol than the other solvents. Hence, from the study; methanol had the highest yield followed by ethyl acetate and hexane.

Plants are rich sources of a wide variety of secondary metabolites. Preliminary phytochemical analysis of the leaves extract of *S.singueana* revealed the presence of some secondary metabolites which includes alkaloids, carbohydrates, tannins, anthraquinones, cardiac glycosides and triterpenes. Phenolic compounds which are known to have antibacterial activity were revealed in the leaves extract. Flavonoids werepresent in both ethylacetate and methanol extracts; thus it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms.Flavonoid rich plant extracts from different species have been reported to possess antibacterial activity (Mishra, 1995)

The activity of flavonoids depends largely on their chemical structure base on the position of the hydroxyl group**.** They are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection (Pandey, 2007)**.** It has been shown that flavonoidshave direct antimicrobial activity and a synergistic activity with antibiotics (Cushnie and Lamb 2011.). Flavanoids have also been showed to suppress bacterial virulence factor in numerous *in-vitro*and a limited number of *in-vivo*studies.Phytoalexin, an isoflavonoid, accumulation in high concentrations has great importance of the resistance mechanism against pathogenic microbes (Ebel*et al.*,2004). The presence of flavonoids in the leaves extracts of *S. singueana* plant indicates that it might have antioxidant properties thereby helping the body to fight against diseases (Ogunleye and Ibitoye, 2003). Flavonoids which are widespread in the plant kingdom, serve specific functions in antimicrobial activities, flower pigmentation, UV-protection, plant defense against pathogens and legume nodulations (Dixon, 1986).

Saponins and Tannins were present in the methanol extracts of *S. Singueana* leaves. Plants containing saponins are believed to have antioxidant, antiviral, anti-inflammatory anticancer properties (Fellow, 1991). Saponins also are associated with the fight against microorganisms, increasing the efficacy of certain vaccines and destroy blood and lung cancer cells (Philips *et al.,* 2005). It was also reported that saponinsareactive against six strains of *E. coli* compared to standard drugs erythromycin (Micheal *et al*., 2012). Nnanga *et al*., (2015) was of the opinion that antimicrobial activity depicted by crude extracts of plants was due to the presence of saponins, flavonoids and tannins. In another study by Enwa *et al*., 2014, it was reported that the presense of secondary metabolites such as alkaloids; flavonoids, tannins, terpenoids, glycosides, saponins and anthraquinones were responsible for the antimicrobial activities observed. Alkaloids were found to be present in all the extracts. Alkaloids have bitter taste and toxic to other organisms

(Gupta *et al.,* 2010) and hence inhibit the growth of microorganism. The antimicrobial activity of the leaves of the plants *S.singueana*may be due the presence of its secondary metabolites such as alkaloids and flavanoids.

Tannin constitutes to certain properties, such as a sharp, unpleasant, astringent sensation in the mouth due to their binding of salivary proteins (Oates *et al.,* 1980). The defensive properties of tannins have been attributed to their ability to bind proteins. The tannins can activate the herbivore's digestive enzymes by binding proteins and so creating complex aggregates (Clausen *et al.,* 1992). It also acts as antimicrobial agents.

The above result of phytochemical screenings can be related with the result obtained from the preliminary phytochemical screening from leaf and seed extracts of *Senna alata* L. Roxb (Archana *et al.,* 2012) It was revealed that *Senna alata* leaves contain alkaloids, flavonoids, anthraquinones, tannins, saponins, terpenes, glycosides and phenols.

p- anisaldehydeis a general spraying reagent for detecting and identifying a wide range of natural and synthetic products . In this study, the use of p-anisaldehydegave various degrees of separations of phytochemicals present in the extracts. Purple, green and dark blue coloured spots observed from the three extracts showed that of *S. singueana* leaf extract is a rich source of steroids/ triterpenes. Specific detecting reagents used (ferric choride, aluminium chloride and Lieberman bucchard) confirmed the presence of phenolic compounds, flavonoids and triterpenes present in the extracts.

These phytochemicals triterpenes, anthraquinones, tannins and flavonoids revealed in this study is in accordance with the work of (Ayo*et al*., 2014) where it was stated that Senna species have been of key interest in phytochemical and pharmacological research due to their excellent

medicinal values. They are rich sources of polyphenols, anthraquinones derivatives, flavonoids and polysaccharides.

Archana *et al.,* (2012) also reported that thin layer chromatographic analysis of the leaf extract of *Senna alata* (Fabaceae) resulted in identification of 4 spots in benzene extract, 3 spots each for chloroform, methanol, aqueous extracts and 2 spots for petroleum ether. Archana *et al.,* (2012) concluded that since the plants contains high amount of these bioactive compounds, it is reliable to possess large number of medicinal values such as anticancers, antimutagenic, antioxidant, antifungal, laxative, and antibacterial activity.

Phytochemical screening of the extracts showed steroids and triterpenoids as the major compound in *S. singueana* hexane extract. This was further confirmed by isolation and purification of pooled fractions obtained from column chromatography (CC). Fraction SS5, on purification using preparative thin layer chromatography (PTLC), a single purple coloured spot obtained is indicative of the present of triterpenoids. The plant*S. singueana* had been reported to contain terpenes and sterols (Adzu *et al.,*2003).It has been documented that some pentacyclic triterpenes possess anti-oxidative, anti-inflammatory, anti-cancer and vasodilatory activities (Channa *et al.,* 2003). These findings suggest that these pentacyclic triterpenes are potent agents for diseases prevention and/or alleviation.The retention factor (Rf) value of the compound was calculated to be 0.56 (100% n- hexane). The structure of this compound couldn't be determined as the weight is very minute (less than 0.1kg). Rf value of a compound can give a reflection on the polarity of the compound and the Rf can also provide corroborative evidence as to the identity of a compound.Compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity (Aniekan *et al.,* 2014). This is because, the stronger a compound is bound to the adsorbent, the slower it moves up the TLC plate, hence, non

polar solute moves faster and has high Rf values while polar solute moves slower with low Rf values.

A zone of observable inhibition in mm of growth of each organism served as a criterion for declaring an extract sensitive and was indicated by a clear zone around the well. From this study, it was deduced that the antibacterial activity of *S. singueana* leaf extracts was predominantly against Gram positive organisms

For the Gram negative organisms (*Escherichia coli, Neisseriagonorrhoea, Shigella dysentery, Campylobacter jejenum, Pseudomonas aeruginosa, Salmonella typhi and Proteus mirabis*) tested, only *Escherichiacoli* and *Samonella typh*i were sensitive to all the extracts against the standard drug ciprofloxacin while the tested gram positive organisms(*Staphylococcusaureus and Streptococcus feacalis*) were both sensitive to all the extracts. This could be because Gram positive cell wall is simple while that of Gram negative bacteria cell wall is complex and contains peptidoglycan, and an outer envelope of lipopolysaccharide, phospholipid and protein which makes it difficult for antimicrobials especially crude extracts to penetrate and bring about the desired effects (Jawetze *et al.,* 1978).

These findings were consistent with those of Singh *et al* (2000), who observed that *Senna species* containing anthraquinone, flavonoids and reducing sugar showed considerable antimicrobial activity against Gram positive microorganisms. This study is also in accordance with the previous report by Chukwuemeka *et al*(2012) on the evaluation of the antibacterial activity of herbal ointments formulated with methanolic extract of *Senna alata* (Fabacea). It is likely that one or a combination of the secondary constituents identified through phytochemical screening could be responsible for observed antimicrobial properties of the extracts. This is more

so since tannins,flavonoids, and saponins which are present in the extract, are plant metabolites well known for their antimicrobial properties (Tsechesche, 1971).

Among the fungi strains (*Candida albican, Candida krusei and Candida tropicalis*) tested, *C krusei* was resistance to all the three extracts used while C. *albican* and C. *tropicalis* were both sensitive to the three extracts. Ethyl acetate extract displayed the highest activity at 23mm zone of inhibition followed by methanol extract at 20mm and hexane extract 18 mm compared to the standard drug fluconazole at 32mm for *C. albican.* From this above result, it could be deduce that *S.singueana* extact can be effective in the management and treatment of infections caused microorganisms such as *E.coli, S. typhi,Staph. aeruginosa, Strep.areus, C. albican* and *C. tropicalis*

Minimum Inhibition Concentration (MIC) is the lowest concentration expressed under defined *in-vitro* conditions which prevent the growth of bacteria within a defined period of time (Ncube *et al*., 2008). MBC is the lowest concentration of an antimicrobial agent under defined *in vitro* conditions that reduces the number of organisms in a medium containing defined inoculum within a defined period of time by 99.9 % (Ncube *et al*., 2008). The closer the MIC is to the MBC, the more bactericidal the compound (Tripathi, 2013). MIC of hexane extract was 5mg/ml against all test pathogens, ethyl acetate ranges between 1.25mg/mL to 2.5mg/mL while methanol extract was at 2.5mg/mL for all test pathogens. Antimicrobials with low MICs are more effective than those with high MICs, as only a low dosage is necessary to eradicate microbes.Hexane extract has an MBC value of 10mg/ml for all test pathogens, ethyl acetate extract MBC ranges between 2.5 – 10mg/mL while methanol extract MBC value was at 10mg/ml. it was observed that higher concentration was required for MBC than MIC. This is because an increased concentration of the extract is needed to kill the microorganisms by 99.9%.

From this study, ethyl acetate extract with the lowest MIC value of 1.25mg/mL and lowest MBC value of 2.5 mg/mL is the most effective antimicrobial extract of *S. singueana* leaves.

It was observed that there was an increased activity of extracts against the microbes when the extract was combined synergistically with the standard drug. Although *N. gonorrhea* and *Ckrusei* remained unsusceptible, the other Gram negative organisms used in this study which were not susceptible to just the extract alone became susceptible with an increased additive activity to the combination of the extract with the standard drug. This study shows that the combination of the extract with standard drugs may be useful in the fight against emerging microbial drug resistance.

CHAPTER VI

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The plant used in this study was collected and identified as *Senna singueana* (Fabacea) and was giving a specimen voucher number of 6863. The macroscopical studies of the leaf of *S. singueana* showed that the shape of the leaf is elliptical, size was about 2.52 – 3.10 cm in length and 1.65 – 2.20cm in breadth, obtuse shape apex with reticulate venation.

Microscopically, the transverse section through the midrib showed some features which includeunicellular trichomes, calcium oxalate, vascular bundles consisting of xylem and phloem, spongy parenchyma, upper and lower epidermis. Paracytic type stomata were viewed in the lower epidermis but absent in the upper epidermis.

Chemomicroscopy indicates the presence of cellulose, lignin, suberin, aleurone grains, calcium carbonate, starch, calcium oxalate crystals, mucillage and tannins while calcium carbonate was absent.

Physicochemical parameters studied showed the average moisture content to be 6.03 %. Total ash 7.33%, acid insoluble ash 1.50 % and water soluble ash of 4.50 %. The alcohol and water soluble extractive values investigated using the powdered leaves were 36.67% and 31.33 % respectively.

The percentage yields obtained from the extraction of 500g powdered leaves of *S. singueana* plant were 2.60% (13.06g), 8.80% (44.22g) and 17.20% (85.90g) for hexane, ethyl acetate and methanol respectively.

Preliminary phytochemical screenings of the crude extracts revealed the presence of some phytochemicals such as alkaloids, saponins, carbohydrates, tannins, anthraquinones, cardiac glycosides, flavonoids, triterpenes, and proteins.

Thin layer chromatography using p –anisaldehyde as detecting agentshows that hexane extract developed in hexane:ethyl acetate 19:1 produced 8 spots, ethyl acetate developed in hexane:ethyl acetate 7:3 produced6 spots and methanol extract developed in chloroform:methanol 4:1produced6 spots. Chromatograms of the extracts were developed using specific reagents, all the extracts tested positive to Liebermann-Buchard sprayrevealing the presence of triterpenes and steroids, only ethyl acetate and methanol extracts tested positive to ferric chloride reagent revealing the presence of phenolic compounds, only methanol and ethyl acetate extract tested positive to bontragers reagent revealing the presence of anthraquinones., only ethyl acetate and methanol extracts revealed the presence of flavonoids.

A compound indicative of triterpenoid was isolated using preparative TLC of a fraction obtained from column chromatography. The compound has Rf value of 0.56 (100% hexane). The weight of this compound was less than 0.1g; hence the structure of this compound couldn't be determined.

The antimicrobial activity of the *S. singueana* leaves extract showed that the plant has significant antimicrobial activity against some Gram positive and Gram negative pathogens such as *Staphylococcus aureus, Streptococcus feacalis, Escherichia coli, and Salmonella typhi* but no activity against *Neisseriagonorrhoea and Candida krusei*

Synergistic activity between the plant extracts and the standard drug were observedagainst the pathogens (*Campylobacter jejenum* and *Proteus mirabis)* that were previously resistance to the extract alone as well as the drug (ciprofloxacin) alone.

The minimum inhibitory concentraction (MIC) value of the extract ranged from 2.5 -5mg/mL while minimum bactericidal/fungicidal concentration (MBC/MFC) ranged from 2.5 -10mg/mL for the extracts.

6.2 Conclusion

Having established the pharmacognosy features of the leaves of *S. singueana*, the study shows that leaves contains phytochemicals or compounds whichhave antimicrobial activity.The leave extracts have high potential as antimicrobial agent when used synergistically with standard antibiotics. The result of the study justified the use of the plant extracts in the treatment of diseases of microbial origin in herbal medicine. This report may serve as a footstep to use this plant as a new source of medication. This could lead to developmentof local pharmaceutical industries, thereby enhanced self-reliance and reduced drug importation

6.3 Recommendations

Further work needs to be done to:

1. isolateand identify the constituents responsible for the antimicrobial activities in the leaf and other parts of the plant;However, further studies are necessary to elucidate the mechanism of action behind these effects.
2. evaluate acute and chronic toxicity of the leaf and different parts of the plant for proper recommendation on use of the drug; and

3. determine other biological and pharmacological activities on the leaf and other parts of the plant.

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APPENDIX:

Appendix A: details of the calculation procedure of the physical constants determination

- Moisture Content

(i) Weight of powder sample = 3.00g

(ii) Constant weight of empty crucible = 48.01g

(iii) Initial weight of crucible + powder = 51.01g

(iv) Constant weight of crucible + powder after heating =50.88

51.01 – 50.88 = 0.13g

0.13 /3 = 0.043

0.0433 x 100 = 4.33%

This was repeated twice and values were 7.00% and 6.80%

Average value = $\frac{4.33 + 7.00 + 6.08}{3}$

= 6.03% moisture content

- Ash values

(i) Weight of powder sample = 2.00g

(ii) Constant weight of empty crucible = 49.94g

(iii) Weight of crucible + ash = 50. 04g

(iv) Weight of total ash = weight of crucible + ash + constant weight of empty crucible

Total ash value = 50. 04 – 49.94 = 0.1g

$$\frac{0.10}{2.00} \times 100$$

This procedure was repeated twice and the ash value was

= 7.33%

- Acid insoluble ash

  (i) Initial constant weight of an empty heated crucible = 49.88g

  (ii) Final weight of crucible with sample residue = 49.91g

  Weight of residue (the acid insoluble ash) = 0.03g

  $$\text{Acid insoluble ash value in \%} = \frac{0.03}{2.00} \times 100$$

  = 1.5%

- Water soluble ash

  (i) Initial constant weight of an empty heated crucible = 49.93g

  (ii) Final weight of crucible with sample residue = 50.02g

  Weighed of residue (water soluble ash) = 0.09g

  $$\text{Water soluble ash value \%} = \frac{0.09 \times 100}{2.00}$$

  = 4.5%

Extractive Values

Alcohol Extractive Value:

(i) Weighed of powdered drug = 5.00/g

(ii) Weight of an empty evaporating dish = 38.44g

(iii) Weight of evaporating dish + 20 ml of drug sample =38.81g

$$38.81 - 38.44 = 0.37g$$

$$\frac{\text{Weight of extract in 20 ml x 5}}{5} = \frac{0.37 \text{ x } 5\text{x } 100}{5}$$

$$= 37.00\%$$

Same procedure was repeated twice using different evaporating dish to obtain 35.00 % and 38.00%.

$$\text{Alcohol Soluble Extractive value} = \frac{37.00\% + 35.00\% + 38.00\%}{3}$$

$$= 36.66\ \%$$

Water Extractive Value

(i) Weighed of powdered drug = 5.00g

(ii) Weight of an empty evaporating dish = 38.44g

(iii) Weight of evaporating dish + 20 ml of drug sample =38.74g

38.74 – 38.44 =0.30g

$$\frac{\text{Weight of extract in 20 ml x 5}}{5} = \frac{0.30 \times 5}{5} \times 100$$

30.00 %

Same procedure was repeated twice using different evaporating dish to obtain 33.00% and 31.00%

$$\text{Water soluble extractive value} = \frac{30.00 + 33.00 + 31.00}{3}$$

= 31.33%

Percentage Yield of Exracts

(weighed powder sample = 500g)

Hexane extract: weight of empty bottle = 193.78g

Weight of bottle + extract = 206.84g

Weight of extract = 206.84 – 193.78 = 13.06g

$$\% \text{ yield of hexane extract} = \frac{13.06}{500} \times 100$$

$$= 2.61\%$$

Ethyl acetate extract: Weight of empty bottle =190.03g

Weight of bottle + extract = 234.25g

Weight of extract = 44.22g

$$\% \text{ yield of hexane extract} = \frac{44.22}{500} \times 100$$

$$= 8.84\%$$

Methanol extract: Weight of empty bottle =115.70g

Weight of bottle + extract = 201.60g

Weight of extract = 85.90g