ANTIBACTERIAL ACTIVITY OF STEM BARK CASHEW PLANT

BY

IBRAHIM KHADIJAT OYINKANSOLA

HND/23/SLT/FT/0373

BEING A RESEARCH WORK SUBMITTED TO THE DEPARMENT OF SCIENCE LABORATORY TECHNOLOGY, INTITUTE OF APPLIED SCIENCE, KWARA STATE POLYTECHNIC, ILORIN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN SCIENCE LABORATORY TECHNOLOGY (BIOCHEMISTRY UNIT).

JULY, 2025

CERTIFICATION

This is to certify that this original work carried out and reported by Matric Number HND/23/SLT/FT/0373 of the Department of Science Laboratory Technology, Biochemistry Unit, Institute of Applied Science (IAS) Kwara State Polytechnic. And it has been Approved in Partial Fulfillment of the Requirements of the Award of Higher National Diploma (HND) in Science Laboratory Technology.

_____ _____

MR. ADEKUNLE J.A **DATE**
(Project Supervisor)

_____ _____

MRS. SALAUDEEN K.A **DATE**
(Head of Unit Biochemistry)

_____ _____

DR. USMAN ABDULKAREEM **DATE**
Head of Department (H.O.D)

_____ _____

EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this project work to Almighty Allah and to my parents Mr. and Mrs. Ibrahim thanks for their support.

ACKNOWLEDGEMENT

First and foremost, I give all glory and praise to Almighty Allah for granting me the strength, wisdom, and perseverance to successfully complete this project, for his boundless mercy and grace through my days in kwara state polytechnic.

My deepest gratitude goes to my beloved parent Mr. and Mrs. Ibrahim who stood by me through every step of this journey .your unwavering belief, sacrifice and relentless support have been my greatest strength. I will forever cherish the impact you have made on this path .

To my siblings, Temitope, Abdul Ganiu, thank you for your constant encouragement and support. Your emotional and financial support helped lighten my burden during this journey, and I am truly blessed to have you all by my side.

To my amiable and best supervisor ever in person of Mr. Adekunle J.A for his unwavering support, patience and unceasing ideas that have helped me tremendously at all time in writing of this project. Mr. Adekunle was available to answer all my questions, provide constructive criticism and offer encouragement, even during challenging times, he is always there for me like a father .his dedication to my academic growth has been truly inspiring .i pray Almighty Allah enrich and grant you longetivity sir .

My appreciation also goes to the head of biochemistry unit ,in person of Mrs. Salaudeen K.A and the head of department ,science laboratory technology in kwara state polytechnic ,Ilorin in person of Dr. Usman Abdulkareem for their support and encouragement and to the entire Lecturers in Science Laboratory Technology .May Almighty Allah bless you all. Ameen

This project is not just a requirement for my degree it is a symbol of growth, resilience, and the power of support from those who stood by me.

TABLE OF CONTENT

Chapter Four

4.1 Results and discussion

Chapter Five

5.1 Conclusion

Abstract:

The aim of this study was to investigate the relative antibacterial activity and phytochemical properties of ethanolic and water extracts of Anacardium occidentale (cashew) stem bark. The phytochemicals were screened using qualitative methods. Qualitatively analyzed phytochemical constituents in the stem bark extracts include carbohydrates, alkaloids, flavonoids, saponins, tannins, sterols, anthraquinone, terpens and phenol. The microorganisms assayed for the antibacterial activities using the agar well diffusion were Escherichia coli, Salmonella Typhi, Bacillus subtilis and Staphylococcus aureus, studies on the susceptibility pattern and the zones of inhibition exhibited by the extracts shows a certain degree of inhibitory effects against the test organisms. Ethanolic extract of A. occidentale stem bark was effective against B.subtilis at concentration of 100mg/ml and 50 mg/ml only, S. aureus and S. Typhi at concentration of 100 mg/ml only, and E. coli at concentration of 100 mg/ml only while in aqueous extract of A. occidentale stem bark, there was effect against S. aureus at concentration of 100 mg/ml and 50 mg/ml, followed by B. subtilis at concentration of 100 mg/ml and 50 mg/ml, S. Typhi and lastly E.coli both at the concentration of 100 mg/ml. Considering the diameter of the zone of inhibition, it was noticed that there was little or no difference between the diameters of both extracts. Although, these results suggest an important ethno-pharmaceutical potential of A. occidentaleas a source of compounds with broad-spectrum antimicrobial activity that can be used in the pharmaceutical industry its low activity may be due to low concentration of the extracts.

CHAPTER ONE

1.1 Introduction:

Cashew plant parts can be used for several medicinal purposes. Every part of cashew has some important medicinal properties. The cashew nut is essential for physical and emotional well-being. It is an energetic diet and the best food medication for many illnesses. Nuts protects from malignant growth, coronary illness, circulatory strain, and various degenerative infirmities connected to aging. Cashew kernel contains proteins, carbohydrates, vitamins, and fats which help gain energy. Cashew nuts are used for many purposes like blood sugar, weight loss, cancer, cold and flu, aging, urinary disorders, digestive disorders, and bone relaxation. In comparing cashew with other nuts, Cashew kernels have good properties, including proteins, carbohydrates, fibers, and minerals, then almond, hazelnut, and walnuts. There are some strong benefits of Cashew for health. They boost the immune system, act as an antioxidant, remove gallstones, and are beneficial for anemia. They are good sources of healthy fats that are necessary for our body to absorb fat-soluble vitamins.

1.2 STATEMENT OF PROBLEM

The basis for using plants as medications is their chemical constituents' capacity to induce biochemical and physiological effects in living systems, these molecules, also known as phytochemicals or secondary metabolites, have become more popular in the domains of biochemistry, pharmacology, medical sciences, and

microbiology (Ujowundu et al., 2010). Many plant species and their parts, like the fruits, leaves, roots, seeds, barks, and flowers, contain a variety of bioactive compounds that may have a range of medicinal benefits. Natural products play a significant role in the development of medications in the pharmaceutical sector and make up more than 50% of all modern clinical pharmaceuticals

1.3 JUSTIFICATION OF THE STUDY

Extracts from various plant components, particularly the stems, roots, fruits, and leaves, have all been utilized to treat inflammatory conditions, oxidative stressrelated illnesses, and infectious diseases (Olajide et al., 2019). Synthetic antibiotics cause microbial resistance over time, which renders bacteria resistant to medication actions. As a result, using plant-based compounds may be a viable option

1.4 AIM

.The aim of this study was to investigate the relative antibacterial activity and phytochemical properties of ethanolic and water extracts of Anacardium occidentale (cashew) stem bark.

1.5 OBJECTIVE OF THE STUDY

I collection and preparation of sample

Ii screening of extracts for phytochemicals:

Iii microbiological media test

CHAPTER TWO

Literature review

Cashew is an evergreen perennial plant belonging to the family Anacardiaceae. This family consists of 400-600 species. Among the eight species in the genus Anacardium, the only cashew is valuable due to its nutritious kernel. Cashew is a tropical tree present in South America and

Brazil. Plant height varies from 5 to 14 m. The trunk is usually short and irregular, starting branches close to the ground. Leaves are green that are placed in a spiral pattern towards the end of the stem. Leaves become mature after 20-25 days. Flowering can occur at any time; individual flowers are short in size consisting of five yellowish-green sepals and five white to reddish petals. In 2011, about 4.7million tons of raw nuts were produced worldwide, which were distributed between Asia and Africa, where 1.8 million cashew apples were produced [1].

Cashew has been cultured essentially, and whole fruit is used for medicinal and food purposes, e.g., apple and kernel. Cashew gained its importance during World War II due to the utilization of its significant by-product, the cashew nut shell liquid (Cordeiro; Rico, Bulló et al. 2016). The Cashew nut has nutritious properties with a pleasant flavor. Cashew kernels have shown low-density lipoprotein cholesterol levels and coronary risk diseases. Cashew part contains proteins and fats. The proteins include lysine, cysteine, arginine tyrosine, valine, and many vitamins likevitamin C, E, D [2].

Cashew gum has been used widely for many health-related issues. These are less in saturated fatty acids and more in unsaturated fatty acids. Its health benefits have been used to decrease the risk of cardiovascular diseases, oxidative stress, inflammation, high cholesterol, and diabetes [3].

Cashew nuts are used for several medicinal purposes and have great importance related to health, as evidenced by research [4]. These are used for obesity, diabetes, heart disease, urinary disorders, digestive disorders, and many other clinical applications like bone relaxation, cold and flow, etc. It also has importance in Cancer, and protects from aging [5].

2.2 CASHEW COMPOSITION:

The nuts of Cashew are present in the regions of north-eastern Brazil, similar to the kidney seed shape appearance that holds the lower part of this, the product of the tree. It is a favorite dried organic product that has excellent and brilliant taste. During the 16th century, the Portuguese familiarized cashew with India and Africa. The Cashew tree has great importance, as its valuable wood has analgesic effect, the cashew itself didn't acqui re notoriety in the start of the twentieth century. Now India is the biggest country in making, processing, and exporting cashew bits on the earth. It is used as a mixed drink or in foods to distribute bread rolls, frozen yogurts, and chocolates. It can also be utilized for natural products, juice, liquor, desserts. The cashew nutshell fluid is extracted from shell comprises as destructive astringent oil valuable in brake coating, paints, and plastics [6].

2.3 CASHEW REFINING:

These nuts are to be prepared to remove palatable bit which is profoundly nourishing. In India, it is collected during long periods of April and May and promoted rapidly from that point. The external surface of cashew is thick and slightly thin internal surface. The pieces that are obtained during preparation are of various evaluation like wholes, parts, and bits, etc.

2.4 ROLE OF CASHEW NUTS IN HEART DISEASES:

As nuts have been selected that they are not suitable food due to their high-fat substance. But recent studies suggest that nuts might be beneficial for the heart. About 66% of cholesterol comes from the liver, not from food, its creation being animated by soaked fat. Soaked fat itself can be dangerous for the heart. Soaked fats are discovered prevalently in synthetic items, including dairy items. However, people can reduce their cholesterol level by using food containing low-soaked food and more unsaturated fats. Recently, tree nuts have been developing a great medical advantage. For example, previous studies from epidemiological and clinical

examinations have shown that tree-nut is helpful for the heart diseases.

Cashew part that is less in soaked fat and more in monounsaturated fat lessens the general degrees of cholesterol and low-density lipoproteins (LDL) - the claimed "bad cholesterol" that prompts coronary disease and builds high-density lipoproteins (HDL), hence assisting in making the heart more sound [7]. The Cashew part contains fiber, carrying more fiber into the diet brings down the level of cholesterol and the danger of heart disease prominently, which is known as

heart nibble. The fiber in the digestive tract lessens the assimilation of cholesterol from food consumption. Ordinary use of these nuts, as a feature of a low-soaked fat eating routine, can bring down the danger of coronary disease overall by advantageously influencing the cholesterol levels in blood and can reduce the risk of having a subsequent respiratory & cardiovascular failure[8].

2.4.1 CASHEW NUTS FOR DIABETES :

Daily utilization of food that is rapidly increasing the sugar level in the blood leads to developing heart diseases and diabetes as Cashew nuts contain high mono saturated fat that is beneficial for decreasing the blood glucose level and increase insulin production. Thus, diabetes can be managed by cashew pieces. They are essential for type 2 diabetes. They are lower in sugar and higher in fiber; when these factors are combined, they decreased the blood glucose level and prevent the development of type 2 diabetes [9].

2.4.2 CASHEW NUTS FOR RHINITIS:

As Cashew parts are rich in vitamin B, an ordinarily occurring cell strengthening which has invulnerability high power. Consequently, burning through cashew parts during winter and cold season will improve an

individual’s insusceptibility [10].

2.4.3 CASHEW NUTS FOR OBESITY:

Harvard University declared that a healthy food contains copious fat from the nuts of tree and olive oil has a strong agent for weight reduction as a small calorie, kept for a low eating regimen and a great benefit for weight reduction [11]. These nuts are rich in supplements and fiber that they will, in general, fulfill appetite on less energy than other snacks [12]. Cashew extract causes significant reduction in total cholesterol, triglycerides, LDL and VLDL cholesterol [13].

2.4.4 PROTECTION FROM CANCER:

This disease can likewise get destroyed from nuts. Selenium - rich cashew bits are helpful for lung, liver, skin, cerebrum, and gastrointestinal malignancy. Due to significant fiber content likewise assists with fighting malignancy. They act as an antioxidant and prevent the growth of cancer cells by removing the free radicals from the body. A class of flavonoids called Proanthocyanidins fight tumor cells and prevents them from further division. High copper content and proanthocyanidins in cashew nut fight copper content in cashew helping to prevent colon cancer [14].

2.4.5 EYE PROTECTION:

Today’s women are

selecting to work at home by using mobile phones and laptops continuously, which damage their eyes. Zeaxanthin is a pigment that is present in Cashew, by the consumption of Cashew, will protect the retina from damaging the UV rays and hence protects the eye.

2.4.6 FOR SKIN:

We all want fresh, flawless, and glowing skin and avoid cosmetic products for damaged skin. By eating a few Cashews daily, we can be protected from acne and damaged skin. This is the most beneficial effect of Cashew nut for charming and glowing skin[15-22]

2.4.7 HELPFUL FOR AGEING:

Vitamin E has extra ordinary boosting power. A new investigation conducted on 65 years old people found that Vitamin E supplementation appeared to stop the decrease in insusceptibility related to aging. 100g of cashew pieces contain 46mg of Vitamin E, in this way protecting from aging as cashew contains free radicals that keep the body free from wrinkles and lines in the face. Cashew is used in skin remineralization and in the treatment of premature aging [23].

2.4.8 RENAL ROLE:

Potassium is an essential element to protect the human renal system. As it contains sodium and potassium, so, it can be used to treat dehydration and essential minerals, which are suitable for the kidneys. 28.35gm of cashew portion gives 0.00015kg of potassium. Potassium is a common mineral is acquire on a regular basis excepts from delicate coconuts and cashew nuts [13-17].

2.4.8 ROLE IN DIGESTIVE DISORDERS:

Cashew contains 1.30% fiber. Dietary fiber is beneficial for stomach-related issues. It maintains water, soothes the stool, and removes the blockage. Thus, it is beneficial for hemorrhoids, varicose veins, hiatal hernias, and diverticulosis.

2.4.9 CASHEW FOR BONES AND NEURALGIA:

About 22.3% of magnesium is present in one-fourth cup of cashews. It contains calcium, magnesium which are essential for nerve and muscle tone. It acts like a natural calcium channel blocker that keeps calcium from rushing into the nerve cell and enacting the nerve. By regulating the calcium entrance, that will keep the nerves loose.

Recent studies have shown that magnesium decreases the recurrent attack of headache, lowers the circulatory strains, cardiovascular failures, manages hormonal influences in women during menopause, and lowers the seriousness of Asthma [38].

2.5 ROLE OF CASHEW KERNEL:

Cashew kernels are purified from raw cashew. These are soft, white, and meaty but change the color and taste upon roasting. They are attached with shells and classify them carefully by food companies around the world. These are dried to lose the skin and then peeled off [18]. Cashew part contains protein (18.22g), carbohydrates (27.13g), and fat (46.92g) per 100g. These have lower fat content than other nuts containing oleic acid, which is essential for the cardiovascular system [29].

The lipid parts of Cashew are rich in unsaturated fats, which include oleic

Acid and polyunsaturated fats. This is free from cholesterol and contains a fixed amount of monosaturated fat, which is best for lowering the cholesterol level [40]. However, cashew has the most powerful benefits for health, including heart, nerve functioning, and muscle, maintain bone strength and oral health. These are good source of vitamins and dietary fats, which are good for our health to absorb fat-soluble vitamins [21].

These are classified into three types; white/scorched wholes, pieces, and splits. They are low in sugar and rich in proteins, fibers, and healthy fats. They also contain important minerals and vitamins like calcium, magnesium, and copper, which are essential for the production of energy, brain health, immunity, and

strengthen the bone. In addition, it contains calcium, phosphorus, copper, zinc, iron, magnesium, and sodium. As Cashew parts give the energy of 611KCals/100g, and this is an equal amount that of almond (612Kcal/100g). In addition, it contains minerals like

Calcium, Phosphorus, Sodium, Potassium, Magnesium, Iron, Copper, Zinc, and Manganese. It is highly rich in starch. Oligosaccharides of cashew part are, for the most part, galactosyl sucrose.

These kernels give an oil that can be used as a mechanical and chemical antidote for irritant poison [22].

2.6 CASHEW APPLE:

This is the fruit of cashew tree. It is red or orange in color. These are rich in vitamin C, so can be used for diseases that can be developed due to the deficiency of vitamin C, like for skin. These are preserved in glass jars. Cashew apple is a pseudo-organic product that is a delicious natural and nutritious. The fleshy part of cashew is known as cashew apple. These contain sugars, tannins, phenols, amino acids, ascorbic acid, minerals and fiber. The cashew apple has a sweet flavor having delicate skin. Cashew apple gives an anti-scorbutic property. Hence the juice of this apple can be used as diuretic, for the treatment of renal diseases, and for cholera. Cashew apple juice can be used for pharyngitis and chronic dysentery. The brandy of cashew apple can be used to relieve the pain in Neuralgia and rheumatism. The amount of ascorbic acid, solvent solids, decreasing sugars and all out acids were found to change among outskirts and focus of the cashew apple. As Cashew apples are rich in amino acids that has a property like aspartic acid, alanine, proline, leucine, and glycine [23].

2.7 CASHEW GUM:

Cashew gum (CG) is a biopolymer extracted from the exudate of

Anacardium occidentale, a typical tree of Brazil's north eastern locale. The gum chain is made out of galactose (72%), with side-chains of arabinose (4.6%),

glucose (14%), rhamnose (3.2%) and uronic acid (4.7%). CG properties were discovered to be like those of gum Arabic. Fundamental oils are unstable, vanishing effectively, and can deteriorate when presented to light, heat or potentially pressure. The encapsulation of fundamental oils intends to safeguard and secure their useful properties, notwithstanding give a controlled delivery in a given medium. Nuts are energy-thick food varieties, for the most part because of their high fat substance. These food varieties are low in immersed saturated fats (SFAs) and high in unsaturated fats. Also, nuts contain large amount of fiber, folate, minerals, and cancer preventing agents. Because of their dietary creation, broad exploration has been completed on nuts and wellbeing results, for example, diminished danger of

Cardiovascular diseases and related risk factors like oxidativestress

And irritation, highncholesterol and diabetes. Considering the observed advantages of nut utilization on heart wellbeing, the US Food and Drug Administration (FDA) delivered a wellbeing guarantee perceiving that these food sources may diminish the risk of coronary diseases from the leafy

foods and utilized in culinary that point forward, nuts have been fused into rules for smart dieting in a few countries. Tree nuts are characterized as dry natural products with one seed in which the ovary divider turns out to be hard at development. Peanuts (Arachis hypogea), albeit organically

delegated vegetables, have a comparable supplement profile to tree nuts and subsequently are normally remembered for this gathering.

Selenium is a fundamental micronutrient with cell reinforcement limit that guides in distinctive physiological cycles like invulnerable framework

Balance, substantial metal and xenobiotic detoxification, and thyroid

Chemical guidelines

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.1.1 Gathering and identifying plant sample:

Anarcadium occidentale (Cashew) shrub bark was obtained and collected at Oke-ose, Ilorin Kwara state. The plant was recognized in the University of Ilorin, which houses the department responsible for researching medicinal plants and traditional medicine. The plant part was thoroughly cleaned under running water before being let to air dry for two weeks in a well-ventilated area. The ground-up plant sample was held until required in a secure container. Collection of test organisms: Escherichia coli, Salmonella Typhi, Bacillus subtilis, acnd Staphylococcus aureus were included in the clinical sample given by the Department of Microbiology, University of Ilorin.

3.1.2 Preparation of ethanol extracts:

The ethanol-aqueous extract was made using ethanol that was 70% ethanol. A 500 g dry plant sample and 2500 ml of ethanol were mixed and left at room temperature for 48 hours. The extract was dried by evaporation after filtering. The plant extracts were stored for future use in an airtight container.

3.1.3 Preparation of water extracts:

With 800 ml of distilled water, 500 g of crushed plant material was steeped and heated for 20 minutes. After cooling to room temperature, the extract's supernatant was decanted and centrifuged for ten minutes. Using glass microfibre filter paper, the supernatant was filtered before being dried at 45 °C for a certain period of time until all the water had evaporated. A bottle that was airtight was used to prepare the plant extract for use.

3.2 Screening of extracts for phytochemicals:

Nine parameters were examined during phytochemical screening, including carbohydrates, alkaloids, steroids, phenols, flavonoids, terpenoids, anthraquinones, tannin, and saponin.

3.2.1 Determination of the carbohydrates:

Three grams of each plant extract were heated for 3 minutes in a water bath with 50ml of distilled water added. After filtering the mixtures while they were still hot, the cool filtrates were collected, and these were used in the subsequent experiments. Using 2 ml of the aforementioned plant samples and 3–4 drops of Molisch's reagent, the Molisch's test was conducted. After concentrated sulfuric acid was added in little amounts, a lower layer formed. A purple color ring in the liquid's interphase indicates the presence of carbohydrates. After shaking, the mixtures were allowed two minutes to stand. It was thinned down with 5 mls of water. When a purple precipitate appears, carbohydrates are present.

3.2.2 Determination of alkaloids:

Three grams of the powdered ingredients and 50 ml of methanol were combined, macerated, and evaporated to dryness. The leftovers and 10 ml of 1% aqueous hydrochloric acid were mixed in a water bath. A 1 ml portion of each from the mixture was treated with Mayer's reagent and Dragendorff's reagent. By checking for turbidity or precipitation, the reagents were employed to evaluate if an extract contained alkaloids.

3.2.3 Determination of anthraquinone derivatives:

Borntrager's Test: About 0.5 g of the powdered plant samples were placed in a test tube along with 10 ml of chloroform, and the mixture was violently agitated for 5 minutes. The extracts were filtered, and the filtrate was agitated before being mixed with an equivalent amount of ammonia solution. It was believed that the bright pink colour in the top aqueous layer was a marker of free anthraquinones.

3.2.4 Determination of sterols and terpens:

About 5 g of the plant samples were dissolved in 10 ml of anhydrous chloroform, filtered, and the filtrates were divided into two parts for further analysis. Lieberman-Burchard Test: The two plant samples' initial portions of the chloroform solutions were mixed with 1 ml of acetic anhydride after 1 ml of concentrated sulphuric acid was added down the test tubewalls to generate a lower layer. Steroids were detected by the formation of a reddish violet tint at the liquid interface and a green color in the chloroform layer.

3.2.5 Salkowski's Test:

Carefully mixing 2 ml of concentrated sulphuric acid into the second part of the solutions of the two plant sample samples caused the acid to create a lower layer. A sign that terpenoids were present was the development of a reddish-brown color during the interphase.

3.2.6 Determination of the saponins:

Foam test: A 20 ml solution of the extracts diluted to a 1 ml concentration in distilled water was agitated in a cylinder for 15 minutes. Saponins are present when stable foam develops.

3.2.7 Determination of tannins:

A beaker containing 3 g of each plant's sample was combined with 50 ml of distilled water and heated for 3 minutes. Filtration was performed on the hot mixtures while they were still hot, and the test was conducted using the cooled filtrates.

3.2.8 Test for ferric chloride:

The cooled filtrates were mixed with a few drops of 10 % iron III chloride ($FeCl_3$). Blue black or blue green colour was thought to be a sign of tannin content.

3.2.9 Determination of flavonoids:

Five grams of the materials were fully detanned in acetone. Warm water was used to wash away the leftovers after the acetone evaporated on a water bath. The mixtures were filtered, and the filtrates were then applied to the tests that came next. Test with lead acetate: To a 10 % lead acetate solution, 5 ml of

detanned water extract was added. The presence of flavonoids is indicated by reddish brown bulky precipitate.

3.2.10 Determination of phenol:

The materials were dissolved in a solution of water, ethanol, and a few drops of neutral ferric chloride solution—a solution made by mixing de-ionized water with ferric chloride. After a stable brown precipitate had developed, sodium hydroxide was added to the mixture. There is phenol present when red or blue coloration appears.

3.3 Microbiological media used for the test:

Mueller Hinton Agar and Mueller Hinton Broth were utilized as the study's medium. The manufacturer's instructions were followed in the preparation of all the media. Preparation of inocula: On nutrient agar slopes, stock cultures were maintained. The active cultures for the research were created by transferring a loopful of cells from the stock cultures into test tubes filled with Mueller-Hinton broth (MHB) and allowing them to grow for a whole night at 37°C. When cultures were diluted with new MHB and the findings were compared to 0.5 McFarland standards, values corresponding to 1.5×10^8 colony forming units of bacteria were discovered. Preparation of stock solution and serial dilution of the extract: About 0.4 g of the extracts and 4 ml of Mueller-Hinton broth were weighed into sterile vials using sterile Pasteur pipettes to produce a concentration of 100 mg/ml. A vortex shaker was used to blend the liquid, and it was given time to thoroughly dissolve. Two millilitres of the original stock solution was serially diluted into four bottles containing 2 ml of Mueller hinton broth in order to reach concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml. In-vitro antimicrobial susceptibility assay of the extract: Mueller-Hinton agar (MHA) was

used for the test of antibacterial activity. Standardized cultures of each microbe, equivalent to 0.5 McFarland standards, were dispensed into 20 ml of sterilized MHA kept at 45 ° C, poured into Petri dishes, and gently swirled to ensure a uniform dispersion of the organisms under aseptic conditions. The mixture was then allowed to gel for an hour. For each plate with bacterial isolates, an 8mm-diameter well was made with the requisite labels using a sterile metallic cork borer. The bottoms of the wells were sealed with 10 µL of MHA. After that, 100 µL of varied extract concentrations were carefully pipetted into each well using a sterile micropipette. The wells were then placed in the safety hood for optimal agar diffusion before being incubated at 37□ for 24 hours. Inhibitory zones could be observed on the plates, and their dimensions were measured in millimeters using a transparent meter ruler (mm). On duplicates, experiments were conducted. Further work included organism viability control (OVC) and medium sterility control (MSC) (OVC). Chloramphenicol 10 µg was used as the standard medication in the control setting.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

Anacardium occidentale (cashew) stem bark ethanol and aqueous extract results of preliminary phytochemical screening are shown in Table 1. Anacardium occidentale's ethanol extract was found to include a carbohydrate, anthraquinone, sterol, terpene, tannin, flavonoids, and phenol, but no alkaloids or saponin. The aqueous extract of Anacardium occidentale, which also included carbohydrate, alkaloids, anthraquinone, saponin, tannin, terpen, flavonoids, and phenol, however did not contain sterol.

Table 1: Phytochemical screening of ethanolic and aqueous extracts of Anacardium occidentale stem bark

Phytochemicals	ESBE	WSBE
Carbohydrates	+	+
Alkaloids	-	+
Anthraquinone	+	+
Sterols	+	-
Terpens	+	+
Saponin	-	+
Tanin	+	+

Flavonoids	+	+
Phenol	+	+

Key:+ =present, - =absent, ESBE=ethanol stem bark extract, WSBE=water stem bark extract

The antibacterial activity of an ethanol extract of A. occidentale is shown in Table 2, and all test species are inhibited by the extract at a dose of 100 mg/ml. All test organisms show resistance at concentrations of 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml, respectively, whereas only Bacillus subtilis was inhibited at a dose of 50 mg/ml. The zone of inhibition for the control (chloramphenicol) was the largest for all of the test organisms. The findings of the aqueous extract of A. occidentale's antibacterial activity are shown in Table 3. At a dose of 100 mg/ml, the extract inhibited every test organism. It inhibits Bacillus subtilis and Staphylococcus aureus at a dosage of 50 mg/ml, but all test organisms show resistance at concentrations of 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml, respectively. For all of the test species, the control's zone of inhibition was larger (chloramphenicol).

Table 2: Antibacterial activities of ethanolic extract of Anacardium occidentale (cashew) stem bark

Concentrations (mg/ml)/Zone of inhibition (mm)						
Organisms	100	50	25	12.5	6.5	Control
E. coli	2	-	-	-	-	29
S. Typhi	3	-	-	-	-	31
B. subtilis	4	2	-	-	-	32

S. aureus	3	-	-	-	-	28

Key: - = no activity

Table 3: Antibacterial activities of aqueous extract of Anacardium occidentale (cashew) stem bark

Concentrations (mg/ml)/Zone of inhibition (mm)						
Organisms						
E. coli	2	-	-	-	-	29
S. Typhi	3	-	-	-	-	31
B. subtilis	3	2	-	-	-	32
S. aureus	4	2	-	-	-	28

Key: - = no activity

4.2 DISCUSSION

Many plant extracts have antimicrobial properties, and medicines use them as natural substitutes to treat a variety of diseases. Scientific research on plants used as medicines has shown promising phytochemicals that may be produced for the treatment of infectious and non-infectious disorders. As a consequence of the rising interest in the search for antimicrobial agents from natural sources, compounds that may serve as appropriate antimicrobial agents to replace

synthetic ones have been discovered and developed (20). These compounds are far less toxic and offer a wide range of therapeutic applications for viruses, bacteria, fungus, and other human disorders. This has led to the use of medicinal plants in drugs, dietary supplements, and nutraceuticals (32). During the qualitative phytochemical screening of Anacardium occidentale stem bark in this research, anthraquinones, terpenes, tannins, flavonoids, and phenols were identified in both the ethanol and aqueous extracts. All other metabolites that were examined, with the exception of sterols were present in the aqueous extract of the Anacardium occidentale stem bark that was used in this study. Alkaloids and saponins, however, could not be discovered in the ethanol extract. Several studies have identified the occurrence of man metabolites, including phenols, flavonoids, glycosides, tannins, alkaloids, and anthraquinones.The study plant is commonly used in traditional medicine to cure a range of illnesses (15). The ethanol or aqueous extracts of A. occidentale had no discernible impact on the clinical bacteria under study. The antibacterial activity shown in this study may be caused by the phytochemical extract components that regulate the bioactivity of the extracts (12). For example, it has been shown that flavonoids may dissolve the bacterial cell wall, which affects the complete functioning of microbial cells (18). However, the ethanol extract of A. occidentale stem bark is only effective against B. subtilis at concentrations of 100 mg/ml and 50 mg/ml, S. aureus and S. Typhi at concentrations of 100 mg/ml only, and E. coli at concentrations of 100 mg/ml only. In contrast, the aqueous extract of A. occidentale stem bark was effective against S. aureus at concentrations of 100 mg/ml and 50mg/ml, followed by B. subtilis at concentration of 100 mg/ml, and 50 mg/ml, S. Typhi and lastly E. coli both at the concentration of 100 mg/ml. In a research, it was discovered that ethanol and ethyl acetate-based extracts of A. occidentale leaf and bark inhibited the development of several microorganisms. Given the comparatively low activity of the extracts seen in the present study compared to that reported by Ngari et al. (13), we can hypothesize that the difference may be caused by the greater concentration of extracts (200 mg/ml) used in their work. The ethanol and aqueous fruit extracts of the A. occidentale fruit's antimicrobial activities mean zone width of inhibition for S. aureus was reported. Between the sizes of 11mm and 28mm, S. aureus was found on several extracts, whereas E. coli ranged from

17 to 29 mm in size. In a different investigation, it was discovered that ethanol and aqueous stembark extracts from A. occidentale have antibacterial effects of on S. aureus and E. coli. Our study demonstrates that B. subtilis in ethanol and aqueos extracts and S. aureus in aqueous extracts, at doses of 100 mg/ml, were more effective. However at 50 mg/ml, these two species showed a decrease in activity. Nevertheless, it was discovered a significant difference between the diameters of the inhibition at a dose of 200 mg/ml. While comparing the zones of diameters, it was shown that there was little to no difference between the diameters of the two extracts. A cold and hot water extract of A. occidentale was shown by Aderiye and David (14) to have a substantial antibacterial effect against methicillin-resistant S. aureus, and E. coli O167:H7. In the cashew stem bark ethanolic extract, B. subtilis had the highest zone of inhibition of 31 mm), followed by E. coli and S. Typhi having (29 mm), and S. aureus (23 mm) having the least zone of inhibition. The control, chloramphenicol, had the highest zones of inhibition (32 mm) against B. subtilis, followed by S. Typhi having (31mm), E. coli (29 mm), and S. aureus.

CHAPTER FIVE

CONCLUSION

Anacardium extracts of the stem bark of occidentale (Cashew) were highly concentrated in a number of secondary metabolites, both in ethanol and water. Anthraquinolones, carbonate, tannins, terpenes, and flavonoids were all present in both extracts. Unquestionably, this is what gives the extract of A. occidentale its antibacterial effects (cashew) against a wide range of pathogenic bacteria, as revealed by prior study and also by this one, but at very low activity. The ethanolic and aqueous extracts could possess antibacterial properties that are useful against pathogenic microbes. Secondary metabolites were present, which helped antibacterial properties of extracts of the stem bark of A. occidentale, which supports the plant's long history of usage in traditional folk medicine to treat a range of illnesses. Together, these results indicate that A. occidentale in the pharmaceutical industry, has great potential as a source of compounds with broadspectrum antibacterial action.

REFERENCES.

1. Allen, K. G., L. M. Klevay, et al. (1977). "The zinc and copper content of seeds and nuts."

2. Bes-Rastrollo, M., J. Sabaté, et al. (2007). "Nut consumption and weight gain in a Mediterranean cohort: the SUN study." Obesity 15(1): 107-107.

3. Cardoso, B. R., G. B. S. Duarte, et al. (2017). "Brazil nuts: Nutritional composition, health benefits and safety aspects." Food Research International 100: 9-18.

4. Cordeiro, T. A. "A REVIEW ON THE NUTRITIONAL ASPECTS AND HEALTH BENEFITS OF CASHEW (ANACARDIUMOCCIDENTALE)." Principal's Message: 85.

5. Davis, C. D. (2003). "Low dietary copper increases fecal free radical production, fecal water alkaline phosphatase activity and cytotoxicity in healthy men." The Journal of nutrition 133(2): 522-527.

6. de Oliveira, E. F., H. C. Paula, et al. (2014). "Alginate/cashew gum nanoparticles for essential oil encapsulation." Colloids and Surfaces B: Biointerfaces 113: 146-151.

7. Dendena, B. and S. Corsi (2014). "Cashew, from seed to market: a review." Agronomy for sustainable development 34(4): 753-772.

8. Allen, K. G., L. M. Klevay, et al. (1977). "The zinc and copper content of seeds and nuts."

9. Bes-Rastrollo, M., J. Sabaté, et al. (2007). "Nut consumption and weight gain in a Mediterranean cohort: the SUN study." Obesity 15(1): 107-107.

10. Cardoso, B. R., G. B. S. Duarte, et al. (2017). "Brazil nuts: Nutritional composition, health benefits and safety aspects." Food Research International 100: 9-18.

11. Cordeiro, T. A. "A REVIEW ON THE NUTRITIONAL ASPECTS AND HEALTH BENEFITS OF CASHEW (ANACARDIUMOCCIDENTALE)." Principal's Message: 85.

12. Davis, C. D. (2003). "Low dietary copper increases fecal free radical production, fecal water alkaline phosphatase activity and cytotoxicity in healthy men." The Journal of nutrition 133(2): 522-527.

13. Fetuga, B., G. Babatunde, et al. (1975). feedingstuff potential of cashewnut scrap kernel meal. Proceedings of the Conference on Animal Feeds of Tropical and Subtropical Origin.

14. Fraser, G. E. (1994). "Diet and coronary heart disease: beyond dietary fats and low-density-lipoprotein cholesterol." The American journal of clinical nutrition 59(5): 1117S-1123S.

15. Hu, F. B., J. E. Manson, et al. (2001). "Types of dietary fat and risk of coronary heart disease: a critical review." Journal of the American college of Nutrition 20(1): 5-19.

16. Layokun, S., A. Obawole, et al. (1986). "Investigation of cashew apple juice as a

substrate for single cell protein production." Journal of Food science 51(1): 237-238.

17. Maia, J. G. S., E. H. A. Andrade, et al. (2000). "Volatile constituents of the leaves, fruits and flowers of cashew (Anacardium occidentale L.)." Journal of food composition and analysis 13(3): 227-232.

18. Nagaraja, K. (1987). "Lipids of high-yielding varieties of cashew (Anacardium occidentale L.)." Plant Foods for Human Nutrition 37(4): 307-311.

19. Nayudamma, Y. and C. Koteswara Rao (1967). "Cashew testa: its use in leather industry." Industrial Cashew Journal 4: 12-13.

20. Odunsi, A. (2002). "Effect of feeding reject cashew kernel meal on pre and early-laying performance of pullet." Archivos de Zootecnia 51(196): 423-429.

21. Oliveira, N., R. Leal, et al. (2015). "The importance of the cashew nut (Anacardium occidentale L.) coat: a review." American International Journal of Contemporary Scientific Research 2(8): 09-41.

22. Patil, P. (2017). "Indian cashew food." Integr Food Nutr Metab 4(2): 1-5.

23. Rani, T. J., K. Prasad, et al. (2017). "Evaluation of antiobesity activity of ethanolic extract of cashew apple against high fat diet induced obesity in rodents." The FASEB Journal 31: lb565-lb565.

24. Rico, R., M. Bulló, et al. (2016). "Nutritional composition of raw fresh cashew (Anacardium occidentale L.) kernels from different origin." Food science & nutrition 4(2): 329-338.

25. Santos, B., G. Juliano, et al. (2013). "Modelling the acidification curve of tropical fruits: cashew apple, coconut, kiwi, mango, papaya, red guava, watermelon and yellow melon." International Food Research Journal 20(2): 763.

26. Schirato, G. V., F. M. F. Monteiro, et al. (2006). "O polissacarídeo do Anacardium occidentale L. na fase inflamatória do processo cicatricial de lesões cutâneas." Ciência Rural 36(1): 149-154.