UNIVERSITY OF EDUCATION, WINNEBA

EVALUATION OF PHYTOCHEMICALS, ANTHELMINTIC, ANTIMICROBIAL, ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES OF METHANOL EXTRACTS OF SEED, LEAF, STEM BARK AND ROOT OF *CEIBA PENTANDRA* (KAPOK)

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MASTER OF PHILOSOPHY

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In the University of Education, Winneba

MARCH, 2023

DECLARATION

I, Bernard Fosu, declare that this research work is the result of my own work towards the Master of Philosophy degree. It has neither in whole nor partially presented elsewhere, with the exception of references from other people's work which have been cited and acknowledged accordingly.

SIGNATURE……..

DATE……..

SUPERVISORS' DECLARATION

We hereby declare that the preparation and presentation of this work was supervised in accordance with the guidelines for supervision of thesis/dissertation/project as laid down by the Akenten Appiah-Menkah University of Skill Training and Entrepreneurial Development (AAMUSTED).

Dr. Emmanuel Agyapong Asare (Principal Supervisor)

Signature…….

Date…….

Prof. Emmanuel Dartey (Co-Supervisor)

Signature…….

Date……..

DEDICATION

This thesis is dedicated to my grandmother, Madam Rose Sarpong, my family, friends and colleagues.

ACKNOWLEDGEMENTS

I would like to acknowledge, the help and support of my supervisor, Prof. Emmanuel Dartey, Department of Chemistry Education (AAMUSTED). Special thanks is reserved for Dr. Emmanuel Agyapong Asare, Department of Chemistry Education (AAMUSTED). Dr. Emmanuel Agyapong Asare painstakingly went through my work word by word and then made detailed contribution to it. Last but not least, I am much grateful to the laboratory technicians (KNUST) for their immensed help during my laboratory sessions. I am also indebted in diverse ways to every other person who contributed to the success of this work. I say may God Almighty bless you in all your endeavors.

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ABSTRACT

This study looked at phytochemicals, anthelmintic, anti-inflammatory, antioxidant and antimicrobial properties of extracts of seeds, leaves, stem and root barks of *Ceiba pentandra*. Parts of the plant investigated were soxhlet extracted with methanol. The extracts were phytochemically screened and their anthelmintic activities investigated using *Millsonia ghanensis* (Earthworms). Antimicrobial activity of the extracts were also investigated using *Staphylococcus aureus, Enterococus faecalis, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumonia* via broth dilution method. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and total antioxidant capacities (TACs) of the extracts were also studied. Anti-inflammatory activities of the extracts were investigated via carrageenan-induced foot oedema in a week old chicks. Phytochemical screening of the extracts indicated the presence of alkaloids, flavonoids, tannins, saponins and cyanogenic glycosides. Anthelmintic activity of methanol extracts of the seed, leave and stem bark were effective on the helminths when compared with the results obtained for standard drug (mebendazole). However, the root extract had a reduced activity on helminths as compared with the results of the standard drug (mebendazole). Minimum inhibitory concentration (MIC) of the extracts ranged from 1.5625-12.5mg/mL whilst minimum bactericidal concentration (MBC) were from 6.25-25.0 mg/mL. The half maximal inhibitory concentration (IC_{50}) (µg/mL) recorded in DPPH assay were 31.90 (ascorbic acid), 5.41 (seed extract), 18.18 (leave extract), 6.10 (stem bark extract) and 19.17 (root extract) whilst mean total antioxidant capacity (g AAE/100 g) were 188.71 ± 0.06 (seed extract), 131.56 ± 13.21 (leave extract), 242.18 ± 5.41 (stem bark extract) and 66.37 ± 2.30 (root extract). Median effective dose (ED_{50}) of carrageenan-induced chicks were 33.13 (seed extract), 22.74 (leave extract), 27.08 (stem bark extract), 43.08 (root extract) and 9.171 mg/kg (standard drug). These results indicate that *Ceiba pentandra* has comparable nutraceutical effect to the standard drugs used. Thus *Ceiba pentandra* could be used in herbal drugs formulations to treat earthworm and other microbes induced infections. Thin-layer chromatography and toxicological profile of *Ceiba pentandra* must be investigated to improve its use in herbal medicine formulations.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The World Health Organization (WHO) has estimated that about 80% of people in developing countries depend on traditional medicines for their basic healthcare needs (Boadu *et al.*, 2017). Approximately, 3.3 billon people (42% of the global population) mostly in developing countries use medicinal plants regularly (Ahvazi *et al.*, 2012). Medicinal plants are the pillars of traditional medicine (Dobriyal *et al.*, 1998). Usage of herbal medicines have progressively increased due to people's allergies to certain chemicals use in the formulation of orthodox medicines couple with high rate of adverse drug reactions, (Acheampong *et al.*, 2018). According to the WHO, policies development on the use of traditional medicines by member states have increased (WHO, 2019).

The World Health Organization has encouraged the use of plant medicine in modern public healthcare delivery systems of developing countries (WHO, 2019). The World Health Organization believes that herbal medicines could contribute to attaining Sustainable Development Goal 3. Sustainable Development Goal 3 promote good health and well-being of people of all ages and nations The WHO has also extended its guidelines to assist member states in their quest to formulate national policies on traditional medicine. These according to the WHO would improve efficacies and safety of herbal drugs (WHO, 2019). Plant medicine usage is the oldest form of healthcare known to humanity. These medicines have been used in different cultures throughout history. Plant medicines are the origin of most modern orthodox medicine

(Labe, *et al*., 2020). Globally, medicinal plants have high demand. This demand is continuously growing due to human search for sources of new drugs (Schmelzer and Gurib-Fakin, 2008).

Of the estimated 300,000 plants species existing globally, approximately 15% (45,000) have been investigated for their pharmacological potentials (Osei-Akoto *et al.*, 2020). Thus, there is the need for more research to discover new plant medicines. Several plants have been identified to exhibit antimicrobial, antioxidant, and anti-inflammatory activities (Osei Akoto *et al.*, 2020). Thus, it is prudent to investigate *Ceiba pentandra* (kapok) for its biological activity and determine whether it would offer some medicinal properties for treatment of diseases and ailments.

1.2 Statement of the Problem

Resistance of diseases causing organisms to drugs, high adverse effects of orthodox drugs and the role played by free radicals in disease pathology are involved in acute and chronic disorders such as diabetes, aging, immune suppression and neurodegeneration in human (Acheampong *et al.*, 2018). Thus, it has become increasingly vital to explore local plants in search for new and green remedies with less adverse effects, cost effective, accessible and affordable for treatment of diseases and ailments.

1.3 Objectives of the Study

The main aim of this study is to determine medicinal properties of methanol extracts of the seed, leaves, stem and root barks of *Ceiba pentandra* (kapok).

To achieve these specific objectives would include:

1 Determination of phytochemicals of the methanol extracts of *Ceiba pentandra*.

2 Determination of anthelmintic, anti-inflammatory, antioxidant and antimicrobial properties of the extracts of *Ceiba pentandra*.

3 To analyze functional groups present in the extracts of *Ceiba pentandra* using IR-spectroscopy (FTIR).

1.4 Significance of the Study

This study would provide new information for pharmaceutical settings to produce drugs that would effectively break resistance of micro-organisms to existing drugs. The people in the rural areas of the northern part of Ghana for example, uses *Ceiba pentandra* for medical purposes and as food, this study would help to identify scientific basis for such usage.

1.5 Justification of the Study

To achieve Sustainable Development Goal 3 which seeks to "ensure healthy lives, promotes wellbeing for people of all ages to achieve universal health coverage", the quest to investigate local plants for their medicinal properties has become important. Though West Africa has varieties of plants, research into *Ceiba pentandra* utilization as a source of plant based medicine is widely ignored. A comprehensive study of *Ceiba pentandra* (kapok) would offer new ideas on medicinal profile of the seeds, leaves, stem and root barks of the plant. Investigation into *Ceiba pentandra* could also lead to discovery and uses of the plant in ways beneficial to people especially in areas where *Ceiba pentandra* abound.

CHAPTER TWO

LITERATURE REVIEW

This chapter present relevant information on *Ceiba pentandra,* its phytochemicals, oxidants and inflammatory process in biological systems, some microbes used, helminths and their activities on mammals. Finally it reviews similar works done by other researchers.

2.1 Medicinal Plants

Medicinal plants have provided and continues to provide valuable therapeutic agents, both in orthodox and traditional medicine (Krentz and Bailey, 2005). They are rich bio-resources of drugs for traditional and orthodox medicines as well as nutraceuticals, food supplements, pharmaceutical intermediates and chemicals for synthetic drugs (Ncube *et al.*, 2008). Though medicinal plants have been used to develop new drugs, they continue to play vital role in drug discovery (Raza *et al*., 2001).

Of the estimated 300,000 plants species existing globally, only about 15% have been investigated for their pharmacological potential (Osei-Akoto *et al*., 2020). Thus, there is the need to investigate futher into medicinal plants to discover more therapeutic potentials for combating ailments. Several plants have been found to exhibit antimicrobial, antioxidant, and anti-inflammatory activities (Osei-Akoto *et al.*, 2020). Medicinal plants contain physiologically active ingredients such as alkaloids and glycosides which over the years have been exploited in traditional medicine to treat ailments (Akbarsha *et al*., 1990).

Phytoconstituents employed by plants to protect themselves against pathogenic insects, bacteria, fungi, among others have found applications in medicines for humans (Nascimento *et al.,* 2000). Medicinal plants are also known to have anti-diabetic, anti-ulcer (Jakhetia *et al*., 2010), and anticacinogenesis effects among others (Nascimento *et al*., 2000, Liu, 2004).

Funtumia elastica (Preuss) has a long ethnopharmacological history for treating whooping cough, asthma, blennorhea, painful menstruation, fungal infections, and wounds (Agyare *et al*., 2013). Investigations into antimicrobial and anti-inflammatory properties of ethanol extracts of the leaves and stem bark of *Funtumia elastica* have been done. Phytochemical screening as well as analyses of secondary metabolites in extracts of the plant has also been done. Minimum inhibition concentration (MIC) of ethanol extracts of the leaf and bark of *Funtumia elastica* against test organisms *(Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans, Aspergillus flavus and Aspergillus niger*) ranged from 125 - 1550 µg/mL (leave extract) and 125 - 1750 µg/mL (stem bark extracts). Ethanol extracts of the leaves and stem bark of *F. elastica* showed significant anti-inflammatory activity at 30, 100 and 300mg/kg as indicated by (Agyare *et al*., 2013).

Preliminary phytochemical screening of *Funtumia elastica* bark showed that it contains hydrolysable tannins, sapogenetic glycosides, steroids and saponins while the leaves contain hydrolysable tannins, flavonoids, starch and alkaloids (Agyare *et al*., 2013). Ethanol extracts of the leaves and stem bark of *Funtumia elastica* according to Agyare *et al*., (2013) have antimicrobial and anti-inflammatory properties. These

pharmacological properties of *Funtumia elastica* are responsible for the ethnomedicinal uses of the leaves and stem bark of the plant (Agyare *et al*., 2013).

Griffonia simplicifolia also has ethnomedicinal use for treating depression, fibromyalgia, bladder and kidney problems, insomnia, malaria, obesity, migraine, as an aphrodisia and a remedy for cough (Osei-Akoto *et al*., 2020). Phytochemical screening, antimicrobial and antioxidant activities have been investigated on methanol and petroleum ether extracts of *Griffonia simplicifolia*. Thin layer chromatography, column chromatography and FTIR analysis have again been employed to isolate a single phytochemical component of *Griffonia simplicifolia* (Osei-Akoto *et al*., 2020). The petroleum ether and methanol extracts of *Griffonia simplicifolia* leaves showed antimicrobial activity against test organisms with MICs ranging from 12.5 – 62.5 mg/mL. Mean IC_{50} values for the methanol and petroleum ether extracts of *Griffonia simplicifolia* leaves in the DPPH and H_2O_2 assays ranged from 61.85 ± 0.41 - 94.26 ± 0.82 µg/mL (methanol extract); and 524.61 ± 0.68 - 976.75 ± 4.17 µg/mL (petroleum ether extract).

Total antioxidant Capacities (TAC) (gAAE/100 g) for methanol extract was 36.42 ± 0.38 whilst that of the petroleum ether extract was 18.47 ± 0.56. Phytochemical investigation of *Griffonia simplicifolia* leaves showed that secondary metabolites such as alkaloids, triterpenoids, flavonoids, steroids, saponins, glycosides, phenols, tannins and coumarins were present. These findings reaffirmed the therapeutic properties of *Griffonia simplicifolia* leaves. These therapeutic properties confer on the leaves of *Griffonia simplicifolia* makes it useful in treating bacteria infections and diseases associated with oxidative-stress (Osei-Akoto *et al*., 2020).

2.2 *Ceiba pentandra* (kapok)

Ceiba pentandra is a tropical tree of order Malvales, family Malvaceae and native to Africa (Osuntokun *et al.*, 2017). However, it is cultivated mostly in the Central, Northern and South America, West Africa and some parts of Asia (Anigo *et al*., 2013). *Ceiba pentandra* is a large deciduous known to produce fibre by its fruit (Ecocrop, 2011). A mature *Ceiba pentandra* is about 30-40 meters tall with some varieties such as *caribaea* reaching 70 meters in height (Ecocrop, 2011). The plant has broad straight trunk with almost horizontally spreading branches supported by prickly buttress roots at the base (Ecocrop, 2011). However, some varieties of the plant are covered with round sharp spines (Ecocrop, 2011).

Ceiba pentandra has compound leaves (5-8 leaflets per stalk) and produces large quantities of cluster yellowish-white flowers similar to rose flowers (Orwa *et al.*, 2009). The plant also has silk and dense hairy-like structures on the outer surface of its leaves (Orwa *et al.*, 2009). It has enlongated fruit capsule (10-30 cm) which hang from its branches (Orwa *et al.*, 2009). The fruit has about 120-175 dark brown to black round seeds embedded in a mass of grey woolly hairs (floss) (Orwa *et al*., 2009). *Ceiba pentandra* is a fast-growing tree and produces fruits within 4 to 5 years after its cultivation. Lifespan of kapok is about 60 years (Yunusa *et al.*, 2015).

Under optimal conditions, *Ceiba pentandra* may produce about 330-400 fruits per year (Louppe *et al.*, 2008). Its fruit produces about 15-18 kg of fibre and approximately 30 kg of seeds (Louppe *et al.*, 2008). The fibre has low density, thermal and acoustic insulation properties (Louppe *et al.*, 2008). These properties make it suitable for filling pillows, mattresses and making clothes. *Ceiba pentandra* wood is

light weighted and is suitable for plywood, packaging and among others (Louppe *et al.*, 2008).

In addition to the fibre, the seeds contain about 20-25% of oil which when extracted can be used for cooking, making soap and as a lubricant (Orwa *et al.*, 2009). Flower of *Ceiba pentandra* is an important source of pollen for honey bees (Orwa *et al.*, 2009).

Plate 1: An image of *Ceiba pentandra*

2.3 Phytochemical Constituents of *Ceiba pentandra*

Phytochemicals are produced by plants through primary and secondary metabolism (Labe *et al.*, 2020). Phytochemicals are biologically active compounds found in plants.

They are plant-derived compounds found to be responsible for majority of diseases protection properties of diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine among others (Arts and Hollman, 2005). Phytochemical screening is a process through which secondary metabolites are examined qualitatively (Labe *et al.*, 2020). These metabolites are biosynthesized by enzymes (e.g., Acetyl co-enzyme) and have different functional groups, polarity and react differently with a given reagent (Arts and Hollman, 2005). Major phytochemicals found in *Ceiba pentandra* are alkaloids, steroids, saponins, flavonoids, tannins, unsaturated steroids, triterpenoids and essential oils (Labe *et al.*, 2020). *Ceiba pentandra* is highly medicinal and has large quantities of phytochemicals in its leaves and other parts compared with other plant species (Labe *et al.*, 2020). Leaves of *Ceiba pentandra* contain flavonoid, tannins, terpenoid, saponins and cardiac glycoside, the stem bark contains flavonoid, tannins, saponins, steroid, cyanogenic glycoside and cardiac glycoside whiles its root contains flavonoid, tannins, steroid and cyanogenic glycoside (Labe *et al.*, 2020).

2.3.1 Alkaloids

Alkaloids are compounds comprising of a heterocyclic ring structure and a nitrogen atom (amino or amido in some cases). The nitrogen atom is mostly located inside the heterocyclic ring structure (Wang *et al*., 2009). The nitrogen atoms confer alkaline property on alkaloids. Alkaloids are naturally synthesis by organisms such as animals, plants, bacteria and fungi (Wang *et al*., 2009). They are basic in character and have bitter taste (Wang *et al*., 2009). For example, quinine (an alkaloid) is one of the significantly bitter tasting substances. Known alkaloids have different chemical structures and may protect some plants from destruction by certain species of insects

(Wang *et al*., 2009). Alkaloids also have diverse and important physiological effects on humans and other animals. They are primarily used in medicine because they act as stimulants on the central nervous system. Also, they have pharmacological effects which enable their application as local anaesthetic and stimulants (Wang *et al*., 2009). Cocaine, caffeine, ephedrine, nicotine, analgesic morphine, berberine anti-bacterial and antimalarial drug (quinine) are examples of Alkaloids.

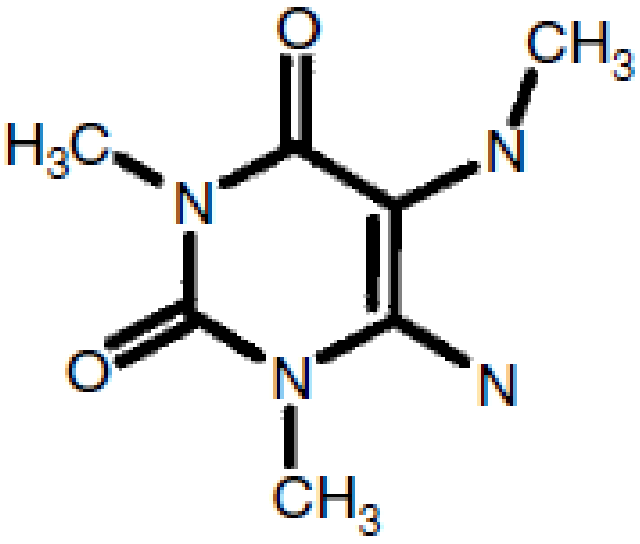


Figure 1: An image showing the structure of caffeine, an example of alkaloid

2.3.2 Flavaniods

Flavonoids are also called bioflavonoid (De Souza *et al.*, 2021). Bioflavonoids are class of polyphenolic secondary metabolites or compounds found in plants. Over 4,000 flavonoids have been found in vegetables, fruits, tea, coffee and some fruit drinks (Delage, 2015). Flavonoids occur naturally and are ubiquitous among vascular plants (Delage, 2015). They occur as aglycones, glucosides and methylated derivatives (Harbone and Baxter, 1999). Small amounts of aglycones (flavonoids without attached sugar) represent considerably proportion of total flavonoid compounds in plants.

Flavonoids have 15-carbon skeleton chemical structure which consists of two phenyls and a heterocyclic ring (De Souza *et al.*, 2021). Based on structure and nomenclature, flavonoids are grouped into three classes viz bioflavonoids, isoflavonoids (3-phenyl-1-4benzopyrone) and neoflavonoids (4-phenyl-1,2-benzopyrone). Flavonoids are ketone(s) containing compounds (Nic *et al.*, 2009). An unsaturated six-membered ring containing one oxygen atom and connected to a benzene ring is either -pyrone (flavones and flavanols) or its dihydroderivative (flavanones and flavan-3-ols).

Position of benzenoid substituent divides flavonoids into other two classes viz flavone (2-position) and isoflavone (3- position). Within each class, flavonoids are characterized as monoglycosidic and diglycosidic (Pretorius, 2003). Glycosidic linkage in flavonoid normally located at position 3 or 7 of the carbohydrate unit. The carbohydrate unit could be L-rhamnose, D-glucose, glucorhamnose, galactose or arabinose (Pretorius, 2003).

Flavonoids are strong antioxidants and effective antibacterial substances against large number of microorganisms (De Souza *et al.*, 2021). They exert their effect by inhibiting membrane-bound enzymes of microorganism (Cowan, 1999). Flavonoids have also showed substantial anticarcinogenic and antimutagenic activities due to their antioxidant and anti-inflammatory properties (Nandakumar *et al.*, 2008).

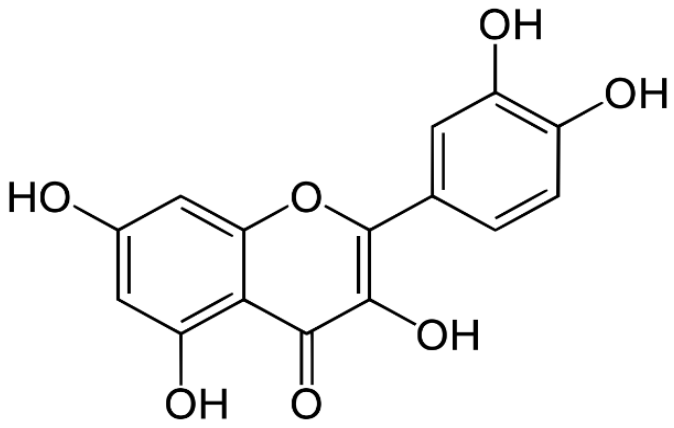


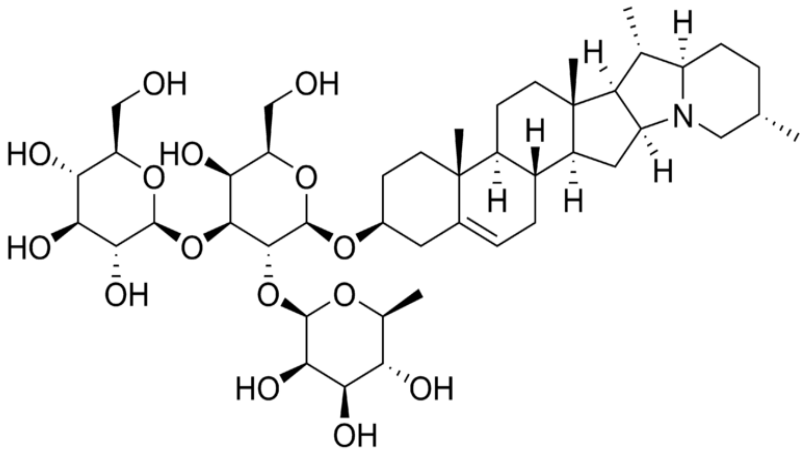
Figure 2: An image showing the structure of quercetin, an example of flavonoid

2.3.3 Saponins

Saponins are organic compounds that form persistent froth when shaken in water (Trease and Evan, 2009). They are a group of secondary metabolites (non-volatile surfactants) widely distributed in plants and marine animals (Vincken *et al*., 2007). Saponins are high-molecular-weight glycosides made up of sugar unit(s) linked to a triterpene or a steroid aglycone (Vincken *et al*., 2007). They are used as bitter sweeteners and detergents. They have emulsifying medical and pharmacological properties which include haemolytic activity, antimicrobial and pesticide (Vincken *et al*., 2007).

Aside their biological properties, saponins are used in pharmaceutical industries as raw material for synthesing steroids such as birth control pills among others (Sparg *et al.*, 2004). They also have intense use in cosmetic industies (Sparg *et al.*, 2004). Saponins possess biological activities such as antioxidant, immunostimulant, antihepatotoxic, anticarcinogenic, antidiarrheal, antiulcerogenic, antioxytoxic, hypocholesterolemia, anticoagulant, hepatoprotective, hypoglycemics,

neuroprotective, anti-inflammatory, inhibition of dental caries and platelet aggregation among others (Guclu-ustundag *et al*., 2007; Rao *et al*., 2000).



Figue 3: An image showing the structure of solanine, an example of saponins

2.3.4 Steriods

Steroids are a group of lipids derived from cholesterol (Saxena *et al.*, 2016). They are low-molecular weight compounds found in or derived from marine, terrestrial, and synthetic sources (Saxena *et al.,* 2016). Examples of steroid includes sterols, bile acids and hormones (gonadal and adrenal cortex hormones) (Saxena *et al.,* 2016).

Steroid and their metabolites play important roles in physiology and biochemistry of living organisms. Synthetic steroids are used extensively as anti-hormones (Moorachian, 2000), contraceptives, anti-cancer, cardiovascular agents, osteoporosis drugs, anti-biotics, anaesthetics, anti-inflammatories and anti-asthmatics (Costa and Zia, 1999). Steroids are derived from cholesterol and thus closely resemble themselves. This close resemblance is due to the fact that all steroids have the same basic perhydro-1, 2-cyclopentenophenanthrene

skeleton (Saxena *et al.,* 2016). However, slight variation may occur in the skeleton or functional groups resulting in various steroids (Saxena *et al.,* 2016).

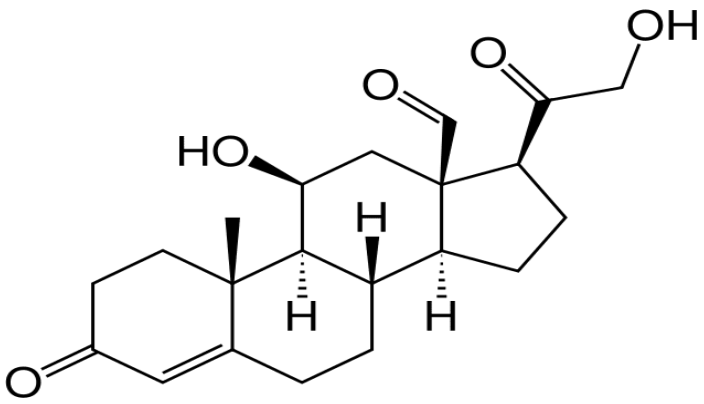


Figure 4: An image showing the structure of steriod

2.3.5 Tannins

On the basis of their structure, two distinct types of tannins (condensed and hydrolysable) have been identified (Teresa and Richard, 2006). Condensed tannins are composed of several flavonoids or proanthocyanins joined together. Properties of condensed tannins are distinct from their building blocks. Hydrolysable tannins are composed of glucose (or rarely, other monosaccharide or polyol) core with several catechin derivatives attached (Teresa and Richard, 2006). These medium to large polymer is widely distributed in plants and fungus kingdoms.

Condensed and larger hydrolysable tannins have most properties in common. However hydrolysable tannins are more susceptible to hydrolysis. Hydrolysable tannins have greater potential to induce toxic effect(s) in exposed population (Zhang *et al.,* 2008). Tannins are also alcohol soluble polymeric phenolic compounds with high molecular weight (Hill, 2003). Smaller tannins are hot

water soluble (Hill, 2003). However, as molecular weight increases tannins becomes far less soluble in any given solvent (Hill, 2003). In cold water, tannins are generally less soluble. Thus, tannin-free extracts can usually be made through cold infusion of crude herbs (Zhang *et al.,* 2008).

Generally, hydrolysable tannins have astringent flavour and their activity depend on their ability to, non selectively bind proteins (Shahat *et al.,* 2013). Tannins pull tissues together as proteins congeal and cause a peculiar puckering sensation in the mouth (Shahat *et al.,* 2013). This property of tannins tends to inactivate bound proteins. Tannins are strong antioxidants and have antitumor and anti-inflammatory properties (Shahat *et al.,* 2013). Tannin-rich herbs are used to slow proteinaceous discharges of all types (Shahat *et al.,* 2013). Most notably, transudates associated with atopic dermatitis skin lesions, diarrhoea, and haemorrhages from the skin or gastrointestinal tract. Structurally, tannins can be divided into four major groups: viz Gallotannins, Ellagitannins, Complex tannins and Condensed tannins (Shahat *et al.,* 2013). Gallotannins have galloyl units and their meta-depsidic derivatives are bound to polyol-, catechin-, or triterpenoid units (Hill, 2003). Ellagitannins have at least two galloyl units coupled to each other by C-C bond and do not have a glycosidic linkage to catechin unit (OkudaandIto, 2011).

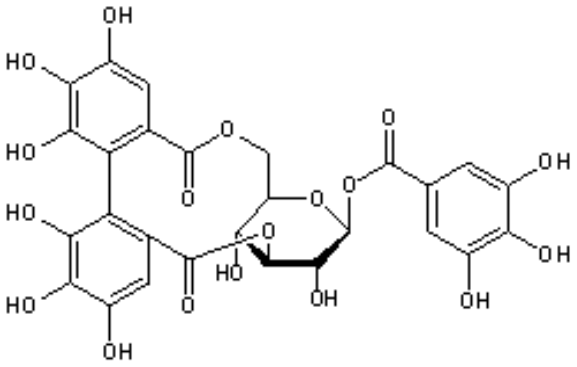


Figure 5: An image showing the structure of corilagin, an example of tannins

2.3.6 Coumarins

Coumarins are phenolic substances composed of fused benzene and α-pyrone rings as indicated (Figure 6) (Swayam *et al.,* 2012). Coumarins and their derivatives constist of oxygen fused with heterocycles and are widely distributed throughout nature (Swayam *et al.,* 2012). Until 2013, over 1,300 coumarins were detected as secondary metabolites in different plants, bacteria and fungi (Venugopala *et al.*, 2013).

In kingdom plantae, coumarins are present in over 30 different families including Umbelliferae, Rutaceae and Clusiaceae (Stefanachi *et al.,* 2018). Coumarin are distributed in seeds, roots and leaves of plants of Umbelliferae, Rutaceae and Clusiaceae (Rosselli *et al.,* 2009).

One of the most important chemical characteristics of coumarins are cyclic esters and open when attacked by a strong base such as NaOH to give yellow solutions of salts of cinnamic acids (Swayam *et al.,* 2012). Their salts are recycled into original coumarins when acidified with sufficient amounts of

strong acid. Coumarins have antithrombotic, anti-inflammatory and vasodilatory activities.

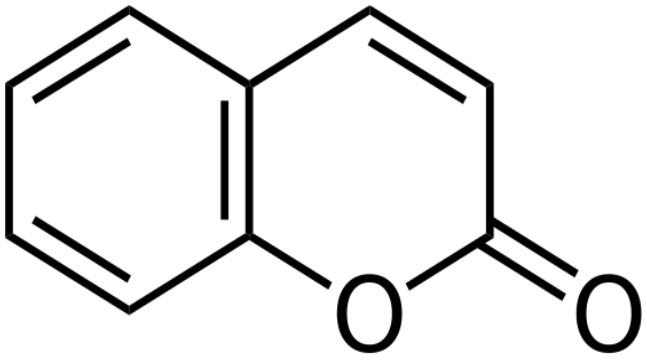


Figure 6: An image showing the structure of coumarin

2.3.7 Quinones

Generally, the term quinone is used for large classes of compounds derived from aromatic quinones through replacement of some hydrogen atoms of the aromatic quinones by other atoms or radicals (Patai *et al.,* 1988). Quinones are class of organic compounds "derived from aromatic compounds such as benzene or naphthalene by conversion of even numbers of –CH= groups into –C (=O)– groups (Patai *et al.,* 1988). This proceeds with necessary rearrangement of double bonds, which result in "a fully conjugated cyclic dione structure" (Pschyrembel *et al.,* 2017). Archetypical member of the class is 1,4-benzoquinone or cyclohexadienedione, called quinone. Other important examples of quinones include 1,2-benzoquinone, 1,4-naphthoquinone and 9,10-anthraquinone. Quinones are oxidized derivatives of aromatic compounds.

Mostly, they are made from reaction of aromatic compounds with electron-donating substituents such as phenols and catechols (Pschyrembel *et al.,* 2017).

The reaction increase nucleophilicity character of the ring and contributes large redox potential needed to break aromaticity (Pschyrembel *et al.,* 2017). Quinones are electrophiles stabilised by conjugation. Depending on the class of quinone and the site of reduction, reduction reaction can either rearomatized the compound or break the conjugation (Pschyrembel *et al.,* 2017).

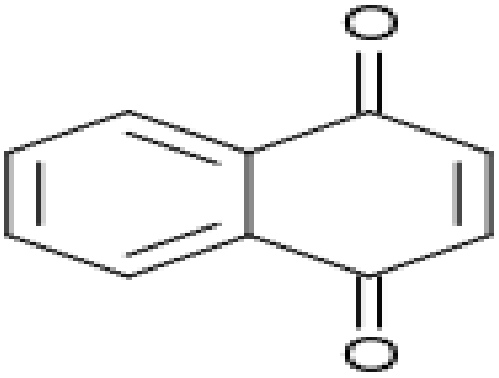


Figure 7: An imaging showing the structure of 1,4-naphthoquinone

2.3.8 Terpeniods

Terpenoids are a class of natural products derived from five-carbon isoprene units. Most terpenoids have multi cyclic structures different from each other by their functional groups and basic carbon skeletons (Christianson *et al*., 2017). Terpenoids are found in living things, and therefore considered to be largest group of natural products. Terpenoids are commercially interesting due to their use as flavours in food and fragrances and cosmetics (Christianson *et al*., 2017). Terpeniods are also essential for quality agricultural products (Christianson *et al*., 2017).

Terpenes are widespread and occur in plants as constituents of essential oils. Terpenoids have isoprene, $CH_2=C(CH_3)-CH=CH_2$ unit as their building block.

They are classified according to the number of isoprene units. They have molecular formula (C_5H_8) n

.

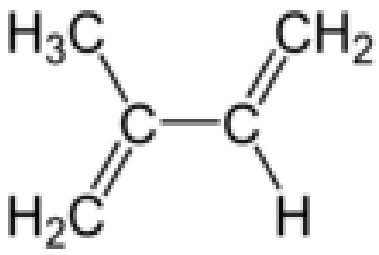


Figure 8: An image showing the structure of Isoprene unit in terpenoids

2.3.9 Glycosides

Glycosides are water soluble non-reducing secondary metabolites (Edeogaetal, 2005). They are also soluble in polar organic solvents. When hydrolysed with acids, bases or enzymes, glycosids yield hydrophilic sugar moiety (glycone) and hydrophobic non-sugar part (aglycone) (Edeogaetal, 2005). They are used in treating heart and skin diseases and also as a purgative (anthracene glycosides) among others.

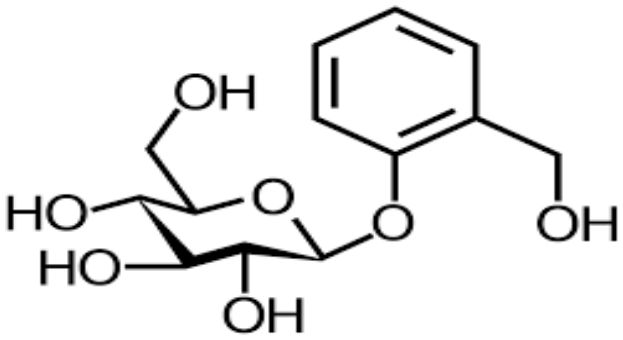


Figure 9: An image showing the structure of glycoside

2.4 Helminths and their Activities in Mammals

Anthelmintics are antiparasitic drugs that expel parasitic worms (helminths) and other internal parasites from mammals (Osei-Akoto *et al.,* 2020). They function either by stunning or killing parasitic worms without damaging the host. Anthelmintics can be

grouped into vermifuges (those that stun) or vermicides (those that kill). Helminthiasis is the condition whereby an organism is infected with helminth (WHO, 2019). It is one of the most serious animal diseases.

Several in vitro studies done on anthelmintic action of plant oils or extracts have reported their toxicity on worms (Gulnaz *et al.*, 2014, Gomes *et al.*, 2016, Osei-Akoto *et al.,* 2020)). Majority of earthworm poisons agitate worms and cause them to flee vicinity of the poisons. When anthelmintic concentration is not enough to kill the parasite, it expel the parasite from its location (Osei-Akoto *et al.,* 2020).

2.5 Inflammatory Process in Biological Systems

Inflammatory is a biochemical reaction of the immune system (Nathan *et al.,* 2010). Inflammatory reactions can be activated by pathogens, damaged cells, and toxic compounds (Nathan *et al.,* 2010). Inflammation include acute and/or chronic inflammatory responses in the heart, pancreas, liver, kidney, brain, intestinal tract and reproductive system (Nathan *et al.,* 2010). An immune system response to harmful stimuli such as (pathogens, damaged cells, toxic compounds or irradiation) among others is referred to as inflammation.

Inflammatory effects function to eliminate injurious stimuli to initiate healing process (Ferrero *et al.,* 2007) and it's is a defence mechanism important to health (Nathan *et al.,* 2010). During acute inflammatory responses, cellular and molecular events interact efficiently to minimize impending injury or infection (Nathan *et al.,* 2010). This alleviation process contributes to restore homeostasis and resolve acute inflammation (Zhou *et al.,* 2016). Nevertheless, uncontrolled acute inflammation

could become chronic and contribute to chronic inflammatory disease such as rheumatoid arthistis, systemic lupus and erythematosus among others (Zhou *et al.*, 2016).

At tissue level, inflammation is characterised by redness, swelling, heat, pain and loss of tissue functions (Takeuchi *et al.*, 2010). These characters originate from local immune, vascular and inflammatory cell responses to infection or injury (Takeuchi *et al.*, 2010). Vascular permeability changes, leucocyte recruitment and accumulation, and inflammatory mediator which are released during inflammatory processes are important microcirculatory events (Chertov *et al.*, 2000). Pathogenic factors, such as infection, tissue injury, or cardiac infarction, could induce inflammation via tissue damage. The inflammatory caused by these organisms may be infectious or non-infectious. Human body produces chemical signals that response to tissue injury and stimulate response to heal damaged tissues. Chemical signals produced by human body activate chemotaxis of leukocytes from general circulation and send them to damage sites to be destroyed (Nathan *et al.*, 2010). The activated leukocytes produce cytokines to induce inflammatory response.

2.5.1 Components of inflammation

Basically, there are five major components of inflammation viz; Pains, Redness, Immobility, Swelling and Heat. Pain is characterised by tender feeling in the inflamed area of body during and after touching. Chemicals that stimulate nerve endings are released, to make the injured area more sensitive to touch. Redness occurs when capillaries in the injured area are filled with extra blood than usual. Immobility is characterised by loss of function cells in the region of the

inflammation. Swelling build-up fluid in the affected region of the inflammation whilst heat causes more blood to flow to the affected area. Thus, this makes the affected area to feel warm when touched.

2.6 Antioxidant Activity

Oxidative stress has received considerable scientific attention as a mediator in the etiologies of many human diseases (Zhang *et al.,* 2015). Oxidative stress is as a results of an imbalance between free radicals such as hydroxyl radical and hydrogen peroxide, among others and antioxidants such as Vitamins C and E, selenium and carotenoids (Zhang *et al.,* 2015). Free radicals play vital role in aging process, disease development and damage of cells (Zielinski, 2000). Antioxidants are the first line of defence against detrimental effects of free radical damage (Zielinski, 2000). They are essential for the maintainance of optimum health via different mechanisms of action. Types of antioxidants includes those generated endogenously by body cells and exogenous agents such as dietary supplements (Mohammed *et al.*, 2015). Even at small levels, antioxidants could inhibit oxidation and thus have different physiological functions in human. Antioxidants are free radical scavengers (Klemchuk, 2000). They react with reactive radicals which render them less active, and less dangerous (Klemchuk, 2000). Antioxidants can neutralise free radicals by either accepting or donating electron(s) to remove unpaired radical. They protect cells and organs of the human body against harmful effects of oxidative stress. This is achieved by defensive mechanisms via synergistical enzymatic and nonenzymatic reactions (Ames *et al.,* 1993). Suitable physiological levels of antioxidants readily absorb, eliminate free radicals, and chelate redox metals.

2.6.1 Oxidants

Oxidants are materials capable of oxidizing certain compounds via lose of electrons (Zhang *et al.,* 2015). Oxidants have unpaired electrons and search for electron pairs. They are produced in humans as by-products of healthy human cellular metabolism (Ames *et al.,* 1993). While about 98% of oxygen consumed by a cell is transformed into water, the remaining 2% escape as free radicals (Pourmorad *et al.,* 2006). Oxidants cause extensive damage to DNA, proteins, and lipids (Pourmorad *et al.,* 2006).

According to (Ames *et al.,* 1993) damages to DNA, proteins and lipids caused by oxidants contribute to aging and degenerative diseases such as cancer, cardiovascular disease, immune system decline, brain dysfunction, and catarac). Over the past decade, connection between oxygenated metabolites and human diseases have been studied. Research indicates that severity of diseases caused by oxidants can be prevented, delayed or reduced by improving antioxidant defence mechanisms of the human body through improved nutrition (Langseth, 1995).

2.6.2 Effects of free radicals on humans

Free radicals are extremely reactive, highly unstable, and can destroy living cells (Kumar *et al.,* 2011). A higher member of free radicals (Reactive Oxygen Species (ROS)) produced in human body can change DNA structure, alter proteins, lipids, sugars and activate several stress-induced transcription factors (Kumar *et al.,* 2011). DNA damage is often caused by free radicals through their reaction with DNA. Free radicals can also activate adenosine diphosphate (ADP-ribose) synthetase which can make DNA to fragment and cause death of cell. ROS cause fragmentation of peptide

chain, alteration of electrical charge of proteins and oxidation of specific amino acids (Kumar *et al.*, 2011).

2.6.3 Reaction mechanism of antioxidants in biological systems

Antioxidants fight oxidative damages caused by free radicals through several mechanisms. Reactive oxygen species (ROS) such as O_2^-, OH^- and H_2O_2 are irreversibly formed during metabolism. Cell-produced intracellular antioxidant enzymes (dismutases and catalases) offer important protective mechanisms against free radicals (Shih *et al.,* 2007). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), thioredoxin reductase and hemeoxygenase are the most essential antioxidant enzymes (Shih *et al.,* 2007). Superoxide dismutase (SOD) convert O_2^- into hydrogen peroxide (H_2O_2), which is converted into water by CAT or GPx, via Fenton reaction. Peroxides formed during metabolism are removed by glutathione transferase (GST) and glutathione peroxidase (GPx). Glutathione reductase (GRd) regulates reduced glutathione (GSH) and oxidizes GSH to glutathione equivalents (GSSG) (Shih *et al.,* 2007). Glutathione reductase (GRd's) plays an important role in raising level of GSH which maintains oxido-redox condition of organism (Shih *et al.,* 2007). Superoxide (cellular free radical) is added in significant amounts to lower levels of antioxidants which increase rate of peroxidative processes. Though O_2^- unreactive to biomolecules, it helps in the production of stronger OH^-, $ONOO^-$ and O_2^- in phagocytes via NADPH oxidase during pathogen-killing process (Shih *et al.,* 2007). Negatively charged oxygen (O_2^-) is also a by-product of mitochondrial respiration (Wadhwani *et al*., 2012). Hydroxyl radical (OH^-) is exceedingly active and sufficiently toxic to biological molecules such as lipids, proteins and DNA than other radical species. Normally, OH^- is considered

to have been formed from Fe^{2+} or Cu^{+}/H_2O Fenton reaction via incubation $FeSO_4$ and H_2O_2 in aqueous solution (Fenton, 1894). Thus, antioxidant activity such as OH^- scavenger can be obtained by direct scavenging of OH^- generated by chelation of free metal ions (Krishnamurthy, Wadhwani, 2012).

2.7 Microbes

Microbes are tiny living organisms found in the global environments. They are minute and cannot be seen with naked eye. Microbes are found in water, soil, and in the air. The human body is home to millions of microbes. Some microbes cause diseases in human whilst others are important to life (Kelly, 2017). Bacterias, viruses, protozoa and fungi are the most common microbes. These are tiny living organisms responsible for diseases such as toxoplasmosis and malaria among others (Kasper *et al.,* 2015). Microbes such as viruses, bacterias and fungi are called pathogens when they cause diseases. An infection occurs when microorganism enters human body and cause harm (Sehgal *et al.*, 2020). The microorganism use the body of the infected person to sustain itself, reproduce, and colonize the host's body. In biological systems, ailments occur when few microbes (less than 1%), invade the system and cause diseases (Andreae *et al.,* 2008). Studies have reported that microbes contribute to many of non-infectious chronic diseases including cancers and cardiovascular disease (Andreae *et al.,* 2008; Kasper *et al.*, 2015).

2.7.1 Viral infections

Millions of different viruses exist and their infections occur when they invade a host. However about 5000 types of viruses have been identified by researchers (Breitbart *et al.*, 2005). Viruses contain genetic code, protein and a protective fat molecule. They

invade a host and attach themselves to a cell and reproduce using host cells (Wu, 2020). Not all viral diseases have symptoms, and in several cases, the human body successfully resist them. Viral disease such as cold sores are caused in certain nerve cells by viruses. They cause blisters in people with weak immune system (Breitbart *et al.,* 2005). When viruses invade cells, they release genetic materials which force the cells to replicate and multiply them.

Viruses may remain dormant for a period before multiplying again. When the cell die, it releases new viruses, which infect new cells (Breitbart *et al.,* 2005). Though not all viruses destroy cells of their host. A person with a viral disease may appear to have fully recovered. However, the individual may get sick again when virus reactivate (Wu, 2020). Therefore, it is difficult to fight viruses with medication.

To be protective of viruses, the immune system can be "trained" by vaccine to better prepared the body to fight the virus (Andreae *et al*., 2008; Kasper *et al.*, 2015). Viral infection such as common cold, occurs due to rhino virus, corona virus and adeno virus whilst encephalitis and meningitis, are also caused by enteroviruses, herpes simplex virus (HSV), and West Nile virus. Warts and skin infections are caused by human papillomavirus (HPV) whilst herpes simplex virus (HSV) causes Influenza, measles and liver inflammation (viral hepatitis).

2.7.2 Bacterial infections

Bacteria are single-celled microorganisms, also referred to as prokaryotes. Most bacteria are not dangerous to humans. Several bacteria live on or in human body, and help humans to stay healthy (McCutcheon, 2021). For instance, lactic acid bacteria in

the bowel help humans to digest food. Whilst other bacteria help immune system to fight germs (McCutcheon, 2021). Certain bacteria are also needed in food such as yogurt, sauerkraut or cheese production. An estimated 1% or less of bacteria have been found to cause for diseases. For instance, tuberculosis is caused by bacteria known as mycobacterium tuberculosis. Bacteria infections can be treated with antibiotics, medicines that kill bacteria or prevent them from multiplying (Andreae *et al.*, 2008; Kasper *et al.*, 2015).

There are trillions strains of bacteria. Of these, only a few causes diseases in humans (Sender *et al.*, 2016). Some bacteria live in human body and in areas such as the gut and the airways, without causing harm (McCutcheon, 2021). Some examples of bacteria infections include meningitis, otitis media, pneumonia, tuberculosis, gastritis, eye infections, urinary tract infections (UTIs), diarrhoea, colds, tonsillitis and sexually transmitted infections (STIs) among others.

2.7.3 Fungal infection

A fungus is a multicellular parasite that decomposes and absorbs organic matter using an enzyme. Best-known fungi include yeast, mould and edible fungi such mushrooms (Andreae *et al.*, 2008; Kasper *et al*., 2015). Though, fungi are single celled, they mostly reproduce by spreading single celled spores.

Structurally fungi are enlongated and cylindrical, with small filaments branching from the main body (Andreae *et al*., 2008). Most fungal infections develop in the upper layers of the skin, whilst others develop in the deeper layers of the skin. Diseases causing fungi are called mycoses and they include athlete's foot or fungal

infections of the nails, valley fever or coccidioidomycosis, histoplasmosis and candidiasis among others (Andreae *et al*., 2008; Kasper *et al.*, 2015; Hawksworth *et al.,* 2017). Fungal infections can sometimes cause lung inflammations, or mucous membranes inflammation in the mouth or on reproductive organs of affected individual and could be life-threatening for people with weakened immune system (Andreae *et al*., 2008; Kasper *et al.*, 2015; Hawksworth *et al.,* 2017).

2.7.4 Route of transmission of microbial infections

Infections occur, when microbes invade the host via portals of entry (NIAID, 2000). Microbes enter the human body through four sites viz respiratory tract (example influenza virus which causes the flu), gastrointestinal tract (example *vibrio cholerae* which causes cholera), urinogenital tract (example *escherichia coli* which causes cystitis) and surface of the skin (example *Clostridium tetani* which causes tetanus) (NIAID, 2000). Microbes must reach their targeted site in the human body before they can cause ailments. They become attached to the target site and cause infections. When they are not dislodged from the target sites, they multiply rapidly and survive attack by the immune system of the host (NIAID, 2000).

2.7.5 Pseudomonas aeruginosa

Pseudomonas aeruginosa is an encapsulated, gram negative, aerobic-facultatively anaerobic, rod shaped bacteria that cause disease in plants, animals and humans (Diggle and Whiteley, 2020). It was first isolated by Carle Gessard (Irish) in 1882 from wound infections of soldiers whose bandages had blue and green colour (Palleroni, 2010). *Pseudomonas aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity and intrinsically advanced antibiotic resistance

mechanisms (Diggle and Whiteley, 2020). *Pseudomonas aeruginosa* is associated with lots of serious illness including hospital acquired infections such as ventilator associated pneumonia and sepsis syndromes among others (Diggle and Whiteley, 2020). Whilst malignant externl otitis, endophthalmitis, endocarditis, meningitis, pneumonia and septicemia are examples of infections caused by *Pseudomonas aeruginosa* (Castro, 2022). Persons infected with *Pseudomonas aeruginos* have fever, chills, fatigue, malaise, irritability, fast but shallow breathimg, cough, rashes, stiff neck, back and joint pains and wound with green pus and among others as symptons (Castro 2022). Though *pseudomonas aeruginosa* is naturally present in the environment, they can be spread through contact with infected person and surfaces (Diggle and Whiteley, 2020). Frequent handwashing with soap under running water, hand sanitizer application and regular disinfection of the environment are recommendable preventive measures against *Pseudomonas aeruginosa.*

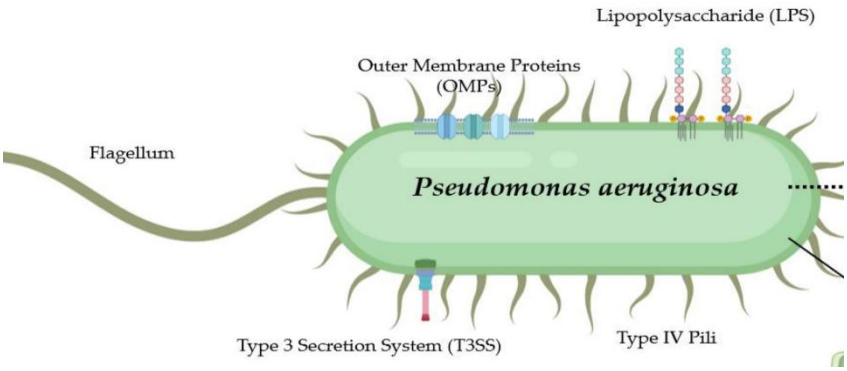


Figure 10: An image of *Pseudomonas aeruginosa*

2.7.6 Salmonella typhi

Salmonella typhi was identified in 1880 by German pathologist Karl Eberth and was first cultured in 1884 by Georg Gaffky (Barnett, 2016). *Salmonella typhi* is a gram-negative bacterium responsible for typhoid fever in humans (Barnett, 2016). Infected individuals often have gradual onset of a high fever over several days (Newton,

2014). Other symptoms of *salmonella typhi* infestation include loss of appetite, cough, diarrhea, stomach pain and weakness (WHO, 2008). People may carry the bacteria without being affected. However, such individuals are able to spread the disease to others (WHO, 2008). Typhoid fever is spread by consumption of foods or water contaminated with feaces of an infected person (WHO 2008). Clean water comsumption, in take of hot food, proper hands washing before touching ready to eat food and vaccination are some of the recommendable preventive measure of typhoid fever (Barnett, 2016).

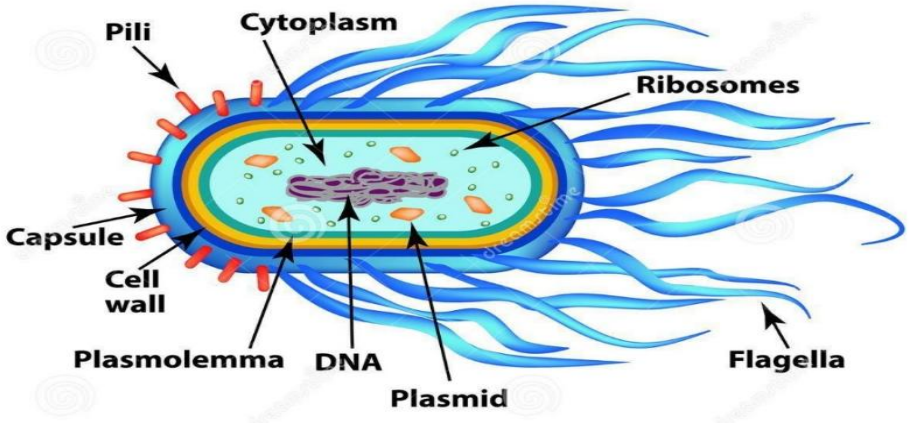


Figure 11: An image of *salmonella typhi*

2.7.7 Staphylococcus aureus

Staphylococci aureus are non-motile, non-sporing, gram positive bacteria (Tracey *et al.,* 2022). They were discovered by Scotish surgeon Sir Alexander Ogston in 1880. This organism is often found as a commensal of the skin, skin glands, and mucous membranes, particularly in the nosal cavity of healthy individuals (Crossley and Archer, 1997). *Staphylococcus aureus* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome, through production of different toxins (Winn, 2006). Infections caused by *Staphylococci* are pyogenic. Conjunctivitis and impetigo are other manifestations of staphylococcal infections (Hugo *et al*., 1992; Parry *et al.*,

2004). They grow in yellow colonies on nutrient rich media and thus referred to as the yellow staphylococci (Winn, 2006).

Approximately 20 – 30% of *Staphylococci* are *S. aureus* carriers and they could be dangerous when it enters the human body (Heyman, 2004). The most common infection by *Staphylococci aureus* is boil. In infected people, the skin over the infected area become red and swollen. *Staphylococci aureus* infections could be also be dangerous when they enter the blood, and that could lead to sepsis or even death (Tracey *et al.,* 2022. *Staphylococci aureus* spread through contact with infected person and contaminated surfaces (Tracey *et al.,* 2022).

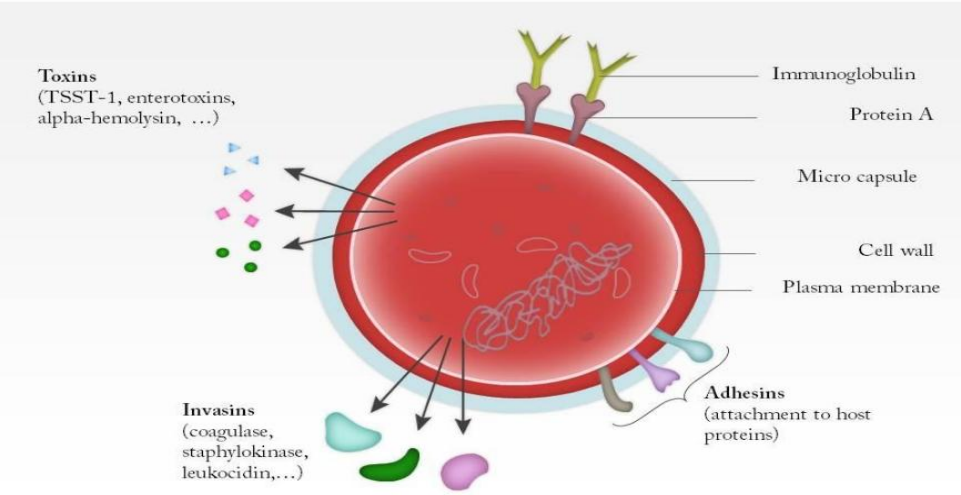


Figure 12: An image of *Staphylococci aureus*

2.7.8 Klebsiella pneumoniae

Klebsiella is a genus of nonmotile, gram-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule (Ryan and Ray, 2004). It is a community acquired pulmonary infection that was observed in chronic alcoholics (Carpenter, 1990). They are often found in soil, vegetation and water, where they contribute to biochemical and geochemical processes (Ryan and Ray, 2004). These bacteria are a

major component of the micro flora in several types of stressed nonclinical environments (Ryan and Ray, 2004). *Klebsiella spp*. can also be found in a wide range of mammals for example humans, dogs and cats among others (Gordon and Gibbon, 1999). *Klebsiella* cause significant numbers of dangerous community acquired infections, such pyogenic liver abscess, pneumonia, and meningitis among others (Shon *et al*., 2013).

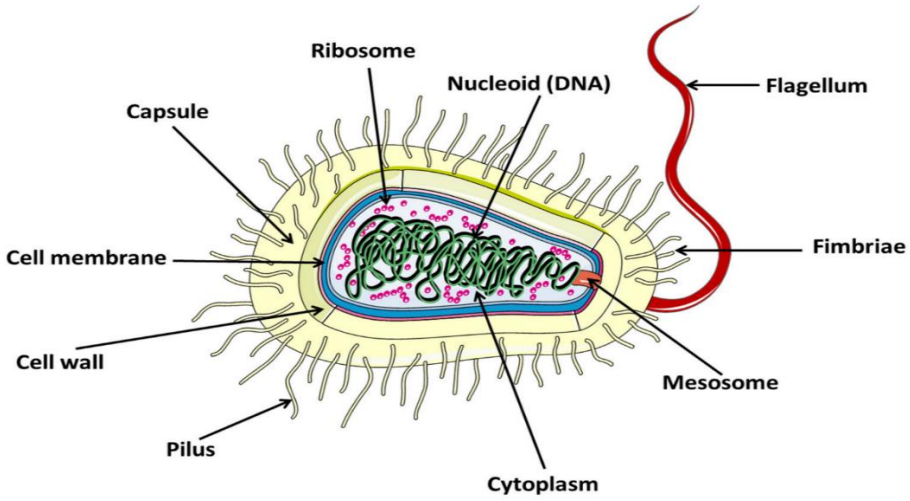


Figure 13: An image of *Klebsiella pneumoniae*

2.7.9 Enterococcus faecalis

Enterococcus faecalis is a gram-positive coccus which was first discovered in human fecal flora in 1899 (Lebreton *et al*., 2014). Approximately, 90% of enterococcal infections in humans are caused by *Enterococcus faecalis* (Kayaoglu and Orstavik, 2004). These bacteria thrive in nutrient-rich, oxygen-depleted environment of the intestinal tract (Mundt, 1963). *Entorococcus faecalis* are capable of causing infections such as endocarditis, sepsis, surgical wound infections, and urinary tract infections (Lebreton *et al*., 2014). Globally, fatality caused by their infections is between about 10% and 20% (Jett *et al*., 1994; Richards *et al*., 2000).

The organisms are normal human commensals adapted to the nutrient-enriched, oxygen-depleted, ecologically complex environments of the oral cavity, gastrointestinal tract, and vaginal vault (Mundy *et al*, 2000). Their infections spread from person to person through poor hygiene. Symptoms of *enterococcus faecalis* infections include fever, chills, fatigue, headache, nausea, vomiting, abdominal pain and burning sensation when urinating (Lebreton *et al*., 2014).

2.8 Spectroscopic Techniques for Structure Determination

Spectroscopy is the observation of interaction of electromagnetic radiation with matter. It originated from the study of visible light dispersed by a prism as per the wavelengths of different colours present in the visible region (John, 2006). Electromagnetic spectrum is range of frequencies of electromagnetic radiation and their respective wavelength and photon energies (John, 2006). It covers electromagnetic radiations with frequencies ranging from below 1Hz to above 10^{25} Hz. This frequency range is divided into separate bands, and the radiations within each frequency band are called by different names.

Beginning at low frequency (long wavelength) is radiowave, follow by microwave, infrared, visible light, ultraviolet, x-rays and gamma rays at high frequency (short wavelength) end of the electromagnetic spectrum (John, 2006, Skrabal, 2012).The electromagnetic radiations in each band have different characteristics such as how they are produced, interact with matter and practical applications (John, 2006, Skrabal 2012).

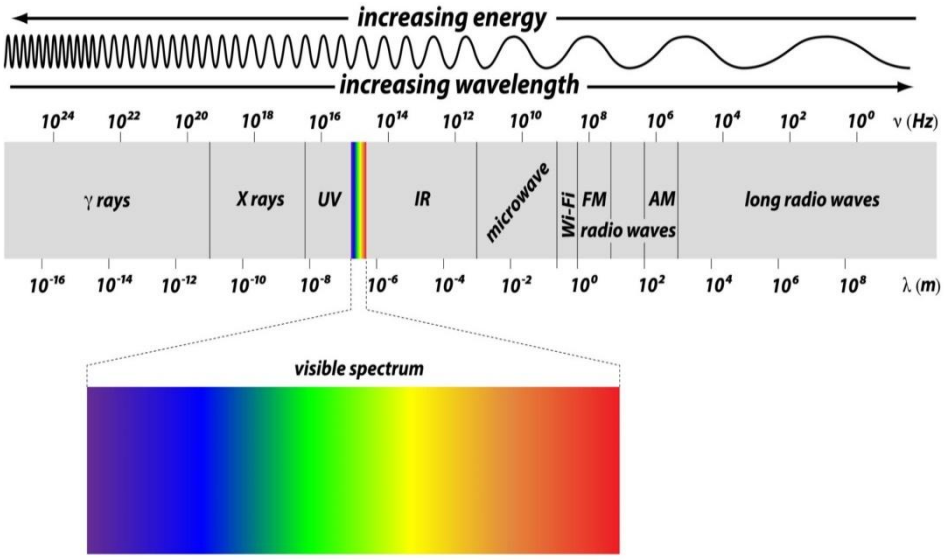


Figure 14: An image of electromagnetic spectrum

For example a dried extract fraction of *Griffonia simplicifolia* was subjected to (FTIR) analysis (UATR Two, PerkinElmer) to determine the functional groups present. The regions between 4000 cm^{-1} and 400 cm^{-1} were scanned, then followed by baseline correction. FTIR analysis was performed on the purified component *Griffonia simplicifolia* obtained from petroleum ether extract using column chromatographic separation. The results obtained showed wave numbers around 2955 - 2849 cm^{-1}, 1461 cm^{-1} and 1377 cm^{-1}. These positions of the spectrum are characteristic of aliphatic C-H, C-C, and/or C-O stretching and bending vibrations. These functional groups indicate the presence of highly saturated compound of the identified secondary metabolites such as steroids and triterpenes. The findings were corrobulated by the results obtained from the phytochemical screening test (Osei-Akoto *et al.,* 2020).

2.8.1 Fourier transform infrared spectroscopy (FTIR)

Infrared region of the electromagnetic spectrum compasses wavelengths between the visible and microwave regions of the electromagnetic spectrum. The IR region has wavelength ranging from 700 - 1000 nm and associated frequency ranging from 1.9 x 10^{13} - 1.2 x 10^{14} Hz (Sharma, 2017). The IR region is subdivided into Near-IR, Mid-IR and Far-IR regions. IR radiation have lower energy than UV radiation thus, no transfer of electrons is possible within this region (Sharma, 2017).

Absorption of IR radiation is due to molecular vibrations and rotations. Molecule absorbs IR radiation and vibrates when dipole moment of the molecule changes upon interaction with IR radiations (Sharma, 2017). Thus, absorption of IR is observed only in polar compounds. When frequency of radiation matches the vibrational frequency of the molecule, the molecule is in resonance with the radiation and the absorption result in production of absorption band (Sharma, 2017).

2.8.2 Applications of FTIR-spectroscopy

FTIR-spectroscopy application is used to determine functional groups present in a compound. Thus, different molecules with different combination of atoms create unique IR spectra. Therefore, IR technique can be utilized for qualitative identification of substances since, intensities of peaks produce in FTIR spectrum are proportional to quantity of molecules present in the sample. The FTIR spectrum is used for quantitative analysis. Infrared spectroscopic technique is also commonly used in analysing organic and inorganic compounds in research and industries (Sharma, 2017). The technique is again used in quality control in chemical and

pharmaceutical industries (Sharma, 2017), and in forensic science to solve criminal and civil cases (Sharma, 2017).

CHAPTER THREE

METHODOLOGY

This chapter presents the sampling procedure, sample preparation, equipment, chemicals and apparatus used in this study.

3.1 Chemicals Used

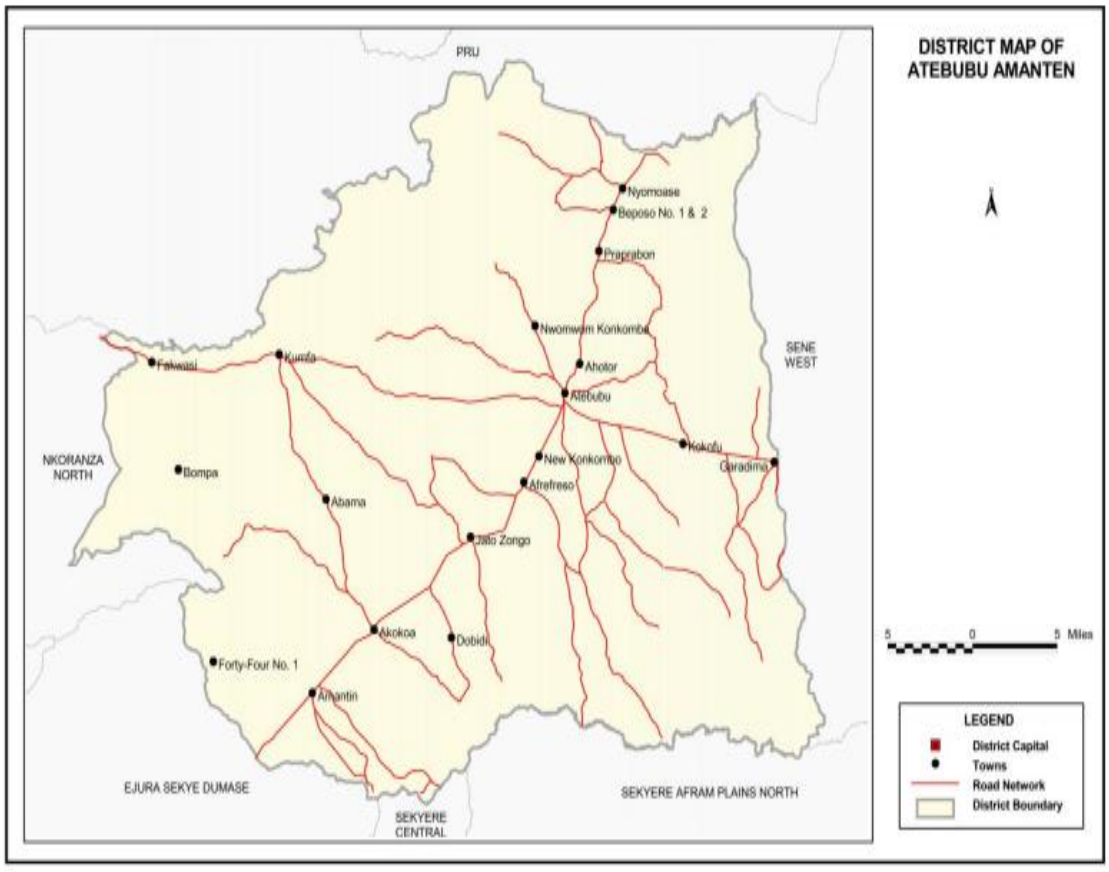
Chemicals used were methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetic anhydride, ammonia solution, ammonium molybdate, ascorbic acid, benzene, 95% concentrated tetraoxosulphate (VI) acid, ethyl acetate and ferric chloride. They also include 35% hydrochloric acid, chloroform, iodine solution, potassium iodide crystals, sodium chloride, sodium hydroxide, distilled water and isopropanol. Potassium hydroxide (KOH), HCl solution, ethanol, nitric acid, nutrient broth and nutrient agar supplied by Oxoid Ltd, Basingstoke, (UK) were also used.

3.2 Equipment/Apparatus Used

Equipment and apparatus used were analytical balance (500 ± 0.20 g), measuring cylinders (250 mL), 500 mL wash bottle, volumetric flasks (250 mL), air-tight containers, beakers (250 mL), boiling tubes, bunsen burner, desiccator, electric mill (Model BLG-450P, Binatone), soxhlet extractor, filter paper (Scharf, 12.5 cm), funnel, hot water bath, hot- air oven, incubator, micropipette, refrigerator, spatula, syringes, test tubes and spectrophotometer (V-730 UV-Vis Spectrophotometer, Jasco, USA).

3.3 Collection of *Ceiba Pentandra* Sample

Fresh leaves, seeds, stem and root barks of *Ceiba pentandra* were collected from the Lambussie-Karni District in the Upper West Region and Atebubu Amanten in the Ahafo Region of Ghana. The samples were transported to the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, at Kwame Nkrumah University of Science and Technology, Kumasi – Ghana for authentication and screening. They were kept in the Department of Crop Science, Akenten Appiah-Menka University of Skills Training and Enterpreneurial Development in the Asante-Mampong municipality of the Ashanti Region of Ghana prior to sample preparation and subsequent utilization.



Source: Ghana Statistical Service, GIS

Figure 15: Map of Atebubu-Amanten district

3.3.1 Collection of Earthworm species (Millisonia ghanensis)

Millsonia ghanensis (Earthworms) were collected from a water-logged area behind Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The worms were collected by hand picking from the soil into a glass bottle. Their average length (13.50 cm), weight (4.60 g) and circumference (3.10 cm) were determined. The worms collected were authenticated at the Zoology Unit, in the Department of Theoretical and Applied Biology, KNUST, to acertain their identity.

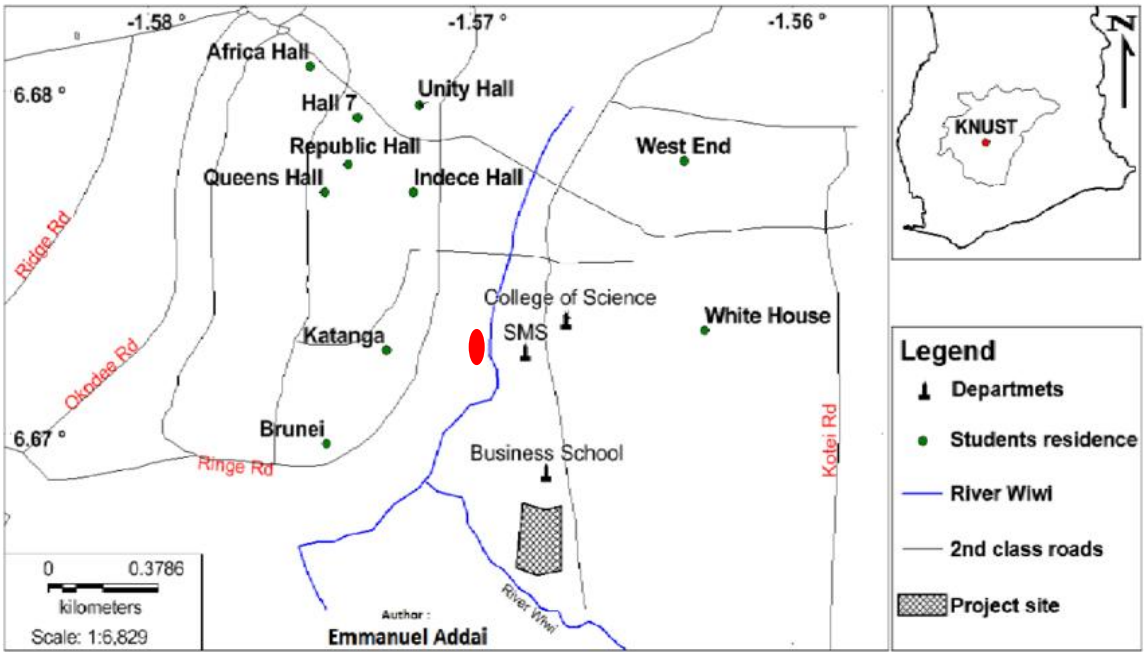


Figure 16: Map of KNUST

3.4 Preparation of *Ceiba pentandra* Extracts

Plant samples were rinsed thrice each with copious amounts of sterilezed water. The samples were air-dried for 4 weeks, after which they were milled (Model BLG-450P, Binatone) and then sieved through a 2 mm pore size sieve to obtain fine powder of the leaves, seeds, stem and root barks. One hundred and fifty grams powdered of each

sample was soaked in 500 mL of methanol and then extracted using soxhlet apparatus. The extract obtained for each plant part was concentrated to dryness at 50 ^{o}C using rotary evaporator (Buchi Rota vapour R-114). The concentrated extracts were properly labeled and then stored in a refrigerator at 4 ^{o}C, until required for usage.

3.5 Preparation of Reagents/Solutions

3.5.1 Preparation of 0.6 M sulphuric acid (H_2SO_4) solution

To prepare 0.6 M sulphuric acid solution, 32.60 mL of 98% sulphuric acid was poured into a 1000 mL volumetric flask previously filled to half its volume with 500 mL of distilled water. Each flask and content were swirled gently to ensure uniform dissolution of the acid. The mixtures were then made to the 1000 mL mark with distilled water.

3.5.2 Preparation of 0.5 M hydrochloric acid (HCl) solution

In preparing 0.5 M hydrochloric acid solution, 43.10 mL of 36% hydrochloric acid was poured into a 1000 mL volumetric flask previously filled to half its volume with 500 mL of distilled water. The flask and its content were swirled gently to ensure uniform dissolution of the acid. The mixture were then made to the 1000 mL mark with distilled water.

3.5.3 Preparation of 0.5 M sulphuric acid (H_2SO_4) solution

To prepare 0.5 M sulphuric acid solution, 27.20 mL of 98% sulphuric acid was poured into a 1000 mL volumetric flask previously filled to half its volume with 500 mL distilled water. The flask and its content were swirled gently to ensure uniform

dissolution of the acid. The mixtures were then made to the 1000 mL mark with distilled water.

3.5.4 Preparation of 1 M hydrochloric acid (HCl) solution

To prepare 1 M hydrochloric acid solution, 8.60 mL of 36% hydrochloric acid was poured into a 100 mL volumetric flask previously filled to half its volume with 50 mL distilled water. The flask and its content were swirled gently to ensure uniform dissolution of the acid. The mixture was then made to the 100 mL mark with distilled water.

3.5.5 Preparation of 2 M ammonia (NH_3) solution

In preparing this solution, 13.50 mL of 28% ammonia was poured into a 100 mL volumetric flask previously filled to half it capacity with distilled water. The flask and the content were swirled gently to ensure uniform dissolution of the ammonia. The mixture was then made to the 100 mL mark with distilled water.

3.5.6 Preparation of 1 M sodium hydroxide (NaOH) solution

In preparing 1M sodium hydroxide solution, 4.0 g of sodium hydroxide pellet was dissolved with 100 mL of distilled water in a 100 mL volumetric flask. The flask and its content were shaken gently to ensure uniform dissolution of the sodium hydroxide pellets.

3.5.7 Preparation of 1% gelatin solution

To prepare 1% gelatin solution, 1 g of gelatin and 0.5 g of sodium hydroxide pellets were weighed and dissolved with 100 mL of distilled water in a 100 mL volumetric flask. The solution was then autoclave at 15 psi for 15 minutes to sterilise it.

3.5.8 Preparation of 5% ferric chloride solution

In preparing 5% ferric chloride solution, 5 g of iron chloride hexahydrate was weighed and dissolved with 100 mL of distilled water in a 100 mL volumetric flask. The flask and its content were shaken gently to ensure uniform dissolution of the salt.

3.5.9 Preparation of 12 mg/mL stock solution of the extracts and standard drug

A stock solution of concentration (12 mg/mL) was prepared for each of the extracts (seed, leave, stem and root barks) and mebendazole (obtained from Panacea Pharmacy, Asafo) used as a standard drug. To prepare these, 1.2 g of each of the extract was dissolved in 100 mL of distilled water in a 100 mL volumetric flask. The flasks and their contents were shaken gently to ensure uniform dissolution. For the standard drug, a stock solution of concentration 12 mg/mL was prepared by dissolving 1.2 g of the standard drug in 100 mL distilled water in a 100 mL volumetric flasks. The flasks and their contents were shaken gently to ensure complete dissolution of the content.

3.5.10 Preparation of 6, 3, 1.5 and 0.75 mg/mL solutions of extracts and standard drug

To prepare 6 mg/mL solution of each extract, 50 mL of the 12 mg/mL stock solution previously prepared for each extract was poured into a 100 mL volumetric flask. The solution was then made to the 100 mL by addition of distilled water. The flask and its contents were swirled gently to ensure uniform dissolution of the extract. The procedure was repeated in preparation of the 6 mg/mL solution of the standard drug. The procedure was again repeated in the preparation of 3, 1.5 and 0.75 mg/mL solutions of both the extracts and the standard drug using 50mL of 6, 3 and 1.5 mg/mL solutions of the extracts and the standard drug respectively.

3.5.11 Preparation of 28 mM sodium phosphate solution

To prepare this solution, 4.62 g of sodium phosphate salt was weighed and dissolved with 1000 mL of distilled water in a 1000 mL volumetric flask. The flask and its content were shaken gently to ensure uniform dissolution of the salt.

3.5.12 Preparation of 4 mM ammonium molybdate solution

In preparing 4 mM solution of ammonium molybdate solution, 4.70 g of ammonium molybdate was weighed and dissolved with 1000 mL of distilled water in a 1000 mL volumetric flask. The flask and its content were shaken gently to ensure uniform dissolution of the salt.

3.5.13 Preparation of 0.2 M buffer solution of phosphomolybdenum (pH 8.5)

To prepare this solution, 10mL of 0.6 M of 98% sulphuric acid, 109 mL of previously prepared 28 mM sodium phosphate solution and 10mL of previously prepared 4 mM ammomium molybdate solution were put into a 250 mL beaker. The mixture was mixed thoroughly to obtained phosphosmolybdenum buffer solution of required concentration and pH.

3.5.14 Preparation of 100 µg/mL stock solution of ascorbic acid

To prepare this solution, 0.1 g of ascorbic acid (obtained from Panacea Pharmacy, Asafo) was weighed using electronic balance and dissolved in 1000 mL of distilled water in a 1000 mL volumetric flask. The flask and its contents were gently shaken to obtain a uniform dissolution of ascorbic acid.

3.5.15 Preparation of 50, 25, 12.5 and 6.12 µg/mL ascorbic acid solution

The 50 µg/mL solution of ascorbic acid was prepared using previously prepared (100 µg/mL) stock solution of ascorbic acid. To do this, 50 mL of the 100 µg/mL stock solution of ascorbic acid was poured into a 100 mL volumetric flask. This was then made to 100 mL by adding distilled water. The flask and its content were the swirled gently to ensure uniform dissolution. The procedure was repeated in the preparation of 25, 12.5 and 6.12 µg/mL ascorbic acid solution by diluting 50 mL of 50 µg/mL ascorbic acid solution to 100 mL to obtain 25 µg/mL ascorbic acid solution. The 6.125 µg/mL ascorbic acid solution was also prepared by diluting 50 mL of 25 µg/mL solution to 100 mL in a 100 mL volumetric flask.

3.5.16 Preparation of 500 µg/mL test solution of extracts

Fifty milligram of each extract of the seed, leave, stem and root barks of *Ceiba pentandra* were weighed into separate 250 mL beakers and then dissolved with 50 mL of distilled water. The mixtures were transferred into separate 100 mL volumetric flasks. Each of the mixtures was then made to 100 mL with distilled water. The flasks containing the individual solutions of the extracts were shaken gently to ensure uniform dissolution of each of the extract.

3.5.17 Preparation of 0.1 mM DPPH solution

To prepare a 0.1 mM DPPH solution, 1.9 mg of DPPH was dissolved in 100 mL of ethanol in a 100 mL volumetric flask. The flask and its content was shaken gently to ensure uniform dissolution of the DPPH. The solution was then kept in the dark for 30 minutes to prevent oxidation of the solution by light.

3.5.18 Preparation of 200 µg/mL stock solution of extracts

A 200 µg/mL stock solution of the seed, leaves, stem and root barks extracts of *Ceiba pentandra* were prepared by weighing 20 mg of each of the extracts into separate 150 mL beakers. Each weighed extract was dissolved separately with 50 mL of distilled water. Each mixture was then transferred into separate 100 mL volumetric flask and then made to 100 mL mark with distilled water. The flasks and their contents were shaken gently to ensure uniform dissolution.

3.5.19 Preparation of 100, 50, 25 and 12.5 µg/mL solutions of extracts

To prepare 100 µg/mL solution of each extract, 50 mL of previously prepared 200 µg/mL stock solution of each extract was put into separate 100 mL flask. The content of each flask was then made to 100 mL. The content of each flask was then shaken gently to obtain uniform mixtures of the extracts. The procedure was repeated in the preparation of 50, 25 and 12.5 µg/mL solutions of the extracts by diluting 50 mL of 100, 50, and 25 µg/mL solutions of the extracts to obtain the required concentrations of each extract.

3.5.20 Preparation of nutrient agar

To prepare nutrient agar solution, 28 g of nutrient agar powder was weighed into a 250 mL beaker and then dissolved with 100 mL of distilled water to obtain a homogeneous mixture of the agar. The agar solution was transferred into a 1L diffusion bottle and enough distilled water was added to make 1L of agar solution. The diffusion bottle was plugged firmly with cotton wool and then sterilised in an autoclave at 121°C and 15 psi for 15 minutes.

3.5.21 Preparation of nutrient broth

To prepare nutrient broth, 13 g of nutrient broth powder was weighed into a 1L beaker. Five hundred milliliter of distilled water was added in smaller volumes. On each addition, the mixture was stirred continuously with a clean glass rod to ensure a thorough mixing. Five hundred milliliter of freshly boiled distilled water was then added to the mixture and stirred continuously again to obtain a 1L solution of nutrient broth. Ten milliliter each of the freshly prepared nutrient broth solution was poured into different test tubes, plugged firmly with cotton wool and then sterilised in an autoclave at 121°C and 15 psi for 15 minutes.

3.5.22 Preparation of ciprofloxacin solution

The reference antibacterial drug, (ciprofloxacin obtained from Panacea Pharmacy, Asafo) was prepared by dissolving 50 µg of the drug in 10 mL of distilled water and then serially diluted with distilled water to obtained the required concentration (50, 25, 12.5, 6.25, 3.125 1.5625, 0.78125 and 0.39065 mg/mL)

3.6 Photochemical Screening of Extracts

Phytochemical screening of the extracts for secondary metabolites were performed using the methods described by Trease and Evans (2009).

3.6.1 Wagner's test for alkaloids

A half gram (0.5 g) of each extract was dissolved separately in 5 mL of previously prepared 0.5M hydrochloric acid solution. The mixture was filtered under gravity using Scharf filter paper (1). The filtrates were collected and then treated with Wagner's reagent. A reddish-brown precipitate obtained indicated the presence of alkaloids as documented in a study by Trease and Evans (2009).

3.6.2 Test for flavonoids using alkaline reagent

A half gram (0.5 g) each of the extracts was dissolved in a 50 mL distilled water. When completely dissolved, the mixtures were then filtered under gravity through Scharf (12.5 cm) filter paper. Two milliliter of previously prepared 1M sodium hydroxide solution was added to 5 mL of the filtrates. One milliliter of previously prepared 0.5 M H_2SO_4 was added and the mixture was then shaken gently to obtain a uniform dissolution. Formation of intense yellow coloured precipate indicated the presence of flavonoids as indicated in a study by Trease and Evans (2009).

3.6.3 Froth test for saponins

A half gram (0.5 g) each of the extracts was dissolved in separate 20 mL distilled water in a 100 mL beaker. The mixtures were stirred continuously with a clean glass rod until the extracts were completely dissolved. The mixtures were put into separate 250 mL graduated cylinders and then shaken gently to ensure uniform dissolution of each extract. These were set aside for 15 minutes during which was a foam layer formed. Formation of these layers indicated the presence of saponins as recorded by Trease and Evans (2009).

3.6.4 Libermann Burchard's test for steroids

A half gram (0.5g) each of the extracts was dissolved in 5 mL of chloroform put into different 100 mL beaker. Each mixture was stirred continuously to obtain uniform mixtures of extracts and chloroform. The mixtures were filtered under gravity through 12.5cm Scharf filter paper. To 2 mL portion of each filtrate, 1mL acetic anhydride was added and the mixtures boiled at 50^{o}C for 15 minutes. The boiled mixtures were set aside to cool to room temperature. When cooled, 2 mL of 98% concentrated sulphuric acid was added to each of the boiled filtrate. Mixtures shaken

gently and then set aside for a brown ring to develop. Formation of brown ring indicated the presence of steroid as reported by Trease and Evans (2009).

3.6.5 Gelatin test for tannins

One percent of previously prepared gelatin solution containing 1g sodium chloride was added to 0.5 g of each of the extract. The mixtures were shaken gently and then set aside until a white precipitates were formed. Formation of white precipitates indicated the presence of tannins as documented by Trease and Evans (2009).

3.6.6 Test for coumarins and quinones

Thirty milligram of each extract was put into a different test tube and covered with 12.5 cm Scharf filter paper moistened with 1M NaOH. The mixtures were placed into boiling water bath at 50^{o}C for 20 minutes. After 20 minutes, the filter papers were removed and then examined under UV light. Production of yellow florescence under UV light indicated the presence of coumarins. To test for quinones, 0.5g of each of the extract was treated with 2 mL of 1M hydrochloride solution previously prepared. Progressive formation of yellow colour indicated the presence of quinones as indicated by Trease and Evans (2009).

3.6.7 Test for anthraquinone glycosides

A half gram (0.5 g) of each of the extracts was hydrolysed with 10mL of previously prepared 1 M HCl solution, followed with 2 mL of previously prepared 5% ferric chloride solution. The mixtures were immersed in boiling water. After 5 minutes have elapsed, they were removed and then allowed cool to room temperature. The samples were extracted with 5mL of benzene and the benzene solutions were allowed to stand until benzene-water layer was formed. The benzene layers of each of the

mixture was separated and then treated with 2mL of previously prepared 2M ammonia solution. Development of pink colour in the ammonia layer indicated the presence of anthraquinone glycosides as reported by Trease and Evans (2009).

3.6.8 Test for terpenoids

Half a gram (0.5g) of each of the extracts was dissolved in 2 mL of chloroform, followed by 3 mL of 98% concentrated H_2SO_4. The mixtures were shaken gently and then set aside for 10 minutes. Development of reddish-brown colour at the interface of the mixture confirmed the presence of terpenoids as documented in a similar study by Trease and Evans (2009).

3.6.9 Keller-Killiani test for cardiac glycosides

Half a gram (0.5g) of each of the extracts was dissolved in 5 mL distilled water in a 500 mL separating funnel. These were followed with 2 mL of glacial acetic acid and 5 drops of previously prepared 5% ferric chloride solution. The mixtures were gently swirled after which 5 mL of 98% concentrated sulphuric acid added. The mixtures were swirled gently again and then allowed to stand for 10 minutes. Formation of a brown colouration with a violet ring below and a greenish ring above as reported by Khan *et al*. (2011), indicated the presence of cardiac glycoside.

3.7 In Vitro Anthelmintic Assay of Extracts and Standard Drug (Mebendazole)

In this study, extracts of the parts of *Ceiba pentandra* investigated were tested on *Millsonia ghanensis* (Earthworms) to accertain their efficiencies as dewormer.

3.7.1 Anthelminthic activities of extracts and standard drugs assay

In vitro anthelminthic activities of the extracts against *Millsonia ghanensis* (Earthworms) were examined. The anthelminthic assays of both extracts and the standard drug (mebendazole) were carried out using a standard methods as used by Ajaiyeoba et al., (2001). Previously prepared stock solutions (12.0 mg/mL) of each of the extracts and the standard drug (mebendazole) were used. From the stock solutions, four other concentrations (0.75, 1.50, 3.0, and 6.0 mg/mL) were prepared for each of the extract and the standard drug. Mebendazole was used as reference standard drug whilst sterilesed distilled water was used as a negative control. The test solutions of the extracts and the standard drug were prepared fresh at the start of the experiment.

Four of the worms were released into separate petri dishes each containing 50 mL of the test solutions. These were set aside and the time taken for paralysis and death of each of the worms to occur were recorded. Paralysis time was noted when there was no movement of any sort by the worms, except when the worms were shaken vigorously. Death time of the worms was also noted and recorded when the worms neither moved when shaken vigorously or dipped into warm water at 50 ^{o}C. Death time of the worms was followed by immediate fading of body colour as indicated by Osei-Akoto *et al.,* (2019). The experiment was done in triplicate and the results obtained expressed as Mean ± Standard Error of the mean (SEM) as used in similar studies by Ajaiyeoba, *et al.,* (2001); KrDeb *et al*., (2013) and Osei-Akoto *et al.*, (2019).

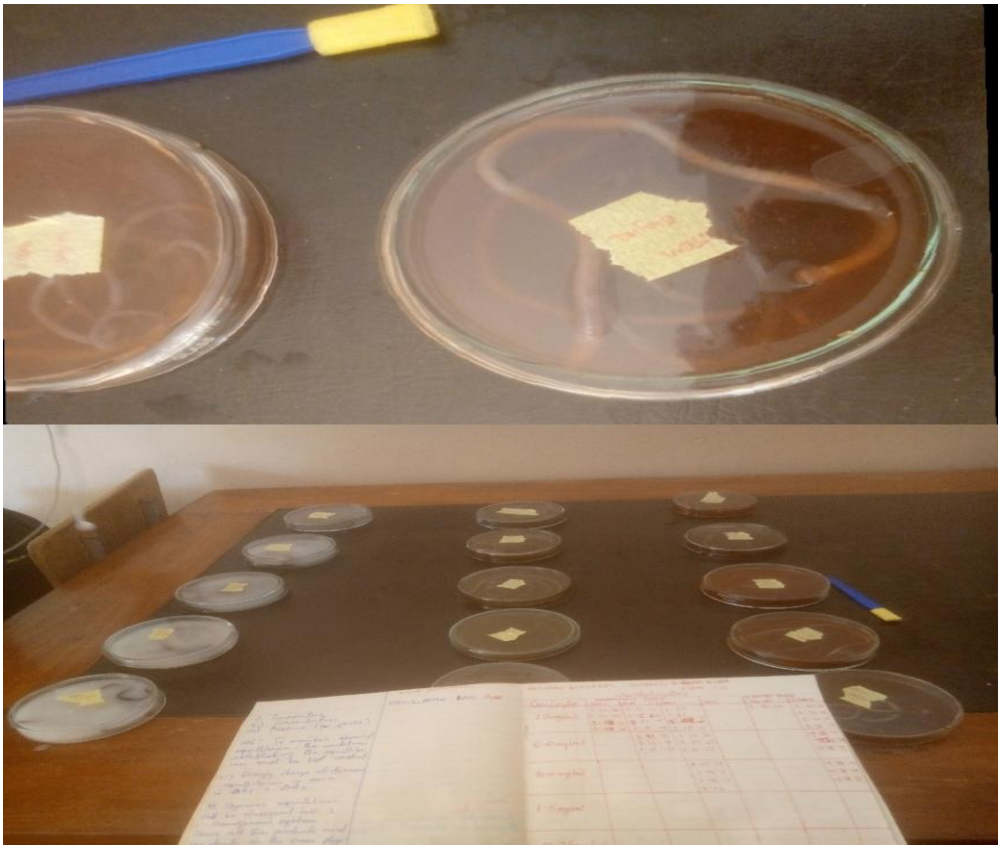


Plate 2: An image of anthelmintic assay set up

3.8 In Vivo Anti-Inflammatory Activity of Extracts of *Ceiba pentandra*

Carrageenan-induced inflammatory in a week-old chicks was done as described by Agyare *et al*., (2013). Responsiveness of the chicks to the seed, leave, stem and root barks extracts of *Ceiba pentandra* and anti-inflammatory standard drug (diclofenac) were determined through measurement of percentage change in volume of the feet of the chicks with respect to time.

3.8.1 Handling and treatment of chicks

A week old chicks *Gallus gallus* (Strain: Shaver 579) were purchased from Darko Farms Company Limited in Kumasi, Ghana. The animals were maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of

Science and Technology in Kumasi, Ghana. The chicks were housed in stainless steel cages and fed with commercial poultry diet produced by GAFCO in Tema, Ghana, water and libitum. They were maintained under laboratory conditions of (28 – 30°C), relative humidity (60 – 70%) and light-dark cycle. The chicks were kept in the laboratory for six days and then used on the 7th day. Procedures and techniques used in caring for the chicks were in accordance with the National Institute of Health guidelines for the care and use of laboratory animals (2022). The protocols for the study were approved by the Department of Pharmacology ethics Committee of KNUST in Kumasi, Ghana.

3.8.2 Carrageenan-induced edema in a week old chicks

A week old chicks (weight between 30 - 55 g) were put into 7 groups. Their initial foot volumes were measured via water displacement plethysmography using an electronic Von Frey plethysmometer (Model 2888, IITC Life Science Inc, Canada). Two percent of carrageenan (0.01mL) was injected into the plantar of the right foot to induce inflammation. Inflammation produced in each chick was also measured via water displacement plethysmography using an electronic Von Frey plethysmometer (Model 2888, IITC Life Science Inc, Canada). Increased in foot volumes were then calculated as [inflammated foot volume – original foot volume of the chicks].

The chicks in groups 1, 2 and 3 were orally dosed with 30, 100 and 300 mg/kg respectively of the extracts and their foot volumes measured hourly for 5hours. The chicks in group 4, 5 and 6 were also dosed orally with 10, 30 and 100 mg/kg respectively of diclofenac (standard drug) used as positive controls. Foot volumes of the positive control chicks were also measured hourly for 5 hours. However, chicks in

group 7 were not administered neither standard drug nor extracts. They were given 2 mg/kg normal saline solution. Five hours were allowed to elapse after the standard drug (diclofenac) and the extracts were administered to elapse to ensure that both the standard drug and the extracts administed were efficiently absorbed. Percentage change in foot volume after induction, and treatment of inflammation were noted and recorded. In order to simulate traditional use of *Ceiba pentandra* and to avoid the difficulty associated with parenteral administration of herbal preparations, oral administration of the extracts and standard drug was chosen.

3.9 Antioxidant Assay of Extracts of *Ceiba pentandra*

Antioxidant assay is both qualitative and quantitative chemical investigation used in determination of free radicals quenching or scavenging capacity of an extract or an established drug. In this study, total antioxidant capacity (TAC) and 2,2-diphenyl-1-picrylhydrazy (DPPH) free radical scavenging assay of the extracts were investigated.

3.9.1 Determination of total antioxidant capacities of the extracts of Ceiba pentandra

Method described by Prieto *et al.*, (1999) was used to study total antioxidant capacities of the extracts obtained from parts of *Ceiba pentandra* collected. This method involved reduction of the yellow-green phosphomolybdic acid, (Mo (VI)) to its blue complex phosphomolybdenum, (Mo (V)) by the extracts. Ascorbic acid was used as standard reference antioxidant drug whilst distilled water was used as blank. The Test solutions containing (50, 25, 12.5 and 6.125 µg/mL) of ascorbic acid were prepared from a previously prepared 100 µg/mL stock solution of ascorbic acid via serial dilution using distilled water. Ten milliliters mixture of each of the extracts were further prepared using 5 mL of a previously prepared 500 µg/mL test solution of

each extract and 5 mL of phosphomolybdenum in series of test tubes. The mixtures were incubated in a water bath at 95 °C for 90 minutes. After 90 minutes, the solutions were allowed to cool to room temperature and their absorbances then measured in triplicate, using UV-visible spectrophotometer (V-730 UV-Vis Spectrophotometer, Jasco, USA) at 695 nm.

The measured absorbances of the ascorbic acid test solutions were ploted against their respective concentrations using Microsoft excel 2007 version to obtain calibration curve for the ascorbic acid. Absorbances of the extracts solutions were also measured and then used on the absorbance – concentration plot of the ascorbic acid solution to obtained concentrations of the extracts using the linear equation obtained from the calibration curve of the ascorbic acid. Concentrations recorded for the extracts solution were expressed in gAAE/100 g ascorbic acid as used in similar studies by Prieto *et al.*, (1999), Torey *et al.* (2010) and Sharadanand *et al.*, (2014).

3.9.2 Free radical scavenging assaying of 2,2-diphenyl-1-picrylhydrazy (DPPH)

Antioxidant activities of the extracts were determined using DPPH-free radical scavenging assay protocol described by Mahdi-Pour *et al.,* (2012). Quantitative determination of free radical scavenging properties of the extracts were carried out in a 250mL universal bottle. Two hundred microgram per milliliters (200 µg/mL) of previously prepared extracts each was serially diluted to 12.5, 25, 50 and 100 µg/mL. One milliliter of each diluted extract was mixed with 1mL of DPPH solution and the mixture incubated at 37°C for 30 minutes. Absorbance of each of the mixtures was measured at 517 nm using a spectrophotometer (V-730 UV-Vis Spectrophotometer, Jasco, USA) whilst an absolute ethanol (99.5%) was used as a negative control.

DPPH free radicals scavenging activity of each of the extract was calculated using Equation 1:

DPPH scavenging activity (%) $= \frac{A_0 - A}{A_0}$ x 100 %, …equation 1

where A is the absorbance of the extract and A_0 is the absorbance of absolute ethanol (99.5%) used as the negative control.

Ascorbic acid was used as a positive control and the measurements made in triplicate. Concentration required for 50% DPPH inhibition (IC_{50}) was then calculated from the plot of percentage residual of DPPH against concentrations of the extracts as described by Sánchez-Moreno *et al.* (1998); Aiyegoro and Okoh, (2010), Torey *et al.* (2010), Basma *et al*. (2011) and Mahdi-Pour *et al.*, (2012).

3.10 Antimicrobial Assaying of Extracts of *Ceiba pentandra*

The extracts were tested for their antimicrobial activities on five different microbes known to be responsible for most infections. The microbial strains used (*Staphylococcus aureus and Enterococus faecalis* (Gram-positive) and *Pseudomonas aeruginosa*, *Salmonella typhi and Klebsiella pneumonia* (Gram-negative)) previously isolated, identified and were provided by the Pharmaceutical Microbiology Section of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Science, KNUST, Kumasi-Ghana. The strains were cultured in a nutrient broth and incubated overnight at 37 °C (Plate 2).

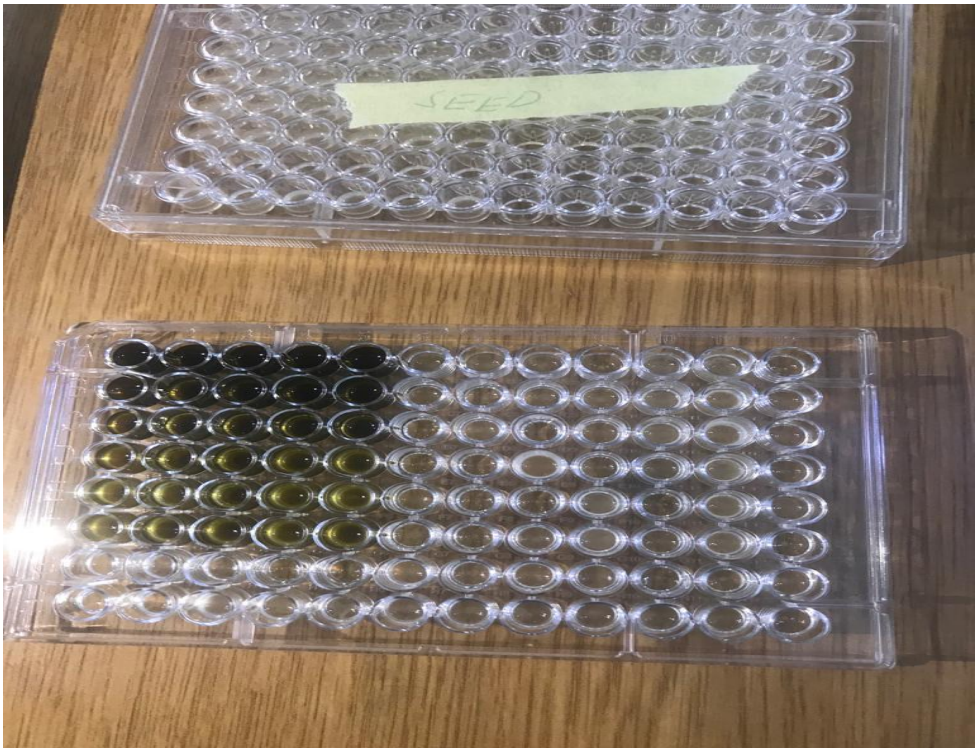


Plate 3: A picture showing antimicrobial assay set up

3.10.1 Determination of minimum inhibition concentration (MICs) of extracts

Minimum inhibitory concentrations (MIC) of the extracts were determined using 96 well microtitre plate via micro dilution method as described Osei-Akoto *et al.*, (2019). The plates were initially filled with 100 µL of a double strength nutrient broth and 10 µL of a 24 hour suspension of the organisms. Fifty microliters of the extract and 40 µL of sterile distilled water were added to each of wells. The contents of the wells were adjusted 200 µL by addition of nutrient agar to obtain the required concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125 1.5625, 0.78125 and 0.39065 mg/mL). The plates were then incubated at 37ºC for 24 hours. The MIC was determined as the lowest concentration of each extract that inhibited growth of the microbes. This was observed by the absence of purple colouration upon the addition of 10 µL of 125 mg/mL MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) to the contents of the well. The experiment was carried out in triplicates.

3.11 Fourier Transform Infrared Spectroscopic (FTIR) Analysis

The dried fractions of the extracts of the parts of *Ceiba pentandra* were subjected to FTIR analysis using UATR Two, PerkinElmer to determine the functional groups present in each extract. 10 mg of dried fractions of the extracts were encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The dried fraction sample of each extract was loaded into the instrument. The regions between 4000 and 400 cm^{-1} were scanned, followed by baseline correction as indicated in a similar study by Ashokkumar *et al.*, (2014).

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter presents nature and percentage of the different extracts obtain after extraction of the parts of *Ceiba pentandra* used with methanol. It also looks at the results obtained for phytochemical screening, anthelmintic, anti-inflammation, antioxidants and antimicrobial activities of the extracts. Finally, the chapter discusses the results obtained in the experiment and compared with results obtained in similar studies by other researchers.

4.1 Extraction of Plant Material

Soxhlet extraction was performed on the seed, leaves, stem and root barks of *Ceiba pentandra* using methanol as extraction solvent. The extract of the seeds was sticky brown-black with 6.37% yield. The extract from the leaves was also sticky with a deep green colouration with 6.27% yield. The stem bark extract had a dark-brown colouration granule with 6.85% yield whilst the root extract had a light brown colouration granule with 5.73% yield.

The percentage yields of the extracts were below 9.43% and 18.60% extracted from fruits of *Ocimum basilicum* using ethanol and n-hexane as extraction solvents respectively as reported by Osei-Akoto *et al.* (2020). However percentage yields recorded in this work were above 4.72% and 2.13% extracted from roots of *sclerocarya birrea* using ethanol and distilled water respectively as reported by Osei-Akoto *et. al* (2020) when soxhlet extraction protocol was used. Percentage yield (14.52%) of the extract obtained from leave of *Griffonia simplicifolia* when methanol

was used as soxhlet extraction solvent and reported by Osei-Akoto *et. al* (2020) was above (6.37, 6.27, 6.85 and 5.73%) recorded in this study whilst 6.72% reported by Osei-Akoto *et. al* (2020) was within 6.37, 6.27 and 6.85% obtained in this work. These outcomes implie that solvent polarity and extraction techniques determine percentage yield of the extracts. The yield of the extracts were root (5.73%) < leave (6.27%) < seed (6.37%) < stem barks (6.85%). The nature and colour of the extracts obtained after soxhlet extraction were noted and presented (Table 1).

Table 1: Nature of extracts obtained from various parts of *Ceiba pentandra*

Plant part	Nature of extract	Colour of extract
Seed	Sticky	Brown-black
Leave	Sticky	Deep green
Stem bark	Granule	Dark brown
Root	Granule	Light brown

After soxhlet extraction with methanol, mass of the extracts obtained from parts of *Ceiba pentandra* investigated and their corresponding percentage yields were also recoreded and the data presented (Table 2).

Table 2: Percentage yield of extracts from the various parts of *Ceiba pentandra*

Solvent used	Plant part	Mass of sample (g)	Mass of extract (g)	% of extract/ (w/w)
Methanol	Seed	150	9.56	6.37
	Leave	150	9.41	6.27
	Stem bark	150	10.28	6.85
	Root	150	8.60	5.73

4.2 Phytochemical Screening of Extracts

The extracts obtained from parts of *Ceiba pentandra* investigated were taken through standard tests required in screening process to determine their phytochemical properties. After phytochemical screening, the extracts were found to contain alkaloids, flavonoids, tannins, coumarins and cyanogenic glycoside (Table 3). Phytochemical screening test conducted on the seed extract indicated the presence of eight secondary metabolites viz alkaloids, flavonoids, tannins, saponins, terpenoids, coumarins, cardiac active glycoside and cyanogenic glycoside. The leave extract had nine secondary metabolites viz alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, coumarins, cardiac active glycoside and cyanogenic glycoside. The stem bark extract also had alkaloids, flavonoids, tannins, saponins, steroids, quinones, coumarins, cardiac active glycoside and cyanogenic glycoside whilst the root extract contained alkaloids, flavonoids, tannins, steroids, quinones, coumarins and cyanogenic glycoside (Table 3).

The presence of alkaloids, tannins, saponins, steroid and cardiac glycoside in the leaves extract and tannins, saponins, steroid and cardiac glycoside in the stem bark extract corresponded to the findings of Osuntokun *et al.,* (2017) where antimicrobial and phytochemical properties of crude extracts of leaf and stem bark of *Ceiba pentandra* were assessed. The occurance of flavonoid, tannins, steroids and cyanogenic glycoside in the root extract of *Ceiba pentandra* also corresponded with the findings of a work done on the plant by Labe *et al.* (2020).

Phytochemicals such as terpenoids, flavonoids, tannins, alkaloids and steroids have been shown by Tiwari *et al.*, (2011) and Boniface *et al.*, (2016) to exhibit

pharmacological activities such as wound healing, anti-inflammation, anticancer, antioxidant, immunomodulation, antidiarrhoeal, antimicrobial, antidepressant and antiplasmodial. Flavonoids and tannins have also been reported by Kessler *et al.*, (2003) to have antioxidant activity on human nutrition and health through scavenging, chelating and termination of free radicals.

Table 3: phytochemical screening of the methanol extract

Phytochemicals	Seed	Leave	Stem	Root
Alkaloids	+ve	+ve	+ve	+ve
Flavonoid	+ve	+ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	+ve	-ve
Steroid	-ve	+ve	+ve	+ve
Quinones	-ve	-ve	+ve	+ve
Terpenoids	+ve	+ve	-ve	-ve
Anthraquinones	-ve	-ve	-ve	-ve
Coumarins	+ve	+ve	+ve	+ve
Cyanogenic glycoside	+ve	+ve	+ve	+ve
Cardiac glycoside	+ve	+ve	+ve	-ve

Legend: +Ve = presence of a phytochemical and -Ve = absence of a phytochemical

4.3 Anthelmintic Activity

Tannins, alkaloids and terpenoids are reported to be responsible for anthelmintic activities exhibited by plants (Osei-Akoto *et al*., 2019). Anthelmintic assay of the extracts was performed on adult earthworms (*Millsonia ghanensis*) of average length

(13.50 cm), average weight (4.60 g), average circumference (3.10 cm) and brown in colour to investigate whether the extracts obtained in this work can function as effevtive dewormer. Earthworms were used because their anatomical and physiological semblance to human intestinal roundworms (*ascaris lumbricoides*) and also they are readily accessible (Danquah *et al.*, 2012).

The methanol extracts obtained from parts of *Ceiba pentandra* investigated and the mebendazole used as standard drug were applied on *helminths* (earthworms) to determine paralysis and death times of the extracts and mebendazole.

The results obtained indicated that both paralysis and death times are functions of concentrations of the extracts and the standard drug (Tables 4, 5, 6 and 7). As concentration of the extracts and the standard drug decreased, longer periods were needed for paralysis and subsequently death to occur.

In the negative control experimental set up, no paralysis and death of the worms were observed in the course of the experiment which lasted for 24 hours. The shorter the time taken for an extract or a standard drug to either paralyzed or kill the helminths the more effective the extract or the drug.

Paralysis and death times of the worms during anthelmintic activity studies of the seed extract and the standard drug were noted, recorded and the data presented in Table 4.

Table 4: Paralysis and Death time of anthelmintic activity of different concentrations of seed extract of *Ceiba pentandra*

Conc (mg/mL)	Mebendazole	Seed Extract	CL 95%	Mebendazole	Seed Extract	CL 95%
	Paralysis time/mins		F-value	death time/mins		F-value
12	206.0 ± 7.12	204.0 ± 7.00	1.02	394.0 ± 21.26	288.0 ± 9.05	2.35
6	337.0 ± 6.00	328.0 ± 3.79	1.58	437.0 ± 5.03	411.0 ± 8.30	1.65
3	361.0 ± 4.24	360.0 ± 4.24	1.00	486.0 ± 0.10	528.0 ± 14.67	146.7
1.5	407.0 ± 0.10	403.0 ± 2.06	20.6	588.0 ± 7.00	570.0 ± 13.99	2.00
0.75	459.0 ± 4.36	464.0 ± 5.89	1.35	700.0 ± 12.34	634.0 ± 9.57	1.29

Critical F-value at 95% is 9.28

Mean paralysis time for different concentrations of the methanol extracts of the seeds of *Ceiba pentandra* ranged from 204.0 ± 7.00 (at 12.0 mg/mL of extract) - 464.0 ± 5.89 minutes (at 0.75 mg/mL of extract) (Table 4). For the standard drug (mebendazole), mean time of paralysis ranged from 206.0 ± 7.12 (at 12.0 mg/mL of the standard drug) – 459.0 ± 4.36 minutes (at 0.75 mg/mL of the standard drug). The results presented (Table 4) indicate that the methanol extract of *Ceiba pentandra* appears to be more effective against the helminths than the standard drug. This is because of the reduced time of paralysis (204.0 ± 7.00 minutes) and death (288.0 ± 9.05 minutes) were observed for the seed extract compared to that of the stardard drug (206.0 ± 7.12 minutes) and (394.0 ± 21.26 minutes) for time of paralysis and death respectively. Efficacy of both the extract and the standard drug were found to decrease as their concentrations decreased (Table 4). This pattern was expected since at high

concentrations active compounds of the extract and the standard drug required to combat the worms also increases. For example at 12.0 mg/mL of extract, mean paralysis was 204.0 ± 7.00 minutes.

This increased to 328.0 ± 3.79 minutes (37.80% increase) when extract concentration was decreased to 6.0 mg/mL. Since shorter paralysis time indicates high efficacy of an extract or drug against target organism, both the extract and standard drug were very effective against *helminths* at high concentrations. Paralysis time recorded for the seed extract of *Ceiba pentandra* and standard drug corresponded with 11.85 ± 0.71 (at 5000 µg/mL of ethanol extract) – 116.38 ± 2.19 minutes (at 250 µg/mL of ethanol extract), 27.90 ± 0.42 (at 5000 µg/mL of n-hexane extract) – 215.34 ± 4.09 minutes (at 250 µg/mL of n-hexane extract) and 94.04 ± 2.57 (at 5000 µg/mL of mebendazole) – 238.97 ± 7.46 minutes (at 250 µg/mL of mebendazole) recorded by Osei-Akoto *et al.*, (2020) when extracts of *Ocimum basilicum* fruits and mebendazole were used on *Eudrilus eugeniae*.

Paralysis and death times of the worms during anthelmintic activity studies of the leave extract and the standard drug were noted, recorded and the data presented (Table 5).

Table 5: Paralysis and Death time of anthelmintic activity of different concentrations of leave extract of *Ceiba pentandra*

Conc (mg/mL)	Mebendazole	Leave Extract	CL 95%	Mebendazole	Leave Extract	CL 95%
	Paralysis time/mins		F-value	death time/mins		F-value
12	206.0 ± 7.12	136.0 ± 2.50	2.85	394.0 ± 21.26	306.0 ± 0.10	212.6
6	337.0 ± 6.00	216.0 ± 19.67	3.28	437.0 ± 5.03	411.0 ± 9.53	1.89
3	361.0 ± 4.24	290.0 ± 4.00	1.06	486.0 ± 0.10	474.0 ± 4.50	45.0
1.5	407.0 ± 0.10	353.0 ± 5.50	55.0	588.0 ± 7.00	575.0 ± 16.74	2.39
0.75	459.0 ± 4.36	404.0 ± 1.20	3.63	700.0 ± 12.34	637.0 ± 5.20	2.37

Critical F-value at 95% is 9.28

Mean paralysis time for different concentrations of the extract of the leaves of *Ceiba pentandra* ranged from 136.0 ± 2.50 (at 12.0 mg/mL of extract) - 404.0 ± 1.20 minutes (at 0.75 mg/mL of extract) (Table 5). For the standard drug (mebendazole), mean time of paralysis ranged from 206.0 ± 7.12 (at 12.0 mg/mL of the standard drug) – 459.0 ± 4.36 minutes (at 0.75 mg/mL of the standard drug). The results presented (Table 5) indicate that methanol extract of the leaves of *Ceiba pentandra* was effective against the helminths since reduced times were observed for both the paralysis and death of the worms.

Effectiveness of the extract and the standard drug decreased as their concentrations decreased (Table 5). For example at 12.0 mg/mL of the leaves extract, mean paralysis time was 136.0 ± 2.50 minutes. This increased by 37.04% to 216.0 ± 19.67 minutes

when extract concentration was decreased to 6.0 mg/mL. Shorter periods recorded for paralysis indicate high efficacy of the leave extract against target organism. Both the leave extract and the standard drug were effective against the target organisms only at high concentrations. At 12.0 mg/mL and 3.0 mg/mL of both the leave extract and the standard drug, difference existed in the death time of the target organism. (Table 5). Results obtained for the leave extract and the standard anthelmintic drug used in this study corresponded with the mean paralysis times 11.85 ± 0.71 (at 5000 µg/mL of ethanol extract) – 116.38 ± 2.19 minutes (at 250 µg/mL of ethanol extract), 27.90 ± 0.42 (at 5000 µg/mL of n-hexane extract) – 215.34 ± 4.09 minutes (at 250 µg/mL of n-hexane extract) and 94.04 ± 2.57 (at 5000 µg/mL of mebendazole) – 238.97 ± 7.46 minutes (at 250 µg/mL of mebendazole) recorded by Osei-Akoto *et al.*, (2020) when extract of the fruits of *Ocimum basilicum* and mebendazole used as a standard drug were investigated on *Eudrilus eugeniae*.

The results obtained in this work also, corresponded with that recorded by Ajaiyeoba *et al.*, (2001) when methanol extracts of the leaves of *Buchholzia coriaceae* and *Gynandropsis gynandra* were applied to earthworms to analysis their anthelmintic activity.

Time of paralysis and death of the worms during anthelmintic activity investigation of the extract of stem bark and the standard drug were also recorded and the data presented (Table 6). Tabulated F-values presented were compared with critical F-value (9.28) at 95% to established differences in the effective times for both paralysis and death of the organism.

Table 6: Paralysis and Death time of anthelmintic activity of different concentrations of stem bark extract of *Ceiba pentandra*

Conc (mg/mL)	Mebendazole	Stem Extract	CL 95%	Mebendazole	Stem Extract	CL 95%
	Paralysis time/mins		F-value	death time/mins		F-value
12	206.0 ± 7.12	113.0 ± 1.73	4.12	394.0 ± 21.26	140.0 ± 3.00	7.09
6	337.0 ± 6.00	189.0 ± 12.26	2.04	437.0 ± 5.03	235.0 ± 0.58	8.67
3	361.0 ± 4.24	226.0 ± 18.43	4.35	486.0 ± 0.10	278.0 ± 1.50	15.0
1.5	407.0 ± 0.10	295.0 ± 5.25	52.5	588.0 ± 7.00	325.0 ± 3.50	2.00
0.75	459.0 ± 4.36	304.0 ± 5.69	1.31	700.0 ± 12.34	387.0 ± 11.00	1.12

Critical F-value at 95% is 9.28

Mean paralysis time for the different concentrations of the extracts of the stem bark of *Ceiba pentandra* ranged from 113.0 ± 1.73 (at 12.0 mg/mL of extract) - 304.0 ± 5.69 minutes (at 0.75 mg/mL of extract) (Table 6). For the standard drug (mebendazole), mean time of paralysis ranged from 206.0 ± 7.12 (at 12.0 mg/mL of the standard drug) – 459.0 ± 4.36 minutes (at 0.75 mg/mL of the standard drug). The results presented (Table 6) indicate that the methanol extract of the stem bark of *Ceiba pentandra* was effective against the target organisms (helminths). Effectiveness of both the extract and the standard drug decreased as their concentrations decreased (Table 6). For example at 12.0 mg/mL concentration of the stem bark extract, mean paralysis time was 113.0 ± 1.73 minutes. This increased by 40.2% to 189.0 ± 12.26 minutes when extract concentration was decreased to 6.0 mg/mL (Table 6). The inverse relation

observed between concentration of the extract and the standard drug and effective paralysis and death times of the target organism establish high efficacious nature of the extract against the target organism. The effectiveness of the extract against the target organism followed a similar pattern exhibited by the standard drug. Both the extract and the standard drug showed an inverse association between both paralysis and death times of the target organism (helminths).

At 1.5 mg/mL concentration of both the stem extract and the standard drug, a significant difference in the paralysis time of the target organism were observed. Least significant difference (LSD) of 112 minutes was recorded. Also at 3.0 mg/ml of both the stem bark extract and the standard drug differences in the death time of the target organism with least significant difference of 208 minutes was observed (Table 6). In this study, paralysis time obtained for the stem extract of *Ceiba pentandra* and the referenced standard drug (mebendazole) corresponded with 11.85 ± 0.71 (at 5000 µg/mL of ethanol extract) – 116.38 ± 2.19 minutes (at 250 µg/mL of ethanol extract), 27.90 ± 0.42 (at 5000 µg/mL of n-hexane extract) – 215.34 ± 4.09 minutes (at 250 µg/mL of n-hexane extract) and 94.04 ± 2.57 (at 5000 µg/mL of mebendazole) – 238.97 ± 7.46 minutes (at 250 µg/mL of mebendazole) recorded by Osei-Akoto *et al.*, (2020) when extract of the fruits of *Ocimum basilicum* fruits and mebendazole used as a standard drug were investigated on *Eudrilus eugeniae*. Results obtained in this work (Table 6), also corresponded flavourably with that recorded by Ajaiyeoba *et al.* (2001) when methanol extract of the stem bark of *Buchholzia coriaceae* and *Gynandropsis gynandra* were administed to *Pheretima posthuma*.

The data documented in this study also corresponded well with that reported by Tagoe *et al.* (2021) when ethanol extract of the stem bark of *Albizia ferruginea* was applied to *Pheretima posthuma*.

In this study, time of paralysis and death of the worms during anthelmintic activities of the root extract and the standard drug were recorded and presented (Table 7). The F-test at 95% was estimated in the request to investigate differences in the paralysis and death times of both the standard drug and the root extract of *Ceiba pentandra*

Table 7: Paralysis and Death time of anthelmintic activity of different concentrations of root extract of *Ceiba pentandra*

Conc (mg/mL)	Mebendazole	Root Extract	CL 95%	Mebendazole	Root Extract	CL 95%
	Paralysis time/mins		F-value	death time/mins		F-value
12	206.0 ± 7.12	257.0 ± 2.38	2.99	394.0 ± 21.26	313.0 ± 4.50	4.72
6	337.0 ± 6.00	335.0 ± 4.50	1.33	437.0 ± 5.03	413.0 ± 7.80	1.55
3	361.0 ± 4.24	375.0 ± 4.11	1.03	486.0 ± 0.10	458.0 ± 9.20	92.0
1.5	407.0 ± 0.10	441.0 ± 3.77	37.7	588.0 ± 7.00	503.0 ± 10.31	1.47
0.75	459.0 ± 4.36	466.0 ± 6.29	1.44	700.0 ± 12.34	550.0 ± 1.53	8.07

Critical F-value at 95% is 9.28

Mean paralysis time for different concentrations of methanol extracts of root of *Ceiba pentandra* ranged from 257.0 ± 2.38 (at 12.0 mg/mL of extract) - 466.0 ± 6.29 minutes (at 0.75 mg/mL of extract) (Table 7). For the standard drug (mebendazole), mean time of paralysis ranged from 206.0 ± 7.12 (at 12.0 mg/mL of the standard drug) – 459.0 ±

4.36 minutes (at 0.75 mg/mL of the standard drug). The results presented (Table 7) indicate that methanol extract of *Ceiba pentandra* was effective against the helminthes as reduced times were recorded compared to that of the standard drug.

Effectiveness of the extract and the standard drug also decreased as their concentrations decreased (Table 7). For example, when 12.0 mg/mL of root extract was used, mean paralysis time was 257.0 ± 2.38 minutes. This increased to 335.0 ± 4.50 minutes (23.28% increase) when concentration of the extract was decreased to 6.0 mg/mL. Shorter paralysis time recorded for the root extract indicates high efficiency of the root extract against the target organisms. This finding was comparable to that of the standard drug against the same target organism.

At 1.5 mg/ml of both the root extract and the standard drug, a difference in the paralysis time of the helminthes was recorded at 95% confidence interval. The least significant difference (LSD) recorded at this concentration was 34 minutes. Also at 3.0 mg/ml of both the root extract and the standard drug significant differences of 28 minutes in the death time of the helminthes was recorded (Table 7). Results obtained for the seed extract of *Ceiba pentandra* corresponded with that 11.85 ± 0.71 (at 5000 µg/mL of ethanol extract) – 116.38 ± 2.19 minutes (at 250 µg/mL of ethanol extract), 27.90 ± 0.42 (at 5000 µg/mL of n-hexane extract) – 215.34 ± 4.09 minutes (at 250 µg/mL of n-hexane extract) and 94.04 ± 2.57 (at 5000 µg/mL of mebendazole) – 238.97 ± 7.46 minutes (at 250 µg/mL of mebendazole) recorded by Osei-Akoto *et al.* (2020) when extracts of the fruits of *Ocimum basilicum* and mebendazole used as a standard drug were investigated on *Eudrilus eugeniae*. In this work, the results

obtained also corresponded well with that recorded by Osei-Akoto *et al.,* (2020) when ethanol extract of the root of *Sclerocarya birrea* were applied on earthworms.

4.3.1 Comparison of effectiveness of standard drug and methanol extracts of parts of Ceiba pentandra investigated

The results presented (Tables 4, 5, 6 and 7) indicate that methanol extracts of the parts of *Ceiba pentandra* used in this study have potential to paralyse and kill helminths. Effectiveness of methanol extracts of the seeds, leaves, stem and root barks of *Ceiba pentandra* was compared with that of the standard drug (mebendazole). This was done in order to determine possibility of substituiting mebendazole with extracts from *Ceiba pentandra* due to its availability, accessibility, economical affordability and possibility of less adverse effects compared with the standard drug (mebendazole).

The extracts obtained from the parts of the plant investigated (seeds, leaves, stem and root barks) and mebendazole (standard drug) had concentration dependent paralysis and death of the helminths as reported by Shivkar *et al.* (2003). The extracts and mebendazole (standard drug) were effective at higher concentrations and had a decreased effectiveness as concentration decreases (Tables 4, 5, 6 and 7). The effectiveness of the extracts and mebendazole (standard drug) as anthelmintic agents were comparable at higher concentrations. This observation corresponded with those made by Shivkar *et al.* (2003) and Gulnaz *et al.* (2014). Times of paralysis of the extracts and mebendazole (referenced standard anthelmintic drug) used in this study were similar. At higher concentration (12.0 mg/mL) the extracts and the standard drug showed activity against the investigated helminths at stem bark (113.0 ± 1.73) < leave

(136.0 ± 2.50) < seed (204.0 ± 7.00) < mebendazole (206.0 ± 7.12) < root (257.0 ± 2.38) minutes.

Whilst the time of death of the helminths at higher concentration (12.0 mg/mL) were stem bark (140.0 ± 3.00) < seed (288.0 ± 9.05) < leave (306.0 ± 0.10) < root (313.0 ± 4.50) < mebendazole (394.0 ± 21.26) minutes (Tables 4, 5, 6 and 7). The order of anthelmintic activities observed in this work corresponded favourably with that recorded by Gulnaz *et al.* (2014) which had time of paralysis (44.50 minutes) and time of death (58.0 minutes) when 100 µg/mL ethanol extracts of *Randia uliginosa* leaves were tested on *Pheretima pasthuma* (length range 5-7 cm). Observation made in this work corresponded with paralysis time (90 minutes at 10 mg/mL) and death time (60 minutes at 100 mg/mL) when aqueous extracts of leaves of *Colotropis procera* were investigated on earthworms by Shivkar *et al.* (2003). The mode of action of some anthelmintics such as piperazine citrate is to paralyse of the target helminths (worms) such that they are expelled in faeces of humans and animals (Ajaiyeoba *et al.*, 2001; Osei-Akoto *et al.*, 2020). The methanol extracts obtained from the parts of *Ceiba pentandra* exhibited high potency against the target organisms (earthworms). The extracts also caused death of the helminths at a much higher rate than the mebendazole (standard drug).

4.4 Carageenan Induced Chick Model Assay for Anti-Inflammatory Activity Investigation

Volume of the right foot of a week old chicks increased gradually as they were induced with carrageenan (0.01mL). Increased in foot volume of the chicks attained a peak after an hour of carrageenan induction. Mean average percentage increase in foot

volume was (24.25 ± 1.53 %). The extracts of parts of *Ceiba pentandra* used were administed orally to the chicks at 300, 100 and 30 mg/kg whilst that of diclofenac (standard drug) dosed at 100, 30 and 10 mg/kg. For the diclofenac, maximum inflammatory inhibition effect within the 5 hour period was 100 > 30 > 10 mg/kg (Figure 16) whilst that of the seed were 300 > 100 > 30 mg/kg (Figure 16). ED_{50} of diclofenac (standard drug) was 9.17 mg/kg whilst that of seed was 33.13 mg/kg (Table 8). Maximum inflammatory inhibition effect of the leave extract was 300 > 100 > 30 mg/kg with ED_{50} 22.74 mg/kg.

Though 300 mg/kg dose of the stem bark extract showed maximum inflammatory inhibition effect, 30 mg/kg concentration of the stem bark extract exhibited a higher inflammatory inhibition than that exhibited by 100 mg/kg of the stem bark extract (Figure 18). ED_{50} of The stem bark extract was 27.08 mg/kg. Maximum inflammatory inhibition effect of the root extract was 100 > 300 > 30 mg/kg (Figure 19) with ED_{50} of 43.08 mg/kg. Both the extracts and the diclofenac exhibited anti-inflammatory effect on the carrageenan induced chicks. The anti-inflammatory response observed for both the extracts and the diclofenac (standard drug) were dose-dependent as reported in a similar study by (Osei-Akoto *et al.*, 2020).

Maximum inflammatory inhibition effect concentrations were 100 mg/kg (diclofenac), 300 mg/kg (seed, leave and stem bark) and 100 mg/kg (root). Anti-inflammatory response induced by 300 mg/kg of the methanol extracts of *Ceiba pentandra* (except that of the root) compared favourably, with that exhibited by 100 mg/kg of diclofenac. Anti-oedematous effect (ED_{50}) exhibited by 100 mg/kg of diclofenac differed from that exhibited by 300 mg/kg of methanolic extracts of the seeds, leaves and stem bark

of *Ceiba pentandra*. (Table 8). The results obtained for the treatment of carrageenan induced edema showed a time-dependent inhibitory response for both the extracts and the diclofenac (standard drug). Percentage volume of the feet of the chicks decreased with time as a response to the anti-inflammatory activity of both the extracts and the diclofenac (Figures 15, 16, 17, 18 and 19).

Results obtained for the treatment of carrageenan induced edema using extracts of *Ceiba pentandra* and diclofenac (standard drug) indicate that effective dose for 50% of the chicks population (ED_{50}) were diclofenac (9.17 mg/kg) < leave (22.74 mg/kg) < stem bark (27.08 mg/kg) < seed (33.13 mg/kg) < root (43.08) (Table 8).These results corresponded favourably with that recorded by Abotsi *et al*., (2017) when extract of *Albizia Zygia* (dosed at 30 – 300 mg/kg) significantly inhibited inflammation caused by carrageenan induced foot edema in a week old chicks. ED_{50} of *Albizia Zygia* extracts were 232.9 ± 53.33 mg/kg (preemptive) and 539.2 ± 138.28 mg/kg (curative). Results of this study also corresponded with that reported by Borquaye *et al*., 2017 when anti-inflammatory activities of extracts of *Oliva species, Patella rustica* and *Littorina littorea* collected from coastal shorelines of Ghana were investigated on carrageenan induced foot edema in a week old chicks. ED_{50} (mg/kg) were 119.80 ± 1.15 (ethanol), 80.72 ± 1.18 (ethyl acetate) and 16.61 ± 1.70 mg/kg (methanol) extracts of *Littorina littorea*. ED_{50} values were 36.83 ± 1.30, 10.16 ±1.47 and 73.46 ± 1.19 mg/kg for ethanol, ethyl acetate and methanol extracts of *Oliva species* respectively whilst that for ethanol and ethyl acetate extracts of *Patella rustica* were 82.70 ± 1.15 and 43.25 ± 1.49 mg/kg respectively. Pattern of anti-inflammatory response of the different dosage of diclofenac (standard drug) on the chicks expressed

as percentage change in the foot volume of the chicks against time was plotted and graph presented (Figure 15).

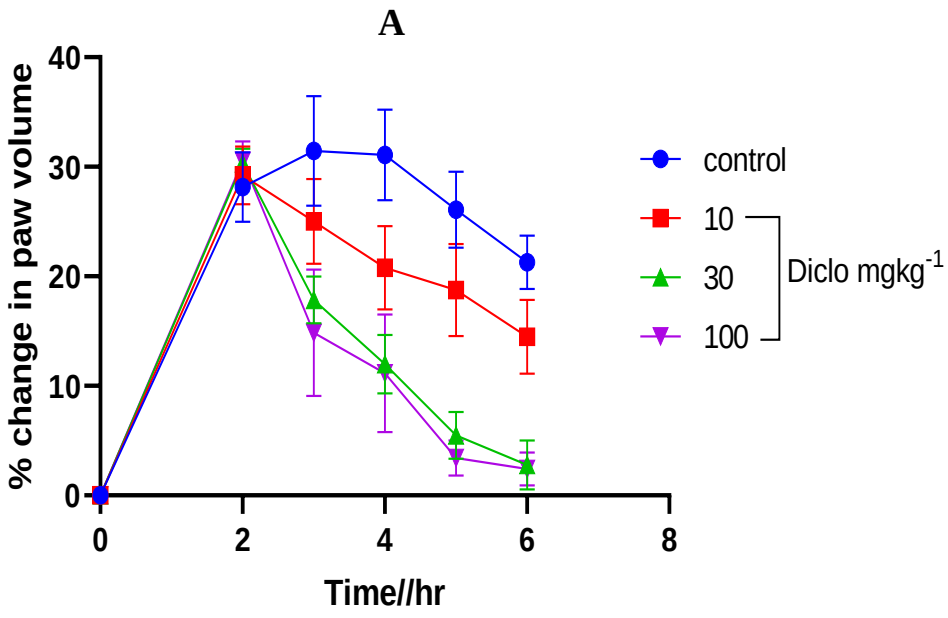


Figure 17: A graph (A) showing effect of diclofenac on carrageenan-induced edema

The data obtained for anti-inflammatory response induced by 100 mg/kg of diclofenac was also plotted and the graph presented (Figure 16). From the graph (Figure 16), there was a gradual reduction in percentage foot volume of carrageenan induced edema from 30.55 - 0.30% in a 5 hour interval. Anti-inflammatory response induced by 30 mg/kg resulted in a decreased in percentage foot volume of the chicks from 30.55 - 2.79% whilst that induced by 10 mg/kg of the diclofenac resulted in percentage foot volume reduction from 30.55 - 14.47% in a 5 hour interval. Order of anti-inflammation induced by the diclofenac was 100 > 30 > 10 mg/kg. Pattern of anti-inflammatory response of the different concentrations of the seed extract of *Ceiba*

pentandra on the chicks expressed as percentage change in the foot volume of the chicks against time presented (Figure 16).

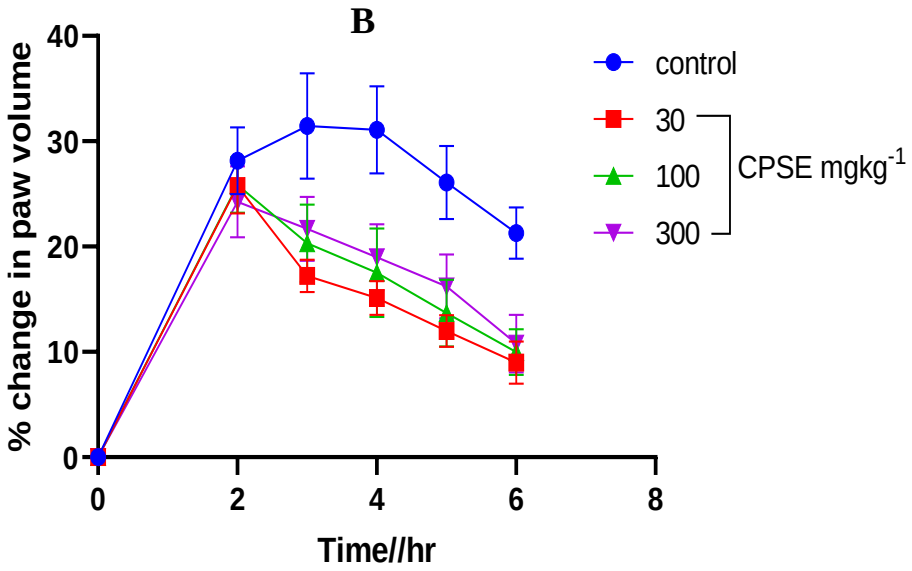


Figure 18: Graph (B) showing effect of *Ceiba pentandra* seed extract (CPSE), on carrageenan-induced edema

Different concentrations (300, 100 and 30 mg/kg) of the seed extract of *Ceiba pentandra* showed anti-inflammatory effect. Anti-inflammatory response induced by 300 mg/kg of the seed extract caused a decreased in mean percentage of foot volume of carrageenan induced chicks from 17.27 - 7.38% within a 5 hour period. The extract administered at 100 mg/kg also resulted in a decreased in the mean percentage foot volume (25.13 – 14.61%) whilst 30 mg/kg extract produced a reduction in foot volume (18.29 - 10.45%) of carrageenan induced chicks within 5 hours after its administration to the chicks.

Inflammatory inhibitory effects exhibited by the extracts were concentration dependant (300 > 100 > 30 mg/kg). The pattern of anti-inflammatory response of the

different concentration of the leave extract of *Ceiba pentandra* on the chicks expressed as percentage change in the foot volume of the chicks against time presented (Figure 17).

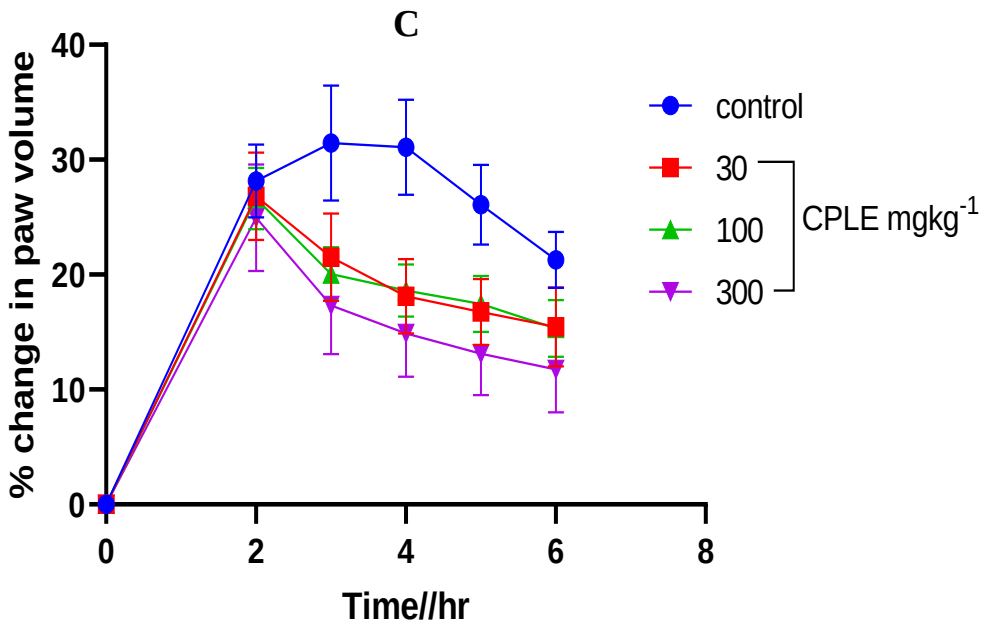


Figure 19: Graph (C) showing effect of *Ceiba pentandra* leave extract (CPLE) on carrageenan-induced edema

The leave extract of *Ceiba pentandra* also exhibited anti-inflammatory effect at 300, 100 and 30 mg/kg concentration administed to the carrageenan induced edema. Anti-inflammatory response induced by 300 mg/kg of the leave extract caused reduction in the foot volume of the carrageenan induced chicks from 20.79 - 11.76% within a 5 hour period after administration of the extract. The extract administered at 100 mg/kg to carrageenan treated chicks produced decreased in mean percentage foot volume from 22.61 - 15.32% whilst that administed at 30 mg/kg had 25.67 - 15.46% decrease in foot volume of the carrageenan treated chicks within the same period (5 hours). The

pattern of inflammation inhibitory effect exhibited by the leave extract of *Ceiba pentandra* was similar to that exhibited by the standard drug (diclofenac) and the seed extract of *Ceiba pentandra* (300 > 100 > 30 mg/kg). The pattern of anti-inflammatory response of the different concentrations of the stem bark extract of *Ceiba pentandra* on the chicks expressed as percentage change in the foot volume of the chicks against time presented (Figure 18).

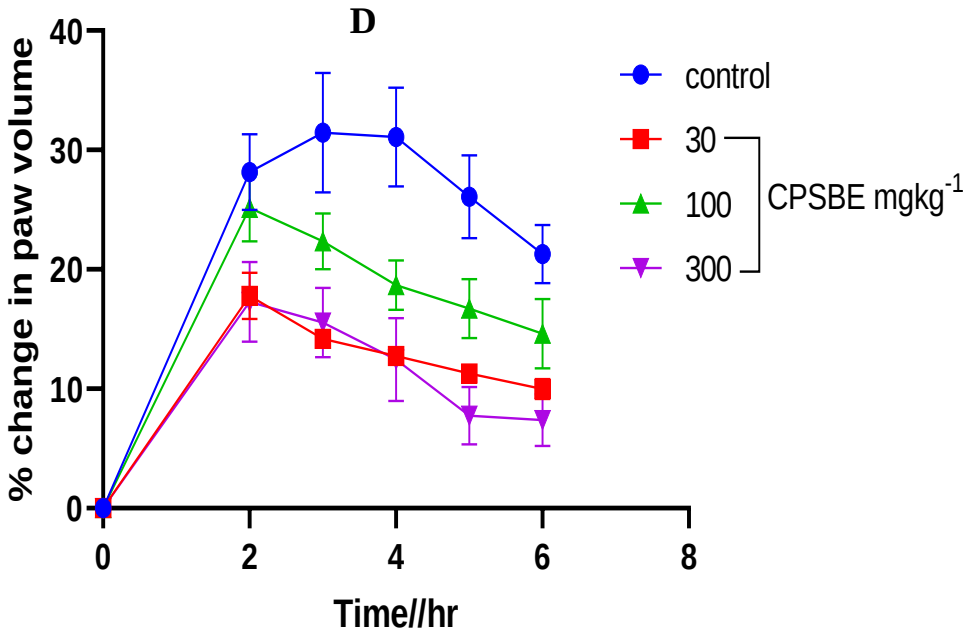


Figure 20: Graph (D) showing effect of *Ceiba pentandra* stem bark extract (CPSBE) on carrageenan-induced edema

The stem bark extract of Ceiba pentandra also had anti-inflammatory property. Pattern of anti-inflammatory property showed by the stem bark extract of *Ceiba pentandra* was concentration dependant (300 > 30 > 100 mg/kg). Anti-inflammatory response induced by 300 mg/kg of the stem bark extract resulted in a reduction in mean percentage foot volume of the carrageenan induced chicks from 17.27 - 7.38% within

a 5 hour period after it was administed to the chicks. Stem bark extract administered at 100 mg/kg produced percentage decreased in the mean foot volume of the carrageenan treated chicks from 25.13 - 14.61%. At 30 mg/kg, 17.78 - 9.98% reduction occured in foot volume of the chicks within the same period (5 hours) after treatment with this concentration (30 mg/kg) of stem bark extract. The pattern of anti-inflammatory response of the different concentration of the root extract of *Ceiba pentandra* on the chicks expressed as percentage change in the foot volume of the chicks against time presented (Figure 19).

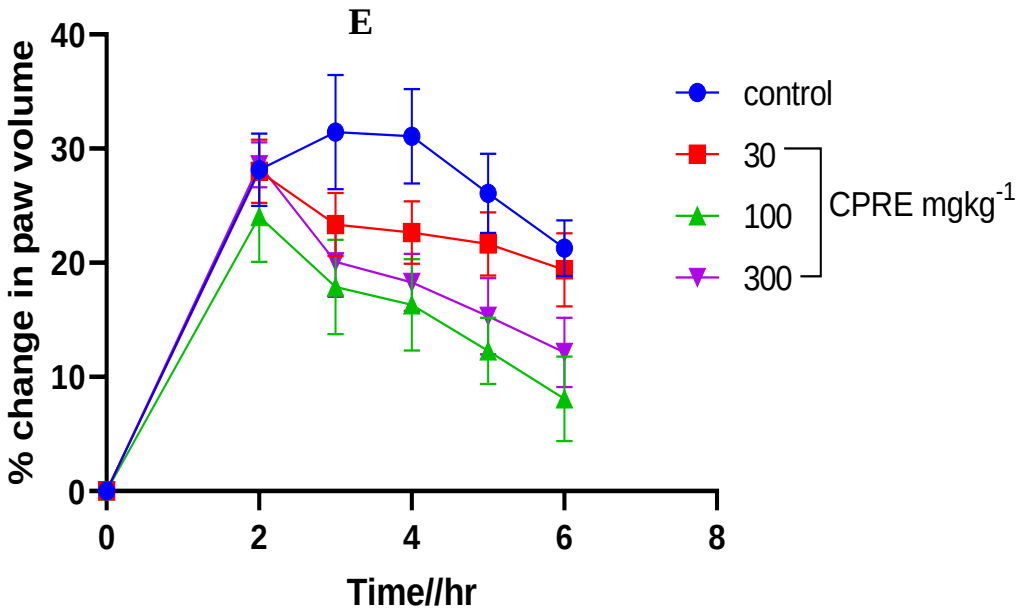


Figure 21: Graph (E) showing effect of *Ceiba pentandra* root extract (CPRE) on carrageenan-induced edema

The root extract of *Ceiba pentandra* exhibited anti-inflammatory activity similar to those exhibited by the standard drug (diclofenac), the seed, leave and stem methanol extracts of *Ceiba pentandra*. Anti-inflammatory response induced by the root extract

caused a reduction in mean percentage of the foot volume of carrageenan induced chicks from 23.47 - 12.16% within a 5 hour period after the carrageenan treated chicks were administed with 300 mg/kg of the root extract. Similarly, 100 mg/kg of the root extract after 5 hours of administration resulted in a decreased in the percentage mean foot volume of the carrageenan treated chicks from 20.28 - 8.09% whilst 30 mg/kg of the extracts produced a decreased in percentage mean foot volume from 25.44 - 19.37% after 5 hours of administration to the chicks. Anti-inflammatory response to the chicks was 100 > 300 > 30 mg/kg of the extracts. ED_{50} of the anti-inflammatory response of the methanol extracts obtained from parts of *Ceiba pentandra* investigated and the standard drug were recorded and the data presented (Table 8)

Table 8: Table of ED_{50} values of the methanolic extracts of various part of *Ceiba pentandra* and the standard drug

Extract / standard drug	ED_{50} Values (mg/kg)
Seed	33.13
Leave	22.74
Stem bark	27.08
Root	43.08
Diclofenac	9.171

4.5 Antioxidant activity of the extracts from the parts of *Ceiba pentandra*

Antioxidant assays seek to evaluate ability of plant extracts to scavenge free radicals (Prieto *et al.* (1999) and Mahdi-Pour *et al.* (2014). Antioxidant properties of methanol extracts of the seed, leaves, stem bark and root of *Ceiba pentandra* were assessed. In order to establish capacity of the extracts to scavenge free radicals as done in a study by Osei-Akoto *et al.,* 2020. Total antioxidant capacity assay and DPPH radical

scavenging assay were used as documented by Prieto *et al.* (1999) and Mahdi-Pour *et al.* (2014).

4.5.1 Total antioxidant capacity (TAC) using phosphomolybdate assay

Total antioxidant capacity assay was used to quantify total number of antioxidants in each of the plant extract. The assay was done using ascorbic acid (vitamin C) as a regular antioxidant via phosphomolybdenum test. Antioxidant power of the test extracts reduced Mo^{+6} to Mo^{+5}. This was observed through formation of a green phosphomolybdenum (v) complex as indicated by Leelaprakash *et al.*, (2011).

Absorbances of the complexes formed were measured at 695 nm using UV-visible spectrophotometer (V-730 UV-Vis Spectrophotometer, Jasco, USA). Estimated total antioxidant capacity of the test extracts was 188.71 ± 0.06 gAAE/100g (seed), 131.56 ± 13.21 gAAE/100g (leaves), 242.18 ± 5.41 gAAE/100g (stem bark) and 66.37 ± 2.30 gAAE/100g (root) (Table 9). The results (Table 9) indicate that 1.0 g of ascorbic acid functions as 18.871 mg (seed extract), 13.156 mg (leaves extract), 242.18 mg (stem bark extract) and 6.637 mg (root extract) of *Ceiba pentandra*. Unknown concentrations of extract were estimated by extrapolating a plot of their absorbance against concentration of the standard solution using the calibration curve (Figure 20).

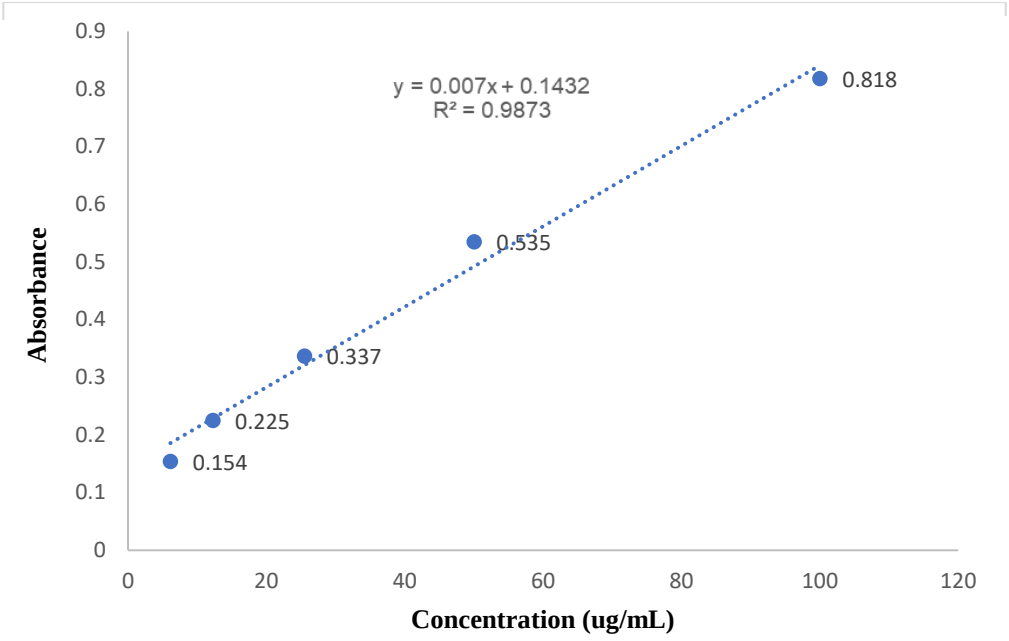


Figure 22: A calibration curve showing absorbance of TAC action against concentration of Ascorbic acid

Concentrations of ascorbic acid (6.25 - 100 µg/mL) showed antioxidant activity and mean absorbance (0.154 ± 0.03 to 0.818 ± 0.02) at 695 nm wavelength (Figure 20). The results obtained were expressed as gram ascorbic acid equivalent per 100 grams (gAAE/100g). The TAC had direct relationship to concentration of the extracts (Figure 20). A study by Shoaib *et al.* (2019) indicates that secondary metabolites such as phenols, carotenoids and terpenoids of medicinal plants have strong antioxidant properties. Such plants according to Osei-Akoto *et al.* (2020) can be used to treat diseases associated with oxidative stress. Also, some reported phenols, vitamin E and vitamin C help delay aging by neutralizing actions of free radicals (Ames *et al.,* 1993). These suggest that antioxidant potential of *Ceiba pentandra* may have resulted from carotenoids, terpenoids, and phenols contents of the extracts. Thus *Ceiba pentandra* can treat diseases originating from oxidative stress as indicated by Ames *et al.*, (1993) and Osei-Akoto *et al.,* (2020).

Table 9: Total antioxidant capacity (TAC) of methanol extract of the part of *Ceiba pentandra* used

Sample	TAC (gAAE/100g)
Seed	188.71 ± 0.06
Leaves	131.56 ± 13.22
Stem bark	242.18 ± 5.41
Root	66.37 ± 2.30

High TAC values indicate that the extracts have a better or strong activity to scavenge for free radicals. Stem bark extract (242.18 ± 5.41) had the highest TAC followed by seed (188.71 ± 0.06), leave (131.56 ± 13.22) and root (66.37 ± 2.30). The capacities of the extracts to scavenge for free radicals was stem bark (242.18 ± 5.41 gAAE/100g) > seed (188.71 ± 0.06 gAAE/100g) > leaves (131.56 ± 13.22 gAAE/100g) > root (66.37 ± 2.30 gAAE/100g). Capacities of the extracts used were better than that (36.42 ± 0.38 gAAE/100g) and (22.56 ± 2.24 gAAE/100g) obtained for *Gliffonia simplicifolia* and *Sclerocarya birrea* respectively and reported by Osei-Akoto *et al.,* (2020). Free radicals scavenging abilities of methanol extracts of the seed, leave and stem bark of *Ceiba pentandra* were also better than (0.085 ± 2.59 x 10^{-5} mgAAE/25g) *Xanthosoma colocasia* (kontomire), (0.146 ± 3.63 x 10^{-5} mgAAE/25g) *Solanum torium* (kwawu nsusua) and (0.102 ± 0.38 x 10^{-5} mgAAE/25g) *Allium asialonicum* (shallot) reported by Acheampong *et al.* (2016).

4.5.2 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay

2,2-diphenyl-1-picryhydrazyl (DPPH) is a free radial with purple colouration. Antioxidants have the ability to reduce DPPH to DPPH-H by proton transfer (Jose *et*

al., 2012). This result in a colour change of DPPH from purple to yellow. Ability of the extracts used to scavenge DPPH radicals was also investigated and the obtained data presented (Table 10).

The results of DPPH scavenging assay (Table 10) show that, half maximum inhibition concentration (IC_{50}) were 5.41 μg/mL (seed), 18.18 μg/mL (leave), 6.10 μg/mL (stem bark), 19.17 μg/mL (root) compared with 31.90 μg/mL when ascorbic acid was used as a standard drug. Half maximum inhibitory concentration (IC_{50}) is the concentration which will inhibited 50% of the free targeted radicals (Aykul *et al*., 2016). Therefore, concentration above maximum inhibition concentration (IC_{50}) is needed for each of the extract to inhibit 50% of free radicals (O^{2-}, OH^{-}, H_2O_2). The results suggest that methanol extracts obtained from the parts of *Ceiba pentandra* used were good DPPH inhibitors when compared to (31.90 μg/mL) ascorbic acid (Table 10).

Table 10: Inhibitory concentration (IC_{50}) of methanol extracts of various part of *Ceiba pentandra*

Extract	IC_{50} (μg/mL)
Seed	5.41
Leave	18.18
Stem bark	6.10
Root	19.17

The results imply that antioxidant activities of the extracts and ascorbic acid (standard drug) were ascorbic acid (standard drug) < root < leave < stem bark < seed (Table 10). Methanol extracts of *Ceiba pentandra* showed better antioxidant activities compared

well with that of ascorbic acid (standard drug). This observation could be due to the presence of flavonoids, phenols and tannins which have been reported to scavenge, chelate and terminate free radicals as indicated by Kessler *et al.,* (2003). The extracts obtained from the parts of *Ceiba pentandra* extracted exhibited useful neutraceutical properties for the treatment of diseases associated with oxidative-stress as indicated in a similar study by Osei-Akoto *et al*., (2020). The extracts of the parts of *Ceiba pentandra* investigated had higher antioxidant activity in the DPPH free radical scavenging assay as indicated (Figure 21).

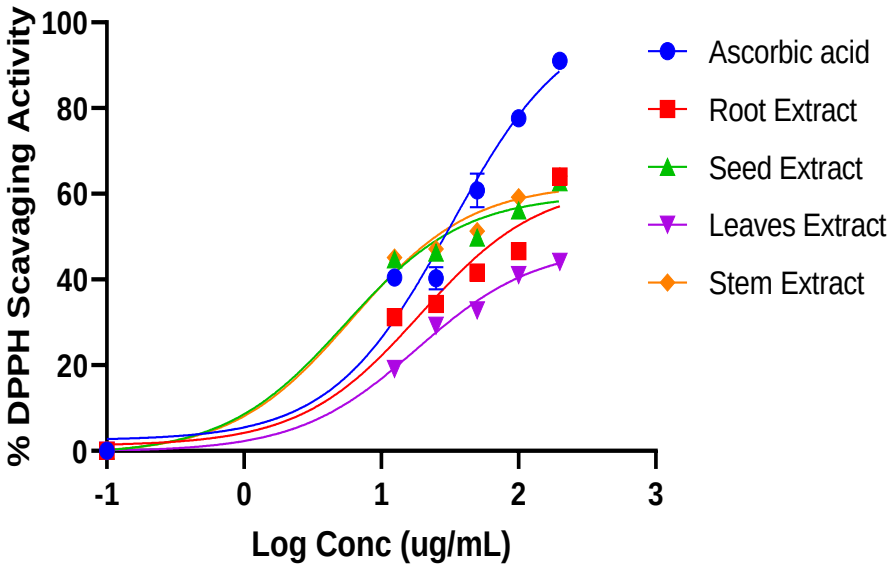


Figure 23: A graph showing DPPH radical scavenging activity of extracts from parts of *Ceiba pentandra* investigated and ascorbic acid.

Maximum inhibition concentration (IC_{50}) for the extracts of the parts of *Ceiba pentandra* used were below (61.85 µg/mL) recorded for methanol extract of the leaf of *Gliffonia simplicifolia* and (24.66 µg/mL) documented for ethanol extract of the root of *Sclerocarya birrea* (Osei-Akoto *et al.*, 2020). The seed, leave, stem and root barks extracts of *Ceiba pentandra* also had a better antioxidant activity than *Xanthosoma*

colocasia (kontomire) (0.0114 mg/mL), *Solanum torium* (kwawu nsusua) (2.4835 mg/mL) and *Allium asialonicum* (shallot) (2.2708 mg/mL) reported by Acheampong *et al.*, (2016).

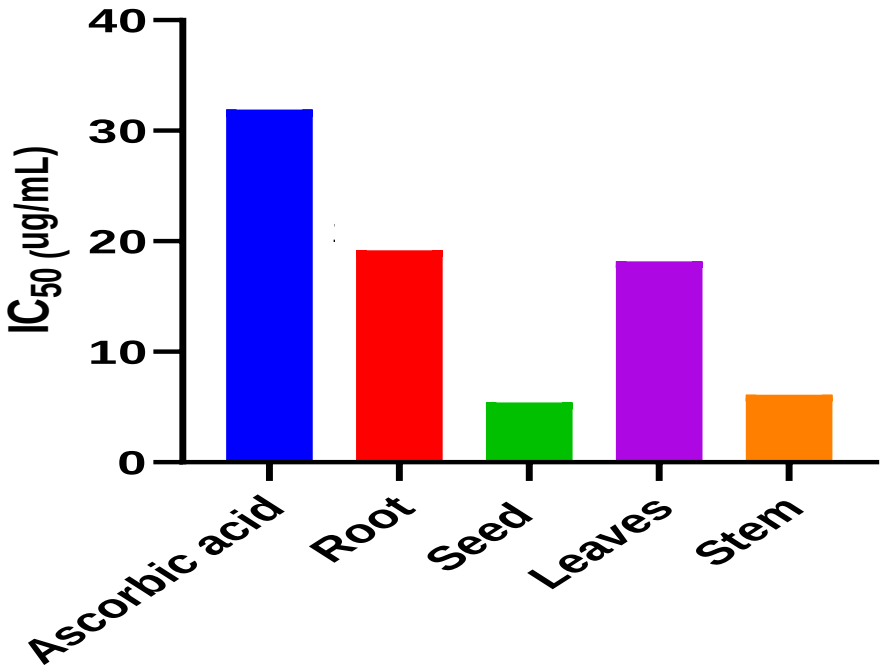


Figure 24: A graph showing IC_{50} values of extracts from parts of *Ceiba pentandra* investigated and ascorbic acid

4.6 Antimicrobial Assay of Methanol Extracts of *Ceiba pentandra*

Antimicrobial properties of the methanol extracts of the parts of *Ceiba pentandra* investigated were studied using broth-dilution assay as used in a study by Osei-Akoto *et al.*, (2019). Five standard microorganisms, (two gram-positive *Staphylococcus aureus and Enterococus faecalis*) and three gram-negative (*Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumonia*) were used. Results of Antimicrobial assay (Table 11) showed that the extracts of the parts exhibited varying inhibitory effects against the five selected microorganisms. Minimum inhibitory concentrations

(MICs) recorded ranged from 1.5625 - 12.5 mg/mL (seed), 3.125 - 12.5 mg/mL (leave), 3.125 - 12.5 mg/mL (stem bark) and 6.25 - 12.5 mg/mL (root). Inhibition responses of the extracts against *P. aeruginosa* were stem and root barks (12.50 mg/mL) < seed and leave (6.25 mg/mL). For S. typhi, MICs of the extracts were root (12.50 mg/mL) < stem bark and leave (6.25 mg/mL) < seed (1.56 mg/mL).

Inhibition responses of the extracts against *E. faecalis* were seed (12.50 mg/mL) < stem and root (6.25 mg/mL) < leave (3.12 mg/mL) whilst that against *S. aureus* were root (12.50 mg/mL) < leave (6.25 mg/mL) < stem bark (3.12 mg/mL) < seed (1.56 mg/mL). Inhibition responses of the extracts against *K. pneumonia* were leave, root (12.50 mg/mL) < seed, stem bark (6.25 mg/mL) (Table 11).

Maximum inhibition effect of methanol extracts of the seed, leave, stem and root barks on the selected microbes were observed for *(S. typhi and S. aureus), E. faecalis, S. aureus and E. faecalis* respectively. According to Fabry *et al*. (1998), extracts with MICs below 8 mg/mL have potent antimicrobial activity. Thus, antimicrobial activities of methanol extracts obtained from parts of *Ceiba pentandra* investigated were moderately effective against the microbes used. Studies have showed that phytochemicals (terpenoids, saponins and tannins) have high antimicrobial properties (Tiwari *et al.*, 2011; Agyare *et al.*, 2013; Acheampong *et al.*, 2016; Osuntokun *et al.*, 2017; Osei-Akoto *et al.*, 2020). Thus, the presence of these phytochemicals in the methanolic extracts of parts of *Ceiba pentandra* used in this work may be responsible for the antimicrobial activities observed.

Table 11: Minimum inhibitory concentrations (MICs) of extracts and reference drugs (mg/mL) against test organisms

Test organism	Minimum Inhibitory Conc (mg/mL)				
	Seed	Leave	Stem	Root	Cipro
P. aeruginosa	6.25	6.25	12.5	12.5	0.390
S. typhi	1.56	6.25	6.25	12.5	0.195
E. faecalis	12.5	3.12	6.25	6.25	0.098
S. aureus	1.56	6.25	3.12	12.5	0.049
K. pneumonia	6.25	12.5	6.25	12.5	0.024

4.6.1 Minimum Bactericidal Concentration (MBC) assay of methanol extracts of Ceiba pentandra

Minimum Bactericidal Concentration (MBC) assay is an accepted parameter for evaluating new antimicrobial agents and it is a research tool used to predict bacteria eradication. Minimum Bactericidal Concentration (MBC) is the minimum concentration of an extract or a drug which will kill a given bacteria. The methanol extracts of the parts of *Ceiba pentandra* used were not only able to inhibit activities of the microbes, the extracts were also able to kill the selected microbes. Results from antimicrobial assay (Table 12) showed that methanol extracts of the parts of *Ceiba pentandra* used had minimum bactericidal concentrations (MBCs) which ranged from 6.25 - 12.5 mg/mL (seed), 6.25 - 12.5 mg/mL (leave and the stem bark) and 250 - 62.5 mg/mL (leave and the stem bark) (Table 12).

Table 12: Minimum Bactericidal Concentrations (MBC) of the extracts and reference drugs (mg/mL) against test organisms

Test organism	Minimum Bactericidal Conc (mg/mL)				
	Seed	Leave	Stem	Root	Cipro
P. aeruginosa	12.5	12.50	12.50	25.0	0.39
S. typhi	6.25	12.50	12.50	25.0	0.20
E. faecalis	12.5	6.25	6.25	6.25	0.10
S. aureus	6.25	12.50	6.25	12.5	0.05
K. pneumonia	12.5	25.00	12.50	12.5	0.02

4.7 FTIR Spectroscopic Analysis of Methanol Extracts of Parts of *Ceiba pentandra* Investigated

Fourier Transform Infra Red Spectrocopy (FTIR) was performed on methanol extracts of the parts of *Ceiba pentandra* used to determine functional groups present in each of the extracts. The FTIR spectra of the extracts showed some prominent absorption for different functional groups present in the extracts (Table 13).

Table 13: FTIR analysis of the extracts from *Ceiba pentandra*

Part of plant	Wavenumber /cm^{-1}	Assignment	Remarks
Seed	3273.65	O-H	O-H alcohol,
	2923.19 - 2853.74	C-H	C-H stretch,
	1743.83 - 1708.39	C=O	C=O carbonyl group,
	1637.33	C=C/Ar-H	C=C/Ar-H stretch,
	1542.60 and 1315.39	N=O	N=O bend/stretch,
	1237.00 - 1046.53	C-O/C-N	C-O/C-N bend/stretch
Leave	3342.55	O-H,	O-H alcohol,
	2923.22 - 2852.91	C-H	C-H stretch
	1712.21	C=O	C=O carbonyl group
	1615.84	C=C/Ar-H	C=C/Ar-H stretch
	1517.79 and 1376.10	N=O	N=O bend/stretch
	1242.21 - 1068.61	C-O/C-N	C-O/C-N bend/stretch
Stem bark	3255.53	O-H	O-H alcohol,
	2918.35 - 2850.31	C-H	C-H stretch,
	1604.95	C=C/Ar-H	C=C/Ar-H stretch,
	1517.68 and 1364.97	N=O	N=O bend/stretch,
	1279.44 - 1043.37	C-O/C-N	C-O/C-N bend/stretch,
Root	3269.67	O-H	O-H alcohol,
	2921.82 - 2852.14	C-H	C-H stretch,
	1604.88	C=C/Ar-H	C=C/Ar-H stretch,
	1517.12 – 1375.43	N=O	N=O bend/stretch,
	1266.77 - 1044.42	C-O/C-N	C-O/C-N bend/stretch

The characteristic absorption bands of the seed extract were exhibited at 3273.65 cm^{-1} (O-H alcohol), 2923.19 – 2853.74 cm^{-1} (C-H stretch), 1743.83 – 1708.39 cm^{-1} (C=O carbonyl group), 1637.33 cm^{-1} (C=C/Ar-H stretch), 1542.60 and 1315.39 cm^{-1} (N=O bend/stretch) and 1237.00 – 1046.53 cm^{-1} (C-O/C-N bend/stretch). The absorption bands of the leave extract were observed at 3342.55 cm^{-1} (O-H alcohol), 2923.22 – 2852.91 cm^{-1} (C-H stretch), 1712.21 cm^{-1} (C=O carbonyl group), 1615.84 cm^{-1} (C=C/Ar-H stretch), 1517.79 and 1376.10 cm^{-1} (N=O bend/stretch) and 1242.21 – 1068.61 cm^{-1} (C-O/C-N bend/stretch).

Also, the absorption bands of the stem bark extract were observed at 3255.53 cm^{-1} (O-H alcohol), 2918.35 – 2850.31 cm^{-1} (C-H stretch), 1604.95 cm^{-1} (C=C/Ar-H stretch), 1517.68 and 1364.97 cm^{-1} (N=O bend/stretch) and 1279.44 – 1043.37 cm^{-1} (C-O/C-N bend/stretch) whilst that of root extracts were observed at 3269.67 cm^{-1} (O-H alcohol), 2921.82 – 2852.14 cm^{-1} (C-H stretch), 1604.88 cm^{-1} (C=C/Ar-H stretch), 1517.12 and 1375.43 cm^{-1} (N=O bend/stretch) and 1266.77 – 1044.42 cm^{-1} (C-O/C-N bend/stretch)..

The FTIR results showed that functional groups such as carbonyl groups(C=O), Alkene/ aromatic C=C double bond, Hydroxyl groups (-OH) and ester groups(C-O) among others were present in each of the extracts.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Summary

This study looked at phytochemicals, anthelmintic, anti-inflammatory, antioxidant and antimicrobial properties of methanol extracts of seed, leaves, stem bark and root of *Ceiba pentandra* commonly known as kapok. These parts of the plant were extracted using Soxhlet extraction technique with methanol as the extraction solvent. The extracts were phytochemically screened and their anthelmintic activities investigated against *Millsonia ghanensis* (Earthworms). The data recorded were compared with that obtained when mebendazole which was used as a standard anthelmintic drug. Data recorded for antimicrobial activity of the extracts were also compared with that recoreded for ciprofloxacin which was used as a standard antibacterial drug. Antimicrobial activity of the extracts were tested on *Staphylococcus aureus, Enterococus faecalis, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumonia* using broth dilution method.

DPPH radical scavenging and Total Antioxidant Capacities (TAC) of the extracts were studied using ascorbic acid as a standard antioxidant drug. Anti-inflammatory assay was carried out on the extracts using diclofenac as a standard anti-inflammation drug. The anti-inflammatory assay of the extracts were determined using carrageenan-induced foot oedema in a week old chicks. The results recorded in each of the activities were compared with that of the referenced standard drug. Infrared spectroscopic analyses of the extracts were also conducted to determine functional groups present in the various extracts.

After extraction, the percentage yield of the parts of *Ceiba pentandra* were 6.37 (seed), 6.27 (leave), 6.85 (stem bark) and 5.73% (root). Phytochemical screening of the extracts indicated the presence of alkaloids, flavonoids, tannins, saponins and cyanogenic glycosides. Anthelmintic activity of methanol extracts of the seed, leave and stem bark were effective on helminths when compared with the results obtained for standard drug (mebendazole). However, the root extract had a reduced activity on helminths as compared with the results of the standard drug (mebendazole). The IC_{50} (µg/mL) recorded in DPPH assay were 31.90 (ascorbic acid), 5.41 (seed extract), 18.18 (leave extract), 6.10 (stem bark extract) and 19.17 (root extract) whilst mean TAC (g AAE/100 g) were 188.71 ± 0.06 (seed extract), 131.56 ± 13.21 (leave extract), 242.18 ± 5.41 (stem bark extract) and 66.37 ± 2.30 (root extract). Carrageenan-induced ED_{50} were 33.13 (seed extract), 22.74 (leave extract), 27.08 (stem bark extract), 43.08 (root extract) and 9.171 mg/kg (diclofenac). MIC of the extracts ranged from 1.5625-12.5mg/mL whilst MBC were from 6.25-25.0 mg/mL. Also, the FTIR results showed functional groups such as carbonyl groups(C=O), Alkene/ aromatic C=C double bond, Hydroxyl groups (-OH) and ester groups(C-O) were present in each of the extracts.

These results indicate that the extracts of *Ceiba pentandra* has comparable nutraceutical effect to the standard drugs used and can be used as a substitute to the standard drugs due to its effectiveness, accessibility, affordability and less adverse effects.

5.2 Conclusion

Results obtained in this work confirms that, *Ceiba pentandra* (kapok) is a promising source of a new anthelmintic, antioxidant and antimicrobial drugs. This was evident in the fact that parts of *Ceiba pentandra* extracted with methanol proved to be potent agents in anthelmintic, anti-inflammatory, antioxidant and antimicrobial activities

when compared with established drugs. Phytochemical screening of the extracts indicated the presence of flavonoids, tannins, alkaloids and Cardiac Glycosides. These phytochemicals as indicated by Arts and Hollman (2005), confer on *Ceiba pentandra* its antimicrobial, anthelmintic, antioxidant and anti-inflammatory activities.

Time of paralysis of helminths used taken into consideration the concentrations of the extracts used in investigating anthelmintic activity were 352 (seed), 280 (leave), 226 (stem bark) and 375 minutes (root) of *Ceiba pentandra* whilst that of the standard drug (mebendazole) was 354 minutes. Extracts of the leave and stem bark were more effective on helminths when compared with the standard drug (mebendazole). However, the root extract had a reduced activity on helminths as compared with the standard drug (mebendazole) whilst the seed extract had similar activity on the helminthes as the standard drug (mebendazole).

In the antioxidants activity of *Ceiba pentandra*, the half-maximal inhibitory concentrations (IC_{50} µg/mL) recorded in DPPH assay were 5.41 (seed), 18.18 (leave), 6.10 (stem bark) and 19.17 µg/mL (root) whilst that of the ascorbic acid (standard drug) was 31.90 µg/mL. Mean TAC (gAAE/100 g) were 188.71 ± 0.06 (seed), 131.56 ± 13.21 (leave), 242.18 ± 5.41 (stem bark) and 66.37 ± 2.30 (root). These results indicate that the extracts of obtained from the parts of *Ceiba pentandra* are better scavenger of radicals than ascorbic acid (standard drug). ED_{50} (mg/kg) values recorded for carrageenan-induced foot method used in assessing anti-inflammatory activity of *Ceiba pentandra* were 33.13 (seed), 22.74 (leave), 27.08 (stem bark) and 43.08 mg/kg (root) whilst that of the standard drug (diclofenac) was 9.171 mg/kg. The standard drug (diclofenac) showed better anti-inflammatory effect than the extracts.

Methanol extracts of the parts of *Ceiba pentandra* used in this study were effective against the microbes (*Staphylococcus aureus, Enterococus faecalis, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumonia)* used in investigating antimicrobial activity of the extracts. Minimum Inhibitory Concentration (MIC) of the extracts ranged from 1.5625 - 12.5 mg/mL whilst Minimum Bactericidal Concentration (MBC) ranged from 6.25 - 25.0 mg/mL against the microbes used. Ciprofloxacin which was used as a referenced standard drug had MIC which range from 0.0244 - 0.3906 mg/mL whilst its Minimum Bactericidal Concentration (MBC) also ranged from 0.0244 – 0.3906 mg/mL. Hence, *Ceiba pentandra* could undoubtedly be useful in herbal medicine formulations.

5.3 Recommendations

Based on the finding of this work, *Ceiba pentandra* could open the floodgate into discovering new and potent agents against microbial resistance to some existing orthodox medicines, free radical degenerative diseases, efficacious anthelmintic drugs and inflammatory triggered diseases among others. Based on the above mentioned potentials of *Ceiba pentandra*, the following recommendations are made:

1 Thin-layer chromatography analyses of *Ceiba pentandra* should be conducted on the extracts to isolate and characterize active compounds and identify their bioactive components.

2 Toxicity study of the extracts should be conducted to evaluate extent to which an exposed tissues, cells or organs would be affected by active compounds of the extracts.

3 In vivo anthelmintic assay should also be conducted and the extracts compared the results with data established by international bodies (such as the WHO) to

help determine the extent to which herbal medicines developed using *Ceiba pentandra* could compete with existing orthodox drugs of established potency.

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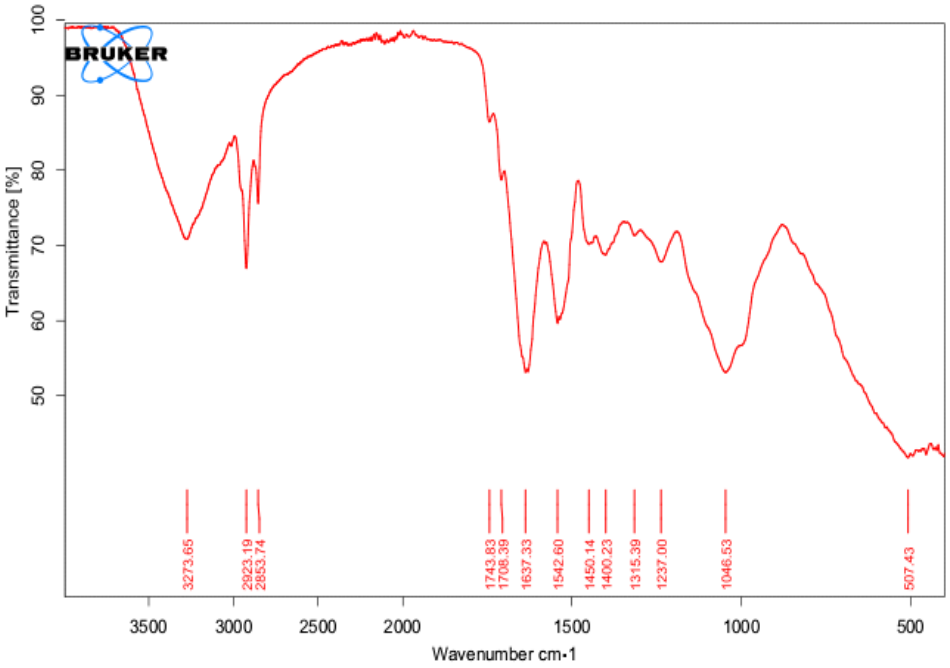
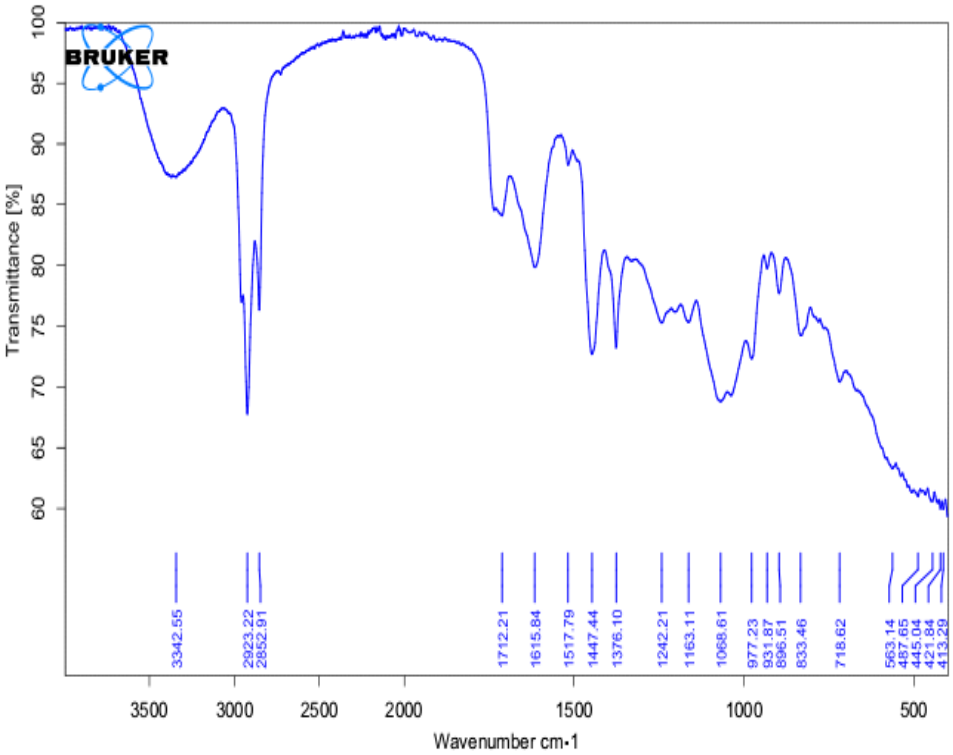
APPENDICES

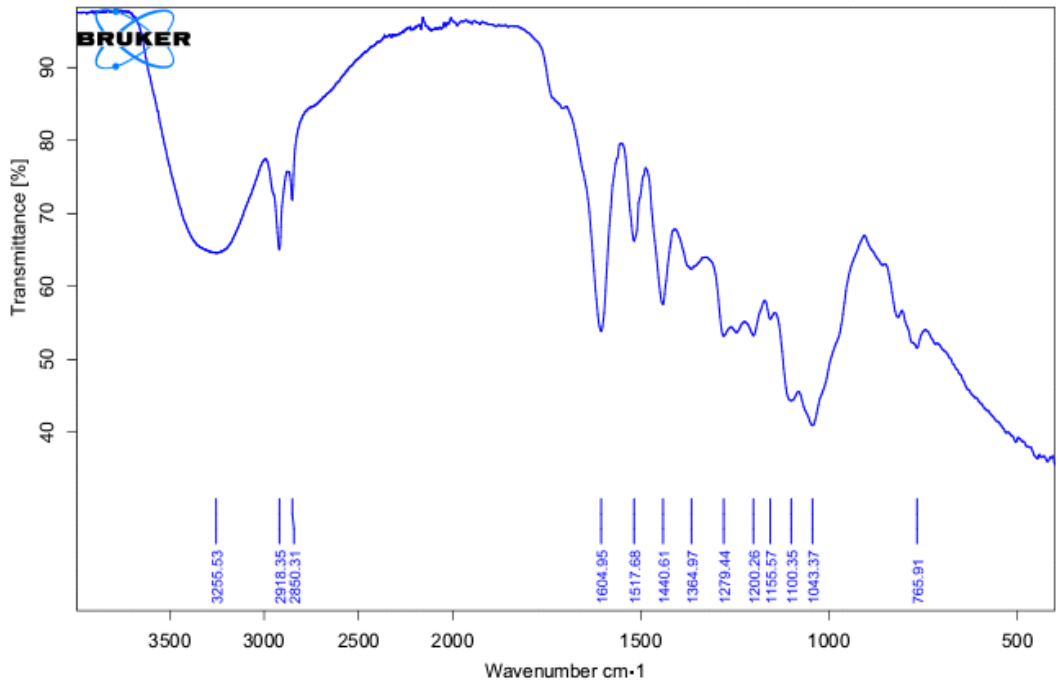
(A) Figure 25: IR Spectrum of methanolic seed extract of *Ceiba pentandra*



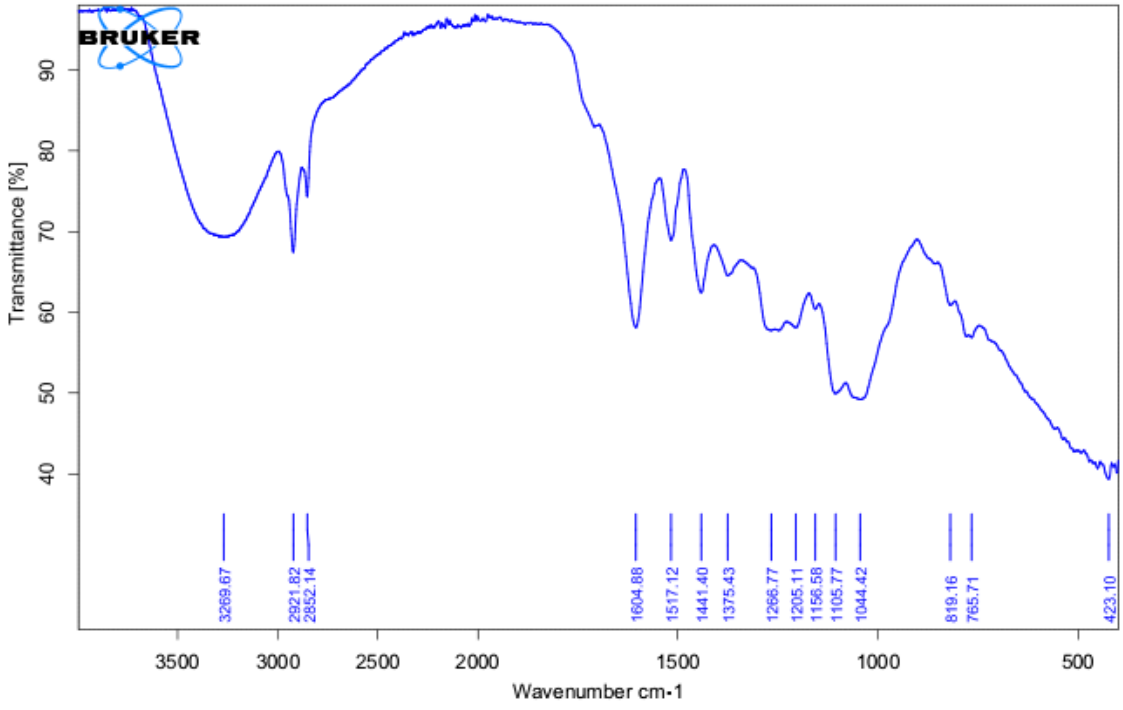
(B) Figure 26: IR Spectrum of methanolic leaves extract of *Ceiba pentandra*



(C) Figure 27: IR Spectrum of methanolic stem bark extract of *Ceiba pentandra*



(D) Figure 28: IR Spectrum of methanolic root extract of *Ceiba pentandra*



(E) Figure 29 Map of Lambussie-Karni District

Source: Ghana Statistical Service, GIS