ANTIMICROBIAL AND WOUND HEALING POTENTIALS OF LEAF AND STEM EXTRACTS OF *CATHARANTHUS ROSEUS*, *MITRACARPUS SCABER* AND *SENNA ALATA* IN RATS

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR AWARD OF MASTER SCIENCE EDUCATIONAL BIOLOGY, DEPARTMENT OF BIOLOGICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA NIGERIA.

30th March, 2011

DECLARATION

I declare that the work in the thesis entitled "Antimicrobial and wound healing potentials of leaf and stem extracts of *Catharanthus roseus, Mitracarpus scaber* and S*enna alata* in rats" has been performed by me in the Department of Biological sciences, Ahmadu Bello University, Zaria, under the supervision of Professor S.P. Bako and Dr S.A. Abdullahi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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The above declaration is confirmed

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Prof. S.P. Bako. Date

Major supervisor

CERTIFICATION

This thesis titled "**Antimicrobial and wound healing potentials of leaf and stem extracts of *Catharanthus roseus, Mitracarpus scaber* and *Senna alata* in Rats**" by **MIDAWA SABASTIAN MALLUM** meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to the scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God almighty giver of life, health and all knowledge. And to my late mother who put in all her effort to see I come to this height but could not wait to reap the benefit. May her soul, rest in perfect peace, Amen.

AKNOWLEDGEMENT

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ABSTRACT

The leaves and stems of *Catharanthus roseus* (L), *Mitracarpus scaber* (L) and *Senna alata* (L) were screened for their antimicrobial and phytochemical properties. Ethanol was used as the solvent of extraction of the plant samples. The leaves and stems extracts of *C. roseus*, *M. scaber* and *S. alata* were investigated at 250 and 500mg for wound healing activities on excision model in laboratory Rats. The antimicrobial activities of the plant samples were tested against standard organisms, *Staphylococcus aureus* (ATCC 02101), *Escherichia coli* (ATTC 11775), *Pseudosomonas aeruginosa* (ATTC 10145) and the laboratory isolates of *Candida albicans* and *Aspergillus niger*. The plant samples were also screened for the bioactive constituents which includes Carbohydrates, Glycosides, Free anthraquinones, Combined anthracene, Cardiac glycosides, Steroids, Triterpenes, Flavonoids, Tannins, Alkaloids and Saponins. The Free anthraquinones were absent in all the plant samples tested. Glycosides were absent in the leaf and the Combined anthracene was absent in the stem extracts of *Mitracarpus scaber*. The stem extracts of *Senna alata* also revealed the absence of Flavonoids, Tannins and Alkaloids while the other phytoconstituents remain the same in all the plant samples. The activities of the extracts showed both bacteriostatic and fungistatic effects. The MICs and the MBC/MFC, ranges from 0.0625 – 0.5mg. The ethanolic extracts of all the plant samples did not exert toxic effects on the Mice used in the toxicity screening. The animals treated with the ethanolic extracts of the plants used in this study showed a significant wound healing response compared to those that received negative (placebo) treatment. The mean wound healing response of the extracts treated groups range from 16.01 – 20.88 days. While the positive was 14.60 days and the negative was 22.91 days. The wound healing properties of the plants may be associated with their antimicrobial activity and also the observed phytochemical. The percentage wound healing contractions along with the antimicrobial activities support the use of the three plant samples in the topical management of wound healing. Thus the traditional claims of the uses of the plants in the treatment of wounds are therefore justified.

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LIST OF ABBREVIATIONS

1	CRLE	*Catharanthus roseus* leaf extract
2	CRSE	*Catharanthus roseus* stem extract
3	DC	De-Condolle
4	L	Linneaus
5	MSLE	*Mitracarpus scaber* leaf extract
6	MSSE	*Metracarpus scaber* stem extract
7	LD50	Median Lethal dose
8	MBC	Minimum bactericidal concentration
9	mg/ml	Milligram per millilitres
10	MFC	Minimum fungicidal concentration
11	MIC	Minimum inhibitory concentration -
12	SALE	*Senna alata* leaf extract
13	SASE	*Senna alata* stem extract

CHAPTER ONE

1.0. INTRODUCTION

Plants have been used for medicinal purposes for long before recorded history. And over the years plants have provided human beings with a source of essentials of life such as food, medicine and raw materials for clothing and shelter (Wambebe., 1996). The use of herbal medicine predates the introduction of antibiotics, and predates social, economic and religious barriers (Akinyemi *et al.,* 2000).

The therapeutic efficacies of many indigenous plants for various diseases have been described by traditional herbal medicine practitioners (Natarajan *et al.*, 2003). Natural products from plants are a source of synthetic and traditional herbal medicine. Herbal medicine sometimes referred to as herbalism, phytotherpy or botanical medicine is the use of herbs for their therapeutic or medicinal values. An herb is a plant or plant parts valued for its medicinal, aromatic or savoury qualities (Wikipedia., 2009). Medicinal plants provide basic raw materials for different industries such as pharmaceutical, cosmetic, perfumery and food (Sachiya *et al.,* 2008).

The presence of various life sustaining constituents in plants has urged scientist to examine various plants with a view to determine their potential wound healing properties (Nayak and Lexley., 2006). They form the basic primary health care system in some parts of the World (Singh and Singh., *2001*). Extracts from the dried or wet flowers and

leaves of plants are applied as a paste on wound in some rural communities. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Alam *et al.*, 2009).

Wounds are the physical injuries that result in an opening and breaking of the skin and appropriate method for healing of the wound is essential for the restoration of the disrupted anatomical continuity and disturbed functional status of the skin (Meenakshi *et al.,* 2006). A wound is one of the major obstacles to the establishment of infections by bacterial pathogens in the internal tissues, when bacteria breach this barrier, infection can result (Giacomette *et al.,* 2000). Wound healing involves a complex series of interactions between different cell types, cytokine mediators and the extracellular matrix. The phases of normal wound healing include haemostasis, inflammation, proliferation and remodelling (Douglas and Alan., 2003).

Catharanthus roseus (L) G.Don (Apocynaceae) also known as *Vinca rosea,* is commonly called Madagascar periwinkle. English name includes Cape Periwinkle, Rose periwinkle or old maid. It is a perennial evergreen herb that was originally native to the island of Madagascar. It has been cultivated for hundreds of years as an ornamental and medicinal plant. It is cultivated mainly for its alkaloid which is having anticancer activities (Jaleel *et al.,* 2006). The two classes of active compounds in *Vinca* are alkaloid and tannins.

It is used locally for dermatitis to treat skin diseases in humans and animals, and has historically been used to treat a wide assortment of diseases. Extracts from the leaves has been used to treat numerous diseases including diabetes and malaria. As an ornamental plant, it is appreciated for its hardiness in dry and nutritionally deficient soil condition; full sun and well drained soils are preferred (Wikipedia., 2009).

Mitracarpus scaber L. (Rubiaceae) which is also known as *Mitracarpus villosus* (SW) DC, is a very common weed of cultivated or fallowed land. Recent studies have shown that alcoholic extracts of the aerial parts of *M.villosus* had *in-vitro* antimicrobial activities against *Dermatophilus congolensis. E*xperiments have proved that ointments containing alcoholic extracts of *M. villosus* used topically had a high efficiency against bovine dermatophilosis and cured tested animals without recurrence (Ali *et al.*, 2003)

Senna alata Linn, (Fabaceae) is native to South America, it is an ornamental shrub which grow well in forest areas of West Africa. It is locally used in many parts of the world for the treatment of several infections, which includes ringworm and parasitic skin diseases (Palanichramy and Natarajan., 1990).

The leaves have been reported to be useful in treating abdominal pains, oedema, gonorrhoea, heart failure and also serve as a purgative (Ogunti and Edeoba., 1993). Decoctions from the leaves, flowers, wood and bark of the plant is reported to be effective in the treatment of skin diseases

such as purities, eczema and allergy (Benjamin and Lamikaria, 1981; Abatan., 1990).

1.2. STATEMENT OF THE RESEARCH PROBLEM

Infectious diseases continue to be a problem that militates against the health of farm animals and man, despite the progress made in the understanding of microbiology and their control (Idu *et al.,* 2006). The incidence of epidemics due to drug resistant microorganisms and the emergence of some unknown disease causing microbes pose enormous public health concern (Idu *et al*, 2006). The traditional ways of using herbs are remarkably similar, either as poultice or extracts from fresh or dried plant products.

The use of synthetic drugs is usually expensive and not easily available, and the emergence of drug resistant strains of microbes is gradually incapacitating current antibiotics. Hence, the need to seek new chemotherapeutics and chemoprophylaxis as agents to combat the disease causing organisms from indigenous sources which may be cheap and also readily obtainable locally.

1.3 JUSTIFICATION

Catharanthus roseus, Mitracarpus villosus, and *Senna alata* are common ornamental plants. They are used locally in the treatment of skin disease such as rashes and itching, eczema, ringworm etc, by applying the leaf sap or by rubbing leaves on skin. The extracts from

these plants has gained traditional claims of wound healing efficacy for long time in history.

Paucity of information exists in the published literature to support the concept about the antimicrobial and wound healing potentials of the plants used in this study. The ethanolic extracts of the plants will be investigated for their antibacterial and antifungal activity and the phytochemical components of each plant extracts will be investigated as a scientific assessment of the claim of therapeutic potency

1.4 AIMS

The aims of this study are to evaluate the ethno therapeutic claims of the antimicrobial activities and the wound healing potential of *C. roseus, M. villosus and S. alata* stems and leaves extract.

1.5 HYPOTHESES

1. There are no phytochemical constituents in the ethanolic extract of *C. roseus, M. scaber* and *S .alata* plants.

2. The concentrations of toxic substances in the plant parts are too high for medicinal uses.

3. The leaf and stem extracts of *C .roseus, M. scaber,* and *S. alata* has no anti-microbial efficacies.

4. The extracts of *C. roseus, M. scaber,* and *S. alata* have no significant wound healing potentials

1.6 OBJECTIVES

This study is designed with the following objectives:

1. To determine the phytochemical constituents of the stems and leaves of C. r*oseus, M. scaber* and *S. alata* plants ethanolic extract.
2. To determine the median lethal dose (LD50) of the extracts using mice.
3. To investigate the antimicrobial activities of the plant extracts.
4. To evaluate the wound healing potentials of the extracts of *C. roseus, M. scaber* and *S. alata* in rats.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. HERBAL MEDICINE:

Herbalism is a traditional or folk medicine practice based on the use of plant extracts. Herbalism is also known as botanical medicine, herbal medicine, phytotherapy or herbalogy. Sometime the scope of herbal medicine is extended to include fungi and bee products, as well as minerals, shells and certain animal parts (Acharya and Shrivastava., 2008).

Many plants synthesize or produce substances that are useful to the maintenance of health in humans and animals. According to Lai (2004), some of these substances (particularly alkaloids) serve as plant defence mechanism against predation by microorganisms, insects and herbivores. And that many of the herbs and spices used by man to season food yield some useful medicinal compound (Tapsel, 2006).

2.2. ANTHROPOLOGY OF HERBALISM

People all over the world used hundreds to thousands of indigenous plants for treatment of different ailments since prehistoric times. The use of herbs to treat diseases is almost universal particularly among non-industrialized societies. The World Health Organization (WHO) estimates that eighty 80% percent of the world's population presently uses herbal medicine for some aspects of primary health care

(Wikipedia., 2009). This is because pharmaceuticals are expensive and the herbal medicine can be grown from seed or gathered from nature.

Huffman (2003), opined that the indigenous healers often claim to have learned by observing, that sick animals change their food preference to nibble at bitter herbs they would normally reject. According to Hulching *et al.* (2003), sick animals tend to forage plants rich in secondary metabolites such as tannins and alkaloids. Researchers from Ohio Wesleyan University found that some birds select nesting materials rich in antimicrobial agents which protect their young from harmful bacteria (Jann., 2004).

2.3. ANTIMICROBIAL ACTIVITIES

Medicinal plants contain bioactive ingredients that play a vital role as antimicrobial agents. Antimicrobial activity is shown as the diameter (mm) of the clear inhibitory zone formed around the discs (Alam *et al.,* 2009). It is defined as the collective activities of antibiotics on microorganisms. In recent years the need to study plants having different values in their medicinal properties has accelerated. The increasing resistance to antibiotics by microorganism has resulted to the search for a lead from new organic molecules from plants with antimicrobial properties (Sofowora., 1983).

Therefore several medicinal plants have been evaluated for possible antimicrobial activities and potential cure from a variety of ailments especially of microbial origin, (Dada *et al.*, 2002). The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds (Lai , 2004). The naturally occurring combination of these compounds can be synergistic and often result in the crude extracts to have great antimicrobial activities than the purified individual constituents.

2.4. ROLE OF PLANT COMPOUNDS IN WOUND HEALING

Wounds are physical injuries that result in the opening or breaking of the skin, and appropriate method for healing of the wound is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (Meenakshi *et al,* 2006). Natural products from herbs have been used in the treatment of various ailments and the healing of wound by man since creation. There are many ethno-botanical literatures that have been described by researchers on the therapeutic efficacy of the medicinal uses of herbs.

Toporcer *et al.* (2006) demonstrates the positive effects of use *Atropa belladonna* on septic wound healing in rats. A study by Shemina *et al.* (2007) showed that, the alkaloids and polyphenols of an *Areca catechu* extracts could be used to enhance the healing of wound, leg ulcers and

skin graft surgery. And also, the recent study by Somashekar *et al.* (2007) showed significant wound healing effects of the alcoholic and

aqueous extracts of *Oscemum sanctum* L in rats. A study by Shivaranda (2006), demonstrated wound healing properties of *Vinca rosea* after topical application in streptozotocin- induced rats.

Glycoprotein from *Aloe vera* (Choi *et al.,* 2001), Oleanolic acids from *Anredra diffusa* (Letts *et al.*, 2006), Curcumin from *Curcuma longa* (Jagitia and Rajanikant., 2004), Phenolic acids from *Chromolaena odorata* (Phan *et al.*, 2001) are some of the few important plant derived wound healing compounds which were tested on animals models.

Based on all available literature the natural products obtained from plants plays an important role in wound healing process. Therefore *C.roseus, M.villosus* and *S.alata* which forms the long list of both medicinal and ornamental plants could be useful for the healing of wounds.

2.5. *CATHARANTHUS ROSEUS* L (Apocynaceae), also known as *Vinca rosea* is native to the Island of Madagascar, and has in history been used to treat a wide assortment of diseases. According to Shivananda., (2006), it has more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat Leukemia, Hodgkin□s disease, Malignant Lymphoma, Nueroblastoma, Wilms tumour and other cancers. The extract of *vinca* has demonstrated significant

anticancer activity against numerous cell types (El-sayed and Cordell., 1981).

The fresh juice from the flower of *C. roseus* made into a tea has been used by Ayurvedic physicians in India for external use to treat skin problems, dermatitis, eczema and acne (Nayak and Lexley., 2006). Its vasodilating and memory-enhancing properties have been shown to alleviate vascular dementia and Alzheimer's diseases (Hindmarch *et al.,* 1991).



Plate. I : *Catharanthus roseus* plant.

CLASSIFICATION

Kingdom:	Plantae
Subkingdom:	Tracheobiota (vascular plants)
Division:	Magnoliophyta (flowering)
Class:	Magnoliopsida (dicotyledons)
Subclass:	Asteridae
Order :	Gentianales
Family:	Apocynaceae
Genus:	Catharanthus
Species:	*Catharanthus roseus.*
Common name:	Rosy periwinkle

2.6. *MITRACARPUS SCABER (ZUCC).*

Mitracarpus scaber (ZUCC) formally known as *Mitracarpus villasus* (SW) DC is native to Africa, and is wide spread in Tropical Africa, West Indies and South America. It belongs to the family Rubiaceae. It is an annual herbal plant that usually grows to about 60cm tall. The inflorescence is

dense clustered together with small white flowers at the leaf axils that are usually thick. *M.scaber* vary in its habitat and reproduces by seed.

It is a common weed in upland areas of savanna. The plant is widely used traditionally among most communities as poultice in the treatment of skin disease such as scabies, eczema, itching and dandruff. *M.scaber* is widely employed in traditional medicine in West Africa for headaches, toothache, venereal diseases, amenorrhoea, dyspepsia, hepatic disease and leprosy (Bisignano *et al.*, 2000).



Plate II: *Mitracarpus scaber* plant.

CLASSIFICATION

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnolipsida
Order	Gentianales
Family	Rubiaceae
Genus	*Mitracarpus*
Species	*Mitracarpus scaber*
Common name:	Gogamasu {Hausa)

2.7. *SENNA ALATA* L (Fabaceae) also known as Ringworm cassia or candle bush, is an ornamental shrub which grows well in the forest area of West Africa (Owoyale., 2005). The plant has been identified as a medicinal plant used in the treatment of many ailments in many parts of the World (Idu *et al.,* 2006). The sap of the leaves is a well known remedy for Ringworm, Scabies, Ulcers, swelling and inflammation conditions and skin parasites (Crockett *et al.*, 1992).

Decoction from the leaves, flowers, barks and wood of the plant is reported to be effective in the treatment of skin diseases such as

Purities, Eczema and Allergy (Abatan, 1990). Other preliminary studies on the therapeutic uses of *Senna alata* is those by Sansores-Peraza *et al,* (2000) and Somehit *et al.,* (2003). According to Idu et al (2006), the extracts of the plants are used in Nigeria to treat dermatophytes, bacterial and fungal infections.



Plate III: *Senna alata* plant

CLASSIFICATION

Kingdom:	Plantae
Sub-Kingdom:	Tacheaophyta (Vascular Plant)
Super-Division:	Spermatophyta (Seed Plant)
Division:	Magnoliophyta (flowering plant)
Class:	Magnoliopsida (dicotyledons)
Sub-Class:	Rosidae
Order:	Fabale
Family:	Fabaceae (pea family)
Genus:	Senna
Species:	*Senna alata*
Common name:	Negro coffee

2.8. PROPERTIES OF THE ORGANISMS USED.

1. *Aspergillus niger:-* These are fungus which, along with other A*spergillus* species causes aspergillosis an infective disease associated with the respiratory tract.

2. *Candida albicans: -* These are yeast that belongs to the class of fungi. It is a dimorphic fungus, that is it can alternate between yeast-like and filamentous form (Dickson, 1998). They form the members of the

natural flora of the skin, mucus membranes, and the gastrointestinal tracts. (kwam-chung and Bennett, 1992). They are the common cause of Vulvovaginitis a virginal disease that is characterised by severe irritation and virginal discharge (Odds, 1988).

There has been wide spread of fungal infection in recent years. One of the factors aiding the spread of the fungal diseases has been the widespread uses of broad- spectrum antibiotics which eliminates or decrease the non-pathogenic bacteria that normally compete with fungi. And another reason has been the increase in the number of individuals with reduced immune responses caused by the acquired immunodeficiency syndrome or by the action of immunosuppressant drugs or cancer chemotherapeutic agents (Sharma and Sharma, 2010)

3. *Staphylococcus aureus:-* These are gram-positive bacteria that are spherical in shape occurring in clusters and are non-motile. They are frequently compared with the bunch of grapes (Brooks and Morse, 1998). They cause skin lesion such as boils, and the nose is the natural home of *S. aureus*. They also form the normal flora harboured in the throat, skins and faces of individuals. Many strains are now resistant to antibiotics such as Penicillin, Streptomycin and Tetracycline (Russel, 1998).

4. *Escherichia coli:-* these are gram-negative, non-spurring and non capsulated rods that belong to the family Enterobacteriaceae. They live in the intestine of man and animals. They are also members of the normal flora in the human intestine (Donnenberg and Kaper, 1992). They are the major cause of enteritis in young infants and young farm animals, causing diarrhoea and fatal dehydration.

5. *Pseudomonas aeruginosa:-* This is another representative group of the gram-positive bacteria. They cause infections especially at burn sites, wounds, pressure sores and ulcers often as secondary invaders.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. PLANT MATERIALS:

The fresh stems and leaves of *Mitracarpus scaber* were collected locally in Samaru, Zaria, while the stems and leaves *of Catharanthus roseus* and *S. alata* were collected from Mubi Adamawa state. Samples from the plant material were taken to the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria for authentication. The voucher deposited in the Herbarium section for future references as follows: *C. roseus,* (19810 *M. scaber* (1074) and S. alata (2421).

The collected stems and leaves of the three (3) plant samples were washed clean with tap water and air dried under the shade for ten (10) days. After which they were ground into a powdered form using a mortar and pestle. The powdered plant samples were put in a black polythene and placed in well labelled envelope separately ready for extractions

3.2. EXTRACT PREPARATION

The cool or maceration methods were used for the extraction using 95% ethanol. The powdered plant material were weighted before their extraction, *Catharanthus roseus* stems and leaves (385.87g and

486.76g), *Mitracarpus scaber* stem and leaf (367.63g and 387.72g) and the *Senna alata* stems and leaves powder weighed 398.28g and 376.21g respectively. The result yielded 18.76 and 24.21g, 24.56 and 26.44g, 28.87 and 23.28g. They were placed in six separating funnels for extraction with 95% ethanol.

They were allowed to stand for 48 hours for filtration after rewashing. The filtrates were then poured into an evaporating dish and placed over a water bath for concentrations to obtain the semi solid extracts at a very low temperature of about 45 degree centigrade. A scraper was used to scrape the dried plant extracts from the evaporating dish into well labelled screwed capped bottles for safe keeping

3.3. PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening were carried out on the crude extracts using the standard methods described by Trease and Evans., (1983), to determine the following bioactive ingredients carbohydrates, glycosides, cardiac glycosides, saponins, alkaloids, tannins, triterpens, steroids, flavanoids, free anthraquinones and the combined anthracene. The activity and quantity of phytochemicals present in extracts can be varying depending upon geographical location of the plant cultivation (Olila *et al.*, 2001).

3.3.1. Test for Carbohydrates

> Molish's test: Few drops of Molisch's reagents were added to a 2ml of the extracts in a taste tube, and a small quantity of concentrated

Sulphuric acid was allowed to run down the side of the taste tube. A purple or violet colour at interface indicates the presence of Carbohydrates.

> Fehling's test: To 5ml of extract, 5ml of a mixture of Fehling solution A and B in the ratio of 1:1 was added and the mixture boiled for a few minutes. A brick red precipitate indicates the presence of free reducing sugars

3.3.2. Test for Glycosides

5ml of sulphuric acid was added to 5ml of extract and boiled for 15 min., it was then cooled and neutralized with 20% KOH divided into two portions. One of the portions was dissolved in distilled water as control, no acid hydrolysis

> Ferric Chloride test: 3 drops of Ferric Chloride were added to the portion. A brown to black precipitate is formed indicating the phenolic aglycone as a result of hydrolysis of glycosides.

3.3.3. Test for Cardiac Glycosides.

> Keller- Killiani's test: 2ml of extract was dissolved in glacial acetic acid containing trace of Ferric Chloride in a test tube. The test tube was held at an angle of 45 degrees, 1ml of concentrated Sulphuric acid added down the side of the test tube, A purple ring colour at interface indicates Cardiac glycosides.

3.3.4. Test for Saponins.

> Frothing test: 2ml of the extract was dissolved in 10ml 0f water. This was then shaken vigorously for 30 seconds and allowed to stand for 30min. A honey comb formed for more than 30min. Indicates Saponins.

3.3.5. Test for Steroids and Triterpenes.

> Lieberman-Burchard's test: 2ml of Acetic Anhydride was added to 2ml of the extract. 1ml of concentrated sulphuric acid was added down the side of the test tube. The colour change was observed immediately and later. Red, pink and purple colour indicates the presence of Triterpene while Blue green indicates Steroids.

3.3.6. Test for Flavonoids.

> Sodium Hydroxide test: Few drops of aqueous NaOH were added to 5ml of extract. A yellow colouration shows the presence of Flavonoids.

3.3.7. Test for Tannins.

0.5g of extract was dissolved in 10ml of distilled water, and filtered. Few drops of Ferric Chloride solution was then added to the filtrate. A blue black precipitate indicates hydrolysable Tannins and green precipitate indicates the presence of condensed Tannins.

3.3.8. Test for Alkaloids

> Meyer's test: Few drops of this reagent were added to 2ml of extract. A whitish precipitate indicates Alkaloids.

>Dragendoff's test: Few drops of this reagent were added to 2ml of extract. A rose red precipitate indicates the presence of Alkaloids.

3.3.9. Test for free Anthraquinones.

> Borntrager's test: A small quantity of the extract was shaken with 10ml of Benzene and filtered. 5ml of 10% Ammonia solution was added to the filtrate and stirred. Production of pink or violet colour indicates the presence of free Anthraquinones.

3.3.10. Test for combined Anthracene.

> Modified Borntrager's test: A small amount of extract was boiled with 5ml of 10% Hydrochloric Acid for 3min; this hydrolysed the glycoside to yield aglycones which is soluble in hot water only. This solution was

filtered hot; the filtrate was cooled and extracted with 5ml of Benzene. The Benzene layer was filtered off and shaken gently with half of its volume of 10% Ammonia solution. A rose red colour indicates combined anthracene.

3.4. TOXICITY STUDIES

Acute toxicity test is usually involved in the estimation of the LD50 that is the least dose which has proved to be lethal (causing death) to about 50% of the tested group of animals (Akhila *et al.*, 2007). The acute toxicity test for this study was carried out on Mice using the methods described by Lork (1983). The oral administration method was used to

administer the extracts using the oral administration canola. The toxicity study was divided into two phases.

In the first phase nine (9) Mice of both sexes were divided into three (3) groups of three mice each. Group A receive 10mg/kg body weight of extract while group B and C received 100 and 1000mg/kg respectively. The animals were observed for sign of toxicity continuously for 2h and then occasionally for a further 4h, and finally over night to note mortality.

In the second phase three (3) Mice were separated, the first received extract at a dose of 1600mg/kg, while the second and third were given

2900mg/kg and 5000mg/kg body weight. The animals were also observed for mortality as done to phase one.

3.5.1. ANTIMICROBIAL SCREENING

The test organisms used were the type culture of S*taphylococcus aureus (*ATCC 02101), *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli (*ATCC 11775), and the clinical isolates of *Candida albicans* and *Aspergillus niger.* Nutrient agar was used for bacteria and the Sabouraud's dextrose liquid media for fungi as the growth media.

3.5.2. PREPARATION OF INNOCULUM

The organisms were stored on Agar slant and kept in the refrigerator. From the slant an overnight (18 hrs) culture was prepared by inoculating a loopful of the organisms onto a Nutrient broth and

incubating at 37°C for 24 hours. Before use, the overnight culture in the Nutrient broth was diluted to 1:5000 for the gram-negative and 1:1000 for the gram-positive organisms, so that each culture will contain about 10^7 colony forming units per ml.

3.5.3. ZONES OF INHIBITION

The zone of inhibition is the radius or diameter over the growth of microorganisms inhibited as a result of the presence of an antimicrobial agent. This is usually carried out on a solid media on a freshly inoculated agar plate. About 20ml of the nutrient agar was prepared, 2ml each was dispensed into sterile Petri-dishes and streaked for even distribution and allowed to solidify.

A cork borer was then used to create a well on the nutrient agar plates for each of the extracts. A drop of pure hot nutrient agar was dropped into the well to seal it and allowed to cool. About 200*ul* of the extracts were dispensed into the well and allowed to stand for 1 hour diffusion time.

A drop of the inoculums (cultured organisms) was added and the inoculated plates incubated for 24 hours at 37°C (for bacteria) and for 48 hours at 30°C (for fungi). Their zones of inhibition were noted and measured using a calliper and a ruler. The entire test for the zones of inhibition was done in duplicates and their mean diameter noted and recorded in millimetres.



Plate IV: Illustrates a representative plate showing the antimicrobial activity of the ethanolic extract from plant sample producing no zones of inhibition against the test organism.

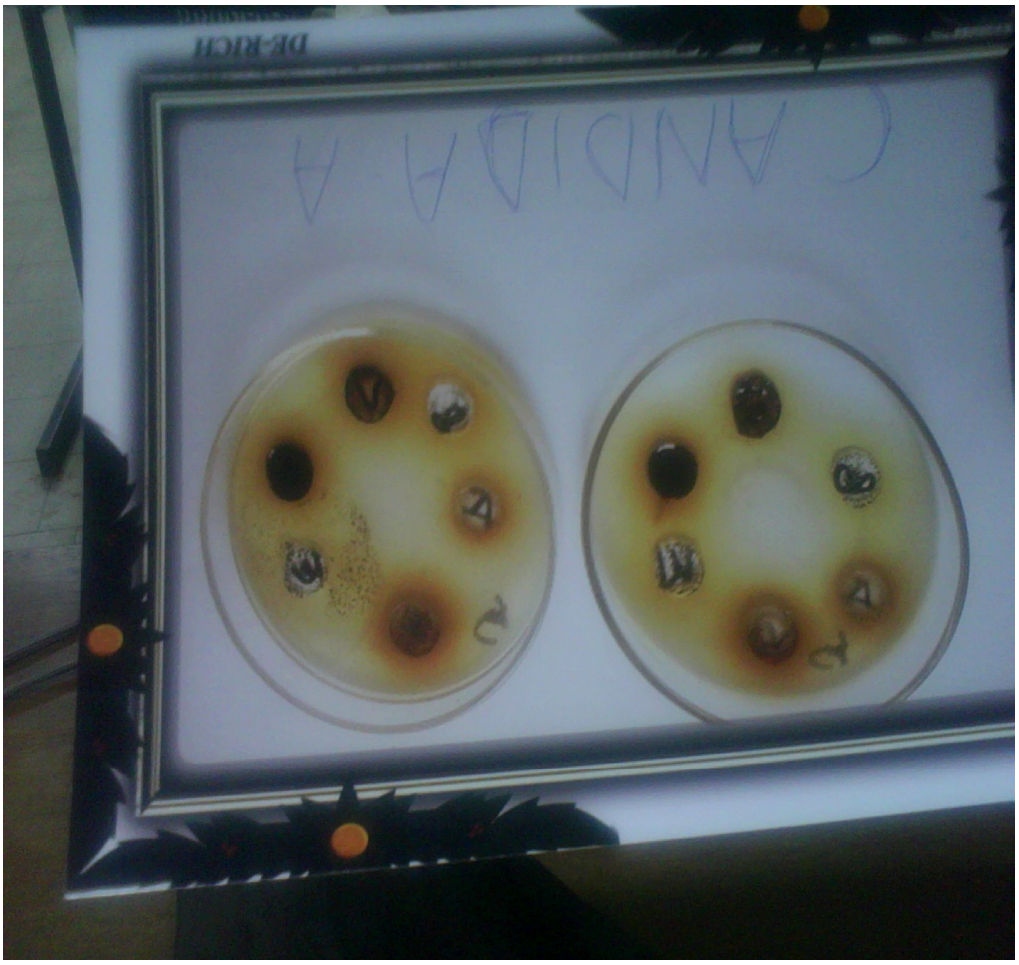


Plate V: Illustrates a representative plate showing the antimicrobial activity of the ethanolic extract that produces zones of inhibition against the test organisms.

3.5.4. MINIMUM INHIBITORY CONCENTRATIONs (MICs).

The tube dilution method employed by Scott (1989), Olukemi and Khandakai-Olukemi (2004) was used. The minimal inhibitory concentrations (MICs) were determined using the standardized culture of the organisms. The sterile Nutrient broth was used for bacteria and Sabouraud□s dextrose liquid media were used for the fungi. Double strength nutrient broth and single strength solution were prepared according to the manufacturer's instructions.

Ten (10) test tubes labelled 1-10 were selected, each tube contained 2mls each of the double strength broth and single strength solution, capped and sterilized by autoclaving at 121°C for 15min. The tubes were removed, allowed cool, and arranged in a row; the first tubes contained double strength broth. To the first tube 2ml of the extracts were added using the sterile pipette and mixed thoroughly. From the first tube 2ml of its content were withdrawn and added to the second tube and mixed properly too. The dilution continued serially to the last tube from which 2ml were withdrawn and discarded. To all the tubes (1-10), a drop of the inoculums (24 hrs culture of the microorganism) was added.

They were incubated overnight, after which they were examined for any sign of turbidity (microbial growth). The tubes that showed no growth represent the MICs. This procedure was carried out for all the test organisms using their culture media.

Antimicrobial agents affect or produce a slight alteration in the normal physiology of an organism. The concentration of an agent that cannot support growth (i.e. inhibit growth) is known as Bacteriostatic concentration and the minimal concentration that are required to produce bacteristatic effect for a given organism is the minimum inhibitory concentration (MIC).

3.5.5. MINIMUM BACTERICIDAL/FUNGICIDAL CONCENTRATIONS.

The minimum bactericidal concentrations (MBCs) and the minimal fungicidal concentrations (MFCs) were determined after the MICs results were obtained. This was done by selecting the tube that shows no growth during the MICs determination. A loopful (sterile wire loop) from the tube containing the media and the extract are inoculated unto a sterile nutrient broth and sabouraud's dextrose liquid of freshly prepared media. These were further incubated for another 24hrs at 37°C for bacteria and 48hrs at 30°C for fungi, after which they were examined for any microbial growth. The lowest concentration at which no growth was observed on the plate gives the MBC and the MFC.

3.6. EXPERIMENTAL ANIMALS

Laboratory rats of both sexes weighing between 100-120g were procured from the Department of Pharmacology and Veterinary Medicine, Ahmadu Bello University, Zaria. They were transported to the

Department of Biological Sciences of the institution and individually housed, and maintained on poultry feed (grower's mesh) and water *ad libitum.* They were left for one week to acclimatize to the new environment.

About 84 Laboratory Rats divided into 14 groups of 6 Rats were used during the experiment. Six (6) groups of 6 Rats were treated with stem extracts, 6 groups of 6 Rats were treated with leaf extracts and the remaining 2 groups of 6 Rats were used as the negative and positive controls.

3.7. EXPERIMENTALLY INDUCED WOUND.

An excision wound model was used to evaluate the wound healing potentials of each of the plant extracts as described by Morton and Malone (1972). The animals were treated humanely during the inducement of the experimental wound. The anticipated area was shaved, marked with a permanent marker and disinfected with 80% alcohol.

A local anaesthesia (Lignocaine) was then used to anaesthetise the animals. A fresh surgical blade was used for each of the animals to completely excise the tissue of the skin to its full thickness. An area of tissue of 2 by 2cm was excised from the depilated side about 5mm from the dorsal back of the Rats. The wounds were humanely induced as shown in plates VI – XI.



Plate VI: The experimental animal after shaving using a Tiger Razor blade.



PlateVII: The animal ready for marking with permanent marker.



Plate VIII: The area of 2x2cm is carefully marked out.



Plate VIV: The animals are light anaesthetised (Lignocaine).



Plate X: Experimental animal with a cut along the marked edge.



Plate XI: The tissue of the skin is cut out to its full thickness using a fresh surgical blade



Plate XII: The wound is left open and measurement taken immediately.



Plate XIII: Some of the Laboratory Rats purchased for the research work



Pate XIV: Sample of the cages used during the experiment



Plate XV: Stock preparation of the extracts used in treatment of the wounds



Plate XVI: Rat with wound contracting and healing.



Plate XVII: A wound about to heal completely.



Plate XVIII: Measurement of the wound area using a centimetre ruler during the wound healing process

3.8. WOUND TREATMENT

The extract treated animals consist of the animals in group 1- 12, that is four (4) groups for each plant. Group 13 form the negative group (untreated group). While the 14th group form the positive group. Animals in the positive control group were treated with a standard drug Spray plus (topical wound spray) purchased from a veterinary store. It is a broad spectrum antibiotic used in the treatment and prevention of local infections of the teat, hoof and skin diseases in animals.

Two (2) concentrations of 500 and 250mg from the leaf and stem extracts of (*C. roseus, M. scaber* and *S. alata*) were prepared for each of the six (6) extracts. The treatment was done by applying the crude extract and the standard drug topically on the wound. The different treatments were applied twice daily from the day of wound excision.

3.9. WOUND MEASUREMENT.

The measurements of the wound area were taken from the day of the excision of the wound and every two (2) days intervals until the epithelisation of the wound is complete. Wound contraction which contributes to wound closure was studied by tracing of the site. A ruler was placed over the wound and the estimate taken to produce a scale calculation.

3.10: DATA COLLECTION

The following parameters were studied.

Epithelisation period – This was monitored by noting the number of days required for the eschar to fall away leaving raw wound behind.

Wound contraction – This was monitored by noting the progressive changes in the wound area. Wounds were traced by placing a centimetre ruler over the wound and the correct estimate was taken. From this percentage of the wound contraction was calculated by taking the initial size of the wound (2 by 2cm) as100%.

Wound healing activity – an excision wound model was used to evaluate the wound healing activity using the ethanol extract from the plant samples.

3.11: STATISTICAL ANALYSIS

The means of the wound area measurements between the test and the control groups were done using student T- test. Data was analysed using analysis of variance (ANOVA) to test for significant differences in terms of the zones of inhibition and wound dressing types (p value was set at< 0.05). Least significant difference (LSD) was used to separate the means.

CHAPTER FOUR

4.0. RESULTS

4.1. PHYTOCHEMICAL ANALYSIS.

The synopsis of the qualitative phytochemical screening carried out on the ethanolic extracts of *C. roseus, M. scaber* and *S. alata* are shown in Table 4: I. Among the eleven (11) bioactive compounds tested, the Free anthroquinones were absent in all the plant extracts. Both the stem and the leaf extract of *C. roseus* contain similar bioactive ingredients. The combined anthracene was absent in the stem of *M. Scaber* as well as glycosides in the leaf extract. Flavonoids, Tannins and Alkaloids were also absent in the stem extract of *S. alata,* while the leaf extracts contained the entire tested compounds except for the free anthraquinones.

4.2. RESULT OF ACUTE TOXICITY STUDIES.

In the acute toxicity screening shown in Table 4:2, Mice of either sex were fed orally with increasing doses of all the six (6) extracts, and the dose up to 5000mg/kg body weight did not produce serious sign of toxicity and no mortality was recorded. The animals were physically active consuming food and water in the regular way after 24 hours of observation. And it is assumed that LD50 values greater than 5000mg/kg are of no practical interest (Lork., 1983). Two levels of concentrations (250 and 500mg) were slated for the wound healing activity i.e. 1/20 and 1/10 of the highest dose used.

Table 4.I: The synopsis of the qualitative phytochemical analysis carried out on the plant extracts.

Plant	*C. roseus*		*M. scaber*		*S. alata*	
Plant part	Leaf	Stem	Leaf	Stem	Leaf	Stem
Constituents						
Carbohydrates	+	+	+	+	+	+
Glycosides	+	+	-	+	+	+
Free anthraquinones	-	-	-	-	-	-
Comb. Anthracene	+	+	+	-	+	+
Cardiac glycosides	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	-
Tannins	+	+	+	+	+	-
Alkaloids	+	+	+	+	+	-
Saponins	+	+	+	+	+	+

KEY:

_ Absence

+ Presence

Table 4:2. The summary of the results of the toxicity studies of the plant extracts.

	No. of Rats	Doses/kg body weight	Mortality					
			CRLE	CRSE	MSLE	MSSE	SALE	SASE
Phase I	3	10mg	0/3	0/3	0/3	0/3	0/3	0/3
	3	100mg	0/3	0/3	0/3	0/3	0/3	0/3
	3	1000mg	0/3	0/3	0/3	0/3	0/3	0/3
Phase II	1	1600mg	0/1	0/1	0/1	0/1	0/1	0/1
	1	2900mg	0/1	0/1	0/1	0/1	0/1	0/1
	1	5000mg	0/1	0/1	0/1	0/1	0/1	0/1

Key:-

CRLE= *Catharanthus roseus* leaves extract.

CRSE= *Catharanthus roseus* stem extract.

MSLE= *Mitracarpus scaber* leaves extract.

MSSE= *Mitracarpus scaber* stem extract.

SALE= *Senna alata leaves* extract.

SASE= *Senna alata stem* extract.

4.3. ANALYSIS OF THE ANTIMICROBIAL ACTIVITIES.

The results presented in Table 4.3 are the zones of inhibition of the ethanol extracts of the Leaf and Stem of *Catharanthus roseus, Mitracarpus scaber* and *Senna alata* plants. The ethanolic extracts of the leaf and stem of C. roseus showed both bacteriostatic and fungistatic effects against all the test organisms, but the stem extract was not active on *Aspergillus niger.*

Mitracarpus scaber leaf and stem extracts exhibited bacteriostatic activities against all the organisms tested, while the leaf extract of *S. alata* was able to inhibit growth of all the organisms. The stem extracts only exhibited bacteriostatic effects on *Staphylococcus aureus* and *Escherichia coli* and was not active on *Pseudomonas aeruginosa.* There were no fungistatic activities shown on *Candida albicans* and *Aspergillus niger.*

Table 4.4: showed the minimum inhibitory concentrations (MICs) of the leaf and stem ethanolic extracts of *Catharanthus roseus, Mitracarpus scaber* and *Senna alata.* The minimum inhibitory concentration values for the plant extracts varied with the types of organisms tested. The most potent were shown against *Staphylococcus aureus, Escherichia coli, pseudomonas aeruginosa* and *Candida albicans* with values of 0.06mg by the stem extract of *M. scaber* and the leaf extracts of *C. roseus* and *S. alata.*

The results of the MBC/MFC for all the extracts are shown in Table 4.5: The leaf and stem extracts of all the plants exhibited bactericidal effects against the tested groups of bacteria except for *M. scaber*, which has no bactericidal effect on *E. coli.* The least MBC values of 0.13mg was shown by the leaf extract of *C. roseus,* against *Staphylococcus aureus, Escherichia coli* and *Mitracarpus scaber* leaf extracts against *Staphylococcus aureus*. The minimum fungicidal effects were shown by the leaf of *M.scaber* and *S.alata* against *Candida albicans* and *Aspergillus niger* with the least value of 0.25mg. All the other extracts did not show any fungicidal activities.

Table 4.3: Zone of inhibition (diameter in mm), shown by the effect of the leaves and stems of *C.roseus, M. scaber* and *Senna alata* extracts on the test organisms.

	Plant					
	C.roseus		*M.scaber*		*S.alata*	
Plant part	Leaf	Stem	Leaf	Stem	Leaf	Stem
Organism						
S.aureu	18b	20b	24a	16b	16b	14b
E. coli	22a	17b	16b	14b	18b	12b
P. aeruginosa	24a	20a	16b	23a	14b	0c
C. albicans	16b	15b	18b	18b	22a	0c
A. niger	14b	0c	18a	15b	20a	0c

Means with the same letter in each column of each plant are not significantly different ($p \leq 0.05$)

Table 4.4: Minimum inhibitory concentration (mg/ml) of the leaf and stem ethanolic extract of *C. Roseus, M. scaber* and *S. alata*.

Test Organisms	C. roseus		M. scaber		S. alata	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
S. aureus	0.13	0.25	0.13	0.06	0.25	0.50
E. coli	0.06	0.13	0.5	0.5	0.25	0.25
P.aeruginosa	0.06	0.25	0.25	0.50	0.25	0.25
C. albicans	0.06	0.50	0.25	0.50	0.06	0.00
A. niger	0.50	0.00	0.50	0.50	0.25	0.00

Table 4.5: Minimum bactericidal /fungicidal concentration (mg/ml) of the leaf and stem ethanolic extract of *C.roseus, M. scaber and S. alata* against the test organism.

Test Organisms	*C. roseus*		*M. scaber*		*S. alata*	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
S. aureus	0.13	0.25	0.13	0.25	0.50	0.50
E. coli	0.13	0.25	0.00	0.00	0.50	0.50
P.aeruginosa	0.25	0.50	0.25	0.25	0.50	0.50
C. albicans	0.00	0.00	0.50	0.00	0.50	0.00
A. niger	0.00	0.00	0.50	0.00	0.25	0.00

4.4. RESULT OF THE WOUND HEALING ACTIVITY.

Significant reduction in the wound area was observed in all the animals treated with ethanol extract compared with those who received the placebo treatment. The wound contraction was measured in centimetre on alternate days for all the test groups until the period of epithelialisation. The mean percentage wound healing effects of the leaf and stem ethanolic extracts of *Catharanthus roseus, Mitracarpus scaber* and *Senna alata* are shown individually in Table 4:6, 4:7 and 4:8

The mean wound healing period (days) of the ethanolic extracts of *Catharanthus roseus, Mitracarpus scaber* and *Senna alata* are shown in Table 4:9. Groups 1, 2, 3 and 4 were treated with *Catharanthus roseus* extracts, groups 5, 6, 7 and 8 were treated with *Mitracarpus scaber* extracts, groups 9, 10, 11, and 12 were treated with *Senna alata* extracts, while group 13 was left untreated (negative control) and 14 was the positive control (treated with standard drug).

The result obtained revealed the existence of anti-microbial compounds in all the plant extracts and the topical wound spray (spray plus). From the analysis of variance (ANOVA), there were significant differences among the wound dressing types. Wounds treated with *Catharanthus roseus* showed a considerable sign of wound healing and significantly ($p \leq 0.05$) heal earlier than the other treatments, as there was fast contraction, epithelialisation and the rapid formation of granular tissue. There was no significant difference between the *C. roseus* extract

treated groups and the positive (drug treated group). The healing rate approximately occurs in 15 - 17 days of the wound treatments.

The mean rate of wound healing of the *M.scaber* and *Senna alata* treated group did not show much significant ($p \leq 0.05$) difference among the wound dressing types, but showed a significant difference when compared to the negative (untreated group). Their mean wound healing rate was between 18 and 19 days, except for group 7 and 11, which has no significant difference with the negative group.

Table 4:6. The mean percentage wound healing effects of the leaf and stem ethanolic extracts of C*atharanthus roseus* in excision wound model

		Days of treatment										
Test Group		3	5	7	9	11	13	15	17	19	21	23
Positive	Mean	23.33 ±2.472	34.17 ±1.537	45.00 ±2.582	60.83 ±3.516	77.50 ±2.141	95.00 ±1.826	0.00. ±0.00	0,00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Crle 250mg	Mean	15.83 ±1.537	23.33 ±1.054	31.67 ±2.472	45.00 ±2.236	55.00 ±2.582	65.83 ±3.516	76.67 ±2.108	88.33 ±1.667	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Crle 500mg	Mean	19.17 ±1.537	30.83 ±2.007	40.00 ±3.162	50.99 ±2.887	68.33 ±2.108	80.83 ±1.537	90.00 ±1.827	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Crse 250mg	Mean	13.33 ±1.054	21.67 ±1.054	27.60 ±2.141	37.50 ±1.708	48.33 ±4.310	65.83 ±4.549	80.00 ±1.291	90.83 ±1.537	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Crse 500mg	Mean	14.17 ±2.386	27.50 ±3.819	40.83 ±3.516	53.33 ±2.472	65.83 ±3.005	77.50 ±1.708	89.17 ±2.007	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Control	Mean	7.50 ±1.708	15.00 ±2.236	27.50 ±1.708	33.33 ±2.472	45.00 ±3.162	51.67 ±2.789	66.67 ±3.575	75.83 ±2.713	85.83 ±1.537	91.67 ±1.667	100.00 ±0.00

N=6, Values are expressed as Mean ± SEM (standard error of mean)

KEY—Crle = *Catharanthus roseus* leaf e/xtract

Crse= *Catharanthus roseus* stem extract

Table 4:7. The mean percentage wound healing effects of the leaf and stem ethanolic extracts of *Mitracarpus scaber* in excision wound model

		Days of treatment										
Test Group		3	5	7	9	11	13	15	17	19	21	23
Positive	Mean	23.33 ±2.472	34.17 ±1.537	45.00 ±2.582	60.83 ±3.516	77.50 ±2.141	95.00 ±1.826	0.00. ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Msle 250mg	Mean	18.33 ±1.054	23.33 ±1.667	29.17 ±1.537	41.67 ±2.108	52.50 ±1.708	61.17 ±1.667	78.33 ±1.667	84.17 ±1.537	91.67 ±1.054	0.00 ±0.00	0.00 ±0.00
Msle 500mg	Mean	17.50 ±2.141	23.33 ±2.789	35.83 ±3.005	48.33 ±3.575	59.17 ±4.362	73.33 ±2.789	81.67 ±2.108	93.33 ±1.054	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Msse 250mg	Mean	15.00 ±1.826	22.50 ±1.708	34.17 ±3.270	41.67 ±4.216	54.17 ±3.007	72.50 ±3.819	85.00 ±2.887	93.33 ±2.108	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Msse 500mg	Mean	16.67 ±1.667	24.17 ±2.386	35.00 ±3.162	47.00 ±3.096	59.17 ±4.167	72.50 ±3.819	83.33 ±2.108	92.50 ±1.118	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Control	Mean	7.50 ±1.708	15.00 ±2.236	27.50 ±1.708	33.33 ±2.472	45.00 ±3.162	51.67 ±2.789	66.67 ±3.575	75.83 ±2.713	85.83 ±1.537	91.67 ±1.667	0.00 ±0.00

N=6, Values are expressed as Mean ± SEM (standard error 0f mean)

KEY—Msle = *Mitracarpus scaber* leaf extract

Msse= *Mitracarpus scaber* stem extract

Table 4:8. The mean percentage wound healing effects of the leaf and stem ethanolic extracts of *Senna alata* excision wound model

		Days of treatment										
Test Group		3	5	7	9	11	13	15	17	19	21	23
Positive	Mean	23.33 ±2.472	34.17 V1.537	45.00 ±2.582	60.83 ±3.516	77.50 ±2.141	95.00 ±1.826	o.00. ±0.00	0.00 ±0.00	0.00 ±0.00	0,00 ±0.00	0.00 ±0.00
Sale 250mg	Mean	11.67 ±2.108	20.00 ±1.826	35.83 ±1.537	42.50 ±1.708	48.33 ±3.073	60.00 ±4.630	78.33 ±2.789	86.67 ±2.108	94.17 ±1.537	0.00 ±0.00	0.00 ±0.00
Sale 500mg	Mean	15.00 ±1.291	25.00 ±2.236	35.00 ±1.826	45.00 ±1.826	60.83 ±2.386	79.17 ±2.386	88.33 ±1.667	95.83 ±2.713	100 ±0.00	0.00 ±0.00	0.00 ±0.00
Sase 250mg	Mean	10.00 V1.826	20.00 ±1.826	33.33 ±2.108	42.50 ±1.708	48.33 ±2.789	58.33 ±2.108	67.17 ±1.537	81.67 ±2.472	92.50 ±1.118	0.00 ±0.00	0.00 ±0.00
Sase 500mg	Mean	15.83 ±1.537	21.67 ±2.108	33.33 ±4.216	46.67 ±4.773	60.00 ±3.651	75.00 ±3.651	84.17 ±2.713	94.17 ±1.537	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Control	Mean	7.50 ±1.708	15.00 ±2.236	27.50 ±1.708	33.33 ±2.472	45.00 ±3.162	51.67 ±2.789	66.67 ±3.575	75.83 ±2.713	85.83 ±1.537	91.67 ±1.667	0.00 ±0.00

N=6, Values are expressed as Mean ± SEM (standard error of mean)

KEY—Sale = *Senna alata* leaf extract

Sase= *Senna alata* stem extract

Table 4.9: Wound healing period (days) in Laboratory Rats.

Animal groups	No. of Animals	Wound dressing types	Healing period (days)
Group 1	6	CRLE250mg/ml	16.31c
,, 2	6	,, 500mg/ml	16.01c
,, 3	6	CRSE250mg/ml	17.46c
,, 4	6	,, 500mg/mi	16.46c
,, 5	6	MSLE250mg/ml	17.75b
,, 6	6	,, 500mg/ml	18.71b
,, 7	6	MSSE250mg/ml	20.88a
,, 8	6	,, 500mg/ml	18.80b
,, 9	6	SALE250mg/ml	19.73b
,, 10	6	,, 500mg/ml	18.73b
,, 11	6	SASE250mg/ml	20.76a
,, 12	6	,, 500mg/ml	19.31b
,, 13	6	NEGATIVE	22.91a
,, 14	6	POSTIVE	14.60c

Mean with the same letter are not significantly different ($p \leq 0.05$)

Key.

CRLE – *Catharanthus roseus* leaf extract

CRSE- *Catharanthus roseus* stem exract

MSLE- *Mitracarpus scaber* leaf extract

MSSE- *Mitracarpus scaber* stem extract

SALE-*Senna alata* leaf extract

SASE- *Senna alata* stem extract

CHAPTER FIVE

5.1: DISCUSSION

All plant parts synthesize some chemicals in themselves which metabolize their physiological activities. These phytochemicals are used to cure disease in herbal and homeopathic medicine (Kesavan *et al.,* 2007). Nowadays, most of the people like to use the traditional methods to cure general diseases (Tyagi and Bohra., 2002).The present research work was focussed on ethanol extracts of the leaves and stems of *Catharanthus roseus, Mitracarpus scaber* and *Senna alata* for their antimicrobial, wound healing activities and phytochemical substances.

5.2: PHYTOCHEMICAL SCREENING

The preliminary phytochemical analysis of the leaf and the stem extracts of the three plant samples used in this research showed the presence of the following bioactive constituents such as carbohydrates, steroids, glycosides, alkaloids, saponnins, tannins, triterpenes and flavonoids. Some of the observed phytochemical constituents present in the plants may be responsible for the wound healing. Tannins have been described to contain a large number of complex substances that are widely distributed in almost all parts of plants. They are usually localized in the various parts of plant such as the leaves, stems, root, and barks (oyi, 2001).

Several researchers have conducted similar type of investigation in different plant species. Aggarwal *et al.* (2002) reported various

phytochemical constituents isolated from *Archyranthes* species (*Archyranthes aspera, A. bidentata* and *A. fauriei*), including steroids, carbohydrates, alkaloids, triterpenes, anthraquinones and amino acids. The preliminary phytochemical screening using different solvents by Kesavan *et al.* (2007) revealed similar bioactive compound which were abundantly found in methanol extracts of the leaves of *Vinca indica.*

Flavonoids Tsuchiya *et al.* (1996) and triterpenes Scortichini and Pia Rossi (1991) are known to promote wound healing due to their astringent and antimicrobial properties, which appears to be responsible for wound contraction and increase the rate of epithelisation.

5.3: ANTIMCROBIAL SCREENING

The antimicrobial activities of the ethanol extracts of *C.roseus, M. scaber* and *S. alata* were tested against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albican* and *Aspergillus niger.* The differences in their anti-bacterial and anti-fungal activities may be as a result of mode of actions and structural properties of the organisms. The result of this study also revealed the antibacterial and antifungal properties of *Mitracarpus scaber* as reported in previous studies (Irodo and Daramola, 1993, Sonago *et al.,* 1996). Bisignano *et al.* (2000), reported that the antifungal activity of *Mitracarpus* methanol extracts against *Candida* may be related to the presence of acetophenone derivatives. The trends of inhibition of the organisms by the extracts may be due to the presence of active chemical

constituents such as alkaloids, tannins, saponins and flavonoids (Adesina *et al*, 2000, Oyi,2002).

The plant samples did not exerted toxic effect on the mice during the toxicity study of the concentrations used. The dose up 5000mg/kg body weight did not produce serious toxic effect and no mortality was recorded. Two levels of concentration were slated for the wound healing activities, that is 250 and 500mg

5.4: WOUND HEALING ACTIVITIES

Wound healing is the body's natural process of regenerating dermal and epidermal tissue. The wound healing potentials of the ethanol extracts of leaves and stems of *C. roseus, M. scber* and *S. alata* were investigated on excision wound model in Rats. The extracts were applied topically twice daily till the complete epithelisation of the wound. There was an accelerated wound healing due to the use of the leaf and stem extracts of *Catharanthus roseus* when compared to both positive and negative control. Shivananda (2006), reported of the presence of tannins, alkaloids and triterpenoids in the flower extract of *Vinca rosea*.

The observed phytochemicals constituent may be responsible for the wound healing activity. Studies have shown that phytochemical constituents like flavonoids, Tsuchiya *et al.* (1996), and triterpenes; Scortichini and Pia Rossi (1991) are known to promote wound healing due to their astringent and antimicrobial properties, which appears to be responsible for wound contraction and increase the rate of

epithelisation. All the plant extracts showed a significant potency in their wound healing activity in the animals treated with all the extracts compared to those that were of the negative control group. From the result of the assessment of wound area, significant reduction of the wound area was observed in all the animals treated with the ethanol extracts compared to those who received the placebo treatment ($p \leq 0.05$). The measurement of the wound area was done on alternate days for all the test groups using a 30cm ruler.

There are many reports of the positive effects of the wound healing potentials of medicinal herbs published in literature. Nayak and Pereira (2006) have shown that an ethanol extract of *C. roseus* flower has properties that render it capable of promoting accelerated wound healing activity. Somashekar *et al. (2007*) studied the wound healing effect of alcoholic and aqueous extracts of *Ocimum sanctum* in rats. Toporcer *et al.* (2008) demonstrated the positive effect of *Atropa balladona* on aseptic surgical wound healing.

The Epithelisation of the wound area was 100% in 14.60 days for the positive and 22.91 days for the placebo control group. The leaf and the stem extracts of *Catharanthus roseus* was able to achieve complete epithelisation in 16.01 and 17.46 days. While complete epithelisation of the wound area for *Mitracarpus scaber* was 17.75 and 20.88 days. For *Senna alata* it occurs between the 18.73 and 20.76 days of the period of treatment.

The wound healing properties of the ethanol extracts of *C. roseus*, *M. scaber* and *S. alata* may be attributed to the antimicrobial effect of the leaves and stems extract and also the phytoconstituents present in them.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

The plants used in this study contain a wide range of bioactive compounds that can be used against both bacterial and fungal microorganisms; therefore the null hypothesis number one was rejected. The concentration of phytochemicals in the plant parts have no lethal effects on the Mice, the second hypothesis was therefore accepted. The leaf and stem extracts of *Catharanthathus roseus, Mitracarpus scaber* and *Senna alata* showed a significant anti-microbial effects on almost all the organisms and significant wound healing potentials. Therefore the null hypotheses number three and four are rejected.

It could be suggested that these plant parts contain a broad spectrum of antimicrobial agents. The result obtained from the antimicrobial studies, the MICS and MBC/MFC values and the accelerated wound healing activities were all promising. Thus, this study provides a scientific rational for the traditional use of the plant in the treatment and management of wounds.

6.2. **RECOMMENDATIONS**

The present study documented the findings that the ethanol extracts of *Catharanthus roseus, Mitracarpus scaber* and *Senna alata* can be used in the treatment of wound.

I wish to recommend that:

> Further pharmacological and clinical studies are required to understand the actual efficacy (pharmacokinetics) of the herbal extracts and the specific phase of wound healing using electron microscopy.

> The roots of these plants could also be investigated for their wound healing potentials.

> The experiment be carried out on larger animals to evaluate its potency on wound healing in man.

> Further research be conducted to identify the active compounds responsible for the biological activity of the plant extracts.

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