ANTIMICROBIAL SENSITIVITY, ANTIOXIDANT ACTIVITY AND MINERAL PROFILE OF THE ETHANOLIC EXTRACT OF THE STEM BARK OF *SPONDIAS MOMBIN* PLANT

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UNIVERSITY OF BENIN,
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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY OPTION)

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CERTIFICATION

This is to certify that this project work was carried out by Ehimwenma Stephanie Edogun with Matriculation Number LSC1507920 of Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This work is dedicated to God Almighty for His unfailing grace, His endless mercies and his love for me. Also to my elder brother Mr. Osaigbovo Edogun.

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My infinite thanks go to God Almighty, the light that shines in my dark days, the best of best and the promise keeper. I love you Lord!

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ABSTRACT

Spondias mombin is a plant used in various medicinal practices and varieties of traditional medicinal practitioners employ it as a raw material in phytomedicine. The aim of the study is to evaluate the antimicrobial sensitivity, antioxidant activity and the mineral profiling of the stem bark of *Spondias mombin* plant. The quantitative estimation of the phytochemical compound and the proximate analysis was done using a well-established method. The phytochemical and proximate analysis of the ethanolic extract of *Spondias mombin* revealed the presence of metabolites and compounds such as carbohydrate (74.404), crude protein (3.563 ± 0.6), flavonoid (16.500µg/ml), saponin (17.868 µg/ml) tannin (26.346 µg/ml), alkaloid (0.8%). The proximate analysis gave a moisture content of (38.20 ± 1.56) and ash content of (11.46 ± 0.27). Some of the compounds identified in the phytochemical analysis were found to possess anti-inflammatory, antioxidant, anti-ageing properties, protection against allergies, ache and joint pain reduction etc. The mineral constituents were also evaluated using a well-established method: sodium (0.80), potassium (65.00), calcium (2.100), magnesium (2.460), manganese (72.85), copper (0.200), zinc (0.100), iron (1.500), nitrogen (0.570) and the ascorbic acid was analyzed using a spectrophotometer, which revealed a high vitamin C content of (1664.714). The antimicrobial activity was checked against some selected bacteria and fungi which revealed high antimicrobial activity against the selected test organisms hence *Spondias mombin* stem bark and its bioactive chemicals are effective in treating various illness and these studies therefore support its traditional medicinal use.

CHAPTER ONE

INTRODUCTION

The entire dependence of man on plants and plant products directly for his basic needs as food, clothing and shelter and indirectly for their beneficial influence on the climate and maintenance of his immediate and remote environment make plants vital to his survival and the basis of his continued existence. Most researchers have reported that plant has a physiological effect on other organisms and that they are also employed as a raw material in the production of orthodox medications. (Osuagwu *et al.,* 2012). Nature has provided medicinal substances and an astounding number of modern pharmaceuticals have been extracted from natural sources, primarily based on their traditional medical applications. In recorded history, medicinal plants have been in use for various purposes primarily for the treatment of man and animal (Erhenhi, 2016). Traditional medicine considers all part of a tree to be therapeutic but only after a plants biological activity has been ethnobotanically reported or scientifically proven does it become a therapeutic/medicinal plant (Atanasov *et al.,* 2015; Anyanwu and okoye, 2017).Medicinal plants usually include a combination of chemical substances that work individually, additively,or synergistically to promote health (Umesh and Veeru,2007). Traditional medicine as defined by World Health Organization (WHO) involve therapeutic practices which have been in existence for hundreds of years before the development of modern scientific medicine and are still in use today. These practices vary, in keeping with the social cultural heritage of different countries (WHO, 2013). Presently, about 80% of the world rely on herbal medicine. Many developing countries of the world look upon native medicinal plants as possible addition to the WHOs list of “essential drugs “ once their value have been clinically proven. A simple first- principle biological technique in the evaluation of plant with no known biological activity is to conduct a screening test (Kasim *et al.,*2014;Balouiri *et al.,*2016).

*Spondias mombin L.*also known as yellow mombin or hog plum is a member of the Anacardiaceae family of plants. It is a fruitful tree that grows in Nigeria forest and savanna regions, Africa rain forest along the coast,Brazil and other tropical forest in the world. This plant grows readily around us in south west of Nigeria (Yoruba) and is commonly used in folk medicine (Erhenhi,2016).In Nigeria, it is known by various names; Ibo uchakuru, Hausa Tsardarmasar, Yoruba Akika etikan", Iyeye, Bini ogheghe, Idoma, Igongo/Ichankla. The tree grows to about 20m in height. The bark is fissured and thick and its lower branches are whorled. It has a deciduous, alternate, pinnate leaves (20-45cm long) and hairy pinkish, pointed leaflets which are inequilaterally and oblique at the base. The fruits, hanged in numerous branched clusters, are aromatic, ovoid and oblong in shape (Uchendu and Isek, 2008). All parts of the plant are reported to be medically useful according to (Ayoka *et al.,*2005,2008) traditional medicinal practitioners in Nigeria utilize the herb to cure a variety of nerve illnesses and its medicinal and traditional use in reproduction have been reported (Ugadu *et al.,* 2014.).Various cultures frequently maintain within their collection of traditional medicine substances valued as drugs for treating diseases (Abbott, 2014; Erhenhi, 2016; Sen and Chakraborty, 2017). The wood is used for making huts, garden poles, axe and hoe handles in tropical Africa. It is also popular for carving amulets, statuettes, cigarette holders and various ornamental objects.

Infusions, decoctions and mixtures are made from hot water and alcohol extracts of this plant leaves, root, and stem bark. The plant is commonly blended with other plants components in Nigeria folk medicines and use to treat disorder like diarrhea, sore throats, cough, leprosy and stomach problems (Olugbuyiro *et al.,* 2013).Additionally, the leaves can be directly squeezed, steeped in alcohol or boiled using water to extract the active ingredients. This leaves are used against convulsion and stomach ache. And also the infusion of the leaves is used for the treatment of cold and cough. In South eastern Nigeria, juice extracts from the leaves are used by the natives to induce delivery in small ruminants with parturient complications (dystocia) arising

from uterine inertia. It had been shown in earlier studies, The decoction or macerated stem bark is used against severe cough, inflammation, muscle and joint injuries, pain reliever etc., with immature palm nuts used for the treatment of fibroid (Uchendu and Isek, 2008; Mahmud *et al.,* 2016). The juice from the fruit is used as a febrifuge (a medication that reduces fever) and for diuretic (any drug that elevates the rate of urination) purpose. The stem bark is fungicidal and showed anti-turmour property when it was administered on Wister Rat (Arumugam*et al.,* 2014).

For example, in Peru(South America), decoctions of the bark and/or leaves are used as a 'child birth aid'. It is also used in postpartum infections of the uterus and following an abortion or miscarriage in women. Women that are pregnant or those seeking to be pregnant are usually advised against the use of the leaf infusion/decoction (WHO, 2013; Nergard*et al.,* 2015; Akour*et al.,* 2016). It had been shown in earlier studies, that butanolic leaf extract of the plant contracts isolated uterine muscle of the rat in a concentration dependent manner (Uchendu, 2004).

The survival and continued existence of man in turn depends on the efficiency with which man, with all the resources and technology available to him, harnesses, develops and utilizes plants and plant products. Many people in rural area have been using medicinal plants for the treatment of various diseases and ailment.

Aim and Objectives

The aim of this study is to evaluate the antimicrobial sensitivity, antioxidant activity and mineral profile of the ethanolic extract of stem back of *Spondias mombin* plant.

Specific Objectives

- To substantiate the antimicrobial activity of *S. mombin*
- To estimate the antioxidant capability of *S. mombin*
- To evaluate the chemical and mineral constituent of *S. mombin*

CHAPTER TWO

LITERATURE REVIEW

Herbal or traditional medicine has been a major aspect of the sociocultural heritage in Africa for hundreds of years even before the advent of conventional medicine (Nwauzoma and Dappa, 2013). It was once believed to be primitive and wrongly challenged by foreign religions dating back during the colonial rule in Africa and subsequently by the conventional or orthodox medical practitioners (Okigbo and Mmeka, 2006; Oyetunji and Opeyemi, 2017). Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in them as sources in the treatment of diseases (Mohana*et al.,* 2008; Adwan*et al.,* 2009; Ajayi and Akintola, 2010; Oyetunji and Opeyemi, 2017). Traditionally, the people of India and China consumed various foods that are considered to be medicinal. Countries like Germany, France and England were the first to consider one's diet more important than both exercise and hereditary factor in people's attempt to achieve good health and this was achieved through nutraceuticals.

2.1 Nutraceuticals

Essentially, a nutraceuticals is a substance that has a physiological benefit or provides protection from chronic diseases. Unfortunately, the definition of nutraceuticals varies from country to country depending on how they are categorized and regulated. At the moment, there is no clear internationally accepted definition of a nutraceutical (Kalra, 2003). Today nutraceuticals have evolved from their traditional background to a highly scientific field where the efficacy and safety of products are backed by evidence, new research and developing technologies. Over the past few years, nutraceuticals have become very popular. They are being used as alternative or supplemental treatment along with pharmaceutical to keep prevents a wide range of diseases.

They have attracted considerable interest because of their potential nutritional values, safety affordability and multiple therapeutic effects and are often seen as an attractive option to conventional treatments. Nutraceuticals can improve health, delay the ageing process, prevent chronic diseases, increase life expectancy or support the structure and functioning of the body. They are also used in the prevention and treatment of mental health issues and disorders. Nutraceuticals can play an important role in the body's various biological processes, which help prevent various diseases and improve overall health and well-being. It helps in preventing the onset of chronic diseases and reduces the complications involved. Evidence suggest that are used in the prevention of cardiovascular diseases, inflammatory based diseases, diabetes, eye disorders, age related macular degeneration, glaucoma, visual disorder etc help with male infertility and dysfunction and prevent drainage to sphere. Nutraceuticals include Lutein, Zeaxanthin, Vitamin C and E reduces the risk of cataracts thereby improving eye health. Omega-3s are important for visual development and retinal function. There are also immune booster nutraceuticals e.g. green tea blue berries, amino acids, and vitamin D. They strengthen the immune system and thus help to prevent diseases. Many nutraceuticals help in the development and regeneration of stem cell and play an important role in reinforcing the body's natural gut defence mechanism (Garima and Manoj, 2016).

2.2 Description of *Spondias mombin L.*

The genus Spondias (Anacardiaceae) consist of about 8-12 species distributed across tropical regions in the world (Silva *et al.,* 2014), including the species Spondias mombin L.,known as cajazeira, and Spondias tuberose Arr.Cam., known as umbuzo. S. mombin is a native to the Amazon region in peru, Brazil, Vanezuella, Bolivia, Colombias, the three Guianas, as well as the southern Mexico, Blize, Costa Rica, and the west Indies (Adedokun *et al.,* 2010; Silva *et al.,*

2014) and *S. tuberosa* is a typical plant that plays an important role in the Caatinga biome of North eastern Brazil, since it blooms even in dry period to furnish edible fruits, largely appreciated by the population (Rhode *et al.,* 2014).

2.3 Description of *Spondias mombin*

Spondias mombin is a type of *spondias.* Linn. is a member of the Anacardiacae family. It can be found in the rain forest and along the seashore. It can grow to be between 15 and 22 meters tall. The bark of the trunk bears deep incisions, which create a brown resinous material. At the end of the branches are the leaves and flowers. The majority of the leaves are stripped from the tree before it begins to blossom. The fruit, a 112-inch long oval yellow plum with a leathery skin and a thin layer of fruit pulp with a very unusual flavor, has a leathery skin and a thin layer of fruit pulp. It is hung in many clusters of over a dozen on the tree (Okwu and Okwu, 2004).

The fruit, which is high in vitamins B1 and C, is largely an oval seed. Seeds and cuttings are the only ways to propagate the plant. Bala (Costa Rica), Jobito (Panama), Jobo blanco (Colombia), a medium-sized tree with long compound leaves that have an odd number of leaflets, ranging from 9 to 19. The leaves are usually alternating, but bunched toward the end of the branches, radiating in all directions like spokes of a wheel. The leaflets are opposite except for the terminal ones. Particularly on young plants, the leaf stalk tends to be reddish towards the outer leaflets. Crushed leaves have faint turpentine-like smell. The trunk and bark are gray, and sometimes have distinctive bur, blunt, gray spines (often more like warts than spines). Jobo corronchoso (Venezuela), Hoeboe (Surinam), Acaiba, Caja, Pau da tapera (Brazil), Ubo (Peru), Hobo (Mexico).

Figure 1: *Spondias mombin* showing the leaves and stem bark

Photo credit: Stephanie

Plate 1: Unripe fruits of Spondias mombin

Table. 2: Ripen fruits of Spondias mombin

Part used: Stem Bark

2.4 Taxonomic Classification

Kingdom:	Plantae
Subkingdom:	Viridiplantae
Infrakingdom:	Streptophyta
Division:	Tracheophyta
Subdivision:	Spermatophytina
Infradivision:	Angiospermae
Class:	Magnoliopsida
Superorder:	Rosanae
Order:	Sapindales
Family:	Anacardiaceae
Genus:	Spondias

2.5 Non-Medicinal Uses of *Spondias mombin*

It is widely utilized for living fences, agriculture, and artisan shelter. The fruits are edible and are known as monkey plums, but the wood is of poor quality and is rarely utilized. The leaves and roots are used as medicine, while the bark is utilized to carve figurines. The yellow *mombin* is less desirable than the purple *mombin*, and it is mostly used to quench the thirst of youngsters and travelers. Ripe fruits are eaten straight from the tree or cooked with sugar. In Costa Rica and Brazil, the extracted juice is used to make ice cream, cool beverages, and jelly. It's used in jams in great amounts in Panama, Peru, and Mexico. The fruit is mostly utilized in Amazon to make wine known as "Vinho de Taperiba." The fruit is converted into a cider-like

drink in Guatemala. Mexicans pickle the green fruits in vinegar and eat them with salt and chilli, similar to how they consume unripe purple *mombin*. Young leaves are used as greens in cooking. The fruits are commonly used as cattle and pig fodder. The tree secretes a glue-like substance. The wood is light in weight, buoyant, flexible, and susceptible to termites and other pests. It is yellow or yellowish-brown with darker markings, light in weight, buoyant, and flexible (Adepoju and Oyewole, 2008). It is widely used in carpentry, as a substitute for cork, for match sticks, match boxes, physician's spatulas, sweet foods sticks, pencils, pen-holders, packing cases, inner sheathing of homes and boats, and as a substitute for cork. It is unsuitable for turnery and polishes poorly. The woody tubercles on the trunk of the tree are chopped off and used as bottle stoppers and seals for stamping sealing wax in Brazil. Saplings are used to make hut poles, garden poles, and axe and hoe handles in tropical Africa. Wood is only used for fuel in Costa Rica and Puerto Rico. In Africa, ashes from burned wood are used in indigo dyeing. The bark is used in dyeing. It is so thick that it is popular for carving amulets, statuettes, cigarette holders and various ornamental objects. Portable water can be derived from the roots in emergency. The flowers worked intensively by honeybees early in the morning Wendakoon *et al.* (2012).

2.6 Ethnomedicinal Uses of *Spondias Mombin*

The juice of the fruit is used as a diuretic and a febrifuge. The astringent bark decoction is used as an emetic, a therapy for gonorrhea and leucorrhea, and a medicine for diarrhea, dysentery, and hemorrhoids. It is thought to remove calcifications from the bladder in Mexico. Wounds are treated with powdered bark. Stomach ache, biliousness, urethritis, cystitis, and eye and throat inflammations can all be relieved by drinking a tea made from the flowers and leaves.

An infusion of the young leaves is used in Belize to treat diarrhea and dysentery. Poultices made from crushed leaves and powdered dry leaves are used to treat wounds and inflammations. The gum is used to expel tapeworms and as an expectorant Rodrignes and Hesse, 2000; USDA, ARS (2002). The leaves of this plant were cited in the literature as abortifacient, antidiarrhoea, anti-microbial, anti-viral; contain a lot of vitamin C; wound-healer, although Osuntokun et al. (2018).could not prove this healing function. Ayoka et al. (2008) report on the plant's various uses based on oral transmission rather than any scientific inquiry. Due to its activity, infusions of its leaves have been used for a long period with no reported side effects Osuntokun and Olajubu, (2014). This plant is part of the 'Living Pharmacy' program developed by the Laboratory of Natural Products University 'Federal de Cearal', Brazil, a project aiming to teach local people how to cultivate and use correctly traditional medicinal plants. One step to preserving this knowledge will be the establishment of 'Forest Reserve' dedicated to the survival of medicinal plants and the healers that use them.

2.7 Phytochemistry of *Spondias mombin*

The constituent phytochemical substance in plant ,such as phenol, tannin, anthraquinones, and flavournoids, are thought to be responsible for the majority of the effects found with *Spondias mobin* extract (Ayoka *et al.,* 2005;Ayoka *et al.,* 2006;Caraballo *et al., (*2004).The leaves of *S.mombin*, according to Igwe *et al.,* (2010), are a potential source of pharmacologically active phytochemicals. From *Spondias mombin*, high-performance liquid chromatography (HPLC) identified carotenoids, phytoene, alpha-trans-beta-carotene, alpha-carotene, betacrytoxanthin (cis and trans), zeinoxanthin, and lutein, according to Hamano and Marcadante (2001). *Spondias mombin* gum exudates, which contain arabinose, mannsone, and rhamnose, are highly soluble in water (Leon-De-Pinto *et al.,* 1995). Ash gums have a high calcium, potassium,

sodium, and magnesium content in their cationic composition. The *Spondias* gums have been found to include arabinofuranose residues as structural characteristics.

Geraniin and galloygeraniin are the two. *Spondias mombin* has a high phenolic and tannin content that can be extracted. Anthraquinones, berberine, flavonoids, naphthoquinones, sesquiterpenes, quassiniods, indole, and quinoline alkaloids may be involved in *Spondias mombin's* anti-malarial activity (Caraballo *et al.,* 2004). *Spondias mombin* includes tannins, saponins, and anthraquinone glycosides that have antibacterial action but not antifungal activity, according to Abo *et al*. (1999). Moronkola *et al.* (2003) found more than 54 chemical elements in the essential oils of *Spondias mombin*, including caryophyllene, delta cadinine, alpha-muurolene, alpha-gurjunene, 5isocedranol, and –cadinene.

Most of the effects observed with extract of *Spondias mombin* may be attributed to the constituent compounds of phenols, tannins, anthraquinones and flavonoids presence in the plant (Ayoka, 2004; Ayoka *et al.*, 2005 and 2006; Caraballo *et al.*, 2004). The presence of these active compounds has been reported for several activities like antibacterial, anti-inflammation, haemostatic activity, anti-microbial, antioxidant, abortifacients, purgatives, hypnotics (Ayoka *et al*., 2005), anti-diarrhoea, anti-helmintic (Ademola *et al*., 2005), anti-malarial (Carabalo *et al*., 2004), wound-healing, enzyme inhibitor (Coates *et al*., 1994), increased capillary permeability in rats, anti-free radical action, anti-aging, reduced glutathione synthesis (Pauly and Fleury, 2002). The phenolic acids composition was observed to cause antibacterial and molluscicidal effect (Corthout *et al*., 1994).

2.8 Antioxidant Activity

Excessive production of free radicals, on the other hand, causes oxidative damage to DNA, lipids, and proteins, leading to cell death, which has been linked to the pathogenesis of cancer, ageing

(Bogdan and Baumann, 2018), ophthalmological diseases (Tezel, 2006), cardiovascular diseases (Lobo *et al.,* 2010), and many general neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and prion disease (Agil *et al.,* 2006).

Reactive oxygen species (ROS) in form of superoxide anion (O2),hydrogen peroxide (H2O2) ,hydroxyl radical,and reactive nitrogen specie (NOS) in the form of nitric oxide radical(NO)are metabolic by-products that can be created by external stimuli (Biriben *et al.,* 2012).A physiological level of ROS is thought to have a role in signal delivery.

Excessive ROS are neutralized in the body via a complex network of antioxidant defenses that is managed and balanced (He *et al.,* 2017). By scavenging free radicals, antioxidants aid to reduce their impact on biomolecules. However, if the body's ability to regulate free radical generation is overwhelmed, an external source of antioxidants must be introduced. An external source of antioxidants appears to be a potential strategy to avoid the harmful effects of excessive ROS exposure. 8 Research has indicated that eating foods high in antioxidant phytonutrients, such as flavonoids and other polyphenols, can help reduce the onset of chronic disease (Pandey and Rizvi, 2009). Also on the rise is the use of antioxidants such as pomegranate, green tea, grape seed, and mushroom extracts in skin care products targeted at avoiding clinical symptoms of photo-aging (Bogdan and Baumann, 2018; Simo *et al.,* 2014). As a result, there has been a surge in interest in naturally occurring antioxidants in foods, cosmetics, and pharmaceuticals. The use of antioxidants for an extended period of time necessitates the use of safe and effective antioxidants, which natural agents represent. Unlike synthetic antioxidants, which have been linked to toxicity (López *et al.,* 2013) and carcinogenic effects with extended usage, natural antioxidants have not been linked to toxicity (Gulcin *et al.,* 2005).

There is a need to find new antioxidants of natural origin to supplement the limited antioxidant arsenals already accessible. *Spondias mombin* is a fructiferous tree native to the West Indies, Southern Mexico, Peru, Brazil, and a number of tropical African countries such as Equatorial Guinea, Cote D'evoir, Nigeria, and Sierra Leone. In Yoruba ethnomedicine, it is referred to as iyeye in Nigeria. The fruit decoction is used as a diuretic and febrifuge, while the bark and leaf decoction is used as an emetic. The leaf of *S. mombin* has been shown to have antidiarrheal, antibacterial, antifungal, and antiviral properties. The flower, leaf or bark is used for wound healings and to treat stomach ache and various inflammatory conditions. Antioxidant activity of the leaf and fruit has been reported (Cabral *et al.,* 2016; Tiburski *et al.,* 2011).

The 70 percent methanolic extract of *S. magnifera* bark, according to Hazra *et al*., (2008), is a potent source of antioxidants. The total antioxidant activity of the 70% methanolic extract was determined from the decolorization of ABTS cat-ion, which was measured spectrophotometrically at 734nm, and compared to the trolox standard. In addition, S. mangifera methanolic fruit extract at concentration of 5μg/ml showed 16% radical scavenging activity compared to the same concentration of vitamin C whichshowedonly5%radical scavenging activity (Chalise *et al.,* 2010; Arif *et al*., 2016) showed that the ethanolic extract of S. mangifera fruits contains large amounts of phenolics, flavonoids,and acid glycosides,suchaspropan-1,2-dioicacid3-carboxyl-β-D-glucopyranosyl-β-D-glucofuranoside.Invitro and invivo studies were conducted to test the effects of ethanolic extract and acid glycoside ease antioxidants against anoxia-stress tolerance, swimming endurance, and Antioxidant Activity. Hazra *et al*., (2008) proved that the 70% methanolic extract of *S. mangifera* bark is a potent source of antioxidants. Total antioxidant activity was assessed in vitro, depending on the ability of the 70% methanolic extract to scavenge ABTS radical cat-ion, and compared to trolox standard, the total antioxidant

activity of the 70% methanolic extract was calculated from the decolonization of ABTS cat-ion which was measured spectrophotometrically at 734nm; the trolox equivalent antioxidant value was of 0.78. In addition, *S. mangifera* methanolic fruit extract at concentration of 5μg/ml showed 16% radical scavenging activity compared to the same concentration of vitamin C whichshowedonly5%radical scavenging activity (Chalise *et al.,* 2010).

According to Arif *et al*. (2016), the ethanolic extract of *S. mangifera* fruits contains high levels of phenolics, flavonoids, and acid glycosides like propan-1,2-dioicacid 3-carboxyl—D-glucopyranosyl glucofuranoside. The effects of ethanolic extract and acid glycoside as antioxidants against anoxia-stress tolerance, swimming endurance, and cyclophosphamide immune suppression were studied in vitro and in animals. The antioxidant activity was compared to that of Geriforte, a commonly used medication.

A UV spectrophotometer set to 517nm was used to conduct an in vitro research against DPPH. In test tubes containing 3ml of methanol and 0.5ml of 1mM DPPH, aliquots of 0.05, 0.5, and 1mg/ml of either the ethanolic extract or the acid glycoside were mixed; ascorbic acid was employed as a standard at the same concentrations, and the reaction mixture was incubated at 37°C for 30 minutes. The radical scavenging activity was calculated; the ethanolic extract and acid glycoside had IC50s of 0.32 and 0.15mg/ml, respectively, whereas ascorbic acid had an IC50 of 0.015mg/ml. The ethanolic extract and the acid glycoside both showed considerable antioxidant activity, according to these findings (Arif *et al.,* 2016).

2.9 Antimicrobial Activity

The long-running fight against human diseases, combined with the emergence of drug-resistant bacteria, has compelled a search for antimicrobial bioactive chemicals in the

environment. Because such chemicals are unique, they may be resistant to microbial resistance and their action may be altered by structural changes.

Spondias mombin has antimicrobial, antibacterial, antifungal, and antiviral activities, according to studies (Olugbuyiro *et al.,* 2013). According to preliminary research, the antibacterial properties of Spondias mombin leaf extract are due to the phenolic acid, 6-alkenylsalicylic acid. Another study discovered that the anacardis acid derivative derived from the plant's hexane extract has beta lactamase inhibiting effects. The anti-malarial effect of *Spondias mombin* has recently been related to a variety of chemicals found in the leaves, including anthraquinones, berberine, flavonoids, naphthoquinones, sesquiterpenes, quassiniods, indole, and quinoline alkaloids (Caraballo *et al.,* 2004). In light of the foregoing, there is no doubt that S. mombin leaves are used to treat a variety of ailments due to their medicinal properties.

Arif *et al.,* (2008) tested the in vitro antibacterial activity of the methanolic and the aqueous extracts of *S. pinnata* bark by cup plate diffusion method at the concentrations of 50, 100, and 150mg.The activity was tested against *Escherichia coli, Salmonella typhimurium, and Vibrio cholerae* and compared with penicillin and streptomycin as standard drugs. The methanolic extract showed a good antibacterial activity against Gram +ve and Gram −ve bacteria, while the aqueous extract showed only a mild antibacterial activity. The resin of *S. pinnata* also showed an antibacterial activity against *Bacillus subtilis* (Arif *et al.,* 2008). The 80% ethanolic extract of S. pinnata fruits showed a strong antibacterial activity against both Gram-positive and Gram-negative bacteria. The antimicrobial activity was tested by disc diffusion method; standard discs of kanamycin (30μg/disc) and blank discs were used as positive and negative controls, respectively (Muhammad *et al.,* 2011). Tapan *et al*. (2014) isolated two new ergosteryl

triterpenes (SP-40, SP-60) from *S.pinnata* bark and tested their antipseudomonal activity by agar disc diffusion method against a moderately resistant strain of *Pseudomonas aeruginosa* MTCC 8158. The tested organism was completely resistant to ampicillin and tetracycline at concentrations of 10 and 30μg/disc, respectively, while exhibiting an inhibition zone of 15mm against streptomycin at 100μg/disc concentration. SP-40 exhibited an inhibition zone of 20mm,which was better than streptomycin at comparable concentrations. SP-60, however, did not show any antimicrobial ctivity against this organism up to a concentration of 200μg/disc. The MIC values of SP-40, thus, were estimated to be between 25 and 12.5μg/disc (Arif *et al.,* 2008).

Olugbuyiro *et al*., (2013) isolated two new phytosterols: stigmasta-9-en-3,6,7-triol and 3-shydroxy-22-epoxystigmastane from the methanolic extract of *S. mombin* stem bark. Both compounds exhibited a marked antimycobacterial activity with 93% inhibition against *Mycobacterium tuberculosis* by a fluorometric microplate AlamarBlue Assay (Olugbuyiro *et al.,* 2013). Furthermore, the methanolic fruit extract of *S.purpurea* showed a strong antimicrobial activity against *E. coli and P. aeruginosa* using the disc diffusion method (Silva *et al.,* 2016). Islam *et al*., (2013) observed similar results when evaluating the antimicrobial activity of *S. dulcis* fruit.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Reagents, Apparatus and Instruments

Ethanol (Analar grade), Folin-Denis reagent, tannic acid**,** Vanillin, Folin–Ciocalteu reagent**,** Sodium Carbonate, Quercetin, Potassium Permanganate Solution, and chromogenic reagent, Analytical Balance**,** UV/Visible Spectrophotometer**,** autoclave, water bath, spatula, crucible, test tube, conical flask, beaker, dry oven, electric blender, sieve, wire loop, petri dishes, incubator, cotton wool, spirit lamp, paper tape, extraction jar, measuring cylinder, mortar and pistle, pasteur pipette and distilled water.

3.2 Sample Collection

The stem bark of *Spondias mombin* was collected from the botanical garden of the Department of Plant Biology and Biotechnology, University of Benin.

3.3 Extraction and Preparation of Sample

The stem of *Spondias mombin* was cut and the bark was pilled and allowed to air-dry away from the sun under room temperature. The dried plant bark was then blended and stored. 250g of the sample was measured and diluted in a solvent of 900ml ethanol, stirred and left to stand for 24hrs with continuous agitation of the soaked sample at 1hr interval. The sample was macerated, concentrated and stored properly. 5g of the extract was weighed into a sterile beaker containing 20ml of ethanol which was allowed to dissolve to form the stock concentration. Varying concentrations were made from the stock concentration by double serial dilutions (100mg/ml, 50mg/ml 25mg/ml 12.5mg/ml, 6.25mg/ml, and 3.125mg/mL).

3.4 Phytochemical Analysis

Phytochemical analysis was carried out on the *S. mombin* in the Organic Chemistry Laboratory, Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City.

3.4.1 Determination of Total Phenolic Contents

The amount of total phenolics in extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi, (1965) with slight modification using tannic acid as a standard. 1.0 ml of extract solution (250 Ug/ml) was added in a test tube. 1.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 5 min, 15.0 ml Na_2CO_3 (20 %) was added and allowed to stand for 2 hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as Ug of tannic acid equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph.

3.4.2 Determination of Alkaloids Content

The total alkaloid content was measured using the method described by Harborne (1973). 5g of the extract was weighed into a 250 mL beaker and 100 mL of 20% acetic acid in ethanol was added and covered to stand for 2 hours. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, washed with 1% ammonia solution, dried and weighed. All samples were analyzed in triplicates.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.4.3 Flavonoid content determination

The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) (Ilahy *et al.,* 2011). Thirty-microliter aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with 90 µL methanol, 6 µL of 10% Aluminum chloride ($AlCl_3$), 6 µL of 1 mol/l Sodium acetate (CH3CO2Na) were added and finally 170 µL of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (Ug Qe/g).

3.4.4 Estimation of total saponins content

Estimation of total saponins content was determined by the method described by Makkar *et al.* based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800mg of vanillin in 10mLof 99.5%ethanol) was added. Then 2.5mLof 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°C for 10min. After 10min, it was cooled in ice cold water and the absorbance was read at 570 nm. 0- 25 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly as test samples. The values were expressed as PPM.

3.4.5 Estimation of tannins content

Exactly 0.20 mL of sample was added to 20 mL of 50% methanol and placed in a water bath at 77 ˚C – 80 ˚C for 1 hour and shaken. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and 20 mL of distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17% Na_2CO_3 were added and mixed. The mixture was allowed to stand for 20 minutes. A series of standard tannic acids solutions were prepared in methanol and their absorbance as well

as samples was read after color development on a UV/Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

3.5 Proximate Analysis

Proximate analysis was also carried out on the stem bark of *S. mombin* in the Organic Chemistry Laboratory, Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City.

3.5.1 Moisture Content.

The weight of dry crucible taken and a known weight of *Spondias mombin* were placed inside the dry crucible. The crucible and the content were placed inside an oven at 105 ^{0}C for 1-2hrs. The samples were placed in the desiccator and the weight is taken. The process was repeated until a constant weight was achieved. Moisture content was estimated as

$$\text{Moisture \%} = \frac{\text{Loss of weight x 100}}{\text{Weight of sample used}}$$

3.5.2 Determination of Ash Content

A silica dish or crucible furnace was Place in a furnace for about 15mins at 350^{0}C. the crucible was remove and cool in the desiccator for about an hour or cool to room temperature and was weigh (w_0). 0.5-2g sample was transferred into the crucible (w_2). The crucible was placed inside the muffle furnace and the temperature was slowly increased from 200^{0}C-450^{0}C. This is to avoid incomplete ashing. Ash sample until it becomes whitish in color. Within a reasonable period, the crucible was removed, cooled, moisten with few drops of distilled water, dry on water bath and was return to the furnace. Crucibles were removed from furnace to desiccator and allow to cool at room temperature. The crucible was re-weigh and content (w_3). Ash content was estimated as

$$\% \text{ Ash} = \frac{w_3 - w_1}{w_2 - w_1} \times 100$$

$$\% \text{ organic} = 100 - \% \text{ Ash}$$

3.5.3 Determination of Crude Fiber

2 g of the leaf sample was weighed into the 100 mL conical flask (W_0). 200 mL of H_2SO_4 was Added and boiled for gently for 5 minutes using cooling fingers to maintain a constant volume. After 5 minutes, it was filtered through the poplin cloth stretched over 9 cm Buchner funnel, It was then rinsed with hot distilled water. The sample was scraped back into the flask with a spatula. 200 mL of NaOH was added and boiled gently for 3o minutes using cooling finger to maintain a constant volume. It was then filtered through a poplin cloth. The residue was washed thoroughly with hot distilled water. It was then rinsed with 10% HCl followed by ethanol. It was finally rinsed with petroleum ether (BP 40 – 60^0C). It was allowed to drain and then the residue was scraped the crucible. The residue was dried overnight in the oven at 105 °C and was cooled in the desiccator. The sample was weighed (W_1) and then ashed in the muffle furnace at 550^0C for 90 minutes. It was finally cooled in the desiccator and weighed again (W_2) (Tel and Hagarty, 1984).

Calculation for percentage crude fiber is done thus:

$$\% \text{ Crude Fiber} = \frac{W_1 - W_2}{W_0} \times 100$$

3.5.4 Determination of Crude Fat

The 250 mL boiling flask was dried in the oven at 105^0C and allowed to cool in the desiccator before weighing (W_2). 2 g of the ground sample was weighed into a labeled porous thimble and was covered with clean cotton wool. 200 mL of petroleum ether was added was added to boiling

flask. The covered porous thimble was placed into the condenser and then the apparatus was assembled. Extraction was done for about 5 – 6 hours and then the porous thimble was removed with care. The petroleum ether was collected in the top container (tube) for reuse. The boiling flask was removed from the water bath when it is almost free of petroleum ether. The boiling flask containing the oil is oven dried at 105 °C for 1 hour. It was then cooled in the desiccator and weighed (AOAC., 1997).

Calculation for percentage crude fat is done thus:

$$\% \text{ Fat} = \frac{W_3 - W_2}{W_1 - W_0} \times 100$$

Where W_0 = weight of empty porous thimble, W_1 = weight of thimble + ground sample, W_2 = weight of empty boiling flask and W_3 = weight of boiling flask + ether.

3.5.5 Determination of Crude Protein

The estimation of crude protein was done by determining the total nitrogen in the samples by Kjeldahl method. The amount of crude protein was obtained by multiplying the nitrogen content by 6.25. This is based on the assumption that all feed protein contains 16% nitrogen and that all the nitrogen in tissue is present as protein. Technical digester which is an alternative method of Kjeldahl digester was used.

0.1 g of the dried leaf sample was weighed into a micro kjeldahl flask. 2 mL of concentrated H_2SO_4 was added followed by 1 tablet of selenium catalyst. This was heated gently in a digester until froting ceased and then it was strongly heated until the solution was clear. It was then filtered into a 100 mL volumetric flask through a Whatman No. 42 filter paper and made up to mark. From the filtrate, 1 mL was taken into 100 mL volumetric flask. 1 mL of alkiali-phenate solution was added followed by 2 mL of sodium potassium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) and 1.5

mL of sodium hypochloride (bleach) and was made up to 25 mL mark. The solution was allowed to stand for 10 minutes so the color develops and it was read in a spectrophometer at 630 nm. A blank was prepared and also subjected to the same processes as the sample. A series of Nitrogen standards of 2, 4, 6, 8 and 10 ppm was prepared and read at the same wavelength. A graph of OD against ppm was drawn and slope reciprocal (SR) was found (Tel and Hagarty, 1984).

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Calculation for Nitrogen content was done thus:

$$\% \text{ Nitrogen} = \frac{\text{IR x SR x FV x CD x 100}}{\text{Wt. x Aliquot x 1,000,000}}$$

Where IR = OD or Instrumental reading, FV = Final volume, CD = Color development, Wt. = Weight of sample used, Aliquot = Volume of solvent used.

Then Crude Protein is calculated thus:

Crude Protein = % Nitrogen x 6.25

3.5.6 Determination of Soluble Carbohydrate

This was obtained as the difference between the sum of percentage of other contents of the leaf samples and 100%. It is then calculated thus:

Soluble Carbohydrate = 100 – (% fat + % ash + % crude fiber + % Crude protein)

(AOAC., 1997).

3.6 Determination of Metals (Fe, Ca, Zn, K, Mn, Mg, Cu, and Na)

1.0g dry and grinded bark of *Spondias mombin* plant extract was weighed into a 300 mL calibrated digestion tube. Concentrated HNO_2 was added in the fume hood and swirl carefully and then placed tubes in the rack. Glass funnel was placed on the calibrated digestion tube and leaved for 8hrs. After pre-digestion, 10ml di-acid mixture was added and swirl carefully. The tubes rack was placed in the block-digester and then a funnel was placed on the neck of the tubes. Temperature setting was slowly increased to 200^0C until the dense white fumes evolve and transparent white contents are left. The tube was lifted out of the block-digester, and carefully placed on a rack holder, and also the tubes were allowed to cool in room temperature. Filtration was done through Whatman No. 1 filter paper, and brought to 50ml volume. Each metal was determined using Atomic Absorption Spectrophotometer except for sodium that was determined with Flame photometer. Each batch of the samples for digestion contained one reagent blank (no plant).

3.7 Determination of Vitamin in Stem Bark of *Spondias mombin.*

3.7.1 Preparation of the Stock and Standard Solution of Ascorbic Acid

This was carried out using the principle of Bulk Scientific method. Standard solution of ascorbic acid was prepared by dissolving accurate weight of 0.01g of standard ascorbic acid in small amount of oxalic acid solution (0.5%) and then completed to 100 ml with the same solution to obtain a concentration of 100 µg/mL. A series of dilutions 1.0, 4.0, 8.0, 12 and 16 µg/ml were prepared from the stock of ascorbic acid solution.

3.7.2 Preparation of Standard Calibration Curve of Ascorbic Acid

Standard calibration curve of ascorbic acid was established by graphing concentrations versus absorbance of ascorbic acid standard solutions by taking 10 ml of each of the standard solutions and put in a test tube, then 1 ml of KMnO4 solution (100 µg/ml) was added. This solution was let to stand for 5 minutes. The absorbance of this standard solutions were read at 530 nm against blank.

3.7.3 Preparation of stem bark of *Spondias mombin* Samples for Analysis by UV-Visible Spectrophotometer (Model: Sheerwood 410)

The sample were accurately taken as 10.0 ml and then transferred into a test tube, and 1.0 ml of KMnO4 (100 µg/ml) was added. The contents of each test tube were mixed well and stand for 5 minutes. The prepared solutions were read at 530 nm against blank by spectrophotometer using a suitable concentration for the analysis.

3.8 Antimicrobial Analysis

3.8.1 Test microorganism

Staphylococcus aereus, Candida ablican, Mucor mucedo and *Penicilium chrysogenum* were obtained from the Pharmaceutical Microbiobiology Laboratory, Faculty of Pharmacy; *Bacillus*

subtilis, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* were obtained from Microbiology Laboratory, Faculty of Life Sciences; *Providencia rettgeri*, *Arthrobacter globiformis*, *Acinetobacter calcoaceticus*, were obtained from Science Laboratory Technology Laboratory, Faculty of Life Sciences; *Aspergillus tamari*, *Aspergillus niger*, *Trichoderma harzianum* and *Escherichia coli* were collected from the Plant Biology and Biotechnology Laboratory, Faculty of Life Sciences, all in the University of Benin, Benin City. Each of these microorganisms was already identified by the various laboratory technologists/scientist before collection. The microorganisms were stored in the refrigerator.

3.8.2 Sterilization of materials

The glass wares used for this study such as, test tubes, conical flask, beakers, pipettes, measuring cylinder e. t. c were thoroughly washed , rinsed with distilled water , then wrapped with aluminum foil and placed in the hot air oven at 160^0C for 1 hour to sterilize . The culture media such as potato dextrose agar, potato dextrose broth, nutrient agar ,agar agar were sterilized in an autoclave at 121℃ for 15 minutes. The inoculating wire loop and cork borer were flame sterilized in a Bunsen flame.the petri dish and Pasteur pipette used were also sterile. The bench tops were also sterilized using alcohol and a Bunsen burner was lighted to create aseptic environment.

3.8.3 Preparation of culture media

The preparation of culture media to the final stage of the identification of zone of inhibition was carried out in aseptic condition. Potato dextrose agar (PDA) was selected for the experiment since it has to do with antifungal property. Thirty-nine (39.0g) as specified by the manufacturer was weighed into a conical flask and 1 L of distilled water was measured and used to dissolve

the media. For Nutrient agar (NA), twenty-eight grams (28.0g) was measured into a conical flask and dissolve in 1 L of distilled water. When a complete dissolution of the media has taken place, a sterile cotton wool and foil paper along sides with a masking tape were used to cork the conical flask to prevent water from entering into it during sterilization and to avoid contamination after sterilization. The media was then taken to the autoclave for proper sterilization at 121^0C for 15 minutes. After sterilization has taken place, the media were allowed to cool between 40-45^0C. Prior to this stage the plates for the experiment have been properly labeled and a drop of the isolates was introduced to the middle of the corresponding plates. The media were poured into each sterile petri dishes and rock to homogenize and allow even distribution of the isolate and also allow the media to solidify.

3.8.4 Antimicrobial Plating

The antifungal activity of the crude aqueous and ethanol extracts was evaluated, using the agar well diffusion method. The fungal isolates grown on the potato dextrose broth and nutrient agar broth were used to seal sterilized potato dextrose agar plates and nutrient agar plate. Three wells (6 mm in diameter) of equidistant from each other were bored on the surface of the inoculated PDA and NA plates using sterile cork borer. Aliquot of 0.2 ml of the crude ethanol extracts was dispensed into each well. The inoculated potato dextrose agar plates were left on the bench top under room temperature for 48 – 72 hour while the nutrient agar plates were incubated at 35 – 37 ^{0}C for 12 – 24 hrs. The zone of inhibition was measured and recorded.

CHAPTER FOUR

RESULTS

Table 1: Analysis of the proximate and mineral composition of *S. mombin*

S/N	Parameter	Unit	Value
1.	Moisture content	%	38.20±1.56
2.	Ash content	%	11.46±0.27
3.	Fat content	%	2.325±1
4.	Crude protein	%	3.563±0.6
5.	Crude fiber	%	8.2480±0.5
6	Carbohydrate	%	74.404
7.	Vitamin C	μg/ml	1664.714
8.	Sodium	g/kg	0.80
9.	Potassium	g/kg	65.00
10.	Calcium	g/kg	2.100
11.	Magnesium	g/kg	2.460
12.	Manganese	g/kg	72.85
13.	Copper	g/kg	0.200
14.	Zinc	g/kg	0.100
15.	Iron	g/kg	1.500
16.	Nitrogen	%	0.570

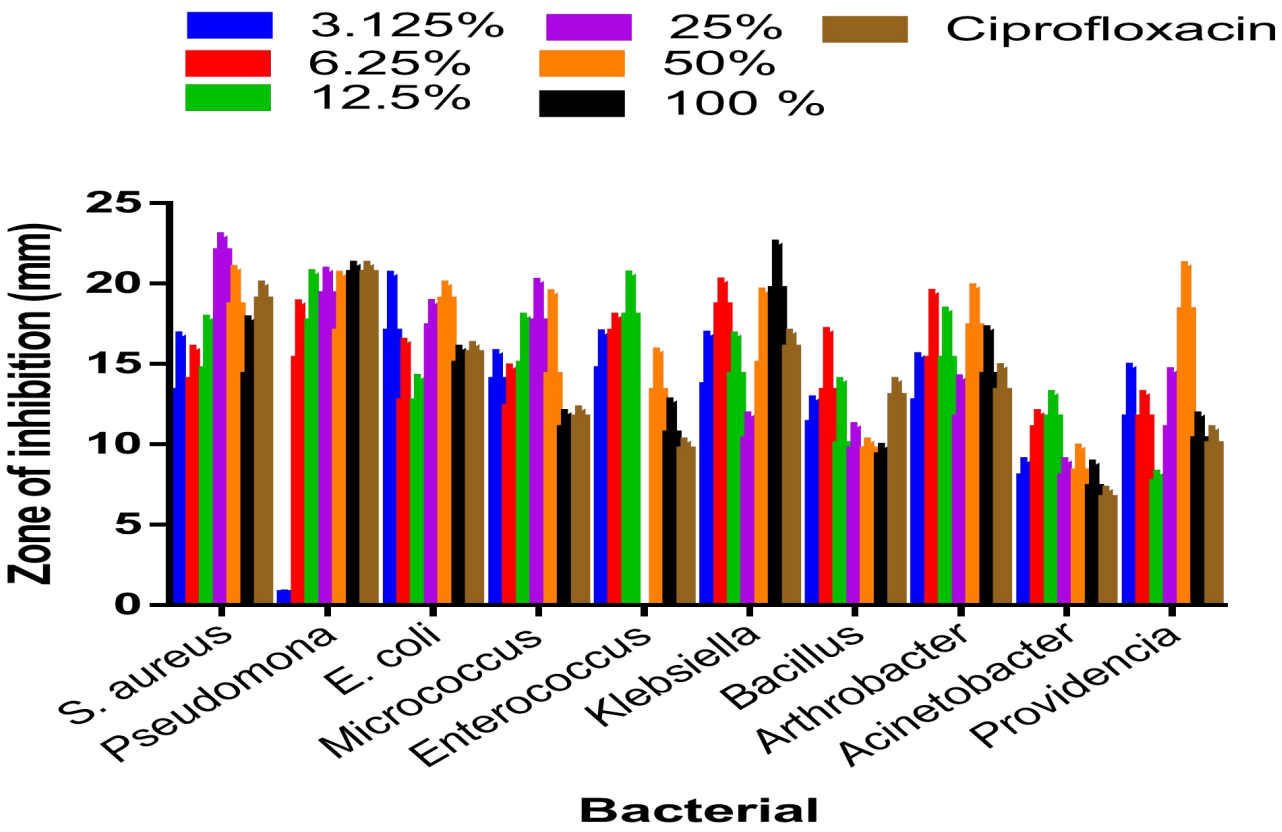

Figure 1: Antimicrobial activity of ethanolic extract of stem bark of *Spondias mombin* plant on selected bacteria using ciprofloxacin as positive control. The result of this study showed that *Spondias mombin* inhibited the growth of all the bacteria at all concentrations ranging from 3.215% to 100%.

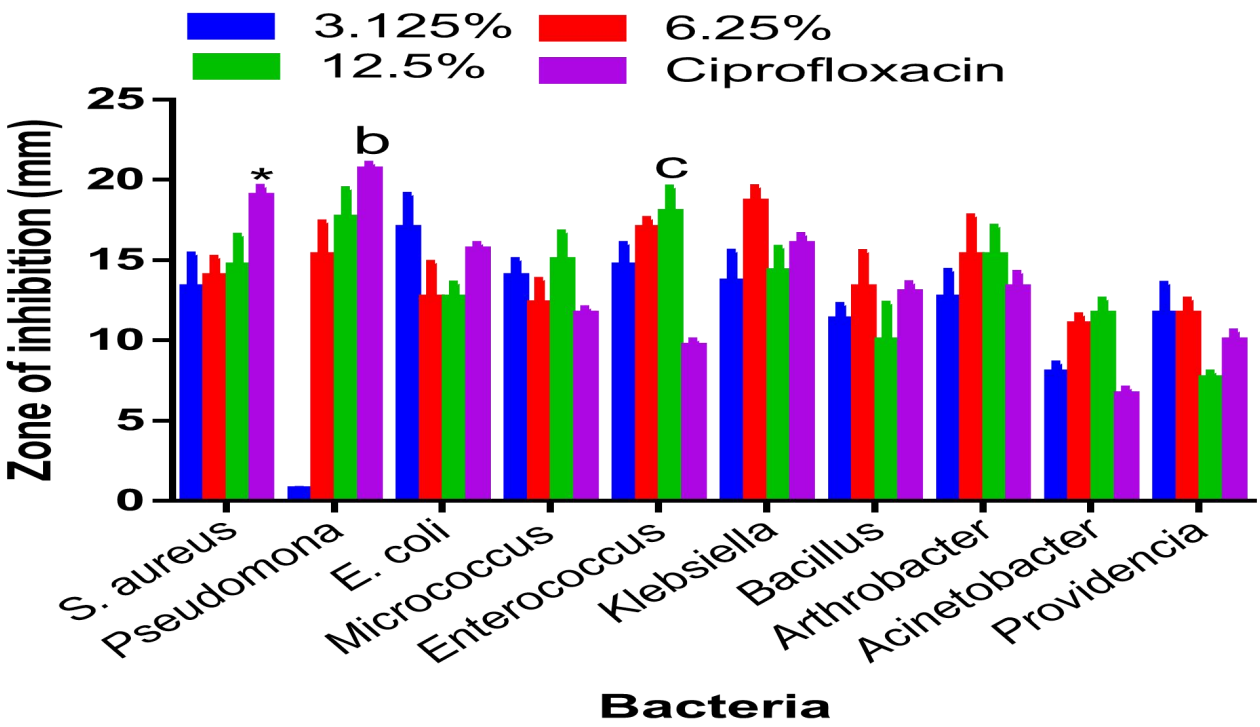

Figure 2: The antimicrobial activity of the ethanol extract of the stem bark of *Spondias mombin* against various bacteria. The ethanol extract of *Spondias mombin* inhibit the growth of *S. aureus, Pseudomonas, E. coli, Micrococcus, Enterococcus ,Klebsiella, Bacillus, Arthrobacter, Acinetobacter, Providencia.* Ciprofloxacin showed a better zone of inhibition to *S. aureus* and *Pseudomonas aeruginosa* ($^{*}p<0.05$; $^{b}p<0.0001$). At 12.5%, *S. mombin* gave a better zone of inhibition against *Enterococcus* ($^{c}p<0.001$). Data are represented by mean SEM where n = 3.

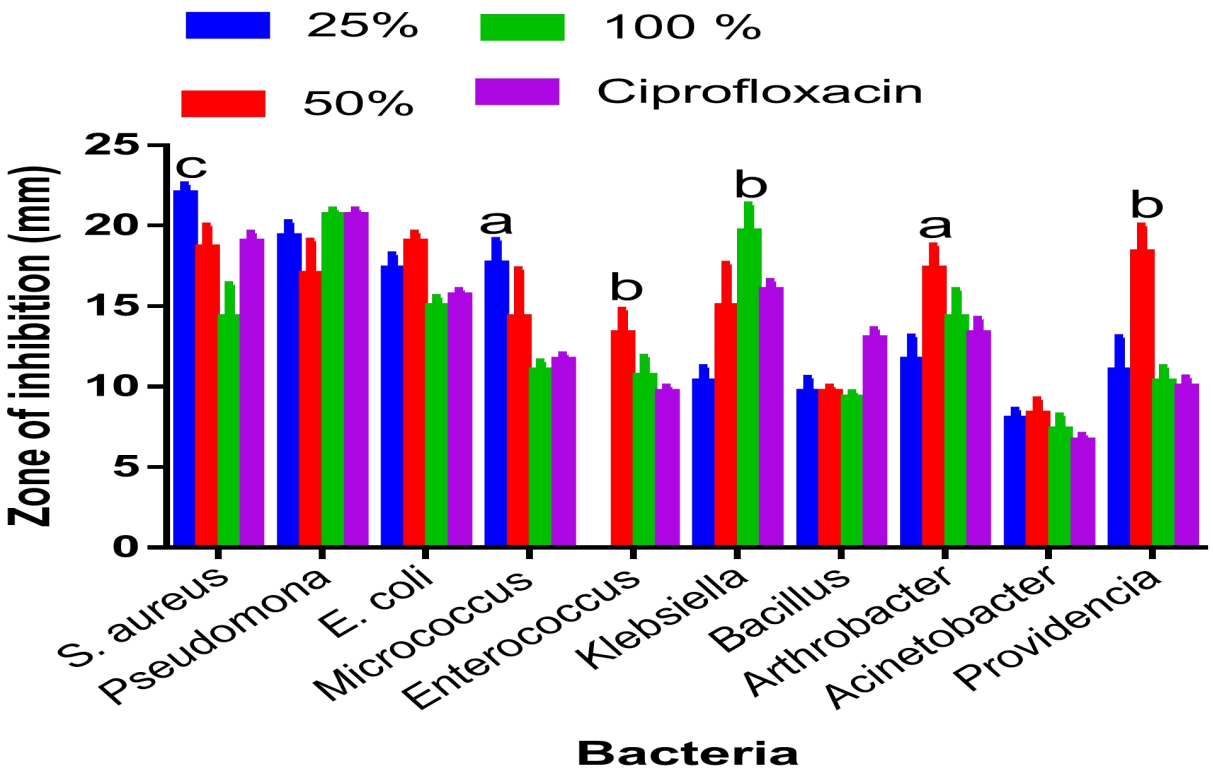

Figure 3: The antimicrobial activity of the ethanol extract of the stem bark of *Spondias mombin* against various bacteria. The ethanol extract of *Spondias mombin* inhibited the growth of *S. aureus, Pseudomonas, E. coli, Micrococcus, Enterococcus, Klebsiella, Bacillus, Arthrobacter, Acinetobacter* and *Providencia*. At 25%, the extract has a better zone of inhibition to *S. aureus and Micrococcus.* 50% of the plant extract has a better zone of inhibition to *Enterococcus, Arthrobacter* and *Providencia*. ($^{b}p<0.0001$; $^{a}p<0.01$). Data are represented by mean SEM, n=3.

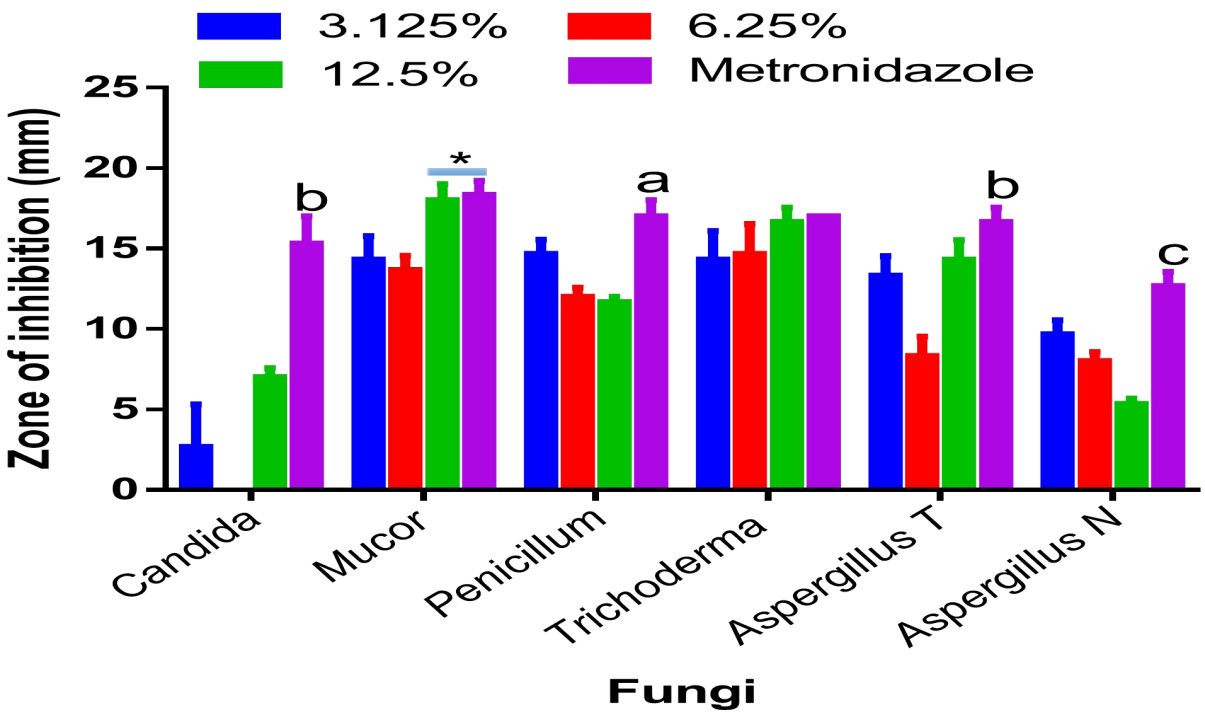

Figure 5: The antifungal activity of the ethanol extract of the stem bark of *Spondias mombin* against various concentratins of 3.125%, 6.5%, and 12.5%. *Spondias mombin* inhibit the growth of Candida, *Mucor, Penicillum, Trichoderma, Aspergillus tamari* and *Aspergillus niger* ($^{b}p<0.0001$;*$p<0.05$; $^{a}p<0.01$; $^{C}P<0.001$), Data are represented by mean SEM, n=3.

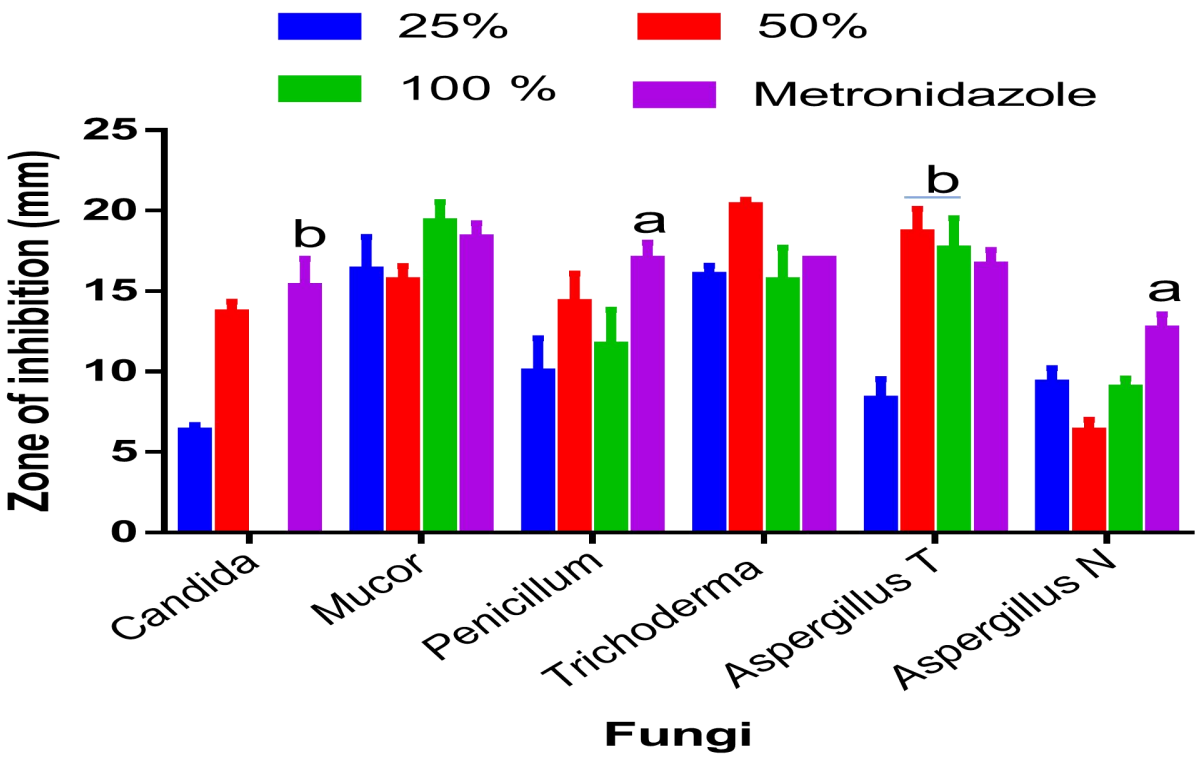

Figure 5: Antifungal activity of the ethanol extract of the stem bark of *Spondias mombin.* At various concentration of 25%, 50%, and 100% *Spondias mombin* inhibit the growth of *Candida*, *Mucor, Penicillum, Trichoderma, Aspergillus tamari* and *Aspergillus niger*. Metronidazole a standard drug gave a better zone of inhibition to *Candida, Penicillum and A. niger* ($^{b}p<0.0001$; $^{a}p<0.01$). However, 50% and 100% gave a better zone of inhibition to *Aspergillus T*, ($^{b}p<0.0001$), Data are represented by mean SEM, n=3.

CHAPTER FIVE

DISCUSSIONS

Medicinal plants of both herbs and trees have been reported in this study to be effective in the management and treatment of various ailments. Some of these plants exist in wide, while others as domesticated plants. The result of this study, determined the stem bark of a local plant (*S. mombin*) used in medicinal practice in Nigeria. However the basic active ingredients in *Spondias mombin* used for treating various ailments are accumulated in the different parts of the plant such as leaves, roots, bark and stem. (Wanbugu *et al.*, 2011). The stem back of *Spondias mombin* has been reported to have significant medicinal and economic value. The result of the phytochemcial analysis of *S. mombin* stem back showed the presence and copious amount of phenolic compounds, flavonoids, saponin, tannin, and alkaloid. As similar to the study of Erhenhi (2016) who reported the presence of alkaloids, saponin, flavonoid, phenol, steroid and anthraquinone at moderate concentration in *Spondias mombin* stem bark .The biological function of flavonoid present include protection against allergies, inflammation, platelets aggregation microbes, ulcer, vineses and tumors (Ferreya *et. al.,* 2012; Kumar and Pandey, 2013; Mierziak *et. al.,* 2014). Flavonoids represent the most common and widely distributed groups of plant phenolics that serve as a flavouring ingredient of spices and vegetables (Vijayashalini *et*. *al*., 2016). Flavonoid has being identified in *Spondias mombin* with antioxidant and anti-ageing properties. They are free radicals scavengers, super oxidant and strong anti-cancer activities. Saponins in this plant bark are responsible for the possession of hemolytic property which uses the medicinal potential of the plant as the basic medicinal agents due to their analgesic, antispasmodic and antibacterial effects. Alkaloids are the most efficient therapeutically significant plant substance, and the pure forms are used to repel parasites and predators when injected by animals. They affect glucagon,

thyroid stimulating hormone and inhibit some mammalian enzymatic activities. They are used as the basic medicinal agents due to their analgesic antispasmodic and antibacterial effects. Moreover, they have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and inhibition of neovascularization (Prabakaran *et. al.,* 2014). Tannins are known to improve wound healing and inflamed mucus membrane. The presence of tannin, flavonoid and saponin as seen in the result give credence to the reported antimicrobial, antifungal anthelminthic properties of *Spondia mombin*. The stem bark extract of *Spondias mombin* in this study have shown to contains naturally occurring antioxidant which are free radicals scavengers, super oxidant and strong anti-cancer activities. The decotion or macerated stem bark of *Spondia mombin* are used for varialis, ulcer, frost bite, burns, severe cough, inflammation, muscle and joint injuries, pain relieve in traditional herbal medicine. Vitamin was present and is effective in the repair of free radical damage to the cells. The mineral constituents analyzed in Table 3 showed the presence of magnesium (2.46), iron (1.5), manganese (72.85), copper (0.2), sodium (0.8), calcium (2.1), zinc (0.1) and potassium (65.0) which are due to high ash content that gives an ideal of the mineral elements present and the content of organic matter in the sample. The protein content was moderate which are source of amino acids for maintenance and for growth, and crude fiber was present in appreciable amount. In this study, the ethanol extract of *S. mombin* was active against all the test organisms. The minimum inhibitory concentration (MIC) for *Enterococcus* was 50% (Figure 3). The minimum inhibitory concentration for *Candida albicans* was 12.5% (Figure 5). Ciprofloxacin was more against *Providencia*, *Arthrobacter* and *E. coli* than the stem bark extract of *S. mombin* while the stem bark extract was more active against *S. aureaus, Pseudomonas*, *Mucor*, *Enterobacter*, *Klebsiella* and *Bacillus* (Figure 3). Metronidazole was more active against *A. niger*, *Penicillium* and

Candida albicans while the leaf extract was more active against *Mucor*, *Tricoderma* and *Aspergillus tamari*.

CONCLUSION

The result of this experiment showed that *Spondias mombin* have phytonutrients that are active against the test microorganisms and can be utilized in providing remedy for infectious diseases caused by the test organisms.

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