PHARMACOGNOSTIC AND ANTIDIARRHOEA ACTIVITY OF LEAVES OF *BAUHINIA RUFESCENS* LAM (CAESALPINIOIDEAE)

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DECLARATION

I hereby declare that this research work was carried out by me under the supervisions of Dr. Abdulkarim Agunu and Prof. K.Y. Musa of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. It has not been presented in any form for a higher degree at any other university or institution. The works of other investigators have been duly acknowledged.

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CERTIFICATION

This thesis entitled "Pharmacognostic and Antidiarrhoea Activity of Leaves of *Bauhinia Rufescens* Lam (Caesalpinioideae)" by Tanko Hajara Yahaya. (Mrs). meets the regulations governing the award of the degree of Masters of Science Pharmacognosy in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This piece of work is dedicated to the three great men that have positively shaped my academic life.

i. My father Aliyu Muhammed of blessed memory

ii. My Uncle DanAzumi Ibrahim of blessed memory

iii. My Beloved Husband Hon Yahaya Tanko.

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ABSTRACT

Bauhinia rufescens Lam (Caesalpinioidae) is a scandent shrub or small tree measuring between 1-3m high but sometimes up to 8m high, often scraggy, stunted and multi-stemmed. The leaves are stomachic, anti-diarrheotic and anti-dysentric. This study is on the pharmacognostic, phytochemical, antimicrobial and anti-diarrhoea (using castor oil induced dirrhoea method), studies of *B. rufescens.* The microscopic studies revealed the presence of anomocytic both at the upper and lower layer, trichomes, calcium oxalate, petiole of 0.57mm, lamina of average length 7mm and width 9.8mm. Quantitative evaluation of the crude plant drug revealed the following parameters, Alcohol soluble extractive of 30.1%, Percentage moisture content: 11%, Total ash value 0.66%, Water insoluble ash value of 6%, Alcohol soluble extractive value of 5.6%. Chemo-microscopy revealed presence of Cellulose, Lignin, Starch, Tannins, Proteins, Mucilage and Calcium oxalate. Preliminary phytochemical studies of the methanolic extract revealed the presence of carbohydrates, saponins, tannins, alkaloids, cardiac glycoside and flavonoid. The methanolic extract was found to be effective against castor oil induced diarrhea on the experimental rats at doses of; 50mg/kg (statistically significant ($P<0.1$) after one hour), 100mg/kg (statistical significance after one hour ($P<0.05$) and after two hours ($P<0.02$)), 200mg/kg (statistical significance after one hour ($P<0.05$) and after two hours ($P<0.02$)). For the antimicrobial screening, The minimum inhibitory concentration (MIC) of *Staphylococcus aureus, Streptococcus feacalis, Escherichia coli, Pseudomonas aeruginosa* and *Candida krusei* were each 2.5mg/ml respectively while that for *Corynebacterium ulcereans*, *Corynebacterium hemolyticum, Shigella dysenteriae* and *Candida albicans* were found to be each 5mg/ml respectively. For the minimum bactericidal concentration (MBC), *Corynebacterium hemolyticum, Shigella*

dysenteriae, and the *fungus Candida albicans* showed the highest MBC of 20mg/ml each, followed by *Corynebacterium ulcereans*, *Escherichia coli,* and the fungus *Candida krusei* with MBC of each 10mg/ml while *Staphylococcus aureus, Streptococcus feacalis* and *Pseudomonas aeruginosa*, showed the least MBC of 5mg/ml. The study revealed that the leaves of *Bauhinia rufescens* contain secondary metabolites that are responsible for its anti-diarrhoeal and antimicrobial activity and thus support the use of this plant by medicinal practitioners in diarrhoeal treatment.

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LIST OF ABBREVIATIONS

b.w.: Body Weight

DMS: Dimethylsufoxide

GC: Gas Chromatography

INF: Interferon

LPS: Lipopolysaccharide

MBC: Minimum Bactericidal Concentration

MIC: Minimum Inhibitory Concentration

MS: Mass Spectroscopy

TLC: Thin Layer Chromatography

TNF: Tumor Necrosis Factor

WHO: World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Brief History of Traditional Medicine

Humans have been concerned with plants through history. For thousands of years, he has used them for one purpose or the other. Early humans recognised their dependence on nature in both health and illness (www.home-herb-garden.com/herbhistory.html). Led by instincts, tastes and experience, ancient men and women treated illness by using plants, animals parts and minerals that were not part of their usual diet. One of the earliest records of the use of herbal medicine is that of chaulmoogra oil from species *Hydnocarpus gaertn*, which was known to be effective in the treatment of leprosy. This information was recorded in the pharmacopoeia of the Emperor Shen Nung of China between 2730 and 3000 B.C. However it was not clear who was the discoverer of this medicinal use of the oil nor when it was discovered (LeStrange, 1972). Similarly the seed of the opium poppy (*Papaver somniferum* L) and castor oil seed (*Ricinus communis L*) were excavated from some ancient Egyptian tombs, which indicated the use of traditional medicine in that part of Africa as far back as 1500 B.C. (Sofowora 2006). Suffice to say that some 3000B.C., man was well aware of the medicinal properties (and probably toxic effects also) of some plants growing around him.

1.2 Traditional Medicine

Traditional medicine is 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 (WHO, 2008). The term herbal medicines is used to cover herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations. Traditional use of herbal medicines refers to the long historical use of these medicines (WHO, 2006). One particular plant or part of a plant can be classified in different categories, such as a medicine, functional/health food, dietary supplement or food, depending on the purpose of its use and the national regulatory framework.

Medicinal plants are known to contain a variety of substances and are used in the treatment of many kinds of ailments in traditional medicine. Most importantly is that they are taken by the majority of the population because they are inexpensive and available (Sofowora, 1982). In some Asian and African countries, 80% of the population depend on traditional medicine for primary health care. In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (e.g. acupuncture) (WHO, 2008).

In practice, the term "traditional medicine" refers to the following components: acupuncture, traditional birth attendants, mental healers and herbal medicine.

During the last decade, there has also been a growing interest in traditional and alternative systems of medicine in many developed countries. One-third of American adults have used alternative treatment and 60% of the public in the Netherlands and Belgium, and 74% in the United Kingdom are in favour of complementary medicine being available within the framework of the National Health Service. A survey among

Member States of the European Union in 1991, identified about 1400 herbal drugs used in the European Economic Community (WHO, 1996).

Medicinal plants are the oldest known health-care products. Their importance is still growing although it varies depending on the ethnological, medical and historical background of each country. Medicinal plants are also important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also when they are used as basic materials for the synthesis of drugs or as models for pharmacologically active compounds (WHO, 1996).

Medicinal plants typically have fewer side effects, and may be safer to use over time since many find the easy availability of herbs appealing (Jeanne, 2011). An example may be seen with herbs and alternative remedies used to treat arthritis. Vioxx, a well-known prescription drug is used treat arthritis, was recalled due to increased risk of cardiovascular complications. Alternative treatments for arthritis, on the other hand, have few side effects. Adjusting the diet to remove vegetables from the nightshade family, reducing white sugar consumption, and adding simple herbs to the diet have few side effects. Most herbal medicines are well tolerated by the patient, with fewer unintended consequences than orthodox drugs. Another advantage of herbal medicine is cost; herbs cost much less than orthodox medications. Research, testing, and marketing add considerably to the cost of orthodox medicines. Similarly, herbal medicines are readily available. Herbs are available without a prescription, and some simple herbs, such as peppermint and chamomile, can be grown at home. In some remote parts of the world, herbs may be the only treatment available to the majority of people (Jeanne, 2011).

1.3 Statement of the Problem

Emergence of more and more multidrug resistant pathogens is reported to be one of the leading causes of death world-wide (Bandow *et al*., 2003). Many infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is very much needed. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of immense therapeutic value (Nostro *et al*., 2000). Even now, approximately 80% of the third world population is almost entirely dependent on traditional medicines for maintaining general health and combating many diseases (Srinivasan, 2005).

1.4 Justification

Research has supported biological activities of some medicinal plants. Now more than ever before due to the various challenges of synthetic drugs of modern medicine, people are increasingly resorting to the use of herbal medicines.

With the onset of scientific research into herbal medicines, it is becoming clearer that the medicinal plants have a potential in today's world, as some diseases are fast becoming resistant to orthodox drugs. According to estimates, only about 20% of the plant flora has been studied and 60% of synthetic medicines owe their origin to plants (Ayurveda, 2005). Folkloric claims coupled with scientific evolution provide us with powerful remedies to eradicate life threatening diseases (Ayurveda, 2005). The pharmacological value of plant's secondary metabolite is increasing due to constant discoveries of their potential roles in health care and as lead chemicals for new drugs development. The use of herbal drugs in the treatment of diarrhoea is a common practice in many African countries. In Nigeria, diarrhoeal infection remains the

number one killer disease among children under five years. While 7-12 month old babies remain the most susceptible (Audu *et al*., 2000). It is estimated that diarrhoea causes 4-5 million deaths annually throughout the world. Eighty percent of these deaths are reported in developing countries including Nigeria (Agunu *et al*., 2005). To combat the problem of diarrhoea in developing countries, the World Health Organisation (WHO) has constituted a diarrheal disease control program aimed at a holistic approach to include all aspects of traditional medical practices, evaluation of health education and preventive approaches (Syder and Merson, 1982; Anon, 1998; Abdullahi *et al*., 2001). There is no doubt that knowledge of medicinal plants activities will help in ascertaining their beneficial or harmful effects on man and animals.

1.5 Hypothesis

1. The plant *Bauhinia rufescens* contain secondary metabolites with antidirrhoea activity.
2. The phytochemicals present in the plant accounts for its antidiarrhoetic properties.

1.6 Aims and Objectives

- To obtain standard parameters of the leaves that can serve as a monograph. This includes macroscopical, microscopical, phytochemical studies and physical constants.
- The evaluation of the folkloric claims of the plant having anti-diarrhoeal and antimicrobial activity.

CHAPTER TWO

LITERATURE REVIEW

2.1 Literature Survey of the family Caesalpinioideae

The family Caesalpinioideae are mainly trees distributed in the moist tropics. Their flowers are zygomorphic, but are very variable. Nodulation is rare in this family, and where it does occur nodules have a primitive structure (Martins *et al*., 2006). This family is found to contain saponins, tannins, mucilage and anthocyanins as secondary metabolites (Trease and Evans, 1992).

2.1.1 Description of the plant *Bauhinia rufescens* Lam (Caesalpinioideae)

Bauhinia is a genus of more than 200 species of flowering plants in the family Caesalpinioideae. The specie, *rufescens* is a shrub or small tree usually 1-3 m high, sometimes reaching 8 m; bark ash-grey, smooth, very fibrous and scaly when old; slash pink; twigs arranged in 1 plane like a fishbone, with thornlike, lignified, lateral shoots, 10 cm long. Leaves very small, bilobate almost to the base, with semi-circular lobes, glabrous, with long petioles, greyish-green, less than 3 cm long. Flowers greenish-yellow to white and pale pink, in few-flowered racemes; petals 5, spathulate 15-20 mm long; stamens 10, filaments hairy at the base. Fruits aggregated, long, narrow pods, twisted, up to 10 cm long, glabrous, obliquely constricted, shining dark red-brown, with 4-10 seeds each. Pods remain on the shrub for a long time.

It is locally called *kargon Allah* or *Matsatsagi*, in Hausa, *Dagbani toha-banggu* in Ghana and "*uncha unha-de-boi*" in Brazil, *kharoub*, *kuikul* in Arabic and *randa* or *jiga*, *jirga* in Wolof (FAO-UNEP, 1983; Burkil, 1999).

2.1.2 Medicinal uses of *B. rufescens*

A cold infusion of the bark is astringent and is used in Nigeria as a wound-dressing and to treat diarrhoea and dysentery. It is rich in tannin and is used locally to tan leather. Trunk and root-barks are prepared as decoctions or infusions in Senegal for treating syphilis and jaundice. The bark, or the root, is also widely used in decoction to treat leprosy and the bark is reported used as a small-pox remedy. The roots is held in Senegal to be febrifugal, diuretic and anti-enteralgic (Burkil, 1999).

The leaves are stomachic, anti-diarrhoetic and anti-dysenteric: though preparations may be variable, macerates are made with millet flour and goats' milk in Senegal. In Mali, the leaves, root and fruit are macerated with millet leaves for dysentery. This is considered by the Bambara to be very effective. Vapour from the boiling pot of the leaves is used in Senegal to treat conjunctivitis. The young leaves are used in Nigeria as an expectorant. Powdered leaves in water are taken internally and used as an external wash for snake-bite in Senegal (Burkil, 1999).

2.1.3 Local uses of *Bauhinia rufescens*

The fruit is eaten by both man and animals. Protein assays is 10-11%. The plant is credited with magical properties in Senegal to ensure the millet harvest and to protect houses from snakes. The wood is light-brown, hard and used in carpentry. The crude bark can be used as tying material, or the fibre can be extracted to produce a cordage (Burkil, 1999).

2.2 Biological Activities of the Genus *Bauhinia*

2.2.1 Antidiabetic Properties

Plants belonging to the genus *Bauhinia* are reported to be important antidiabetic agents; the leaves and stem-bark of these plants are used in different phytopreparations to lower blood glucose levels (da Silva and Cechinel-Filho, 2002; Cavalcanti and Favoreto, 2005; Mali *et al*., 2007).

B. forficata, an Asiatic plant (Miyake *et al*., 1986), is one of the most commonly used plants against diabetes. Though widely studied biologically, its chemical composition has attracted attention due to the isolation of kaempferol-3,7-O-(alpha)-dirhamnoside (kaempferitrin) (1), a flavonoid shown to occur only in the leaves of the plant and, consequently, one that can be used as a chemotaxonomical marker (da Silva *et al*., 2000). Russo *et al*., (1990) evaluated the hypoglycemic profile of the leaf infusion of *B. forficata* against type II diabetic patients and found that the ingestion of an infusion of 3 g of leaves a day, for 56 days, had no hypoglycaemic effect; similarly, Coimbra-Teixeira *et al*., (1992) reported that an alcohol extract from the leaves of *B. forficate* did not lower the glucose blood level in streptozotocin induced diabetic rats. Lino *et al*.,(2004) showed that aqueous, ethanol and hexane extracts of *B. forficata*, evaluated in a model of alloxan-induced diabetes in rats, reduced glucose, triglycerides, total cholesterol and HDL cholesterol levels, validating the clinical use of this plant for the treatment of diabetes mellitus type II. Similarly, Menezes *et al*., (2004) reported that aqueous extracts obtained from *B. monandra* and *B. forficata* leaves exhibited hypoglycemic activity when evaluated in normoglycemic mice. De Sousa *et al*., (2004); showed that kaempferitrin when administered orally, was effective in decreasing blood glucose levels in normal and alloxan-induced diabetic rats, and that this effect was unrelated to reduced intestinal absorption.

Román-Ramos *et al*., (1992) studied the hypoglycemic effect, on *B. divaricata*, and concluded that the decoction of this plant significantly decreased hyperglycemia compared with the control. Lemus *et al*., (1999) evaluated the hypoglycemic activity of a leaf infusion of *B. candicans* in alloxan and streptozotocin-induced hyperglycemic rats, indicated a remarkable hypoglycemic effect in both experimental models, with a greater decrease of glycemia in alloxan diabetic rats. Investigating the hypoglycaemic action of different fractions of the methanol extract of this plant in alloxan-induced diabetic rabbits, showed hypoglycemic activity, along with reduced urinary glucose excretion. The butanol extract exhibited the highest activity and also reduced plasma glucose levels in normal, as well as glucose-loaded rabbits (Fuentes *et al*., 2004). Studies carried out with the aqueous leaf extract of *B. megalandra* indicated that it is able to inhibit intestinal glucose absorption, as well as glucose-6-phosphatase and hepatic gluconeogenesis, suggesting that this plant might help control hyperglycemia in diabetic patients (Gonzalez-Mujica *et al*., 2003). Almeida *et al*., (2006) demonstrated that the methanol leaf extract from *B. cheilandra* exhibited considerable hypoglycemic action when tested on glucose loaded and alloxan-induced diabetic rats. A maximum reduction in the glucose level of blood by 215.9 mg/kg on day 10 was shown, corresponding to a reduction of 55.3% compared with the diabetic control.

2.2.2 Antimicrobial Studies

Plants belonging to the genus *Bauhinia* are frequently used in folk medicine to treat infectious diseases, and several experimental studies have confirmed their antimicrobial potential, especially against pathogenic fungi and bacteria. *B. splendens* has activity against pathogenic bacteria, especially gram-positive microorganisms such as *Streptococcus sp.* and *Staphylococcus aureus* and *Salmonella typhimurium*

(Savi *et al*., 1996). On the other hand, both *B. forficata* and *B. microstachya*, proved inactive when tested against pathogenic bacteria such as *Escherichia coli* and *S. aureus* (da Silva and Cechinel Filho, 2002). Evaluation of the antifungal potential of *B. forficata* showed that it is active against dermatophytes such as *Microsporum canis*, *Trychophyton mentagrophytes, T. rubrum* and *Epidermophyton floccosum*, although it does not inhibit several yeasts (Savi *et al*., 1996). Achenbach *et al*., (1988) studied the chemical composition and antifungal effects of *B. manca* collected in Costa Rica; the results showed that several metabolites, particularly the flavonoids (2S)-3′,4′-dihydroxy- 7-methoxyflavan and (2S)-7,4′-dihydroxyflavan, caused significant inhibition of phytopathogenic fungi such as *Botrytis cinerea*, *Claviceps viridis, Coprinus cinereus, Rhizoctonia solani* and *Saprolegnia asterophora*. Similarly, the dichloromethane extract of *B. rufescens* collected in Niger exhibited antifungal activity against *Cladosporium cucumerinum* (Maillard *et al*., 1991). Studies carried out with *B. racemosa*, a plant used in India to treat various pathologies, including infections, confirmed its production of active principles with antimicrobial potential (Ali *et al*., 1995; Kumar *et al*., 2005) and showed that the broad-spectrum antimicrobial activity against a panel of fungi and bacteria, was due to the presence of phenolic metabolites. On the other hand, *B. variegata*, a plant collected in Nepal, was found to have antimicrobial activity against *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphyllococcus aureus* and *Vibrio cholerae*. The largest zone of inhibition (18 mm) was found to be exhibited against *B. subtilis*. For this organism, the minimum bactericidal concentration of the crude extract was 0.39 mg/kg. However, the existence of synergism was verified, when the activity of the extract decreased during purification (Pokhrel *et al*., 2002). The same plant, collected in India, showed remarkable activity against gram-positive

and gram-negative bacteria. Defatted acetone and methanol extracts were most active against all the organisms studied when evaluated by agar well diffusion method (Parekh *et al*., 2006).

2.2.3 Analgesic Potential Studies

The stem extract of *B. splendens* contains active flavonoids and tannins with antinociceptive potential, when evaluated against acetic acid-induced abdominal constrictions in mice (Cechinel Filho *et al*., 1995), and this effect is dose-related, with a long-lasting inhibition (up to 3 h) against the writhing test by intraperitoneal or oral administration, and that it is also active by both routes in the formalin model, attenuating the first and second phases of this experimental model. Extracts and two phytoconstituents from the leaves of *B. microstachya* leaves have also shown antinociceptive effects that are more potent than the reference drugs (Meyre- Silva *et al*., 2001).

2.2.4 Anti-inflammatory

B. guianensis, which showed anti-inflammatory activity when tested against carrageenan, dextran and histamine-induced paw edema in rats, also inhibited the algogenic process in the writhing test induced by acetic acid in mice (Carvalho *et al*., 1999). The leaves of *B. tarapotensis* are traditionally used in Ecuador as an anti-inflammatory agents. Sosa *et al*. (2002) carried out a bioassay-oriented fractionation, using the inhibition of croton oil-induced ear oedema in mice as a model for anti-inflammatory activity, and identified a 2:1 mixture of ursolic and oleanolic acids as that having the strongest antiphogistic effect, with a potency similar to that of indomethacin. Both triterpenic acids have long been recognized as having anti-inflammatory activity, as well as other important biological properties (Liu, 1995; Deepak and Handa, 2000; Cipak *et al*., 2006). The effects of the methanol stem bark

extract of *B. racemosa* on the acute and chronic phases of inflammation were studied in carrageenan, dextran and mediators (histamine and serotonin)- induced paw oedema, and cotton pellet induced granuloma, respectively, showing pronounced anti-inflammatory properties. The antiedema of this extract was compared with 10 mg/kg of indomethacin orally. In the acute phase of inflammation, a maximum inhibition of 44.9%, 43.2%, 44.8% and 45.9% was observed at 200 mg/kg body weight after 3 h of treatment with a methanol extract in carrageenan, dextran, histamine and serotonin-induced paw edema, respectively. The extract also inhibited peritoneal leukocyte migration in mice. In addition, it produced a significant analgesic effect when analysed in the acetic acid induced writhing and hot-plate tests in mice. The extract also produced a morphine- and aspirin-induced analgesic effect in mice (Gupta *et al*., 2005). Yadava and Reddy (2003) isolated a new flavonol glycoside, 5,7,3′,4′-tetrahydroxy-3-methoxy-7-O-alpha-L-rhamnopyranosyl(1→3)-O betagalactopyranoside from *B. variegata*, which showed anti-inflammatory potential. Recently, Rao *et al*., (2008) isolated several known flavonoids together with a triterpene caffeate from the aerial parts of this plant. They were evaluated as inhibitors of some macrophage functions involved in the inflammatory process. Some of them, particularly kaempferol, ombuin and the triterpene caffeate caused significant inhibition of lipopolysaccharide (LPS) and interferon (INF)-gamma induced nitric oxide (NO), and cytokines [tumor necrosis factor (TNF)-alpha and interleukin (IL)-12]. *B. malabarica*, a plant growing in Thailand, produces biologically active products such as racemosol and 10-O-demethylracemosol, which exhibit potent in-vitro anti-inflammatory activity against cyclooxygenase-1 and -2 (COX-1 and 2) enzymes (Kittakoop *et al*., 2000; Songarsa *et al*., 2005). These metabolites have been used as models for the synthesis of several derivatives and to carry out structure-

activity relationship studies. Recently it was demonstrated that the leaves of *B. purpurea* have an analgesic effect when evaluated against the writhing, hot plate and formalin tests, and an anti-inflammatory effect in the carrageenan-induced paw edema test (Zakaria *et al*., 2007). In the hot plate test, the aqueous extract caused prolonged latency at 60 mg/kg for 3 h. When evaluated against both phases of the formalin-induced model, the aqueous extract at 6 mg/kg exhibited the highest activity, which was equieffective when compared with 5 mg/kg morphine in the early phase but with 100 mg/kg acetyl salicylic acid in the late phase. The anti-inflammatory activity of aqueous extract against carrageenan-induced paw edema test in rats was observed at 30 mg/kg, comparable to the 100 mg/kg acetyl salicylic acid between the interval times of 1–4 h. Boonphong *et al*., (2007) isolated several new and known metabolites, especially bibenzyls and dihydrodibenzoxepins, some with anti-inflammatory activity from the roots of this plant.

2.3 Pharmacological and Toxicological Investigations of *Bauhinia*

It is recognized that plants of the genus *Bauhinia* are not toxic (Barata, 2005). This has been confirmed by several experimental investigations using animal models, e.g. Pepato *et al*., (2004) evaluated an orally administered decoction of *B. forficata* for its effects on the toxicity markers amylase (pancreas toxicity), creatine kinase (muscle toxicity), lactate dehydrogenase (muscle and liver toxicity), bilirubin (liver and biliary toxicity) and angiotensin-converting enzyme (renal microcirculation and kidney toxicity), and concluded that the decoction could be used in the treatment of diabetes, because it improves the diabetic condition, without causing tissue toxicity as shown by the biomarkers used. Damasceno *et al*., (2004) determined the effects of diabetes on the defense system against oxidative stress in pregnant female rats, and evaluated the influence of the *B. forficata* extract on the antioxidant system, glycemic control,

hepatic glycogen, cholesterol, triglycerides, total proteins and lipids. The results showed that experimental diabetes induced by streptozotocin in female rats causes repercussions in the antioxidant defense system, with an increase in uric acid and a decrease in glutathione and superoxide dismutase activity, and that treatment of pregnant diabetic female rats with *B. forficata* did not control maternal hyperglycemia, hyperlipidemia or hypercholesterolemia, but it did increase the amount of hepatic glycogen, decreased the uric acid concentration, and increased the glutathione activity. This suggests that the extract of the plant acts as an antioxidant defense system, probably due to the presence of the previously detected flavonoids (da Silva *et al*., 2000; da Silva and Cechinel Filho, 2002; Pizzolatti *et al*., 2003). A recent report demonstrated that the stem-bark extract of *B. racemosa* does not alter the hematological parameters, liver and kidney functions, at doses of 100 and 200 mg/kg body weight in mice, indicating that it is free of toxic effects. However, at a higher dose (400 mg/kg), the treated groups exhibited significant alterations in their haematological profile and hepatorenal functions and metabolism (Kumar *et al*., 2005). Similarly, Reid *et al*., (2006) found that leaf extract of *B. galpinii* showed antimutagenic properties in the presence of metabolic activator S9 and that it did not cause any mutagenic response against *Salmonella typhymurium* bacterial strains in the Ames assay, confirming its non-toxic effects.

2.4 Phytochemical Constituents of the Genus *Bauhinia*

Review of the chemical composition of the genus *Bauhinia* showed a predominance of terpenes, steroids, alkaloids and especially flavonoids (da Silva and Cechinel- Filho, 2002). Pizzolatti *et al*., (2003) isolated several flavonoids from the leaf extract of *B. forficata* (see figure 2.1), including kaempferol (1) and flavonoid glycosides identified as kaempferitrin (2). From the flowers, only the flavonoid

kaempferol was isolated. It is interesting to note that flavonoid kaempferitrin, is present only in *B. forficata*, making it a suitable chemical marker for phytopreparations containing this plant (da Silva *et al*., 2000). Research done by Zhao *et al*., (2005) on the root bark extract of *B. variegata* and reported the isolation of flavonone, together with quercetin and kaempferol. Phenanthraquinone has also been isolated from the stem bark extract of *B. variegata* (Reddy *et al*., 2003).

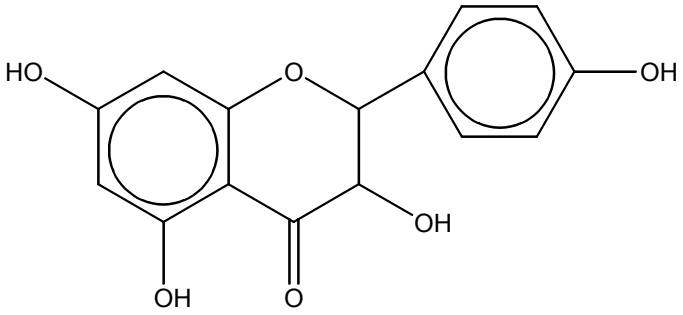
Rao *et al*., (2008), searching for new anti- inflammatory natural agents, isolated kaempferol from the aerial parts of *B. variegate*. The species, *B. microstachya*, showed the presence of phenolic metabolites (da Silva and Cechinel Filho, 2002; Bianco and Santos, 2003).

Studies carried out on *B. ungulata* collected in Brazil resulted in the isolation and identification of the flavonoids quercetin, quercitrin and alkaloids. While, bioassay guided fractionation of the two active fractions of *B. galpinii* led to the isolation of the flavonoid glycosides quercetin-3-O-galactopyranoside. (Neto *et al*., 2008).

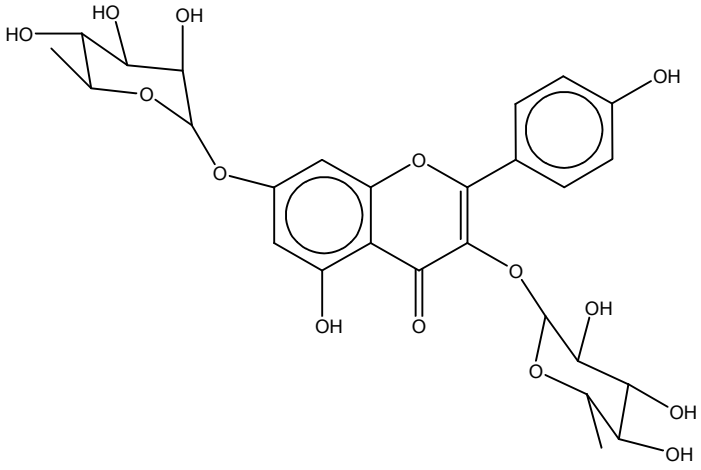
Research carried out on *B. aurea* led to the isolation of several known metabolites, including aromatic acid derivatives, triterpenes and steroids (Shang *et al*., 2006). Shang et at., (2006) also isolated, from *B. aurea*, the flavonoids astilbin, isoastilbin, isoengeletin, neoastilbin, neoisoastilbin and catechins. Studies carried out with *B. malabarica* indicate that besides racemosol derivatives (Kittakoop *et al*., 2000), it produces flavonoids such kaempferol, quercetin, isoquercitrin and quercitrin (Kaewamatawong *et al*., 2008).

Research carried out on volatile oils of seven species of Bauhinia (*B. aculeata, B. alata, B. brevipes, B. forficata, B. longifolia, B. outimouta, B. pentandra, B. rufa* and *B. variegata*) indicated that *B. alata* and *B. outimouta* have no detectable volatile

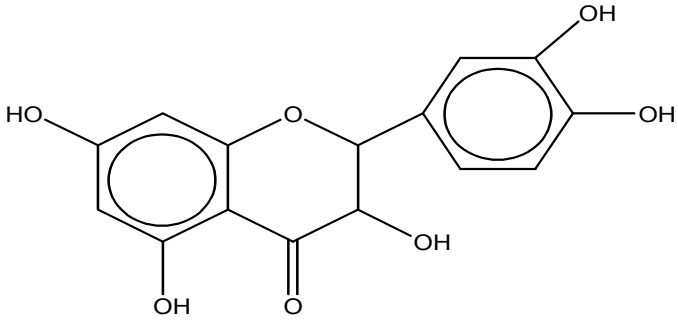
oils, while *B. brevipes* and *B. rufa*, both from savanna ecosystems, yielded 0.25% and 0.30% of volatile oil, respectively, and the remaining species, from mesophytic forests, yielded low amounts of oil. The oils, when analysed by GC/MS, revealed the presence almost exclusively of sesquiterpenes, (Duarte-Almeida *et al*., 2004).



(Kaempferol (1)



kaempferitrin (2)



Quercetin (3)

Figure 2.1 Some flavonoids

2.5 Evaluation of Crude Plant Drugs

The kingdom plantae is made of a variety of plant having different chemical compositions. Yet, not all plants are presently known to be medicinally useful, even members of the same genus may not all be recognised as official drugs. Thus it is necessary to evaluate the correct identity, source, quality and purity of plant drugs. Thus pharmacognostic studies are very important for the identification and determination of potential drugs from plants. Such pharmacognostic studies include the following;

a) Morphological or Organoleptic evaluation
b) Physical characteristics of the drug
c) Microscopic studies
d) Chemical studies
e) Biological studies

Morphological evaluation refers to evaluation of drugs by colour, odour, taste, size, shape, and special features such as touch, texture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Organoloeptic evaluation include conclusions drawn from studies on impressions on organs of senses (Kokate *et al*., 2008).

Determination of various constants of a drug is important in evaluation of its identity and purity. These constants are important because, they are constants for a particular drug but varies from drug to drug. Thus they serve as useful tools for identification of a drug. These parameters of natural drugs include extractive values, ash values, moisture content and other physical constants (Kokate *et al*., 2008).

Microscopical evaluation employs the use of microscope and its accessories in order to evaluate a drug. It allows more detailed examination of a drug and it can be used to identify the organised drug by their known histological characters. The microscope by virtue of its property to magnify objects, permits the minute structure under study to be enlarged and used to confirm structural details of the drugs from the plant origin. The microscope is indispensable in evaluating powdered drugs. Quantitative and qualitative microscopical test are available. The former uses all qualitative characters such as presence of starch grains, calcium oxalate crystals, trichomes and their shapes and sizes. The latter uses some numerical microscopical constants such as stomatal number, stomata index, vein islet number and vein termination number (Kokate *et al*., 2008).

Chemical tests are also very important in the verification of plant drugs. The chemical test is one of the most important in the proper identification of plants because the plants are used as drugs due to their chemical constituents. Some of the qualitative chemical tests and quantitative chemical assays of the various chemical constituents of drugs are already available, for example Vitali's test for tropane alkaloids etc (Kokate *et al*., 2008).

Biological tests are also used as means of evaluating plants drugs. It is used in determining the potency and extent of pharmacological and microbiological activity of the constituents of the drugs. The purity of a crude plant drug can be ascertained ascertained by the quantitative estimation of active chemical constituents present in them (Kokate *et al*., 2008). The method may be useful in determining single active constituent or the group of related constituents present in the same drug.

The chemical character is one of the most important among the pharmacognostic features of plant identification, because the chemical nature of the constituents of plant drugs can be definitely established by various chemical tests. The chemical parameter has therefore received wide attention in pharmacognostic studies. Many plant drugs are now compared and identified based on their chemical constituents.

It must be noted that for proper identification of a plant, using only one of these characteristics may not be sufficient to establish identity of a drug. Thus a combination of two or three different parameters may be necessary to establish the correct identity of a drug.

2.6 Chemical Constituents of Plants

Plants produce a variety of chemical substances of different nature, that not withstanding, not all chemical compounds produced by plants are of equal interest. The secondary metabolites are the most important one in pharmaceutical consideration (Trease and Evans, 1992). However, the active principles of plants deserve special attention, both because of their abundance in the plant due to their pharmacological activity in the living system. The secondary metabolites are found to be the active principles in most cases. Other plants substances such as carbohydrate, fats are of dietetic importance, while gums, starch, calcium oxalate, lignin are important in the identification of drugs and detection of adulterants in them (Trease and Evans. 1992). Chemical characters are important in pharmacognostic studies if they occur in significant quantities in the plant. Chemical constituents in trace amount cannot certainly be taken as taxonomic or identification aids (Gibbs, 1963).

In the search for new drugs to cure existing ailments, or to provide alternatives to the present approach of treatment, pharmacognostic evaluation of drug becomes necessary. The side effects of many drugs especially synthetic drugs are essential consideration in screening plants for their active constituents.

B. rufescens leaves which is traditionally used in Zaria and its environs was evaluated to determine some of its pharmacognostic, anti-diarrhoeal and anti-microbial profiles.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant collection, Identification and Preparation

Leaves of *Bauhinia rufescens* Lam (Caesalpinioideae) were collected around Shika, outskirts of Zaria, Kaduna State, Nigeria. The plant was identified by a Taxonomist (Mal. Umar Galla) at the Herbarium unit, Biological Sciences Department, Ahmadu Bello University, Zaria. Where, a voucher specimen number (427) has been deposited. The sample was air dried at room temperature and then powdered using mortar and pestle. The powders were sieved with sieve No. 40. The powders of the leaf were parked in an air- tight containers and labelled properly until required for further use. For microscopical work, fresh leaves samples were obtained whenever the need arose.

3.1.2 Equipment and apparatus

The equipments and apparatus were those available at the Departments of Pharmacognoscy and Drug Development, Pharmaceutical Microbiology and Pharmacology and therapeutics, in Ahmadu Bello University, Zaria, Nigeria.

3.1.3 Solvents and Reagents

All the solvents and reagents used were of analytical grade.

3.2 Pharmacognostic Studies of The Leaves Of *Bauhinia rufescens*

The macro-morphological, microscopy, chemo-microscopy and physical constants studies of the leaves of *Bauhinia rufescens* were carried out using fresh and dried powdered sample as outlined in the procedures below: -

3.2.1 Macro-morphological examination

The general feature of the fresh leaf of the plant were studied. The lengths of the petiole, the size (length and breath) of the lamina were measured with a ruler. The shape, composition, venation, type of the margin, apex and base of the lamina were observed and noted. Organoleptic character (odour, taste and texture) of the leaf were tested on powdered leaf. Length and thickness were measured with a ruler. The shape, colour and type of fracture of the leaves of *Bauhinia rufescens* were tested on the powdered leaves. Both fresh and dried samples were examined. Length and thickness were measured with a ruler. Odour, taste and texture of the leaves were studied on the powdered drug using standard methods (Evans, 1996).

3.2.2 Microscopical Examinations

Microscopical analysis was conducted on the epidermal peel of the fresh leaf of the plant. The shape, size and wall structure of the epidermal cells; the form, distribution and relation of the epidermal cell to the stomata; the form, distribution and abundance of the epidermal trichomes were observed and drawn. The transverse section of the midrib and lamina were made using sharp razor blade and examined. Powdered leaf was examined and diagnostic characters of the plant were observed and noted (Evans, 1996).

3.2.3 Chemo-microscopical Examinations

Chemo-microscopy was also carried out on the whole and powdered leaf of *B. rufescens*. The presences of cellulose, lignin cutin, suberin, tannin, starch, and calcium oxalate crystals were tested using methods described in Evans (1996) and Brain & Tuner (1975).

3.2.3.1 Cellulose

To the sample on a microscopic slide, two drops of N/50 iodine solution was added followed by a drop of 66% solution of sulfuric acid. A blue stain indicates the presence of cellulose.

3.2.3.2 Lignin

To the sample on a slide, two drops of phoroglucinol was added and allowed to stand for two minutes. It was followed by one drop of concentrated hydrochloric acid and examine for the presence of red or pink stain, which will show the presence of lignifications.

3.2.3.3 Starch

To the samples on a slide, two drops of N/50 iodine solution was added. Presence of blue black stain indicates the presence of starch.

3.2.3.4 Proteins

To the sample on a slide, two drops of pitric acidn was added. Presence of yellow stain indicates the presence of proteins.

3.2.3.5 Tannins

To a small amount of the powdered sample, one drop of ferric chloride solution was added. A blue-black or greenish-black colour shows the presence of tannins.

3.2.3.6 Gum/mucilage

To the sample on a slide, two drops of Ruthenium red solution was added and observed for a red stain which will indicate the presence of gum and mucilage.

3.2.3.7 Calcium oxalate crystals

The powdered leaves were cleared by warming with chloral hydrate solution. Calcium oxalate crystals appear as bright structures. Treatment of the crystals with concentrated hydrochloric acid dissolve them slowly without evolution of gas.

3.2.3.8 Calcium carbonate

To the powdered sample on a white tile, five drops of acetic acid were added. Rapid evolution of gas indicates the presence of calcium carbonate.

3.2.4 Determination of Some Physical Constants

The following physical constants were determined for the powdered leaf based on the procedures outlined by Brain and Tuner (1975) unless otherwise stated.

3.2.4.1 Quantitative extractive values:

From the plant material, 10g was weighed and transferred into a conical flask. 100ml of alcohol was measured, added to the flask and stoppered. The flask and its content were placed on a mechanical shaker for six hours and allowed to stand for another eighteen hours. The content was filtered. 20ml of the filtrate was taken into a clean, dried and previously weighed evaporating dish. The filtrate was evaporated to dryness on a water bath, allowed to cool in a dessicator and weighed. Two more 20ml aliquot of the filtrate were treated similarly and the percentage of the alcohol soluble

extractive value with reference to the air dried material was examined from the formula:-

Alcohol soluble extractive value = weight of residue/weight of sample x 100

The same procedure above was repeated with chloroform-water (0.5% chloroform in water) in place of alcohol to determine the water soluble extractive value.

3.2.4.2 Determination of ash value

The powdered material (2.0g) was weighed and transferred to a clean and dry crucible. The powder was heated until it charred in an oven at 105^{o}C. After 1 hour, it was allowed to cool in a dessicator and weighed. Two more samples were treated similarly. The total ash value was calculated with reference to the air dried material as follows:-

Total ash value = weight of ash/weight of sample x 100

3.2.4.3 Water soluble ash value

The ash obtained from the total ash value determination was quantitatively transferred on to a beaker containing 25 ml of water. The beaker and its content was boiled for five minutes and filtered through an ashless filter paper. The crucible was washed several times with warm water and the washing passed through the filter paper. The filter paper and residue were transferred to the crucible and placed I the oven for an hour. It was cooled and weighed from where the percentage of water soluble ash was obtained from the following formula

Water soluble ash = weight of total ash – weight of residue/weight of total ash x 100

3.2.4.4 Alcohol soluble ash value

Exactly 2.0g of the powdered plant material was treated in similar manner as in the determination of total ash and latter treated with alcohol in place of water in the determination of water soluble ash. The alcohol soluble ash value was obtained from the following formula;

Alcohol soluble ash value = weight of residue / weight of sample x 100

3.2.4.5 Moisture content determination

Exactly 2.0g of the plant material was weighed into a clean, dry and previously weighed metal crucible. The crucible and its content were heated in an oven at 115^{o}C for 1 hour. It was removed from the oven, cooled in a desiccator, and weighed. Heating, cooling and weighing were repeated until a constant weight was obtained. Two more samples were treated similarly. The percentage moisture content was calculated with reference to the original sample from the following formula;

Moisture content = weight of water loss/weight of sample x 100

3.3 Preliminary Phytochemical Screening

Basic phytochemical screening to detect the presence or absence of plant chemical constituents such as alkaloids, tannins, saponins, anthraquinones,, cardenolides, flavonoids, etc were carried out on the powdered leaves using the procedures outlined below;

3.3.1 Test for Anthraquinone and their Derivatives

The plant material (2.0g) was extracted with l0 ml of benzene and filtered. 5ml of 10% ammmonia solution was added to the filterate and shaken. The presence of a

pink, red or violet colour in the ammoniacal (lower layer) phase indicates the presence of free hydroxy anthraquinone. For the anthraquinone glycosides, 2.0g of powered material was boiled with 10ml of dilute sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer was separated and to it, 3ml of 10% ammonia solution was added and mixture shaken. A pink, red or violet colouration in the lower layer shows the presence of anthraquinone glycosides (Sofowora, 1993).

3.3.2 Test for Saponin Glycosides

a. Frothing test

The powdered material (0.5g) was shaken with 5ml of water in a test tube for 30 seconds. A persisted froth suggests the presence of saponins.

b. Haemolysis test

The plant material (1.0g) was extracted with warm water and filtered. 2ml of 1.8% aqueous sodium chloride solution was taken into two separate test tubes. To one of the tubes 2ml of the aqueous plant extract was added, to the other test tubes, 2ml of water was added. 5 drops of animal blood was added to each of the two test tubes and their content mixed by gentle inversion of the tubes. Haemolysis in the tube containing the extract but not in the control tube (the tube containing water only) indicates the presence of saponin glycosides (Brain and turner, 1975).

3.3.3 Test for Steroids and Terpenoids

a) *Salkowski's test*

The powdered drug (0.5g) was extracted with 5ml of chloroform and filtered into a clean and dry test tube. 2ml of concentrated sulphuric acid were carefully added

down the side of the test tube to form a lower layer. A reddish brown colour at the interface indicates the presence of steroidal ring (Sofowora, 1993).

b) *Liebermann - Burchard's test*

The powdered plant material (0.5g) was extracted with 5ml of ethanol and filtered. The filtrate was evaporated to dryness on water bath. The residue was shaken with chloroform and filtered into a clean, dry test tube. 2ml of acetic anhydride was added to the filtrate and shaken. 1 ml of concentrated sulphuric acid was added carefully down the side of the tube to form a lower layer. A brownish-red or violet ring at the zone of contact of the two liquids and the upper layer turning green denotes the presence of sterols and terpenes (Ciulei, 1990).

3.3.4 Test for Tannins

a. *Ferric chloride test*:

The powdered leave (2.0g) was extracted with 10ml of water and filtered. To 2ml of the filtrate in a test tube two drops of ferric chloride solution were added. A green or greenish black precipitate indicate the presence of condensed tannins, while a blue or bluish black precipitate shows the presence of hydrolysable tannins (Evans, 1989).

b. ***Lead acetate test***

To 1ml of the aqueous extract, three drops of lead subacetate solution was added. A coloured precipitate indicates the presence of tannins (Abubakar,1993).

3.3.5 Test for Carbohydrates

The powdered leaves (3.0g) was boiled with 50ml of water for 3minutes on a hot plate. The mixture was filtered while hot and allowed to cool for the following tests:-

a. ***Molisch's test*** (General test for Carbohydrates)

To 2ml of the aqueous extract in a test tube was added three drops of Molisch's reagent followed by another three drops of concentrated sulphuric acid down the side of the test tube which was allowed to form a lower layer. A purple to violet coloured interface indicate the presence of carbohydrates. The mixture, when shaken gently and allowed to stand for two minutes, followed by dilution with 5ml of water would produce a dull violet precipitate-another indication of carbohydrates (Evans, 1989).

b. Barfoed's test for monosaccharide

1ml of Barfoed's reagent was added to 1ml of aqueous plant extract in a test tube and the mixture heated on a water bath for 5 minutes. A red precipitate shows the presence of monosaccharide (Evans, 1989).

c. Fehling's test for free reducing sugars

To 5ml of a mixture (1:1) of Fehling solution A and Fehlings solution B was added 2ml of the water extract and the mixture boiled on water bath for 5 minutes. A brick-red precipitate indicates the presence of free reducing sugars (Evans, 1989).

3.3.6 Test for Alkaloids

The powdered plant material (10g) was weighed in to a beaker. It was moistened with concentrated ammonia solution and allowed to stand for 20mins. 50ml of chloroform was added; the mixture was stirred for 10mins and filtered through a plug of cotton wool. The marc was washed twice with 5ml chloroform and the washings were added to the filtrate. The combined filtrate was evaporated to dryness on a water bath. The residue cooled and dissolved in 20ml chloroform. The chloroform extract was transferred to a separating funnel and shaken with 25ml of 5% sulphuric acid. The two layers were allowed to separate. The chloroform (lower) layer was drained off and discarded. 10ml of chloroform was further added, shaken, drained off and discarded until the acid layer was colourless (Evans, 1989).

The acid layer was made alkaline with concentrated ammonia solution and extracted with 20ml of chloroform. The extract was retained and evaporated to dryness. The residue was dissolved in 10ml of ethanol and the following tests were carried out:-

General test: To 1rnl of the methanol extract in separate test tubes, 2-3 drops of the following reagents were added;

a. ***Wagner reagent***: Light brown to brownish precipitate indicates the presence of alkaloids
b. ***Mayer's reagent*** : White or creamy white precipitate indicates the presence of alkaloids
c. ***Dragendorff's reagent***: Orange or orange-yellow precipitate indicates the presence of alkaloids

d. ***Tannic acid solution***: Buff coloured precipitate indicates the presence of alkaloids

3.3.7 Test for Cardiac Glycosides

The powdered leaves (1.0g) was boiled with 10mI of 70% ethanol on a water bath for 2 minutes. The extract was filtered and diluted with water. 1ml of strong lead subacetate solution was added and filtered. The filtrate was divided into two for the following tests (Evans, 1989):-

a. Keller-killiani Test (for deoxy sugars):

To the first portion of the filtrate was added chloroform, the chloroform layer (lower) was separated and evaporated to dryness on water bath. The residue was dissolved in 3ml of 3.5% ferric chloride solution in glacial acetic acid. The mixture was allowed to stand for a minute before it was transferred to a test tube. 1.5ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer which, on standing, will give a brown colour at the junction of the two liquids and a pale green colour in the upper layer indicates the presence of cardiac glycoside (Brain and Turner, 1975).

b. Kedde's Test For Cardenolides

To the second portion of the filtrate 1 ml of 2% solution of 3,5-dinitrobenzoic acid in methanol was added followed by 2ml of 5% aqueous sodium hydroxide solution. An immediate violet colour indicates the presence of cardenolides in the vegetable drug (Sofowora, 1993).

3.3.8 Test for flavonoid

a. Shinoda's test

The powdered drug (1.0g) was boiled with ethanol on a water bath for 5 minutes, filtered and cooled. The filtrate was diluted with ethanol. Some pieces of magnesium filing were added followed by five drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids (Balba, 1976)

b. Sodium hydroxide test

The powdered drug (2.0g) was completely dettanned with acetone and the acetone discarded. The residual acetone was evaporated on water bath and the residue was extracted with warm water. It was filtered while hot then allowed to cool and used for the following test.. 5ml of the detanned water extract was taken in a test tube and mixed with an equal volume of 10% aqueous sodium hydroxide solution. A yellow solution indicates the presence of flavonoids. On addition of dilute hydrochloric acid, the solution becomes colourless (Brain and Turner, 1975).

c. Ferric chloride test

The detanned water extract (2ml) was diluted with water in the ratio of 1:4 and three drops of 10% ferric chloride solution was added. A green or blue colour indicates the presence of phenolic nucleus (Abubakar, 1993).

3.4 Thin Layer Chromatography (TLC) on Methanolic Extract of leaf of *Bauhinia rufescens*

Thin Layer Chromatography (TLC): stationary phase: Silica gel (60 F_{254}, Pre-coated plates (manufactured by Merck)) of 0.25mm thickness were used. Spotting was done manually using glass capillary tubes. The plates were developed at room

temperature using chromatographic tank. The chromatograms were then allowed to dry and then observed under iodine tank before spraying with 5% sulphuric acid and placed in the oven at 120^{o}C for 2 minutes.

3.5 Castor Oil Induced Diarrhoeal Studies

3.5.1 Animals

Swiss albino mice weighing 18 to 30g maintained at the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria were used for the experiment. The animals were fed with the standard laboratory feeds and water *ad libitum*. This research was carried out according to the rules governing the use of laboratory animals as acceptable internationally.

3.5.2 Acute toxicity study

A total of 13 mice were used for the experiment. In the first phase, three doses of the methanolic extract were administered to three groups each containing three mice. In the second phase, more specific doses were administered to four groups each containing one mouse. The median lethal dose (LD_{50}) value was determined as the geometric mean of the highest non lethal dose and the lowest lethal dose of which there is 1/1 and 0/1 survival (Lorke, 1983).

3.5.3 Effects of castor oil-induced diarrhea in mice

The mice were fasted for 18 hours prior to the commencement of the experiment and were randomly divided into five groups of five mice each. The first group (group I) was administered normal saline, while groups II, III and IV were administered 50, 100, and 200mg/kg respectively of the methanolic extract orally. Group V administered loperamide (5mg/kg). An hour later, castor oil was administered

0.2ml/mouse orally. The animals were placed in individual cages over clean filter papers. After the administration of the castor oil, the cages were inspected for the presence of characteristic diarrhea droppings after every one hour for three hours. The absence was considered as protection from diarrhea (Diurno *et al*., 1996) and the percentage protection calculated (Akah and Offiah, 1996).

3.6 Antimicrobial Studies

3.6.1 Test Organisms

The gram positive bacteria for this research viz; *Streptococcus aureus, Staphylococcus pyogenes, Streptococcus feacalis, Corynebacterium ulcereans, Corynebacterium hemolyticus,* and gram negative bacteria; *Salmonella typhi, Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa, Klebsiella pneumonia* and the fungi *Candida albicans, Candida krusei, Aspergillus flavus* and *Aspergillus fumigates* were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. All the micro-organisms (clinical isolates) were checked for purity and maintained in slants of agar.

3.6.2 Cultivation and Standardization of Test Organism

A loop full of each of the test organisms was taken from the agar slant and sub cultured into test tubes containing 20 ml of sterile nutrient agar (for bacteria) and sabouraud dextrose agar medium (for the fungi). The test tubes were then incubated for 24 hours at 37^{o}C for two days (for bacterial) and 27^{o}C for 2-7 days (for fungi). The growth culture was standardized using sterile normal saline to obtain a density of 10^{6} cfu/ml for bacteria. A sporulated test fungal spores was harvested with 0.05%Tween80 in sterile Normal saline and standardized to 10^{6} spores/ml (Lino and Deogracious, 2006).

3.6.3 Preparation of Culture Media

The prescribed quantities of the dehydrated bacteriological culture media was weighed and hydrated with distilled water according to the manufacturers specification. Where necessary, gentle heat was applied to aid dissolution and the resultant suspensions were dispensed into clean bottles and sterilized at 121^{o}C for 15 minutes in an Adelphi bench autoclave.

3.6.4 Antimicrobial Profile (susceptibility test)

The antibacterial screening was carried out using a modified agar diffusion method described by Lino and Deogracious (2006). The extract (0.2g) was weighed and dissolved in 10mls of Dimethyloxosulphite (DMS) to obtain the initial concentration of 20mg/ml of the extract. Overnight culture of the various bacteria in blood agar and the fungi in sabouraud dextrose agar slant media were sterilized to produce inoculums size of 10^{6}cfu/ml. The medium was seeded with 0.1 ml of standard inoculums of the micro-organism (Mc-Forland 0.5). The inoculums was sprayed evenly by the use of sterile swab over the surface of the medium, the seeded plates were allowed to dry at 37^{o}C and 27^{o}C for the bacteria and fungi respectively for 30mins inside incubator. A standard cork borer of diameter 6mm was used to cut a well at the centre of each seeded medium used and 0.1ml of the solution of the extracts was then introduced into each hole on the surface of the medium of each bacteria. In one medium was introduced 0.1ml of DMS to serve as negative control and in another, Spafloxacin and Fluconazole to serve as positive control for the bacteria and fungi respectively. The medium was incubated at 37^{o}C for 24 hours (for the bacteria) and 27^{o}C for 2-7 days (for fungi) after which the plates were observed for zones of inhibition. The zones of inhibition were measured with a transparent ruler and the result recorded.

3.6.5 Determination of Minimum Inhibitory Concentration

This was done using broth dilution method as described by Vollekova *et al*., (2001) and modified by Usman *et al.*, (2007). In this method, 10ml nutrient broth (prepared according to manufacturers specifications) was dispensed into test tubes and sterilized at 121^{o}C for 10 minutes and allowed to cool. Mc-Forland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was inoculated with each of the test micro-organism and incubated at 37^{o}C for 6 hours to make a turbid suspension of the micro-organisms. After incubations, dilution of the micro-organism in dimethyloxosulphite (DMS) was done until the turbidity ($1.5x10^{6}$cfu/ml) matched that of the Mc-Forland scale by visual comparism. Two fold serial dilution of the extract in the broth was done to obtain the following concentrations; 20mg/ml, 10mg/ml, 2.5mg/ml, 1.25mg/ml and 0.625mg/ml. From the suspension of the micro-organism in DMS, 0.1ml was inoculated into the different concentrations of the extract in the nutrient broth. The broths were incubated at 37^{o}C for 24hrs (for the bacteria) and 27^{o}C for 2-7 days (for the fungi) after which the test tubes wee observed for turbidity. The lowest concentration of the extract in the broth which shows no turbidity represent the MIC. The results after 24 hour were recorded.

3.6.6 Determination of Minimum Bactericidal Concentration (MBC)

Blood agar was prepared according to manufacturer's instruction, sterilized at 121^{o}C for 15 minutes. It was poured into sterile petri-dishes. The plates were allowed to cool and solidify. The contents of the MIC test tubes in the serial dilution were then sub-cultured on to the prepared plates and the plates were then incubated at 37^{o}C for 24 hours (for the bacteria) and 27^{o}C for 2-7 days (for the fungi) after which the plates were observed for growth. The plate without growth represent the minimum

bactericidal concentration. After 24 hours the results were recorded (Vollekova *et al*., 2001 and Usman *et al*., 2007).

CHAPTER FOUR

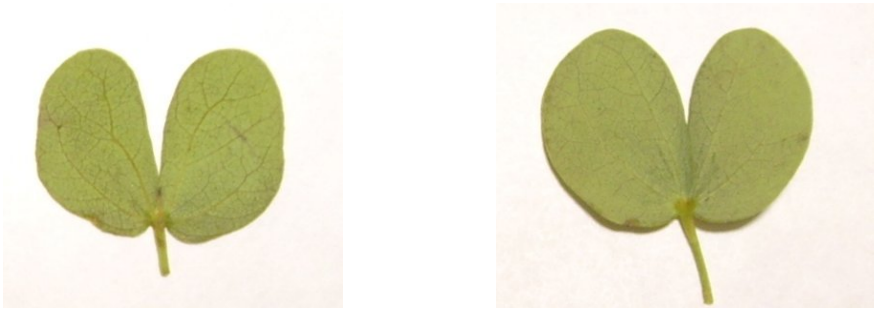
RESULTS

4.1 Picture of the plant and the leaves.

Bauhinia rufescens is a scandent shrub or small tree measuring between 1-3m high but sometimes up to 8m high, often scraggy, stunted and multistemmed. The leaves are arranged in the form of compound alternate small in size (less than 3cm long), bilubate almost to the base with semi circular lobes. The surface of the leaf is rough and the colour is greyish green.



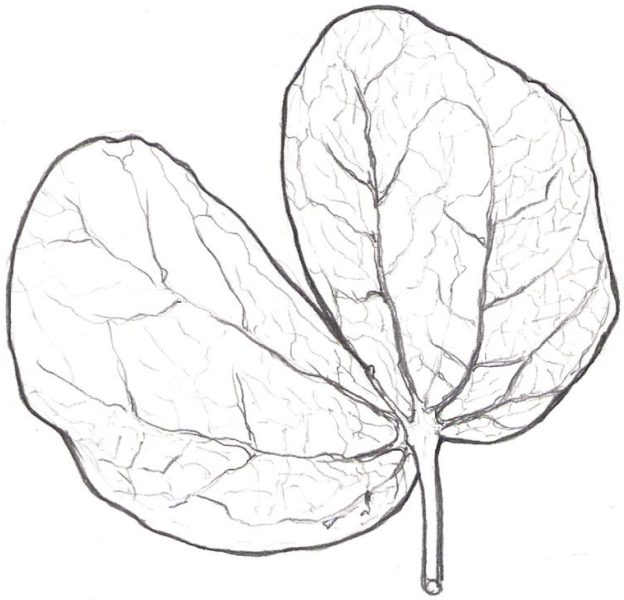
Plate 4.1 *Bauhinia rufescens* in it natural habitat.



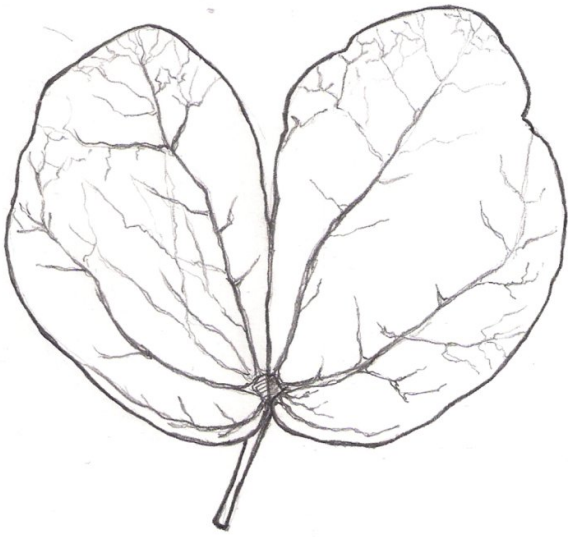
a b

Plate 4.2 Lower surface of leaf(a) Upper surface of leaf (b)

(Magnification : x2.8)



a



b

Figure 4.1 Veneration of leave of *Bauhinia rufescens* (Lower surface of leaf(a)

Upper surface of leaf (b)) (magnification: x5.3)

4.2 Micro-Morphological Examination

Description of microscopical features of the powdered leaf of *B. rufescens*.

Stomata:

Type: anomocytic

Location: upper and lower epidermis

Trichomes:

Type: glandular

Position: lower epidermis

Frequency: few

Calcium oxalate:

Form: cluster crystals or rosette

Location: lower epidermis

Frequency: frequent

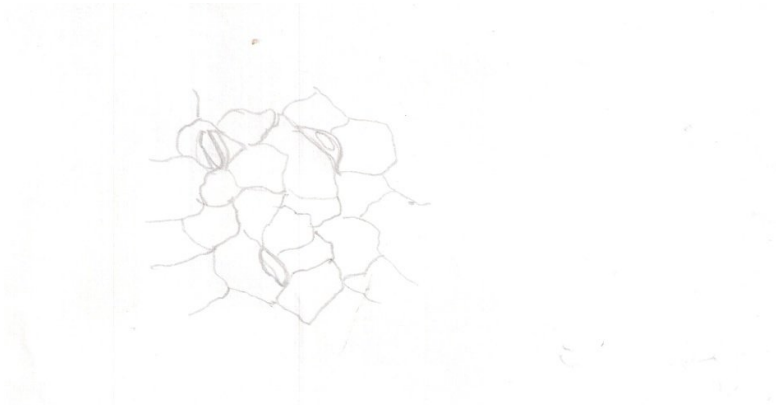
Petiole:

Average 0.57mm

Lamina:

Average (Length): 13mm

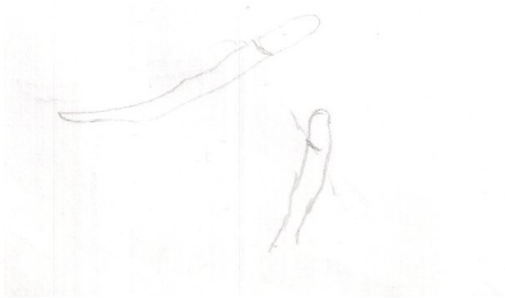
Width: 9.8mm



a



b



c

Figure 4.1: micro – morphological structures of the leave (a = stomata; b = calcium oxalate; c = trichome). See appendix A, B and C.

4.3 Determination of Some Physical Constants of Leaf of *Bauhinia rufescens*

The results of the physical constant of the leave of *Bauhinia rufescens* is presented below;

1.5.1 **Alcohol soluble extractive value**:

The value of alcohol soluble extractive value of the leaves of *Bauhinia rufescens* was determined to be 30.1 ± 0.10%

1.5.2 **Percentage moisture content**:

The percentage moisture content was determined to be 11 ± 0.16%

1.5.3 **Total ash value:**

The total ash value was determined to be 0.66 ± 0.01%

1.5.4 **Water soluble ash value**:

The water insoluble ash value was determined to be 6 ± 0.04%

1.5.5 **Alcohol soluble ash value**

The alcohol soluble ash value was determined to be 5.6 ± 0.05%

Table 4.1: Summary of the results of the Physical Constant determined on the leaves

Parameters	**Value obtained ± SEM (%w/w)**
Alcohol soluble extractive value	30.10 ± 0.10
Moisture content	11.00 ± 0.16
Total ash value	0.66 ± 0.01
Water soluble ash value	6.00 ± 0.04
Alcohol soluble ash value	5.60 ± 0.05

n= 3

Key: SEM = standard error of mean

4.4 Chemo-Microscopical Examination

The results of the chemo-microscopical analysis of the powdered leave are given below;

4.4.1 Cellulose: The appearance of a blue stain on the walls of the cell elements of the fresh and powdered samples upon the addition of N/50 iodine and 66% sulphuric acid shows the presence of cellulose.

4.4.2 Lignin: Many of the fibres, sclerenchymatous cells and vascular elements of the powdered leave gave a red stain when treated with solution of phloroglucinol and concentrated hydrochloric acid, indicating the presence of lignin.

4.4.3 Starch: Blue-black coloration was observed in some of the parenchyma cells of the powdered leave on addition of drops of N/50 iodine.

4.4.4 Tannins: A bluish green coloration observed on treating the powdered leave with ferric chloride solution indicating the presence of tannins.

4.4.5 Proteins: the appearance of yellow colour on the microscopic structure on addition of drops of picric acid indicates the presence of proteins.

4.4.6 Mucilages: the presence of a dark pink coloration on addition of ruthenium red indicates the presence of mucilage.

4.4.7 Calcium oxalate: Calcium oxalate crystals were observed as shining structures in the powdered samples of the leaves.

In summary, the plant contain cellulose, lignin, starch, tannins, oils, proteins, mucilages and calcium oxalate as shown in table 4.2.

Table 4.2: Summary of the chemo-microscopical analysis of the powdered leaf

Constituents tested	**Leaf**
Cellulose	+
Lignin	+
Starch	+
Tannins	+
Proteins	+
Mucilage	+
Calcium oxalate	+

Key: + = presence, - = absence

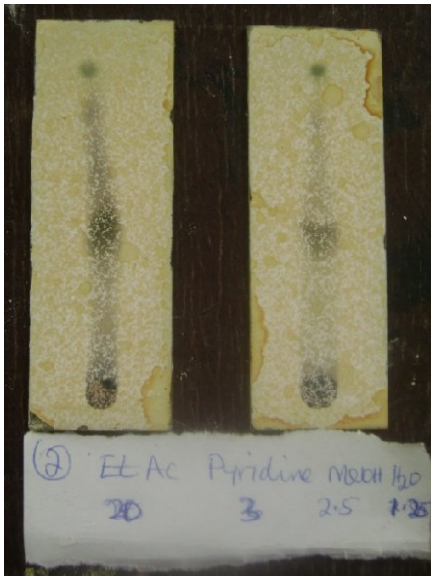
4.5 Thin Layer Chromatography of methanolic extract of the leaves of *Bauhinia rufescence*

Table 4.3 Represents the various solvents systems used for TLC and their rf values.

SN	Solvent system	Number of spots after spraying with 5% H_2SO_4	Number of spots after when placed in iodine tank	Rf value
1	EtAc:MeOH:H_2O(10:1:35)	1	1	0.31
2	EtAc:MeOH:C_5H_5NH:H_2O(10:1:3:5)	2	2	0.5, 0.87
3	$CHCL_3$:EtAc (6:4)	1	1	1



EtOAc:MeOH:H_2O(10:1:35)



EtOAc:MeOH:Pyridine:H_2O(20:3:2.5:1.25)



$CHCL_3$:EtOAc (6:4)

Plate 4.3: TLC of Methanolic Extract of leave of *B. refescens*.

4.6 Phytochemical Constituents Present in Methanolic Extract

4.6.1 Test for Anthraquinone and their Derivatives

There was no pink, red or violet colouration in the lower layer when the benzene extract was treated with ammonium solution thus showing the absence of anthraquinone glycosides.

4.6.2 Test for Saponin Glycosides

Frothing test: After 30 seconds of shaking the extract, a persisted froth was observed indicating the presence of saponins.

Haemolysis test: Haemolysis was observed in the tube containing the extract but not in the control tube (the tube containing water only) thus confirming the presence of saponin glycosides.

4.6.3 Test for steroids and terpenoids

a) ***Salkowski's test:*** There was no reddish brown colour at the interface when the plant powder was extracted with chloroform followed with conc. Sulphuric acid indicating the absence of steroidal ring.

b) ***Liebermann - Burchard's test***: There was no brownish-red or violet ring at the zone of contact of the two liquids when the ethanol extract was treated with chloroform and followed with conc. Sulphuric acid and the upper layer did not turn green denoting the absence of sterols and terpenes.

4.6.4 Test for Tannins

a. ***Ferric chloride test***: A greenish black precipitate was observed when the water extract was treated with ferric chloride solution which indicates the presence of condensed tannins.

b. ***Lead subacetate test***. A dirty white coloured precipitate was observed when the water extract was treated with lead subacetate solution which indicates the presence of tannins..

4.6.5 Test for Carbohydrates

a. *Molisch's test*

A purple to violet coloured was observed at the interface when the water extract was treated with Molisch's reagent and followed with concentrated sulphuric acid, which indicates the presence of carbohydrates. The mixture, when shaken gently and allowed to stand for two minutes, followed by dilution with 5ml of water produced a dull violet precipitate confirming the presences of carbohydrates.

b. Barfoed's test for monosaccharide

A red precipitate was observed when Barfoed's reagent was added to the water extract and heated which indicates the presence of monosaccharide.

c. Fehling's test for free reducing sugars

A brick-red precipitate was observed when Fehling's A and B were added to the water extract and boiled which indicates the presence of reducing sugars.

4.6.6 Test for Alkaloids

a. **Wagner's reagent**: A light brown to brownish precipitate was observed when the alkaloidal extract was treated with Wagner's reagent, indicating the presence of alkaloids.
b. **Mayer's reagent** A white or creamy white precipitate was observed when the alkaloidal extract was treated with Mayer's reagent, indicating the presence of alkaloids.
c. **Drangendorff's reagent:** An orange-yellow precipitate was observed when the alkaloidal extract was treated with Drangendorff's reagent, indicating the presence of alkaloids.
d. **Tannic acid solution:** Buff coloured precipitate was observed when the alkaloidal extract was treated with Tannic acid solution, indicating the presence of alkaloids

4.6.7 Test for Cardiac Glycosides

a. Keller-killiani Test: A brown colour at the junction of the two liquids and a pale green colour in the upper layer was observed when the concentrated sulphuric acid was added down the side of the test tube (containing the chloroform extract dissolved in ferric chloride) which indicates the presence of cardiac glycoside (Brain and Turner, 1975).

b. Kedde's Test: An immediate violet colour was observed when 1ml of 2% solution of 3,5-dinitrobenzoic acid in methanol was added followed by 2ml of 5% aqueous NaOH solution to the aqueous extract indicating the presence of cardenolides.

4.6.8 Test for flavonoid

a. Shinoda's test: A pink or red colour was observed on addition of Mg chips and few drops of conc. HCl acid to the ethanolic extract, which indicates the presence of flavonoids.

b. Sodium hydroxide test: A Yellow solution was observed when NaOH was added to the ethanolic extract, which indicates the presence of flavonoids. On addition of dilute hydrochloric acid, the solution becomes colourless confirming the presences of flavonoids.

c. Ferric chloride test: A green colour was observed when $FeCl_3$ was added to the ethanolic extract, which confirms the presence of phenolic nucleus.

In conclusion, the methanolic extract was found to contain carbohydrates, saponins, tannins, alkaloids, cardiac glycoside and flavonoid with the absence of anthraquinones.

Table 4.4: Phytochemical constituents present in the powdered leaf of *Bauhinia rufescens*

Constituents	**Test**	**Observation**	**Inference**
Anthraquinones	a. Borntrager's test	No change	-
	b. Combined Anthraquinone	No change	-
Saponin	a. Frothing test	Frothing which persist > 30 min	+
	b. Haemolytic Test	Haemolysis of red blood cells	+
Steroid	Salkowski test	Red colour at interphase	-
Triterpenes	Lieberman Burchard's test	Red colour ring	-
Tannins	a. $FeCl_3$ test	Greenish black coloration	+
	b. Lead subacetate	Yellow coloration	+
Carbohydrate	a. Molisch test	Purple colour at interphase	+
	b. Barfoed's test	Brick red precipitate	+

	c. Fehling test	Brick red precipitate	+
Alkaloids	a. Wagner's test	Light brown precipitate	+
	b. Meyer's test	White precipitate	+
	c. Dragendoff's test	Orange yellow precipitate	+
	d. Tannic acid test	Buff coloured precipitate	+
Cardiac	a. Kella-killiani test	Purple ring at interphase	+
glycosides	b. Kadde test	Purple-blue ring	+
Flavonoid	a. Sodium hydroxide	Yellow coloration	+
	b. $FeCl_3$ test	Green coloration	+
	c. Shinoda's Test	A red coloration	+

Key: + = presence, - = absence

4.7 Castol Oil Induce Dirrhoeal

The percentage inhibition after one hour with dose 200mg/kg showed more inhibition. As the time increased, the percentage inhibition decreases.

As shown in table 4.5, the activity after 1 hour was quite remarkable and comparable to Loperamide, i.e. percentage inhibition was high (60%) compared with 100%. All three doses (50, 100 and 200mg/kg) showed inhibition of diarrhoea after 1 hour. After 2 hours, only the 200mg/kg dose showed significant inhibition (60%). As the dose increases, the percentage inhibition increases. As time increases, the effect of the extract on diarrhoea decreases. Thus the drug is more active within one hour of administration.

Table 4.5: Effect of methanolic extract of *Bauhinia rufescens* leaves at different dose levels on castor oil induced diarrhoea.

Group	2ml/kg Castor oil + extract (ml/kg)	***1 hr***		**2 hrs**		**3 hrs**	
		Activity	*% inhibition*	Activity	% inhibition	Activity	% inhibition
I	50	*2/5*[a]	*60*	5/5	0	5/5	0
II	100	*2/5*[a]	*60*	3/5[a]	40	3/5	40[a]
III	200	*1/5*[b]	*80*	2/5[c]	60	4/5	20
IV	Normal saline	*4/5*	*20*	5/5	0	5/5	0
V	Standard (loperamide)	*0/5*	*100*	0/5	100	1/5	80

Key: a= significance at P<0.1, b = significance at P<0.05 and c = significance at P<0.02

4.8 Antimicrobial Studies

4.8.1 Zone of Inhibition

The extract showed high zone of inhibition (29mm) against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* and *Streptococcus feacalis* (both 28mm) while the growth of *Corynebacterium hemolyticus* was least inhibited (21mm) by the extract as shown in table 4.5. For the fungi, *Candida krusei* growth was highly inhibited (25mm) followed by *Candida albican (20mm).* The extract had no activity on *Streptococcus pyogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa,* and the fungi *Aspergillus flavus and Aspergillus fumigates*. Spafloxacin showed no activity against *Corynebacterium hemolyticus, Salmonella typhi* while Fluconazole showed no activity against the *Aspergillus spp*.

Table 4.5 Zone of inhibition of the methanolic extract on some micro-organisms.

Micro-organisms	Zone of inhibition (mm)		
	Methanol fraction	**Spafloxacin**	**Fluconazole**
Gram positive			
Staphylococcus aureus	28	40	NA
Streptococcus pyogenes	0	37	NA
Streptococcus feacalis	28	32	NA
Corynebacterium ulcereans	24	36	NA
Corynebacterium hemolyticus	21	0	NA
Gram negative			
Salmonella typhi	0	0	NA
Escherichia coli	25	37	NA
Shigella dysenteriae	23	32	NA
Pseudomonas aeruginosa	29	0	NA
Klebsiella pneumonia	0	30	NA
Fungi			
Candida albican	20	NA	32
Candida krusei	25	NA	40
Aspergillus flavus	0	NA	0
Aspergillus fumigates	0	NA	0

Key: NA = Not Applicable.

4.8.2 Minimum Inhibitory Concentration (MIC)

The MIC of *Staphylococcus aureus, Streptococcus feacalis, Escherichia coli, Pseudomonas aeruginosa* are 2.5mg/ml while that for *Corynebacterium ulcereans*, *Streptococcus feacalis*, *Shigella dysenteriae* and *Candida albicans*, are shown in table 4.6. The MIC for the fungus *Candida albicans* is 5mg/ml while that for *Candidas krusei* 2.5mg/ml.

Table 4.6: Minimum Inhibitory Concentration (MIC) of the methanolic extract on some micro-organisms

	Concentration (mg/ml)					
Micro-organisms	20	10	5	2.5	1.25	0.625
Staphylococcus aureus	-	-	-	MIC	+	+
Streptococcus feacalis	-	-	-	MIC	+	+
Corynebacterium ulcereans	-	-	MIC	+	+	+
Corynebacterium hemolyticum	-	-	MIC	+	+	+
Escherichia coli	-	-	-	MIC	+	+
Shigella dysenteriae	-	-	MIC	+	+	+
Pseudomonas aeruginosa	-	-	_	MIC	+	+
Candida albicans	-	-	MIC	+	+	+
Candida krusei	-	-	_	MIC	+	+

Key: - = no turbidity, + = turbidity

4.8.3 Minimum Bactericidal Concentration (MBC) of Methanolic extract

Corynebacterium hemolyticum, Shigella dysenteriae, and the fungus *Candida albicans* showed the highest MBC of 20mg/ml each, followed by *Corynebacterium ulcereans*, *Escherichia coli,* and the fungus *Candida krusei* had MBC *of 10mg/ml* while *Staphylococcus aureus, Streptococcus feacalis* and *Pseudomonas aeruginosa*, had the least MBC of 5mg/ml as shown in table 4.7.

Table 4.7: Minimum Bactericidal Concentration (MBC) of methanolic extract on micro-organisms

	Concentration (mg/ml)					
Micro-organisms	20	10	5	2.5	1.25	0.625
Staphylococcus aureus	-	-	MBC	+	+	+
Streptococcus feacalis	-	-	MBC	+	+	+
Corynebacterium ulcereans	-	MBC	+	+	+	+
Corynebacterium hemolyticum	MBC	+	+	+	+	+
Escherichia coli	-	MBC	+	+	+	+
Shigella dysenteriae	MBC	+	+	+	+	+
Pseudomonas aeruginosa	-	-	MBC	+	+	+
Candida albicans	MBC	+	+	+	+	+
Candida krusei	-	MBC	+	+	+	+

Key: - = no turbidity, + = turbidity

CHAPTER FIVE

DISCUSSION

Bauhinia rufescens is a shrug or small tree. Evaluation of the plant shows an anomocytic stomata type located both at the upper and lower epidermis of the leaves. It also contain trichomes at the lower epidermis which are scanty, calcium oxalate at the lower epidermis, petiole of average 0.57 ± 0.04mm, lamina of average length of 13 ± 33mm and width of 9.8 ± 0.79mm.

The physical constant reveals an alcohol soluble extractive value of 30.1 ± 0.10%, moisture content of 11 ± 0.16%, total ash value of 0.66 ± 0.01%, water soluble ash value of 6 ± 0.04% and alcohol soluble ash value of 5.6 ± 0.05%. which can be used to check purity of the crude drug.

The evaluation of physical constants can afford the detection of adulteration of drug. Solvent extraction of any drug gives solution of different compounds, which depend on the nature of the drug and the solvent used. Solvent extraction also gives preliminary information on the quality of the drug, for example, the extraction of a re-dried crude drug or the addition of an exhausted drug to fresh sample, with a solvent would be indicated by a lowering of the value of the solvent extractive. Alcohol soluble extractive value of 30.1±0.10% shows that the constituents of the plant leaves are highly soluble in alcohol. The alcohol soluble extractive value is meant to detect exhausted and already utilized drug which could be fraudulently used as substitutes and adulterants (Elujoba, 1999).

Excess moisture content in crude drug may result in the breakdown of important constituents or may lead to the growth of yeast and fungi during storage. In this

respect the evaluation of moisture content in setting standard for crude drug is very important (Brain and Tuner, 1975). The general requirement of moisture content in crude drug is generally given as 8 – 14% (African Pharmacopeia, 1986) and the values obtain in this study show 11 ± 1.6% showing that the chances of this drug to be degraded by micro-organism is minimal.

Total ash value represents the amount of the residual substance not volatilized on ignition at 450^{o}C when crude drugs are ignited. Both the plant tissue (physiological ash) and other extraneous matter e.g. soil and sand, adhering to the drug surface (non-physiological ash) can produce ash (African pharmacopoeia, 1985). Total ash value is particularly significant in the evaluation of the purity of the drug and depends largely on the absence of foreign organic matter. Direct contamination such as by sand or earth is immediately detected by the ash value. The total ash of any material, normally consist of inorganic mixture of metallic salt and silica (Danmalam, 2000). The total ash value of 0.66±0.01% obtained is very low showing that the plant contains less contaminants and can be used as a parameter for quality control of the crude drug. Total ash usually contains carbonates, phosphates, silicates and silica (Evans, 2001; Wallis, 2002). Low total ash value of *Bauhinia rufescens* may also be due to low (scanty) quantity of calcium crystals of the plant as already confirmed in the microscopical studies.

The results of the macroscopic, microscopic, physical constants and chemo-microscopic evaluations provide a number of information which can be included in a monograph of the plant for proper identification.

The plant contain cellulose, lignin, starch, tannins, oils, proteins, mucilages and calcium oxalate.

The TLC chormatogram revealed two spots using the solvent system ethylacetate : pyridine : methanol : water (20:3:2.5:1.25). All these provides useful information that can be included in the plant monograph.

Phytochemical screening of the methanolic extract was found to contain carbohydrates, saponins, tannins, alkaloids, cardiac glycoside and flavonoid. The phytochemical constituent of the partitioned portions of the stem bark of this plant have been reported to contain aloes, anthraquinones derivatives, cardenolides and cardiac glycosides, flavonoids, resins, saponins and tannins (Usman *et al*., 2007)

Preliminary phytochemical screening consist of performing simple chemical tests to detect the presence of bioactive constituents of a drug or extract. The test are useful in the identification and evaluation of drug samples. The test may be a general test for a particular group of consitituens or may be a specific test for a single type of bioactive compound. Many of the tests involve the production of a coloured solution or a precipitate (Danmalam, 2000).

Most carbohydrates including sugars are wholly or partially soluble in polar solvents thus methanol was used as a solvent for extraction and used to test for carbohydrates. Sugar especially the monosaccharides are found both as free and in combined form in higher plants and are also the component of glycosides an polysaccharides (Harbone, 1973). The presence of reducing sugar in *Bauhinia rufescens* is in agreement with the fact that sugars are found in stems and underground organs of plants (Harbone, 1973)

Bauhinia rufescens contains saponins. Saponins are glycosides with either triterpenoidal or steroidal aglycone which have been detected in over seventy families of plants. They are surface-active agents with soap-like properties and can be detected by their ability to cause foaming in aqueous solutions and to haemolyse red blood

cells. These glycosides are of economic value due to their toxicity to cattle and their sweet taste (Danmalam, 2000).

Phytoconstituents have been found to inhibit bacteria, fungi, viruses and pests (Marjorie, 1999). Tannins, saponins and flavonoids are secondary metabolites of plants which are known to have antibacterial activity (Lewis and Ausubel, 2006, Cowan, 1999). Plants are the hope for new compounds which will be used against resistant organisms as they have made large contributions to human health and well being (Iwu *et al*., 1999). Plants have been reported to be the source of efflux pump inhibitors which will ensure the delivery of antimicrobial compounds into bacterial cells for example, Stermitz *et al*., (2000) reported that *Berberis* plant produces the antimicrobial compound berberine as well as the multiple drug resistance inhibitors 5-methoxyhydrocarpin D (5-MHC-D) and pheophorbide A which facilitated the penetration of berberine into a model Gram positive bacteria. Trease and Evans (1982) reported that the phenolic group provided the astringent and antibacterial properties in tannins, while the –OH radical conferred antimicrobial action to the flavonoids. Further more, it is reported that the oxidative property of the saponins was responsible for their bacteriocidal actions. Thus the antimicrobial property exhibited by the plant *B. rufescens* can be attributed to the presence of the flavonoids, saponins and tannins it contains.

The methanolic extract was found to be effective against castor oil induced diarrhea on the experimental rats at doses of; 50mg/kg (statistically significant ($P<0.1$) after one hour), 100mg/kg (statistical significance after one hour ($P<0.05$) and after two hours ($P<0.02$)), 200mg/kg (statistical significance after one hour ($P<0.05$) and after two hours ($P<0.02$)). In these three doses, the inhibition decreases as time increases.

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract resulting in an excess loss of fluid in the feaces. The secretory component of some diarrhea predominates while other is characterized by hypermotility. Castor oil causes diarrhea due to its active metabolite, recinoleic acid (Chitme *et al*., 2004; Ammon and Thomas, 1974; Watson and Gordon, 1962) which stimulates peristaltic activity in the small intestine thus leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin (Zaval *et al*., 1988). Castor oil also induce diarrhea by increased the volume of intestinal contains by preventing re-absorption of water.

The methanolic extract was more active against gram positive bacteria than gram negative bacteria as shown in the zones of inhibition.

For the gram positive bacteria, the MIC of the *Staphylococcus aureus*, *Streptococcus feacalis* was 2.5mg/ml while *Streptococcus pyogene* and *Corynebacterium ulcereans* had MIC of 5mg/ml. While for the gram negative bacteria, *Shigella dysenteriae* and *Klebsiella pneumonia* had MIC of 5mg/ml while *Escherichia coli* had MIC of 2.5mg/ml. *Candida albicans* had MIC of 5mg/ml while that for *Candida krusei* was 2.5mg/ml. Thus it can be concluded the plant had antibacterial activity on both gram positive and gram negative bacteria with MIC between 2.5mg/ml and 5mg/ml.

Corynebacterium hemolyticus, *Shigella dysenteriae* and *Candida albicans* had MBC of 20mg/ml which is very high. *Staphylococcus aureus*, *Streptococcus feacalis* and *Pseudomonas aeruginosa* had the least MBC of 5mg/ml.

Usman *et al*., (2007) also reported some antimicrobial activity on the stem bark of *Bauhinia rufescens* against *Corynaebacterium spp*. and *Staphylococcus*

aureus (Gram positive bacteria); *Proteus vulgaris, Pseudomonas aeruginosa* and *Shigella dysenteriae* (Gram negative bacteria).

Hugo and Rusell, (1998) reported that "with most bactericidal antimicrobials, the MIC and MBC are often near or equal in value" as has typically been observed in this study. It is also observed from these results that the extract have broad spectrum antibacterial activity against gram positive, gram negative and some fungal strain under study.

The observed anti-diarrhoeal activity in rats coupled with the antimicrobial findings support the observed traditional use of the plant leaf in diarrhoeal treatment.

The demonstration of activity against both Gram positive and Gram negative bacteria is an indication of broad spectrum of activity and thus can be used to source antibiotic substances for drug development that can be used in the control of these bacterial infections.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Pharmacognostic studies of the leaves of *Bauhina rufescens* revealed that it contain; anomocytic stomata located on the upper and lower epidermis of the leaves, trichomes and calcium oxalate are found on the lower epidermis, petiole of average 0.57mm, lamina of average length of 13mm and width of 9.8mm. The physical constant revealed an alcohol soluble extractive value of 30.1%, moisture content of 11%, total ash value of 0.66%, water soluble ash value of 6% and alcohol soluble ash value of 5.6. % . The plant contains cellulose, lignin, starch, tannins, oils, proteins, mucilages and calcium oxalate. These findings are useful information in preparation of the plant monograph.

The methanolic extract was found to contain the following phytochemicals; carbohydrates, saponins, tannins, alkaloids, cardiac glycoside and flavonoid with the absence of anthraquinones.

Leaves of *Bauhinia rufescens* exhibit anti-diarrhoeal property on castol-oil induced diarrhoeal in rats mostly at concentration of 200mg/kg and was very active after one hour of administration. The plant also had antimicrobial activity against *Staphylococcus aureus. Streptococcus feacalis, Corynebacterium ulcereans, Corynebacterium hemolyticum, Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa, Candida albican,* but not in *Candida krusei.*

6.2 Conclusion

This study has provided additional information which are useful in preparation of the monograph of the plant. It has also revealed that the leaves of *Bauhinia rufescens* contain secondary metabolites that are responsible for its anti-diarrhoeal and antimicrobial activity and thus support the use of this plant by medicinal practitioners in diarrhoeal treatment.

6.3 Recommendation

Further studies be done on the leaves of this plant to isolate the compound responsible for the anti-diarrhoeal activity and antimicrobial activities.

It is also recommended that further studies be carried out on the pharmacognostic properties of the stem and roots of this plant.

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APPENDIX

Appendix A : Table showing average length and width of petiole and lamina

	Length of petiole (mm)	Length of lamina (mm)	Width of lamina (mm)
	0.9	12	10
	0.5	13	9
	0.7	14	10
	0.5	13	10
	0.6	13	9
	0.6	12	11
	0.4	15	11
	0.5	14	9
	0.6	12	9
	0.4	12	10
Mean +SEM	0.57 ± 0.04	13 ± 0.33	9.8 ± 0.79

Appendix B

Plates showing stomata and trichomes and calcium oxalate

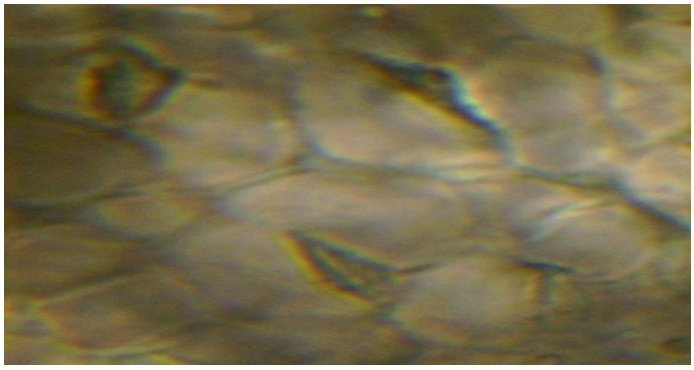


Plate 1 of cells showing stomata

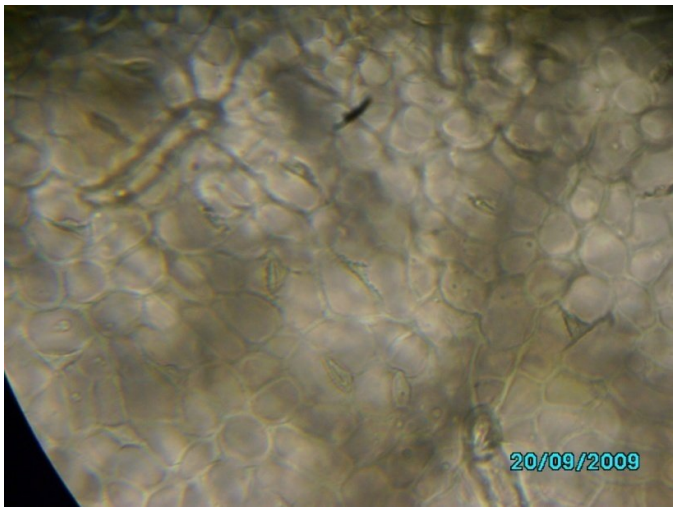


Plate2 showing trichomes

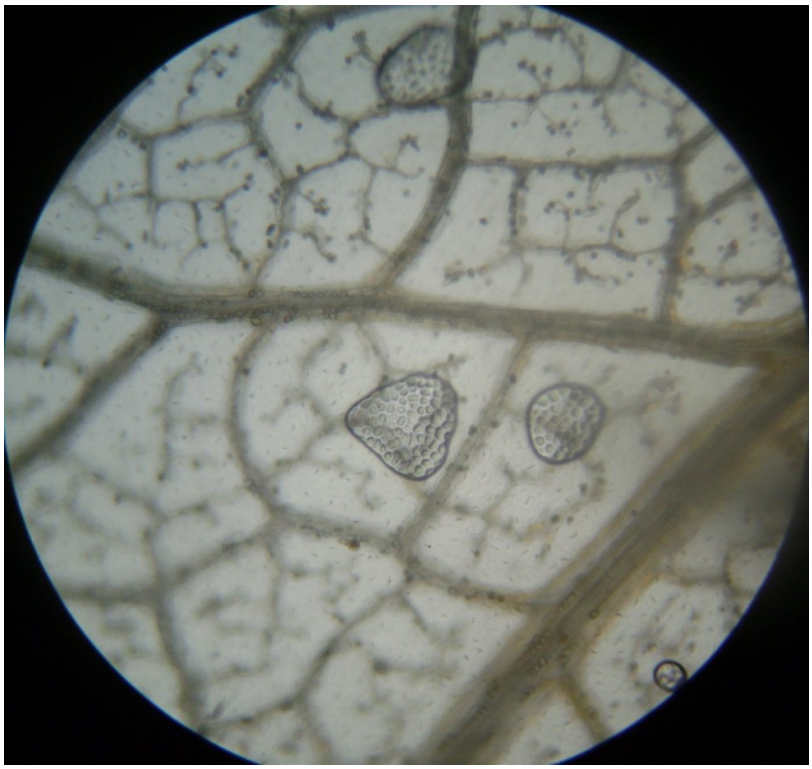


Plate 3 showing calcium oxalate

Appendix C

Plates showing zones of inhibitions of the bacteria

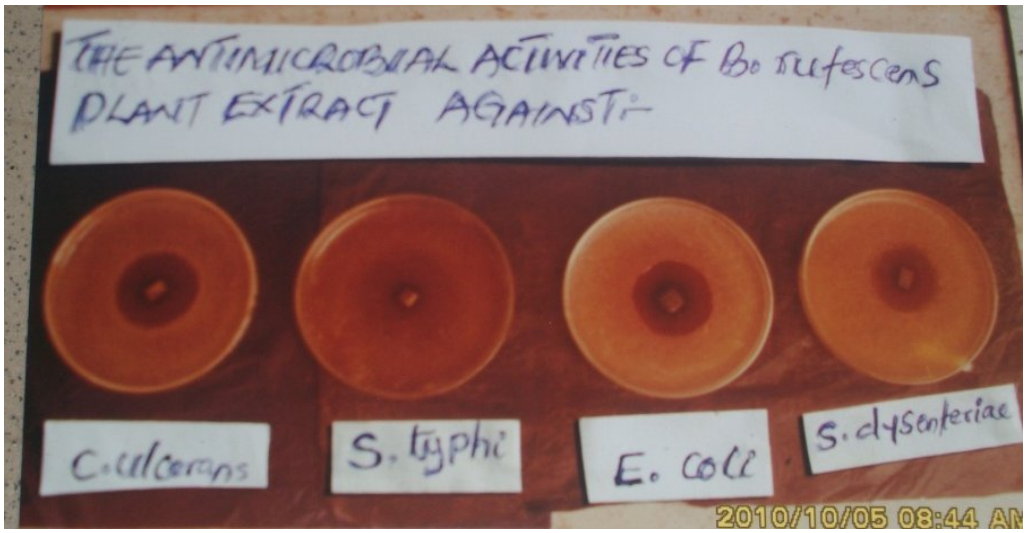


Plate 4 showing zone of inhibition of *C. ulcerans, S. typhi, E. coli* and *S. dysenteriae*



Plate 5 showing zone of inhibition of *C. albicans, K. pnemoniae, C. krusei* and *P. aeruginosa*



Plate 6 showing zone of inhibition of *S. aureus, S. feacalis, C. hymoliticus* and *S. pyogenes*

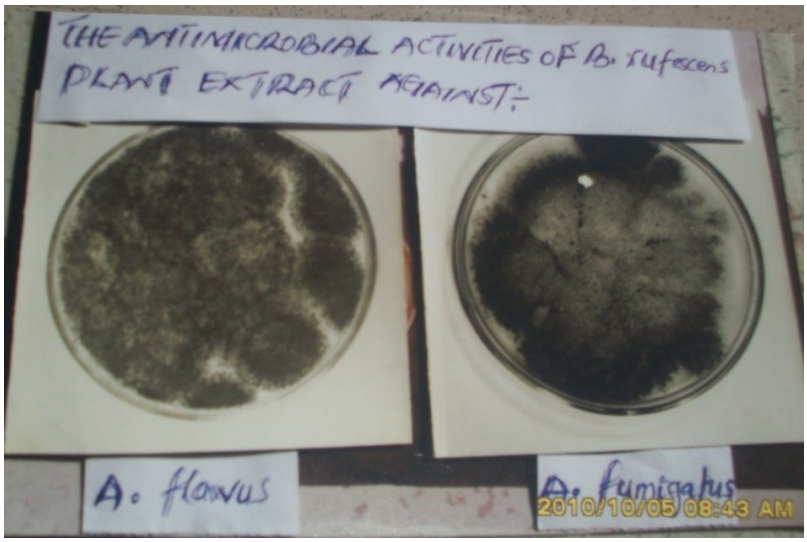


Plate 7 showing zone of inhibition of the fungi *A. flavus* and *A fumigatus*