KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

PHYTOCHEMICAL SCREENING, ANTIMICROBIAL, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF METHANOLIC, AQUEOUS AND PET ETHER EXTRACTS OF THE LEAVES OF *ALLANBLACKIA PARVIFLORA* (A. CHEVALIER)

BY

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DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY OF THE FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES KNUST, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN PHARMACEUTICAL CHEMISTRY

MAY, 2016

DECLARATION

I hereby declare that this thesis is my own work and was carried out by me under the supervision of Dr. Isaac Ayensu and that no previous submission on this topic has been made to this University or any institution for the award of any degree of a university. Related work by others has been duly acknowledged by reference to the authors.

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DEDICATION

To my brother; Simon Onilimor

ACKNOWLEDGEMENT

"O give thanks unto the Lord; for he is good: for his mercy *endureth* forever." Pslam 136:1

Thank you Lord for your numerous grace, mercy and wisdom throughout the period of this research.

My profound gratitude goes to Doctor Isaac Ayensu my supervisor, a man of great resource and influence. Sir, your time and effect spent on me would be rewarded by God. I am forever grateful. I also appreciate greatly the support of all the lecturers of the Department of Pharmaceutical Chemistry, KNUST and Dr. Osei-Djarbeng, lecturer at Kumasi Polytechnic, Department of Pharmacy Technology.

I thank all the technical staff of the Department of Pharmaceutical Chemistry, Department of Pharmacognosy, Pharmacology and Pharmaceutical Microbiology all of the Faculty of pharmacy and Pharmaceutical Sciences, KNUST, for your support offered me in all the experiments carried out at their respective Departments.

To my family and friends, Simon Onilimor, Simon Bangni, and Paulina, may God richly bless you for your support and encouragement.

ABSTRACT

The research has investigated the phytochemical constituents, antimicrobial, anti-inflammatory and antioxidant activities of various extracts of *Allanblackia parviflora*. The Phytochemical screening revealed the presence of flavonoids, tannins, reducing sugars, alkaloids glycosides, anthraquinones, terpenoids, steroids and cardiac glycoside with the absence of saponins.

Antimicrobial investigation involving agar well diffusion and broth dilution methods were used for preliminary examination and MIC determination respectively. The aqueous extract showed the highest activity against *Candida albicans* with zone of inhibition of 15.00 mm and MIC of 10 mg/ml. The methanolic extract showed activity against *Staphylococcus aureus* with a zone of inhibition of 12.00mm and MIC of 5 mg/ml with no activity from pet-ether extract.

Antioxidant activity of the extract was established using DPPH scavenging assay, total antioxidant capacity and total phenol content. The extracts were found to possess DPPH scavenging activity with IC_{50} of 48.97µg/ml for the methanolic extract, 158µg/ml for the aqueous extract and1479 µg/ml for the pet ether extract. The standard (ascorbic acid) gave an IC_{50} of 29.91µg/ml. Total antioxidant capacity values obtained were 14.7953 mg/g, 13.7681 mg/g and 12.6185 mg/g for the methanolic extract, pet ether extract and aqueous extract respectively. Total phenol content values were 21.515mg/g for methanolic extract, 19.1836mg/g for pet ether extract and 15.66mg/g for aqueous extract.

An in vivo carrageenan induced paw oedema method was used to assess therapeutic and prophylactic anti-inflammatory activity of the extracts in chicks.

The methanolic extract exhibited the highest anti-inflammatory activity for therapeutic (300mg/kg -57.2%, 100mg/kg -54.19% and 30mg/kg53.16%) and, prophylactic (300mg/kg-54.60%, 100mg/kg-51.20% and 30mg/kg -37.52%) assessment at the various concentrations respectively.

Pet ether extract and aqueous extracts also gave significant anti-inflammatory activities at percentages of (52.30%, 30.00% and 25.43%), (45.41%, 35.96% and 23.00%) for prophylactic and (.12%, 47.00% and 25.00%), (50.90%, 46.29% and 45.41%) for therapeutic respectively

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Medicinal plants have been broadly utilized by both antiquated and cutting edge men of all societies for treating distinctive diseases. A specific plant handled in various forms can be useful in the cure of extensive variety of infections. The utilization and efficacy of herbal medicine or therapeutic plants by the world populace shows it importance in health delivery (Mathews *et al*., 1999). Ghana is rich in all the three levels of biodiversity, including genetic, species and habitat diversities (Evans 1989). Plant medication is still the backbone of health delivery of around 75-80% of all the populace, mostly in developing nations for essential social insurance due to better social adequacy, better similarity with human body and less symptoms when compares with the orthodox medicine (Tomako *et al*., 2002). Throughout the years, World Health Organization (WHO) acknowledges conventional pharmaceutical as efficacious for curing of both microbial and non-microbial diseases (WHO. 1978). Medicinal plants are utilized and available all throughout the world, however a large portion of these plants are found in tropical nations such as Ghana. These herbal plants are major sources of novel bioactive substances (Tomoko *et al*., 2002).

Reports have demonstrated that medicinal plants contain substances like aldehydes, alkaloids, unsaturated long chain fatty fats, essential oils, phenolics, steroids and other natural products. These substances are conceivably important in therapeutic applications against human and animal pathogens, including microscopic organisms such as bacteria, fungi and viruses and non-pathogenic

disease (Khan *et al*., 2003). In this scientific era, it has become imperative not to create new drugs from naturally occurring biological substances only, but increase potency of the medicinal plant and find ways of making mass production. The way and manner in which drugs are being handled in combating diseases especially in developing countries has resulted in multi-drug resistant strains of microbes for some time now. Notwithstanding this issue, unfavourable impacts on the host including hypersensitivity, immunosuppression, gastrointestinal agitation and hypersensitive responses are now and then ascribed to the utilization of these medications. This has drawn the attention of the scientific community to biologically active compounds derived from medicinal plants since they present less desirable side effects (Samrot, 2010).

More than half of all present day clinical medications are plant-based. This demonstrates plant products assume a noteworthy part in the advancement of medications by the pharmaceutical industry (Baker, 1995). The use of medicinal plants in the area of human health and nutrition has helped in improving the wellbeing of mankind. The world therefore needs new treatment options and effective therapeutic products for both infectious and non-infectious diseases. These treatment alternatives must be efficacious, safe and cost effective for the treatment of these illnesses, particularly in developing nations where monetary limitations, and expanding in resistance by pathogenic microscopic organisms to current anti-microbial and chemotherapeutics is overwhelming. Quite a decent number of medications acquired from herbal plants are being employed for the treatment of one disease or the other. Example L-Dopa from fava beans for the treatment of Parkinsion's, L-Cysteine found in plants is used as eye drops

and tropical antiinfection agents (Carter *et al*., 1989). These medications are gotten from a few sections of the plants, for example, leaves, stems and roots.

Allanblackia species are monoecious plant belonging to the family Clusiaceae located in thick tropical forest of Eastern, Western and Central Africa. It can be found in countries like Ghana, Tanzania Nigeria and Sierra Leone. It can be used for medicine, timber and shade. The fruit can be eaten and the most important use is the oil from the seeds. The tree can grow up to about 30 meters tall, it is cylindrical in shape and its diameter is about 30cm – 80cm.

The pictures below are some of the species

Figure 1.1 Shows; (A) the Allanblackia parviflora plant which is at its growing stage and (B) is Allanblackia floribunda tree bearing the fruit, inserted is the full matured fruit.

The dry seeds contain close to 67-73% of strong white fat which is used generally for cooking and soap making (Sefa, 2006). *Allanblackia* seed oil has been identified and used by Unilever in the area of margarine production,

cosmetics and other food products. In this manner, worldwide interest has been shown in the area of *Allanblackia* seeds production on a large scale. The oil of *Allanblackia* seeds is believed to be higher in quality as compared to other vegetable oils due to its dissolution ability. Lately, the oil has gotten the endorsement from European Union (EU) Novel Food Regulations that ensure its safe usage in food products (Herman, 2009). Due to high interest for the oil there is a concern that collection of the seeds might bring about abuse of this asset in a way, to the point that will weaken regular recovery and additionally biodiversity protection.

Unilever, which at present is the real purchaser of *Allanblackia* oil, estimated that the purchase of the seeds is over 100,000 tons yearly. As a result, the quantity of *Allanblackia* seeds needed by Unilever cannot be supplied by only three countries (Ghana, Nigeria and Tanzania) because Unilever produces 240 tons of *Allanblackia* oil annually and Ghana currently supplies only 110 tons of the seeds, Nigeria 60 tons and Tanzania 450 tons which does not meet the demand (Kattah, 2010). Most of the seed are gotten from the bush, and farmlands which do not meet the demand so there is the need for cultivation of *Allanblackia* plants to augment those from the farms. These endeavours will enhance the proficiency of the *Allanblackia* production, quality and quality of *Allanblackia* product.

Despite the fact that *Allanblackia* species is best known for its oil advantage, there are additionally different possibilities particularly with respect to its bioactive properties. Research has demonstrated that *Allanblackia* species have bioactive properties that can be utilized as part of curing various ailments such

as bacterial infections, fungi infections, inflammations, cancer infections, in addition to having anti-oxidant properties.

There are several species of *Allanblackia* over the world, for example, *Allanblackia parviflora* found in Guinea and Ghana, *Allanblackia floribunda* in Benin, Nigeria, DR Congo and Angola, *Allanblackia stuhlmannii* and *Allanblackia ulugurensis* found in Tanzania. The trees serve as shade for cocoa.

Allanblackia. Parviflora belong to the family Clusiaceae which is used for its oil from the seeds and locally used for medicine. It can grow up to 30 meters tall and 30cm to 80cm in diameter. In Ghana the plant can be found in the forest belt such as Ashanti, Eastern and Western part of the country (Ofori *et al*., 2008; Orwa and Oyen, 2007; Stucki, 2005).

People in Western, Eastern and Central Africa cultivate *Allanblackia* for its seed to sell and make a living, but that does not match the commercial scale demanded by industries. Oil from *Allanblackia* seeds are not too different from the other vegetable oil, it can be kept for a very long time without going bad, it can also be combined with other oils to change physical characteristic. Due to its higher melting point and composition, it makes it good as starting material without changing it to enhance consistency for production. The seeds are eaten when there is food shortage, and the fruits can be made into jellies and jams.

Allanblackia parviflora has not been properly exploited in the area of it medicinal properties as compared to other *Allanblackia* species. Traditionally the bark is used in treating stomach aches, diarrhoea and dysentery. It is also used as mouthwash to relieve the pain of toothaches. The bark or the leaves are also taken for the treatment of asthma, bronchitis and cough. The bark is

pounded and rubbed on the body to relieve painful conditions. Liquid squeezed from the bark is a component of medicine used to treat urethral discharge.

1.2 Problem Statement

The emergence and the spread of multiple drug-resistance to microbial infections and those of synthetic drugs poses a serious problem in dealing with infectious diseases, especially antibiotics. As multiple drug-resistance is on the rise and new infections are escalating, there is a reduction in the number of newly accepted drugs especially antibiotics onto the market. In 2003, about six new antibiotics were approved even though the societal need for these drugs are very high (Donadio *et al*., 2010).

The continual search for these drugs to treat infection, the development of new bioactive compounds from plants and other natural products are in the right direction. Also the cost of synthetic drugs is as a result of the cost of raw materials used and the time spent in developing these drugs. In addition, the decline in discovery of antibiotics is as a result of time frame in using these antibiotics when someone has been prescribed to use them as compare to other drugs used for other chronic diseases (Fischbach and Walsh, 2009). Another cause of decline in antibiotics discovery is due to the restriction of newly developed antibiotics unlike any other newly approved drugs that can be prescribed without restriction.

1.3 Research Justification

In respect of these problems in relation to multiple drug-resistance and the cost of synthetic drugs, it is therefore necessary for new drugs (antimicrobial and non-antimicrobial agents) to be discovered and developed to help in the

reduction of multiple drug-resistance and the cost of synthetic drugs and also reduce the spread of new infection. The search for new bioactive compounds to develop new antimicrobial and non-antimicrobial drugs using herbal plants cannot be ignored. Herbal plants have played important roles in traditional medicine and a lot of bioactive compounds can be found in these plants that can be used to develop new drugs.

In acute microbial infection and chronic diseases there is formation of inflammation, since the body uses this mechanism to respond to invasion of both microbial infection and tissue damage. When that happens, there is the need to prevent the inflammation from getting to chronic stage which leads to other chronic infections, so the development of anti-inflammatory drugs to prevent such situations is very important.

Recently, the search for anti-inflammatory agents that counter the effect of microbial infection and tissue damage is going on. Herbal plants have been given much attention since most of these plants contain steroidal compounds which are a major source of anti-inflammatory and other bioactive compounds that play major roles in developments of analgesic.

Continual exposure to radiation and biological processes such as metabolism in the human body has led to the formation of free radicals which can lead to various illnesses such as cancer. Antioxidants drugs are therefore needed to be developed to help counteract these free radicals to prevent excessive damage to the cells of an organism. Currently, researchers are investigating for antioxidants to help reduce the effect of free radicals. Herbal plants have been given great attention in this search, since most dietary and other constituents of

these plants such as ascorbic acid (Vitamin C), phenols, coumarins, and flavonoids have shown to possess major antioxidant activity (Devasagayam *et al*., 2004).

1.4 Research Objectives

1.4.1 General Objectives

The aim of the research is to investigate the anti-inflammatory antioxidant and anti-microbial potential of methanolic, pet ether and aqueous crude extracts of the leaves of *Allanblackia parviflora*.

1.4.2 Specific Objectives

1. To screen for phytochemical constituents in pulverised and methanolic, aqueous and pet ether of *Allanblackia parviflora* leaves
2. To determine Anti- inflammatory activities of methanolic, aqueous and pet ether extracts of *Allanblackia parviflora* leaves
3. To determine Anti-oxidant and free radical scavenging activities of methanolic, aqueous and pet ether extract of *Allanblackia parviflora* leaves
4. To determine anti-microbial activities of methanolic, aqueous and pet ether extract of *Allanblackia parviflora* leaves
5. To determine minimum inhibitory concentration of methanolic, aqueous and pet ether extract of *Allanblackia parviflora* leaves

1.5 Research Delimitation

The research involves the use of methanolic, pet ether and aqueous extract from the leaves of *Allanblackia parviflora* in determining anti-microbial, antioxidant and anti-inflammatory activities of the plant extracts. The research also seeks to

identify the phytochemical constituents present in the plant and also carry out chromatographic analysis of the plant extract. Time permitting, we may also wish to carry out elementary composition analysis, some spectroscopy analysis and determine the antimicrobial activities of the various isolates from column chromatography. .

1.6 Area of Study

The area of study is pharmaceutical chemistry which include medicinal chemistry, the area that deals with the discovery and development of new compounds either from the synthetic means or from natural products such as plants. The plant is being investigated for its antioxidant and antiinflammatory properties as well as bioactive activities against microorganisms. These activities have been demonstrated in different species of the same plant in other geographical locations.

1.7 Organisation of Study

The present work has been organised into five chapters. Chapter one of the study introduces the problem statement, justification of the study, general objectives and describes the specific objectives of the study, research delimitation and area of study. Chapter two presents a review of literature and relevant research associated with the problem addressed in the study. Chapter three describes the methodology and procedures used for data collection and analysis. Chapter four contains the analysis of the data and presentation of the results.

Chapter five discusses the result of the research findings, implications for practice, conclusion and recommendations for further research.

CHAPTER TWO

LITERATURE REVIEW

For a considerable length of time, medicinal plants have been utilized everywhere throughout the world for treatment of different diseases. In developing nations where infectious diseases are endemic and current human resources and health facilities are grossly deficient and the cost of orthodox medicine is high, people relies on herbal medicines. In Africa, traditional medicine is of considerable worth and more than 70% of the general population allude to traditional healer's concerning wellbeing issues (kamenzy *et al*., 2002). In Ghana, as in other African nations, several roots, leaves, fruit, stem barks of plants are utilized for various medicinal purposes. Some of them have been found to contain numerous agents high in secondary metabolic such as phenols, tannins, alkyl flavonoids, steroids, glycosides, sugars and other volatile oils. These properties make them therapeutically important in their activities (Cowan., 1999; Vastoden and Rabe, 2000). A few plant species have been tried for anti-inflammation and antioxidant properties, however a larger part has not yet been satisfactorily assessed.

2.1 *Allanblackia Species*

In the 19th century, European botanists extensively explored the tropics for fat and oil containing plants. In their quest, many plant species were identified. In 1869, Prof. Daniel Oliver through his effort, the discovery of the genus *Allanblackia oliv* of the Guttiferae family now Clusiaceae in recent literatures was found. The first species published in the genus was *Allanblackia floribunda* found in flora of tropical Africa in Cameroon. In 1895, Engler published a

second specie *Allanblackia stuhlmannii* from east Tanzania and until now nine species are fully known following the revision of Bamps (Renaat, 2003).

Allanblackia species are monoecious plant belonging to the family Clusiaceae located in thick tropical forest of Eastern, Western and Central Africa. It can be found in countries like Ghana, Tanzania Nigeria and Sierra Leone. *Allanblackia* species generally thrive across tropical Africa because these areas are characterized by high humidity and annual rainfalls ranging between 1200-2500mm at altitudes of 400-1800m and commonly found in leached acid soil of pH between 3 and 4. There are nine species that have so far been discovered and are distributed over Africa plus a tenth imperfectly known species from Fernando Po after a taxonomic revision on the genus in 1969. The report from the revision reveals the species as A. floribunda (Oliver (1869) in Journ. Linn. Soc. X: 43) found in Nigeria and Congo-Kinshasa, A. gabonesis (Pellegr. Bamps (1969) in Bull. Jard. Bot. Nat. Belg. xxxix: 356) in Cameroon and Gabon, A. kimbiliensis (Spirl. (1959) in Bull. Jard. Bot. Brux. Xxix: 357) in Congo-Kinshasa (Kivu) and Uganda, A. Kisonghi (Vermoesen (1923) in Man. Ess. Forest. 11), A. marienii (Staner (1934) in Bull. Jard. Bot. Brux. xiii. 110) is also found in Congo-Kinshasa. A. parviflora (A.Chevalier (1909) in Veg. Ut. Afr. Trop. Franc. 5: 163) in West Africa from Sierra Leone to Ghana, A. stanerana (Exell & Mendonça (1936) in Journ. Bot., Lond. Lxxiv. Suppl., 20) in Cameroon, Congo Kinshasa and Angola and finally A. stuhlmanni (Engl. (1897) in Engl. & Prantl, Die Natürlichen Pflanzenfamilien. Nachtr. I. 249) and A. ulugurensis (Engl. (1900) in Engl. Jahrb. xxviii. 435) in Tanzania (Renaat, 2003).

2.1.1 Medicinal Uses of *Allanblackia* Species

Plants have been used since time immemorial to generally improve health and among these *Allanblackia* species are no exception. They are used traditionally in some parts of Africa where they are found to alleviate the suffering caused by certain ailments. In Ghana, the stem bark of the *Allanblackia* specie is used by the local people for the treatment of toothache, diarrhea and generally as a pain reliever (Abiww, 1990). Roots of A. stuhlmannii are used to treat rheumatism, impotence and the oil could be smeared on wounds as well as skin rashes (Munjuga *et al*., 2010).

Research reports have demonstrated that *Allanblackia* species have biological, pharmacological and bioactive properties that can be utilized as part of treating various disease such as bacterial infections, fungi infections, inflammations, cancer infections, in addition to having anti-oxidant properties (Nguemfo *et al*., 2007).

2.1.2 Review of Works done on *Allanblackia* species

Extracts from leaves, roots, fruits and flowers of *Allanblackia gabonensis* have recently been reported to display a broad spectrum activities of about 72% against some bacterial strains and fungi (Fankam *et al*., 2015: Ajibesin *et al*., 2008).

Some *Allanblackia* species investigated over the years have showed significant anti-inflammatory activities as exemplified by the *in vivo* antiinflammatory study conducted by (Nguemfo *et al*., (2007)

Allanblackia floribunda has demonstrated some antioxidant properties. (Boudjeko *et al*., 2013).

Kuete *et al* 2011 isolated some compounds from the bark of *Allanblackia floribunda* which include Allanxanthone, benophenones, 1,7dihydroxyxanthone, xanthones, morelloflavone bioflavonoids and –Oglucoside. They are said to exhibit wide a ranging of pharmacological activities including antioxidant, anti-inflammatory, anticancer and antimicrobial. Allanblackia species such as *Allanblackia floribunda* and *Allanblackia gabonensis* possess antihypertensive, anticancer, anti-malarial and analgesic activity (Yemele *et al*., 2001; Nkengfack *et al.*, 2002; Azebaze *et al*., 2015).

2.1.3 Phytochemistry of *Allanblackia* Species

Not much has been done especially in scientific literature on the phytochemistry of *Allanblackia* species in general but few authors have reported on some phytochemicals present in these species. Phytochemical screening conducted by Ajibesin *et al* (2008) on the leaves, stem bark and root bark of *A. floribunda* indicated the presence of high concentration of tannins and cardiac glycosides, moderate concentrations of flavonoids and terpenes were present with the absence of alkaloids, anthraquinones and pthlobotannins in the various plant parts. Saponins were abundant in the leaves and absent in the root and stem bark. Extracts of *Allanblackia gabonensis* were screened for the presence of major secondary metabolites and the results obtained from the screening indicated the presence of flavonoids, alkaloids, anthraquinones, phenols, tanins and anthocyanines and absence of saponins, triterpenes and steroids (*Fankam et al*., 2015).

Literature search shows that not much work have been done on *Allanblackia parviflora*, a species that is abundant in Africa where plants are normally used

for their therapeutic effects. It is therefore necessary to investigate the phytochemicals that may be present in this plant species and any possible therapeutic properties they may possess.

2.1.4 *Allanblackia Parviflora*

Allanblackia parviflora (family Clusiaceae) is used for its oil from the seeds and traditionally used as medicine. It can grow up to 30 meters tall and 30cm to 80cm in diameter. In Ghana the plant can be found in the forest belt in the Ashanti, Eastern and Western parts of the country (Ofori *et al*., 2008; Orwa and Oyen, 2007; Stucki, 2005).

People in western, eastern and central Africa cultivate *Allanblackia* for its seed to sell and make a living, but that does not match the commercial scale demanded by industries. Oil from *Allanblackia* seeds are not too different from the other vegetable oil. It can be stored for a very long time without going bad, and can be combined with other oils to change physical characteristic. Due to its higher melting point and composition, it makes it good as starting material without changing it to enhance consistency for production. The seeds are eaten when there is food shortage and the fruits can be made into jellies and jams.

Allanblackia parviflora has not been properly exploited in the area of it medicinal properties as compared to other *Allanblackia* species. Traditionally the bark is used in treating stomach aches, diarrhoea and dysentery. It is also used as mouthwash to relieve the pain of toothaches. The bark or the leaves are also taken for the treatment of asthma, bronchitis and cough. The bark is pounded and rubbed on the body to relieve painful conditions. Liquid squeezed from the bark is a component of medicine used to treat urethral discharge.

2.2 Oxidants and Anti-Oxidants

2.2.1 Free Radicals and How They Are Generated

Free radicals are atoms, molecules or ions that possess odd number of electrons. The most common chemical species that can produce free radical in living organisms is oxygen (Halliwell and Gutteridge, 1989). Oxygen (O_2) is obviously, crucial to cell digestion system and generation of energy. The breakdown of oxygen additionally delivers very receptive operators that tend to harm human tissues. There exist numerous ways by which free radicals can be generated and they are normally found in mitochondria since it utilizes most of the oxygen needed by the body. The process leads to the creation of various numbers of short-lived intermediates which include the formation of hydrogen peroxide (H_2O_2), superoxide (O_2-) and hydroxyl radical (‑OH). The oxide and oxyl radicals' each has a free electron in their external orbital which are the responsive oxidants. Hydrogen peroxide is somehow toxic to cells and can add an additional free radical, especially when there is a reduced transition metals which lead to the formation of hydroxyl radicals. Human cell is believed to generate about 327 noxious hydrogen peroxide molecules regularly (Sies, *et al*., 2000). Moreover intracellular development of unpaired molecule can be formed from the environment which include ultraviolet light, ionizing radiation, and toxins from ozone. Cell membranes happen to be the most susceptible to peroxidising responses. For instance, the formation of lipid peroxides which can breakdown to course a response that can develop into mutagen is as a result of the reaction of free radical and polyunsaturated fatty acids (Lippman, 1983). Proteins such as enzymes are also affected by these radical activities. Normally these radicals attack the amino acids and as a result, lead to damage of tissues

and cells in the body which leads to ageing (Stadtman, 1995). Another damage that free radicals can cause to the body is reaction with DNA molecules, which causes the breakage of the strands which can lead to serious damage including death. It has been shown that in a normal cell over 10,000 DNA's undergoes oxidative reaction daily (Ames *et al*., 1993). Despite the fact that repair frameworks redresses a great part of the harm, there is a link between oxidative DNA and ageing, and this is a major cause of maturity in humans (Randerath *et al*., 1995).

2.2.2 Sources of Free Radicals

There are several sources by which free radicals can be formed from. They might emerge from natural processes, for example, metabolic processes. It can likewise emerge from the environment which include; ozone gotten from air contamination, car fumes discharge, tobacco smoke, pesticides and excessive radiation. Hydrogenated oils, harmful metals and browned food when undigested might serves as sources of free radical (Diplock, 1997).

Another way that can bring about free radical formation is xenobiotic reduction under X-rays and γ-rays in the presence of oxygen (Deva Kesavan, 1996).

2.2.3 Ideas of Oxidative Stress

Sies (1986) was able to explain the connection between disease and free radicals. A normal body can prevent the formation of oxidant by production of antioxidant as a defence mechanism. But when the environmental factors such as atmospheric contaminations, cigarette smoking, ultraviolet rays, radiation, and lethal chemicals come in, this carefully kept up equalization is moved for ace oxidants bringing about 'oxidative anxiety'.

2.2.4 Antioxidants

Antioxidants are chemical substances that remove free radicals or any activity by these radicals (Sies, 1996). Living organisms have been invested with enough defensive mechanisms in their cells against any destructive impact of free radicals. The human body uses enzymic processes in the elimination of these free radicals from the cells, examples are glutathione reductase, disulphide bonding glutathione peroxidase, superoxide dismutase (SOD) and thioredoxinthiols. It has been accounted for that vitamin E is a basic supplement which works as a chain-cracking antioxidant that keeps the formation of free radical response in the cell layer of the human body (TAD *et al*., 2004). Also there are non-enzymic processes that can also be used such as vitamin C, flavonoids, carotenoids and other related polyphenols.

2.2.5 Mode of Action of Antioxidants

Antioxidants use various ways of removing free radicals and their activities from the body. They either prevent the radicals from forming or capture the free radicals when they are formed. They can also be used to repair the damage caused by these radicals. They normally use SOD that causes the dismutation of superoxide to H_2O_2 and the enzymes that would convert it to water to prevent it from forming (Sies, 1996; Packer, 1996). Capturing radicals is primarily by radical rummaging. At the repair stage, dietary supplement such as ascorbic acid, glutathione, vitamin E, carotenoids, flavonoids, and so forth can be used. (Sies, 1996; Cadenas and Packer, 1996).

2.2.6 Significance of Antioxidants to Disease

Enzyme co- factors such as zinc, cytoplasmic antioxidant and selenium all play important role in antioxidant activities. Lipid soluble antioxidant such as

vitamin E helps in the breakdown of lipid peroxidation in the membrane of cells (Packer and Ong, 1998; Kagan *et al*. 2002).

The most effective antioxidant normally used as standard to which other compounds can be compared to is vitamin E and C with vitamin E being the most effective. Recommended daily supplement for these antioxidants is about 60 mg. Other compounds that exhibit the properties of antioxidant are betacarotene, lycopene, lutein (Sies, 1996; Packer, 1996; Kagan et al. 2002).

2.2.7 New Therapeutic Trends in Using Antioxidants

Pharmaceutically developed antioxidants are to help deal with infections by using it for prevention and treatment of non-infectious diseases such as stroke, diabetes, atherosclerosis, Alzheimer's illness (AD), Parkinson's ailment, cancer, etc. Therapeutic potentials of antioxidants can also be derived from dietary supplement which help in keeping the aged and their related disease. By combining folkloric knowledge and present day science, the world can make world-class products by expanding on conventional drugs and screening the various plants and microbial sources (Jayaraman, 2003).

2.2.8 Sources of phytonutrients, Antioxidants and Functional Foods

Plants and animal products have been found to give wide range of antioxidants. A number of beverages such as tea can be a good source of antioxidants. The direct utilization of tea can protect the body against various forms of pathophysiological conditions. Tea is especially high in catechins for which epigallocatechin gallate (EGCG) is the most abundant.

In respective of this, research on the antioxidant property of *Allanblackia parviflora* is worth looking into.

2.2.9 Overview of *in Vitro* Antioxidants Assays

There are distinctive receptive oxygen species that are generated as typical physiological activities that happen in living cells. The oxygen reactive species have diverse response mechanism. Thus endeavouring to utilize one technique to assess the antioxidant activity of test samples may be analytically inaccurate.

Different *in vitro* techniques have been developed to evaluate antioxidant activities of compounds from extracts and nutritional supplements. Some of these techniques included; total phenol content assessment method, DPPH radical scavenging assay method, lipid peroxidation assay technique, total antioxidant capacity assay method, TLC autography technique method and ferric reducing power assessment technique among others.

2.3 Inflammation and Anti-Inflammation

2.3.1 Inflammation

Inflammation is a natural response of a disturbed tissue or cell homeostasis (Medzhitov, 2008). When that happens the tissue turns to utilize the proteins in the blood, leukocytes and fluid. This leads to changes in the nearby vasculature that prompt vasodilation, expanded vascular porousness, and increased blood flow.

Infection that are caused by microbial invaders is typically involved as the real culprit that advances inflammation and its related responses. Be that as it may, harm or injury, that is without parasitic disease and introduction to foreign particles or aggravations or pollutants are likewise intense triggers of inflammation (Medzhitov, 2008), giving an indication that this reaction can be advanced as a general adjustment for adapting to damage or break down tissue

(Matzinger, 2002). The reason why infection and injury may summon comparable inflammatory responses is that infection usually occurs after injuring, which suggests that it is favourable to react to injury as though disease happened (Nathan, 2002). The known clarification is that pathogens and injuries can cause harm to tissue or cells that can trigger the comparable reactions (Bianchi, 2007).

2.3.2 Mechanisms of Inflammation

Inflammation comprises of a firmly controlled physiological, behavioural and immunological processes as a result of cytokines. Inflammatory cascade involves identifying of damage or infection.

This is accomplished by identification of pathogen-associated molecule patterns (PAMPs), and are coordinated by communicated pathogens through their molecules. There is also another molecule that signals the damage and is sensed by innate immune system called Damage Associated Molecular Patterns (DAMP). This pattern cannot be identified by innate immune system except adaptive system which play important roles in an inflammatory processes (Janeway *et al*., 2005).

2.3.3 Types of Inflammation

There are two types of inflammation: Acute inflammation and chronic inflammation

2.3.4 Acute Inflammation

When an injury occurs and the body's response to the affected area is fast, the inflammation formed is said to be acute inflammation. The body does this by releasing host defenses like leukocytes and plasma proteins to the affected area.

Three main components of acute inflammation exist: (1) modifications in vascular system that result in an increase in blood flow; (2) structural changes in the microvasculature that allow plasma proteins and leukocytes to leave the circulation; and (3) movement of the leukocytes from the microcirculation. There are various stimuli that triggers acute inflammatory reaction. These are infections by microbes, physical and chemical factor, tissue necrosis, immune reaction etc.

2.3.5 Chronic Inflammation

Inflammatory responses that last for longer periods of time even at times indefinitely, is refered to as chronic inflammation. When that happens the cells or tissues that are damaged can no longer repair itself. Chronic inflammation is the cause of complications in the damaged tissue or damaged cells.

2.3.6 Anti-Inflammation

Natural compounds isolated and developed from medicinal plants have shown promising activities against numerous diseases, including inflammatory effects (Balunas *et al*., 2005). They have shown to possess a broad spectrum of therapeutic effects (Jha *et al*., 2004; Mayer *et al*., 2005).

Natural products with anti-inflammatory effects target and modulate the NFκB signaling pathway (Salminen *et al*., 2008; Folmer *et al*., 2008). Compounds isolated from plants and marine organisms containing terpenoids that possess anti-inflammatory activities makes it worthwhile as a species for research (Salminen *et al*., 2008, Folmer *et al*., 2008). Allanblackia parviflora with anti-inflammatory activity have not been documented in any publication yet. There are two main methods of anti-inflammation assay; *in vivo*, carrageenan-induced

paw oedema inflammation in animals and in vitro, based on the expression of nF-kB and coX-2, as well as the release of TNFα and NO, from LPS-stimulated Raw264.7 macrophages.

2.4 Antimicrobial Activity

Chemical agents that acts or function to inhibit the effect of microbial action by either killing or preventing microbial growth is said to be antimicrobial agents. Most of these agents have been identified and developed over the years. In 1890's, Dr. Paul Erlich used methylene in treating malaria. Also, in the early 1900's there were discovery of organic arsenical for managing Trypanosomiasis, Salvarsan 606(1909) for syphilis and Atebrin (1932) for prophylaxis of malaria. The major breakthrough of antimicrobial agents of clinical importance began in the 1920's when Fleming discovered penicillin from *Penicillium* notatum followed by the isolation of streptomycin from *Stretomycetes griseus* in 1944 and cephalosporin from *Cephalosporium acremonium* in 1945. Basically, almost all potent antimicrobial agents employed today in clinical therapies whether synthetic or natural source are produced or developed from living organisms.

There are various types of antimicrobials depending on the type of pathogen they target, they are antifungal, antibacterial, antiviral etc. The rate of microbial strains resistance to antibiotics and antimicrobial agents is on the ascendancy. The way and manner in which drugs are being handled in combating diseases especially in developing countries has resulted in multidrug resistant strains of microbes for some time now. Notwithstanding this issue, unfavourable impacts on the host including hypersensitivity, immunosuppression, gastrointestinal

agitation and hypersensitive responses are now and then ascribed to the utilization of these medications (Dabur *et al*., 2007). However in developing countries like Ghana the issue is not only attributed to the reason stated above rather the non-availability, side effects as well as the higher cost of these drugs has made the treatment of microbial infections a major problem in the country. As a result most people resort to medicinal plants and other traditional means to alleviate the suffering caused by these infections. These medicinal or traditional preparation are believed to contain inhibitory chemicals that will either kill or prevent the growth of the pathogenic microbes. Plants employed for these activities are said to be called medicinal plants.

2.4.1 Mechanism of Antimicrobials Agents

Antimicrobial agents are said to obstruct chemically with the synthesis of function of vital components of microorganisms in order to treat or prevent infections. The various ways in which these agents exhibit their activities are categorized in accordance with their mode of action. These mechanisms include interaction with cell wall and cytoplasmic membrane, enzymatic activity inhibition and protein synthesis, inhibition of metabolic pathway and nucleic acid interaction (Tenover, 2006).

2.4.2 Overview of *In Vitro* Antimicrobial Assays

Microorganisms that causes microbial infections behaves in a way that using one assay method will not give the right picture of antimicrobial agents, therefore, the agents needs to be quantitatively assessed to help ascertain their activities. Thus endeavouring to utilize one technique to assess the antimicrobial activity of test samples may be analytically inaccurate.

2.5 Phytochemical Constituents

These are naturally occurring chemical compounds found in plants, animals and micro-organisms and are sometimes called secondary metabolite that play important role in living organism. Some of these secondary metabolites are; Alkaloids, Saponins, Reducing sugars, Phenolics, Polyuronoids, Anthracenoside, Flavanoids, Titerpern, steroids, and cynogenic glycosides. Some are reported to be present in some species of *Allanblackia* such as *Allanblackia floribunda*, *Allanblackia gabonensis* and *Allanblackia monticola*, few of these secondary metabolites have been isolated and believe to have pharmacological activity (Azebaze *et al*., 2006; Fankam *et al*, 2015).

2.5.1 Alkaloids

Among the earliest isolated pure compounds of biological importance are the alkaloids. This is due to the simplicity of isolation. Alkaloids are basic in nature and exist in the plant as salt due to the nitrogen present in them. They can be extracted using water or weak acid which can be recovered as a crystalline material (Sofowaora, 1993). Alkaloids are toxic and physiologically active compounds derived from plants. They contain nitrogen in their rings and are heterocyclic in nature. Readily available alkaloids are the tropanes and solanaceaes. Plants with these secondary metabolites are believed to be dangerous, but possess valuable therapeutic properties. *Allanblackia* species have been reported to possess this secondary metabolite, but specific alkaloids have not been isolated from the plant yet (Fankam *et al*, 2015). Alkaloids in nature have been shown to have anti-analgesic, antiinflammatory, antimicrobial effects as well as an anaesthetic properties (Abdel-Hadi, 2014; Lotito *et al*., 2011).

2.5.2 Terpenoids

Terpenoids are the largest secondary metabolite in nature and are divers also. They are referred to as isoprenoids and are isolated from isoprene unit and can be modified in various forms. Apart from their functional groups terpenoids have multicyclic structure that differentiate them based on their isoprene unit. They are lipid in nature and exist in both plants and animals, their roles in traditional medicine is in the area of antineoplastic, antibacterial and other therapeutic effects (Kessler *et al*., 2014). Due to their aromatic properties they are extensively used in various forms. The scent of flowers in plants is due to terpenoids (Evans. 1989). *Allanblackia floribunda* has been reported to have this secondary metabolite (Ajibesin *et al*., 2008).

2.5.3 Amino Acids

Amino acids are the precursors of protein in plants. There are many amino acids in plants, being used by plants to guard themselves against intruders. They are used by humans in cellular functions, some are toxic to humans and some are used to developed drugs like L-Dopa from beans.

Drugs developed from amino acids are used in the management of Parkinson's diseases, eye drops and topical antibodies. Amino acids developed medicine such as L- arganine stimulates hormonal growth. Others present in coffee, liquor ice, sugar beet, are neuro-excitatory. For example L- aspartic acid (Harborme, 1983).

2.5.4 Steroidal Compounds

Steroids are natural compounds, which control the reproductive system of humans, molt insects and aquatic fungi. Therapeutically, steroids are used as

cardiotonics (precursors of Vitamin D, oral contraceptives), possess antiinflammatory properties (corticosteroids) and used as anabolic agents (androgens). Steroidal compounds are plant glycoside with 4-member hydrocarbon ring both in animals and synthetic steroids. In plants, there are two types of steroids which are steroid saponins, and steroidal alkaloids, (Harborne and Baxterv1984). Steroidal secondary metabolites from plants plays an important role both in plant and animals. They are used as medicine ranging from topical antibiotics to relieving dysmenorrheal (Evans, 1989). Research has shown the presence of steroids in *Allanblackia floribunda* (Ajibesin *et al*., 2008).

2.5.5 Phenolic Compounds

Phenols are secondary metabolites with their chemical structures having aromatic ring and hydroxyl group on the ring. They are the most found compound in plants. They are used in food and beverages. Phenols serves as parent structure for most secondary metabolites in plants (Harborne Baxter, 1984). Some of these compounds, isolated from *Allanblackia* specie are Allanxanthone, benzophenones, 1, 7-dihydroxyxanthone, xanthones, morelloflavone bioflavonoids and –O-glucoside which are said to exhibit wide a range of pharmacological activities including antioxidant, anti-inflammatory, anticancer and antimicrobial activity(Kuete *et al* 2011).

2.5.6 Flavonoids

Flavonoids are secondary metabolites and constitutes closes to half of all known phenols that have been discovered in plants. Therapeutically flavonoid plays very important role. It also plays a role in plant pollination of all plant species and gives the plant unpleasant taste to repel herbivores. Flavonoids give plants

fruit and flowers colour. They are used as antioxidants, anticarcinogens, counter stress, help in fighting allergies, and active against virus (Evans, 1989). Certain flavonoids help in protein synthesis, and some are anti- inflammatory agents. Others also show some vaso- protective, diuretic, antispasmodic, antibacterial, and antifungal activity (Harborne and Baxter, 1984). Flavonoids are classified as flavonoid glycosides, flavonals, isoflavones and xanthones. They help in building blood cell walls (Harborne and Baxter 1984). Some of these constituents have been isolated from *Allanblackia floribunda* and *Allanblackia monticola* (Kuete *et al* 2011).

2.5.7 Tannins

Tannins are phytochemical constituent which are made up polyphenols. These compounds are grouped into hydrolysable and non- hydrolysable tannins, and condensed tannins. Tannins are used as antiseptic, but when overused it can be hepatotoxic especially hydrolysable tannins. Harmameli tannin, have pharmacological activity and are found in men's aftershave lotion. Tannins possess antibacterial properties and research is ongoing on their anti-cancer actions (Evans, 1989). Tannins have been reported to be present in *Allanblackia Floribunda* and *Allanblackia gabonensis* (Kuete *et al* 2011;

Fankam *et al*, 2015)

2.5.8 Glycosides

Glycosides are sugar ethers which consist of a sugar group called glycon and aglycon which is a non-sugar component. Glycosides are not phytochemical constituent on their own like terpenoids, phenols, and alkaloids. When these constituents are in their glycosidic states they possess a good therapeutic effect.

Plants containing glycosides are different from each other by the substituents present in them, such as nitrogen, oxygen and sulfur. Their formation and their activities are as a result of interaction of these elements with carbon. The formation of glycosides in major phytochemical constituent is due to the sugar ether which can bind to molecules in many ways. Classification of glycosides is based on the aglycon group and this gives different therapeutic properties and medicinal applications. Some of the therapeutic effect of glycosides are analgesics, antihirheumatics and it is also believed to be a precursor for salicylic acid. Anthraquinone glycosides possesses laxative properties (Evans, 1989).

A. Lactone Glycosides

The fragrant smell of plant is as a result of the presence of lactone glycosides which are called **Coumarins**. Medicinally, coumarin glycoside possess esheamorrhagic, antifungicidal, and antitumor properties. It is also used for treatment of lymphedema. Dicomarol from lactone glycoside is used as an anticoagulant (Farinola *et al*., 2005). Plants that possess Coumarins have properties that prevent animals from grazing them. Coumadins and dicoumerol derivatives are used as rodenticides (Piller, 2005).

B. Cardiac Glycoside

Synthetic cardiac glycoside derivatives are used in the management of heart and cardiac related diseases. In plants they are identified as secondary metabolites and can also be found in animals. They are mainly used in the treatment of cardiac failure, as a result of their arrhythmia effects. Their mode of action is by increasing the cardiac output and thus slowing ventricular contraction and allowing more time for ventricular filling. Drugs with cardiac glycosides are

ouabain and digoxin and often used clinically and experimentally (Doherty, 1985)

Cardiac Glycosides are also triterpenoid groups of compounds found in living organisms. Research on their therapeutic activities on cardiovascular system is still ongoing. However, it has been shown that it has effect on myocardial infarction (Antman and smith, 1985).

2.5.9 Saponins

Saponins are compounds found in living things. They are also class of secondary metabolites. The name saponin is originated from soapwort plant, member of the genus Saponaria because of its roots being used as soap. Saponin possess soapy foam-like in nature when shaken in aqueous solutions. It has hydrophilic glycosides moieties linked to a lipophilic triterpene derivatives. Saponin has a bitter taste. They are found in most plant species and marine organisms (Liener, 1980). Saponins helps plants fight against microbes and also enhance nutrient absorption by plants. Saponins possess cardio-active agent digoxin which is therapeutically useful. They aid in digestion of food in animals, especially those found in oat and spinach. Some also serves as antifeedants to livestock due to their toxic in nature (Riguera 1997). Saponins isolated from *Allanblackia monticola are* stigmasterol-3-Obeta-D-glucopyranoside (Azebaze *et a*l., 2006).

2.5.10 Anthraquinones

They are aromatic organic compounds with a molecular formula $C_{14}H_8O_2$. They are also called anthracenedione or dioxoanthracene and can be viewed as a diketone derivatives of anthracene. Their ketone groups are on the central ring and they belong to the family quinone which are the building block of dyes and also use as bleaching pulp for paper making. They are crystalline solid and

yellow in color, they are not dissolvable in water but inorganic solvents. They have antimalarial properties and also used in the treatment of cancer. (Muller-Lissner, 1993).

CHAPTER THREE

METHODOLOGY

3.1 Materials

3.1.1 Chemicals for Analysis

The following drugs, reagents and chemicals were obtained from the designated sources; tannic acid (Fluka U.K) diphenyl-picryl-hydrazyl (DPPH) (sigma Aldrich USA), sodium carbonate, disodium hydrogen phosphate, ammonium molybdate folin-ciocalteu and ascorbic acid (all from BDH Chemical Laboratory U.K), tragacanth, saline, DMSO, methanol, petroleum ether, ethanol, chloroform, ferric chloride, meyer's reagent, hydrochloric acid, sulphuric acid, diethyl ether, picrate pepper ammonia hydroxide fehling solution I and II, acetone, acetic anhydride were of analytical graded Diclofenac powder and carrageenan, were gifts from Ernest chemists Ltd (Ghana) and University of Greenwich (UK) respectively.

3.1.2 Apparatus and Equipment

Apparatus and equipment employed in the analyses included, calipers (Powerfix IAN56288 Model No: Z22855), autoclave, incubator, water bath, electronic balance, 96-well micro-plates, fume chamber, rotary evaporator(R210 Buchi, Switzerland), petri dishes, micropipette, test tubes, micro-plate reader(Synergy H1, serial number: 271230)., Bunsen burner, separating funnel, beakers, measuring cylinder, media bottles, funnel and conical flasks.

3.1.3 Plant part, Microorganisms, *in-vivo* model, and Media

Leaves of *Allanblackia parviflora*, microbial organisms including *Candida albicans* (clinical strain), *Bacillus subtillis* (NTCC-10073), *Eschariccia coli*

(ATCC-25922), *Entrococcus faecalis* (ATCC-29212), *Klebsiella pneumoniae* (clinical strain), *Pseudomonas aeruginosa* (ATCC-4853), *Salmonella paratyphi* A (clinical strain), *Staphylococcus aureus* (ATCC-25923), *Neisseria gonorrhea* (clinical strain) and *Streptococcus pyogenes* (clinical strain), 7 day old chicks, and media (Mueller Hinton agar and Nutrient Broth).

3.2 Methods

3.2.1 Sample Collection and Authentication

The leaves of *Allanblackia parviflora* were collected from some farmlands in Afosu (Eastern region) and was authenticated by Mr. Osafo Asare at the Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Science. The species was assigned the voucher specimen number KNUST/HMI/2015/LO10 for reference purposes and deposited at the department's herbarium.

3.2.2 Sample preparation

The leaves of *A. parviflora* were cut into small pieces, washed with water, airdried under room temperature for 2 weeks and pulverized into fine powder. 200g of the powdered form was serially extracted with methanol, water and petroleum ether using maceration for 72 hours. Filtration of the extracts was done and concentrated under normal pressure using rotary evaporator (Type R-210 Buchi, Switzerland) to yield 14.4%, 2.21%, and 6.52%, w/w of methanol leaves, aqueous leaves and pet-ether leaves of the crude respectively.

The crude extract was stored in a refrigerator until required.

3.3 Phytochemical screening of *Allanblackia parviflora*

Qualitative test for the screening and identification of bioactive secondary metabolite in the leaves of *Allanblackia Parviflora* were investigated using

standard procedures employed by Ayensu and Quartey, (2015). The methods are briefly described below

3.3.1 Alkaloids

2% sulphuric acid was added to about 0.2g of the powdered sample, boiled and filtered. About 5 drops of Dragendorf's reagent was added to 1ml of the filtrate. The formation of a characteristic orange brown precipitate shows alkaloids presence. A confirmatory test was performed by the addition of 2 drops of Mayer's reagent to 1ml of the acidic extract. The absence of a buff white precipitate confirms the presence of alkaloids.

3.3.2 Anthraquinones

2mls of dilute H_2SO_4 and 5% of aqueous $FeCl_3$ was added to 0.2g of the powdered sample and boiled, for 5 minutes. The hot mixture was filtered, cooled and shaken with equal volumes of chloroform. Separated chloroform layer was then shaken with half its volume of dilute ammonia solution, formation of a pink rose coloration indicate the presence of anthraquinones.

3.3.3 Reducing Sugars

5ml of dilute HCl was used to extract 0.2g of the plant sample on a water bath for about 2 minutes. Several drops 20% NaOH was added to the filtrate after the mixture was filtered. Fehling's solution A and B were added 1ml each to the alkalized filtrate, then heating was done for about 2 minutes on water bath. Formation of brick red precipitate indicate presence of reducing sugar.

3.3.5 Flavonoids

5ml of distilled water was added to about 0.2g of the powdered sample and boiled for about 2minutes and filtered. 20% of NaOH was added to 1ml of the filtrate and the formation of an intense yellow coloration which disappeared

when exposed to fumes of conc. HCl shows flavonoids presence. For confirmatory test, 1ml of lead acetate was added to the filtrate, formation of yellow color confirm the presence flavonoids.

3.3.6 Glycosides

70% ethanol was used to extract 0.2g of the powdered sample, and the mixture was filtered. 1ml glacial acetic acid was added to 5ml of the filtrate and traces of ferric chloride. Concentrated $H_2SO_4$was added. The formation of reddish brown ring at the interface indicate the presence of cardiac glycosides.

3.3.7 Phenols

0.5g was Extracted with ethanol and filtered. 3-4 drops of ferric chloride solution was added. Bluish black color formation indicated the presence of phenols

3.3.8 Phytosterols

Chloroform was added to 0.2g of the pulverized plant sample and filtered.

Drops of acetic anhydride was added to 1ml of the filtrate, boiled and cooled, Conc. Sulphuric acid was then added. Brown ring formation indicate that phytosterols are presence.

3.3.9 Saponins

About 0.2g of plant sample was diluted with 10ml of distilled water and shaken vigorously for about 2 minutes in a test tube and observed for the presence of persistent foam.

3.3.10 Tanins

20ml water was added to 0.5g of the sample and boiled for 5 minutes, cooled and filtered. 10ml of distilled water was added to 1ml of the filtrate and 1 %

lead acetate solution 5 drops was added. White precipitate indicate the presence of tannins

3.3.11 Triterpenoids

0.2g of powdered sample was shaken in chloroform to obtain a chloroformic layer and then filtered.3-4 drops of concentrated H_2SO_4 was added to the filtrate, it was shaken and allowed to stand for 15 munities. Effervesce and a clear reddish brown color at the interface shows terpenoids presence

3.4 *In Vitro* Assessment of Antimicrobial activity

Two methods were carried out for the antimicrobial assessment; nutrient broth dilution method and agar well diffusion method.

10 organisms were employed in the assay including, four gram positive bacteria (*Staphylococcus aureu* (ATCC-25923), *Enterococcus faecalis* (ATCC-29212), *Bacillus subtillis* (NTCC-10073) *and* Streptococcus *pyogenes* (clinical strain)), five gram negative bacteria (Escharicci*acoli* (ATCC-25922), *Klebsiella pneumoniae* (clinical strain), Pseudomonas *aeruginosa* (ATCC4853), S. *paratyphi* A (clinical strain) and, *Neisseria gonorrhea* (clinical strain) and one fungi candida *albican* (clinical strain).

The microbial cultures were obtained from the Microbiology Department of the faculty of Pharmacy and Pharmaceutical sciences (KNUST). The cultures were maintained by sub culturing periodically using nutrient broth.

3.4.1 Agar Well Diffusion Assay

Determination of antimicrobial activity of each plant extract was assessed using the agar well diffusion method employed by Linthoingambi & Singh, 2013.

Culturing of microbes was done using nutrient broth and incubated at 37^{o}Cfor 24 hours. The microorganisms were then subcultured and incubated for another 24hours at 37^{o}C to obtain pure colonies. About 100g of MuellerHinton agar powder was weighed and prepared in distilled water, the media was then sterilized in an autoclave for 1hour at 120^{o}C to prevent contaminations.

The petri dish and other equipment used in preparing media were also sterilized. About 100ml of the prepared media was then poured into the petri dish and allowed to solidify. It was incubated at 37^{o}C for 24hours to eliminate contaminated plates. The subcultured microbes were picked from test tube and spread on the media in the petri dish using micro pipette and sterilized cotton swab and allowed to dry. Wells were then made on the media using a sterilized cork borer (3mm in diameter). Appropriate labels were given to each well based on the extract.

0.2g of the dry crude methanolic and pet ether extract were dissolved in 10ml each of 2% dimethyl sulfoxide (DMSO) and 0.2g aqueous extract was dissolved in 10ml distilled water.

100µL of 0.02g/ml of each extract were inoculated into each well, allowed for the extract to diffuse into the media and subsequently placed in the incubator at 37^{o}C for 24hours. The analyses were done in triplicate.

The effect of the extract on the organisms shows a clear zone of inhibition after incubation, the results are recorded in millimeters by measuring the length of zone of inhibition using ruler. Positive control used was ciprofloxacin at a concentration of 0.0001g/10ml and the negative control was the various solvents.

3.4.2 Broth Dilution Method (Minimum Inhibitory Concentration (MIC))

Broth dilution method also known as MIC was carried out on the extracts that gave antimicrobial activity in the well diffusion method. Determination of MIC was carried out using the broth dilution method as employed by Linthoingambi *et al*., (2013).

In this method, the organisms were prepared from a 24hour nutrient broth culture which was then adjusted to obtain a suspension of 10^8cfu/ml.

The plant extracts of 2% were dissolved in 2% (DMSO), serial dilutions were done to obtain concentrations ranging from0.158mg/ml to 20mg/ml. Sterilized 96-well micro-plates were used. In each well was placed, 100µl of double strength nutrient broth, 80µl of each prepared extract and 20µl of the various cultures organisms.

Incubation of the plates was done for 24hours at 37°C. The growth of the organisms were estimated by adding 20µl of tetrazolium salt solution (MTT), which was further incubated for about 15minute. A dark colour in the well implies the presence of microorganisms, since the enzymes in the live organisms react to form a dark complex with the tetrazolium salt. .The MIC is read as the lowest concentration without the visible growth, which is characterised by a dark colouration. Ciprofloxacin and DMSO were used as positive and negative controls respectively. All experiment were triplicated.

3.5 *In Vitro* Antioxidant Assessment

3.5.1 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging Assay

The free radical scavenging activity was estimated as described by Govindarajan *et al*., (2003).

Concentrations of the crude extracts ranging from 62.5µg/ml to 500µg/ml were prepared for the analysis. 20mg/L concentration of DPPH dissolved in methanol was used.1ml each of the extract and 3ml of the DPPH was used. The reaction mixture was kept at 25^{o}C for 30 minutes. The process was repeated for concentrations of ascorbic acid with concentrations (6.25µg/ml - 50 µg/ml). The DPPH absorbance was then estimated at 517 nm using microplate reader. Radical scavenging activity of the DPPH is calculated accordingly:

% DPPH radical scavenging activity = 1 - [Ao /A] x100……..Equation 3.1

Where Ao and A are absorbance of sample and control respectively.

IC_{50} was estimated (IC_{50} is the drug concentration causing 50% inhibition of the desired activity). The following equation explains the estimation of IC_{50};

$y=a + (b-a)/1+ 10^{logIC50-x.}$

Where y donates the response and x is the logarithm of concentration, a is the maximum parameter and b the minimum parameter. All analysis were done in triplicate

3.5.2 Total Antioxidant Capacity

The reaction is based on the reduction of molybdenum, Mo^{+6} to MO^{+5}, using the test samples and the formation of a complex green color phosphatemolybdate

(Mo^{+5}) at acidic pH (Prieto *et al*., 1999). The solution was prepared by adding Ammonia molybdate (4mM), Disodium hydrogen phosphate (28mM) and Sulphuric acid (0.6M). Test tubes containing 1ml each of the different concentrations of the extract (500µg/ml to 63.5µg/ml) and 3ml of the reagent solution were incubated at 95^{o}C for 90 minutes. The process was repeated for ascorbic acid concentration (50µg/ml to 6.25µg/ml). A blank solution was prepared by adding every other solution with the exception of the extract. The mixture was allowed to cool at temperature of 25^{o}C and measurement of the absorbance of the solutions were taken at a wavelength of 695nm using micro-plate reader. Concentrations of the ascorbic acid was used to construct a calibration curve. A blank solution was prepared. The capacity of antioxidant was measured as mg/g of ascorbic acid equivalent (AAE) to the extract.

3.5.3 Total Phenol

Various concentration of the extracts were prepared (500µg/ml to 62.5µg/ml) and used for the experiment. 0.1ml of each extract was added to 0.5ml of folin-ciocateu reagent, the solution was then incubated for about 15 minutes at a temperature of 25^{o}C. 2.5ml of 2% sodium carbonate was then added and incubated again for another 15minutes at the same conditions. The absorbance for the various concentrations were measured at a wavelength of 760 nm using micro-plate reader.

Tannic acid was used as standard drug. Various concentration of the drug (200µg/ml to 3.125µg/ml) was used and conditions used for the test samples were applied to it. Calibration curve was plotted using the absorbance obtained from tannic acid analysis. The content of total phenol was measured as mg/g of the tannic acid equivalent to the extract**.**

3.6 *In Vivo* Anti-Inflammatory Assessment

The method used in assessing the anti-inflammatory properties of the extracts was the *in vivo* carrageenan foot oedema paw model of inflammation in chicks by (Roach and Sufka 2003) with some modification by Woode *et al.,* (2009) was used and the reference drug used was Diclofenac. In this method, two aspect of the extract was assessed; therapeutic effect of the extract and prophylactic effect of the extract.

150 chicks (7 day old chicks) were acquired, the chicks were divided into six groups for each extract with each group having 5 chicks. The weight of the chicks were obtain and the volume of the extract for each chick was based on its weight; that is the higher the weight the higher the volume of the extract. Concentrations of 300mg/kg, 100mg/kg and 30mg/kg were prepared, using tragacanth as dissolving solvent for the extracts.

Carrageenan (10µl or 0.1ml of a 2% suspension in 0.9% saline) was prepared. Initial volume of the footpad of the chicks were measured and recorded using digital caliper, after which therapeutic and prophylactic analysis were done.

For therapeutic analysis, the carrageenan was administered into the left footpad of the chicks for about 30 minutes to 1hour before the test samples were given. The volume of the footpad of the chicks were measured hourly for six hours after administration of the test samples to determine the effects of the samples. Both the test sample and the standard drug were given orally.

For prophylactic assay, the test samples were given first and from 30 minutes to 1hour the carrageenan was induced. The quantification of the oedema

component was done by taking measurement of the difference in footpad volume before carrageenan injection and at various time point.

The concentrations for the standard drug were 10mg/kg, 30mg/kg and 100mg/kg. One of the group was injected with only carrageenan to use as control. A graph of percentage increase in paw size against time was plotted to determine the effect of the extracts.

CHAPTER FOUR

RESULTS

Investigation of the phytochemical constituents of the powdered sample and crude extracts of the leaves of *Allanblackia parviflora* was carried out. Antimicrobial, antioxidant and anti-inflammatory activities of the crude methanol, pet ether and aqueous extracts were also investigated.

The results below show the summary of the various investigations that were carried out

4.1 Phytochemical screening

Phytochemical screening results of the powdered sample and the crude extracts are summarized in Table 4.1.

Table 4.1: Results of phytochemical screening powdered and crude extracts of Allanblackia parviflora

Test	Powdered Sample	Crude Extract		
		Methanolic	Pet Ether	Aqueous
Flavonoids	+	+	-	+
Saponins	-	-	-	-
Tannins	+	+	-	+
Alkaloids	+	-	-	+
Reducing Sugars	+	+	+	+
Glycosides	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	-	-
Table 4.1 cont'd			-	
Anthraquinones	+	+		+
Cynogenic Glycosides	-	-	-	-
Cardiac Glycosides	+	+	+	+

Key: (+) present (-) absent

4.2 Anti-Microbial Assay

Antimicrobial activities of the leaves of *Allanblackia parviflora* plant were conducted using three solvent extracts. Agar well diffusion and broth dilution methods were employed. For agar well diffusion method, the activity of the samples against the microbes were determined by measuring the length of clear zone of inhibition. For MIC, the least concentration that shows inhibition was taken as minimum inhibitory concentration.

4.2.1 Agar Well Diffusion Method

Assessment of antimicrobial activities were determined using the methanol, pet ether and aqueous extracts. The extracts were used on ten selected microbes (Four-gram positive bacteria, five-gram negative bacteria and one fungus). 20mg/ml of each extract and 0.2mg/ml of the standard drug (ciprofloxacin) were used. Table 4.2 below shows the summarized result of antimicrobial activity of the plant extracts with ciprofloxacin as the positive control. The result were recorded in millimetres.

Table 4.2: Zone of inhibition results

TEST ORGANISMS	PLANT LEAVES EXTRACTS			STANDARD	NEGATIVE
	Methanol (mm)	Pet Ether (mm)	Aqueous (mm)	Ciprofloxacin (mm)	2%DMSO (mm)
E. Coli	10.75	0.00	13.00	15.50	0.00
E. feacalis	10.50	0.00	13.00	16.50	0.00
Staph Aureus	12.00	0.00	13.50	15.50	0.00
Strep	11.00	0.00	10.25	15.50	0.00
B. Subtillis	10.00	0.00	11.50	15.50	0.00
C. albicans	9.00	0.00	15.75	24.50	0.00
Gono	8.50	0.00	10.50	15.50	0.00
S. Typh A	10.75	0.00	13.00	16.50	0.00
Pseudomonas	10.50	0.00	13.50	19.50	0.00
K. pneumonia	10.25	0.00	13.75	15.50	0.00

Key: Ciprofloxacin = Standard drug, 2% DMSO = Negative control

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4.2.2 Broth Dilution Method (MIC)

The minimum inhibitory concentration of the crude extracts that showed zone of inhibitions when agar well diffusion method was used were assessed. The extracts were methanol and Aqueous extracts. The extract were tested against ten microorganisms (four gram positive bacteria, five gram negative bacterial and one fungus). The table below shows the result of minimum inhibitory concentrations (MIC) for the extract and positive control.

Table 4.3: Broth Dilution Method (MIC) results

TEST ORGANISMS	MIC(mg/ml)		
	PLANT EXTRACTS		STANDARD DRUG
	Methanol	Aqueous	
E. Coli	5.00	20.00	0.025
E feacalis	2.50	5.00	0.050
Staph Aureus	5.00	20.00	0.050
Strep	5.00	10.00	0.025
B. subtillis	2.50	20.00	0.0125
C. albicans	5.00	20.00	0.0125
Gono	5.00	20.00	0.050
S. Typh	5.00	20.00	0.050
Pseudomonas	2.50	10.00	0.050
K. pneumonia	5.00	20.00	0.050

4.3 Anti-Inflammatory Assay

The anti-inflammatory activities of methanolic, pet ether and aqueous extracts of the leaves of *Allanblackia parviflora* were evaluated using carrageenan induced paw oedema method as presented by Roach and Sufka (2003). In this experiment, therapeutic and prophylactic effects of the extracts and the standard drug (Diclofenac sodium) were determined and statistically evaluated with respect to the control. The concentrations used for the standard were 10, 30 and 100mg/kg and that of the extracts were 30, 100 and 300 mg/kg. Percentage increased in paw sizes were evaluated and used to plot a time cost curve to determine the increase in foot size with respect to time. Total oedema was also determined using Area under the curve against concentration of each extract and the standard drug. Data are the mean ± SEM of 5 chicks. The p values were also determined using one-way ANOVA and Dunnett's multiple comparison test (*P<0.05, **P<0.01and ***P<0.001) showing the degree of significant. Percentage inhibition of the test samples and standard drug (Diclofenac) were determined using the equation $AUC_{control}–AUC_{concentration}$ of the sample /$AUC_{control}$ *100, where AUC is Area Under the curve. The figures below show the results of anti-inflammatory activities of the extracts and the standard drug of both therapeutic and prophylaxes effects.

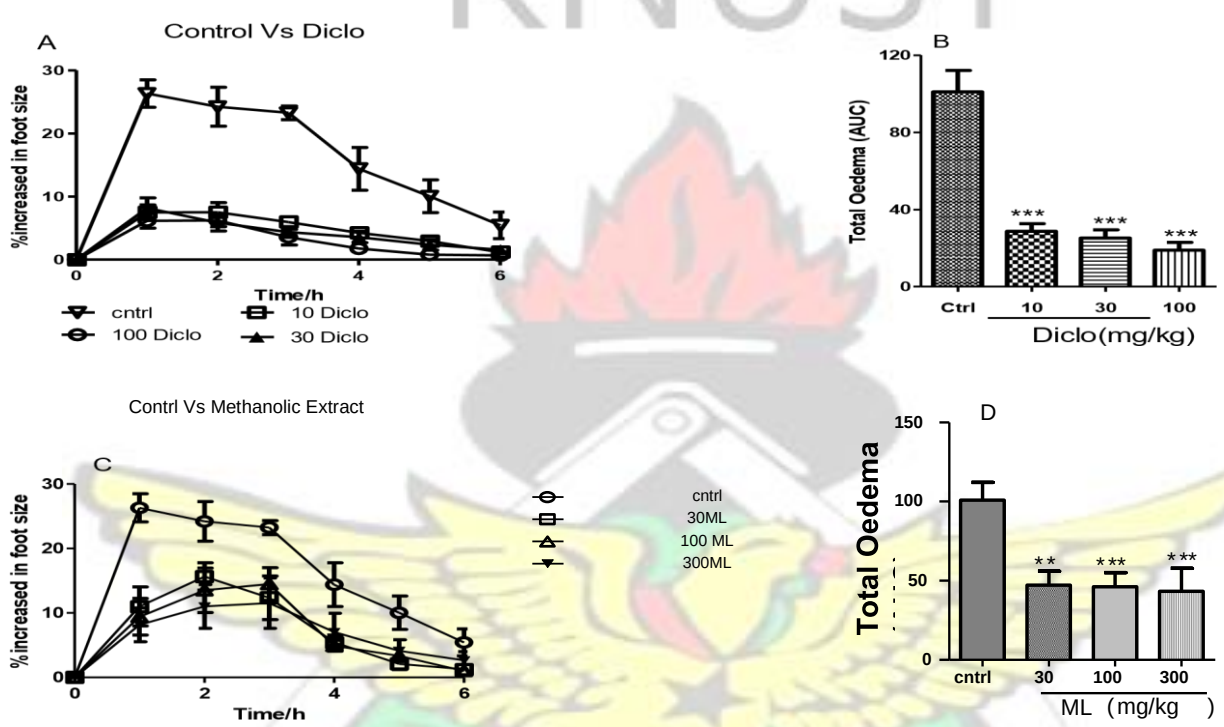

Figure 4.1: Therapeutic effect of standard drug and methanolic extract.

[A] & [C] - Inhibition of carrageenan induced paw oedema by Diclofenac at 10, 30 and 100 mg/kg and the Extract 30, 100 and 300 mg/kg within six hours administration with carrageenan as control. [B] & [D] - Total oedema calculated as Area the under curve with $p < 0.05$ (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) using One Way ANOVA and Dunnett's multiple comparison test. The results are compared with the control.

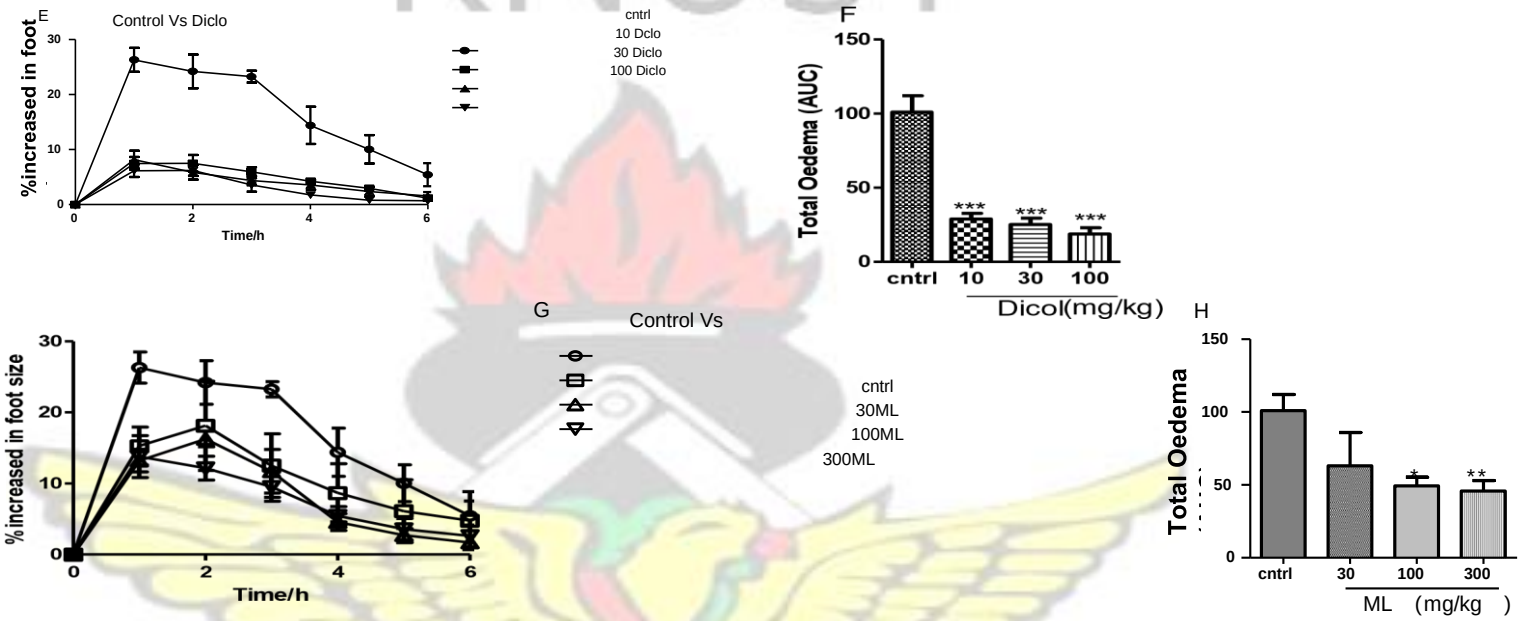

Figure 4.2: Prophylactic effect of standard drug and methanolic extract.

[E] & [G] - Inhibition of carrageenan induced paw oedema by Diclofenac at 10, 30 and 100 mg/kg and the Extract 30, 100 and 300 mg/kg within six hours administration with carrageenan as control. [F] & [H] - Total oedema calculated as Area the under curve with $p < 0.05$ (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) using One Way ANOVA and Dunnett's multiple comparison test. The results are compared with the control.

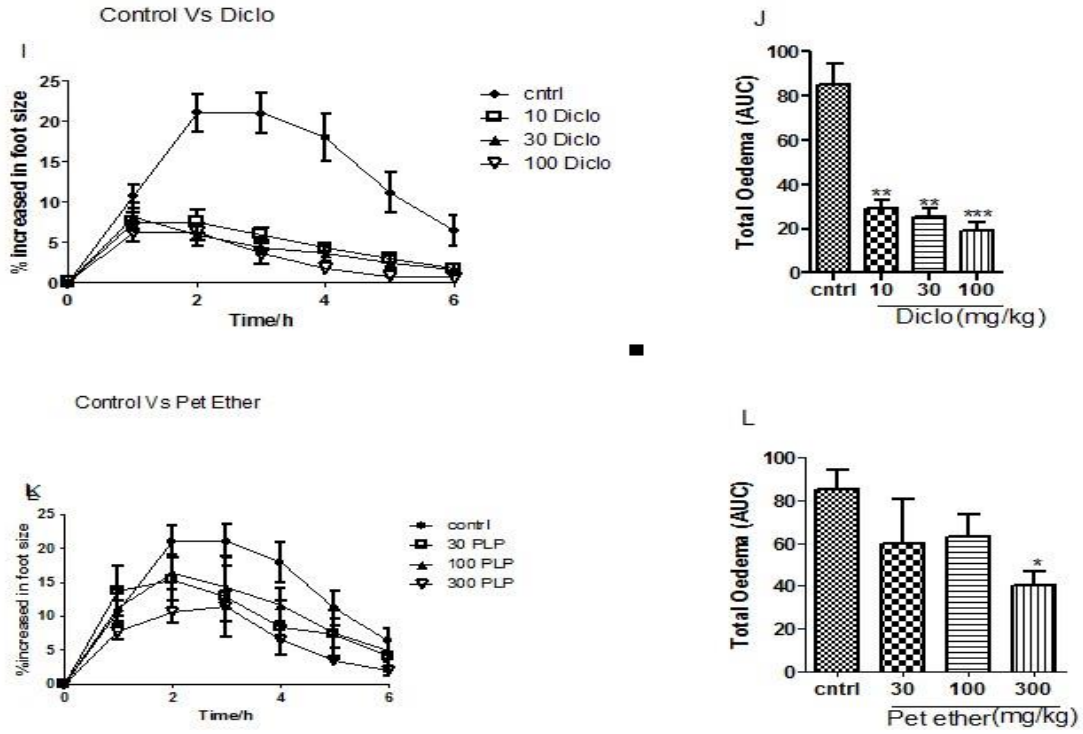

Figure 4.3: Prophylactic effect of standard drug and Pet ether extract.

[I] & [K] - Inhibition of carrageenan induced paw oedema by Diclofenac at 10, 30 and 100 mg/kg and the Extract 30, 100 and 300 mg/kg within six hours administration with control been carrageenan. [J] & [L] - Total oedema calculated as Area the under curve with $p < 0.05$ (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) using One Way ANOVA and Dunnett's multiple comparison test. The results are compared with the control.

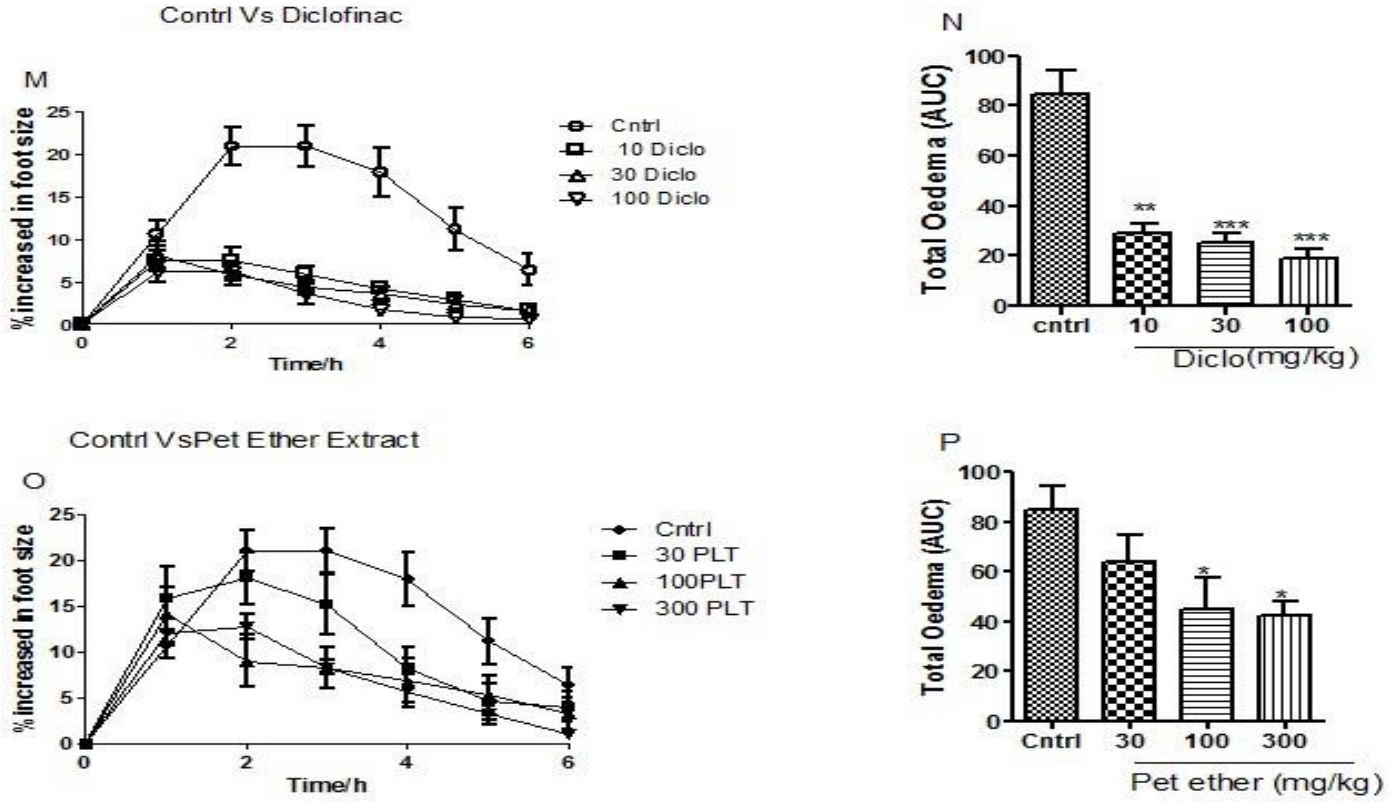

Figure 4.4: Therapeutic effect of standard drug and pet Ether extract.

[M] & [O] - Inhibition of carrageenan induced paw oedema by Diclofenac at 10, 30 and 100 mg/kg and the Extract 30, 100 and 300 mg/kg within six hours administration with carrageenan as control. [N] & [P] - Total oedema calculated as Area under curve with $p < 0.05$ (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) using One Way ANOVA and Dunnett's multiple comparison test. The results are compared with the control.

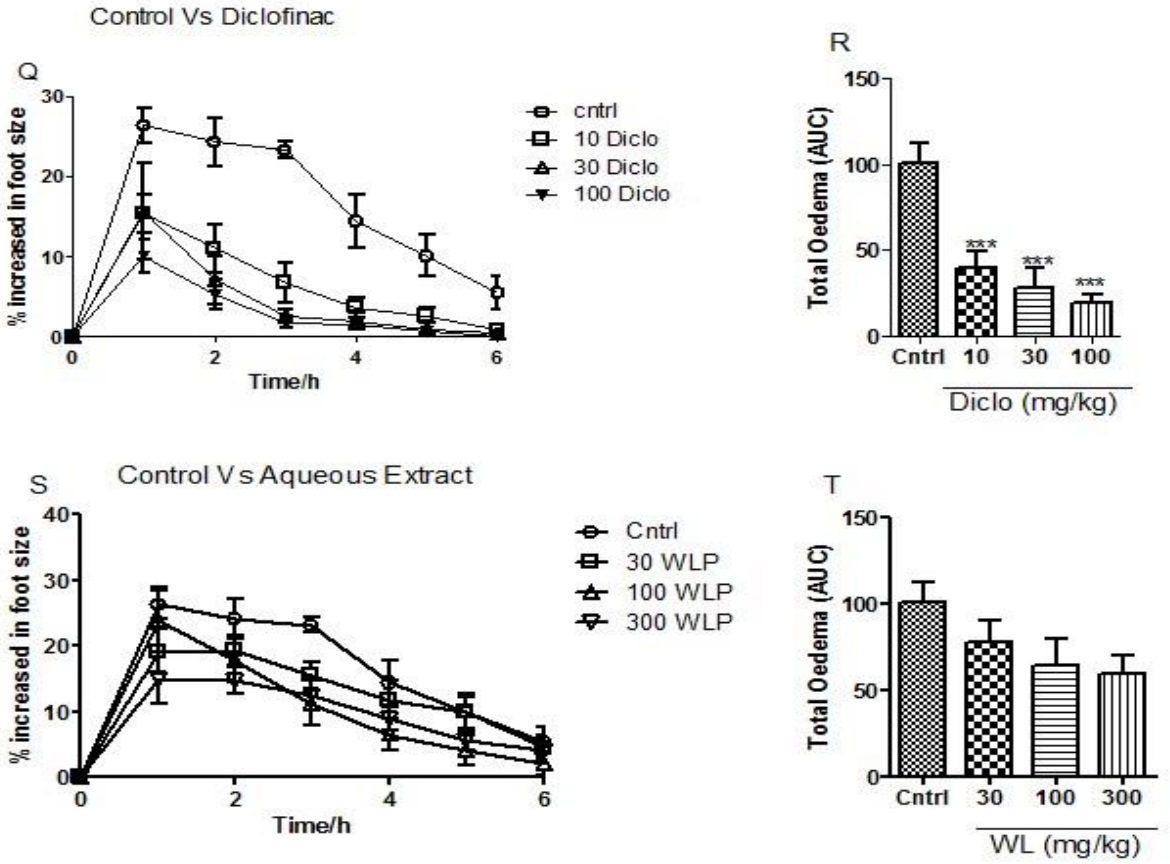

Figure 4.5: Prophylactic effect of standard drug and aqueous extract.

[Q] & [R] - Inhibition of carrageenan induced paw oedema by Diclofenac at 10, 30 and 100 mg/kg and the Extract 30, 100 and 300 mg/kg within six hours administration with carrageenan as control. [S] & [T] - Total oedema calculated as Area under curve with $p < 0.05$ (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) using One Way ANOVA and Dunnett's multiple comparison test. The results are compared with the control.

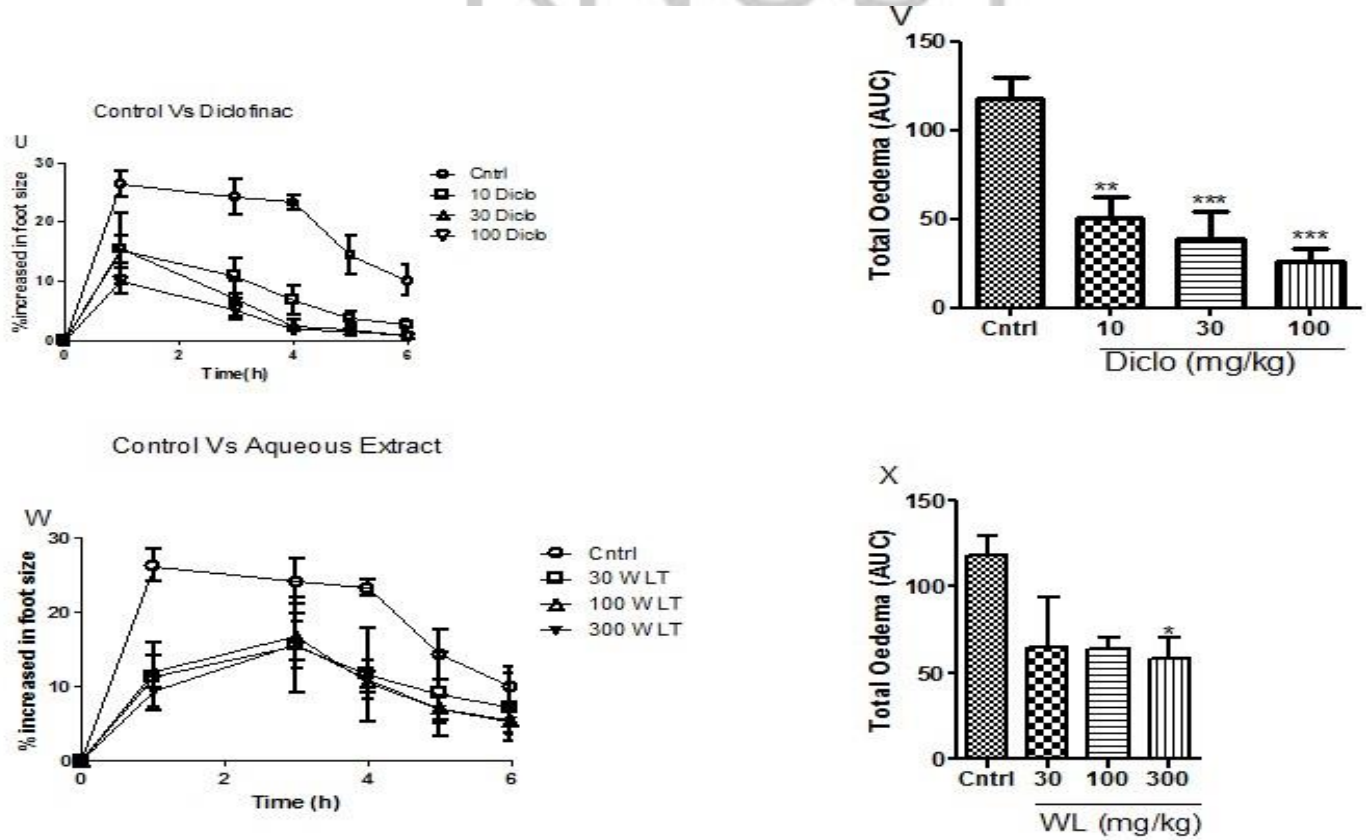

Figure 4.6: Therapeutic effect of standard drug and aqueous extract.

[U] & [V] - Inhibition of carrageenan induced paw oedema by Diclofenac at 10, 30 and 100 mg/kg and the Extract 30, 100 and 300 mg/kg within six hours administration with carrageenan as control. [W] & [X] - Total oedema calculated as Area the under curve with $p < 0.05$ (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) using One Way ANOVA and Dunnett's multiple comparison test. The results are compared with the control.

Table 4.4: Percentage inhibition of therapeutic and prophylactic results

Concentration(mg/kg)	PERCENTAGE (%) INHIBITI(			
	Therapeutic effect			
	Methanol	Aqueous	Pet ether	Diclofenac
30mg/kg	53.16	45.41	25.00	71.50
100mg/kg	54.19	46.29	47.00	75.10
300mg/kg	57.20	50.90	50.12	81.30
	Prophylactic Effect			
30mg/kg	37.52	23.00	25.43	65.80
100mg/kg	51.20	35.96	30.00	70.31
300mg/kg	54.60	45.41	52.30	77.80

4.4 Antioxidant Assay

4.4.1 DPPH Scavenging Activity

DPPH scavenging activities for the three solvent extracts were determined using micro-plate reader at a wavelength of 517nm. DPPH scavenging activity is the ability for the test sample to scavenge free radicals. The DPPH scavenging activity was dependant on the concentration from the result obtained. The IC_{50} values obtained were 48.97µg/ml, 158µg/ml and 1479µg/ml for the methanol, aqueous and pet ether extracts respectively. For the standard drug (Diclofenac) the IC_{50} value was 29.91µg/ml.

The table and the graph below shows the result of the DPPH scavenging activity and percentage scavenging activity against log of concentration.

Table 4.5: DPPH scavenging activity of the extract and the standard drug.

Drugs/ Extract	IC_{50}(µg/ml)
Methanolic	48.97
Aqueous	158.00
Pet Ether	1479.00
Ascorbic acid	29.91

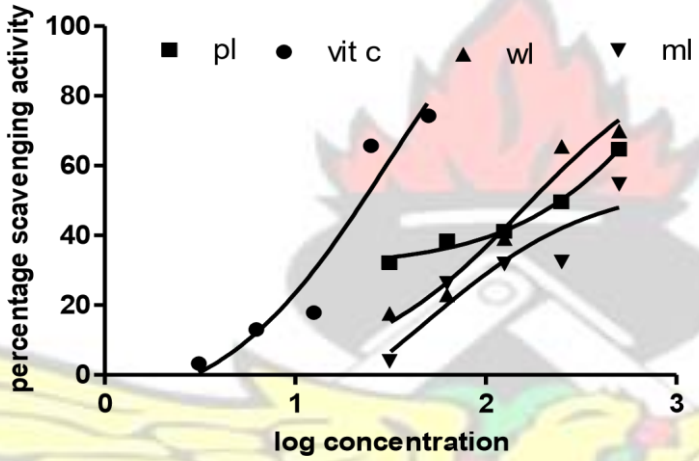

Figure 4.7: The percentage scavenging activity of the test samples and the standard drug.

Where pl = Pet Ether extract, vit c = Vitamin c, wl = Aqueous extract and ml = Methanol extract.

4.4.2 Total Antioxidant Capacity (TAC)

Ascorbic acid was used as standard drug to assay total antioxidant capacity. The ascorbic acid equivalent to antioxidant activity of the extracts is expressed as mg/g of the extract. Total antioxidant capacity is concentration dependant from the result obtained.14.7953mg/g13,17681mg/g and12.6185mg/g is the total antioxidant capacity estimated for the methanol, Pet ether and Aqueous extracts respectively, both expressed as ascorbic acid equivalent.

Table 4.6: Total antioxidant capacity of methanolic, pet ether and aqueous extract of Allanblackia parviflora, expressed as mg ascorbic acid equivalent per g of extract.

Extract	mg/g equivalent of ascorbic acid
Methanol	14.7953
Pet Ether	13.17681
Aqueous	12.6185

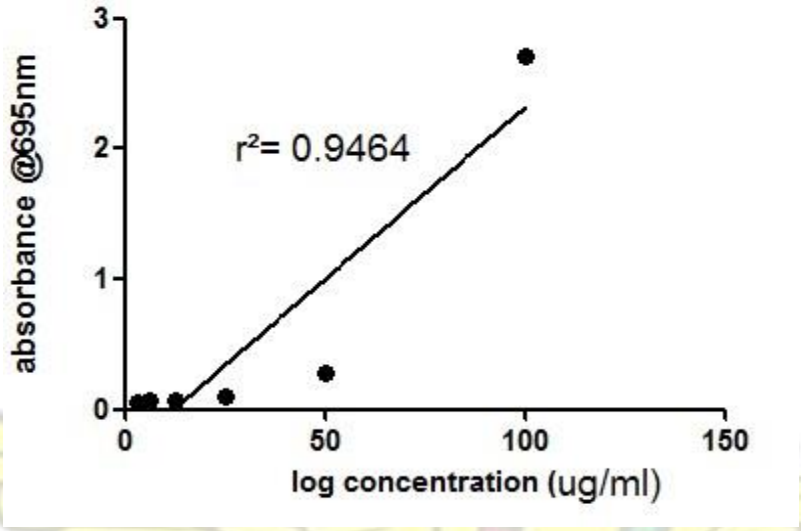

Figure 4.8: Calibration curve for ascorbic acid showing absorbance against log concentration.

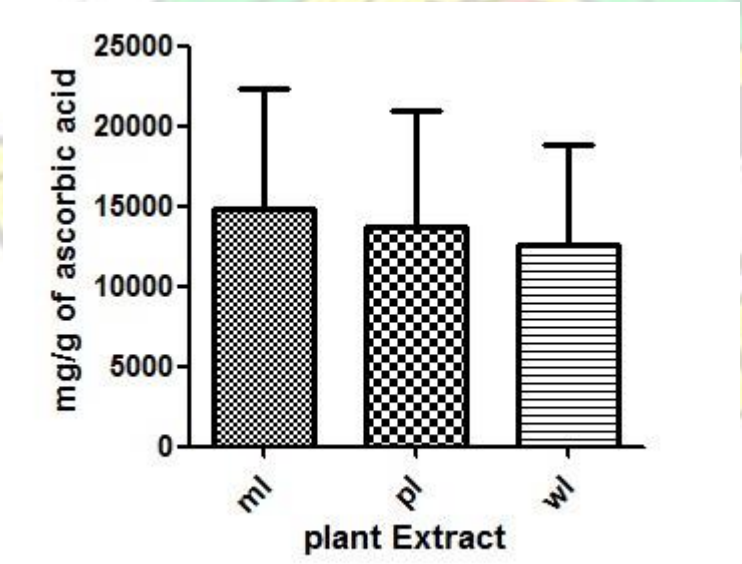

Figure 4.9: Total antioxidant capacity of the extract

4.4.3 Total Phenol Content (TPC)

Folin-ciocaltues reagent was used to assess total phenol content of the extract with tannic acid as the standard. 21.5256mg/g,19.1836mg/g and15.661mg/g of the total phenol content of the extracts were estimated and expressed as mg/g of tannic acid equivalent. The table below shows the total phenol content of the extracts expressed as mg/g of tannic acid equivalent of the extract.

Table 4.7: Total phenol content of the extracts expressed as mg of tannic acid equivalent per g of extract

Extract	mg/g Equivalent of tannic acid
Methanol	21.5256
Pet ether	19.1836
Aqueous	15.661

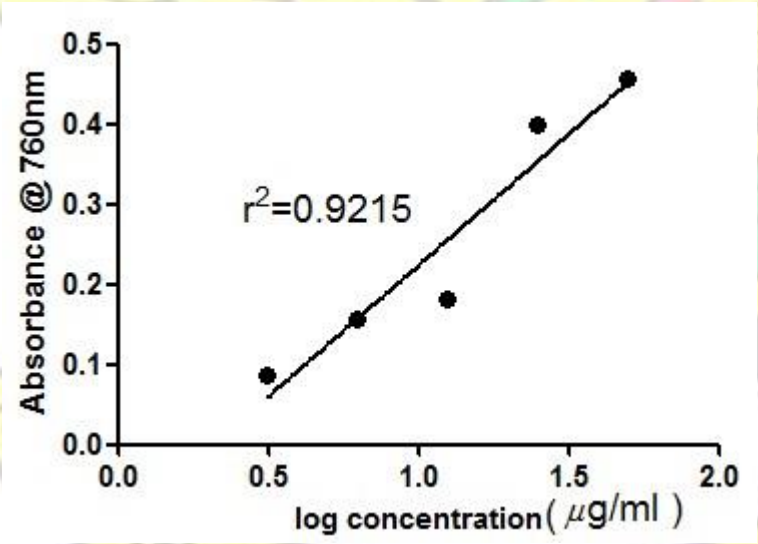

Figure 4.10: Calibration curve for tannic acid for total phenol content

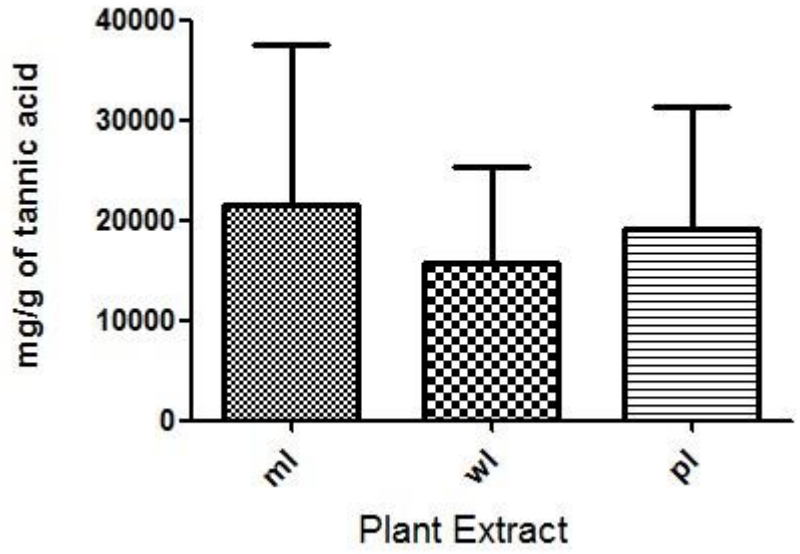

Figure 4.11: Total phenol content of the extract.

4.4.4 Correlation Graphs between TAC and TPC

Correlation graphs of total antioxidant capacity and total phenol content were determined for each extract. The figures below are the correlation curves of various extracts of total antioxidant capacity and total phenol content.

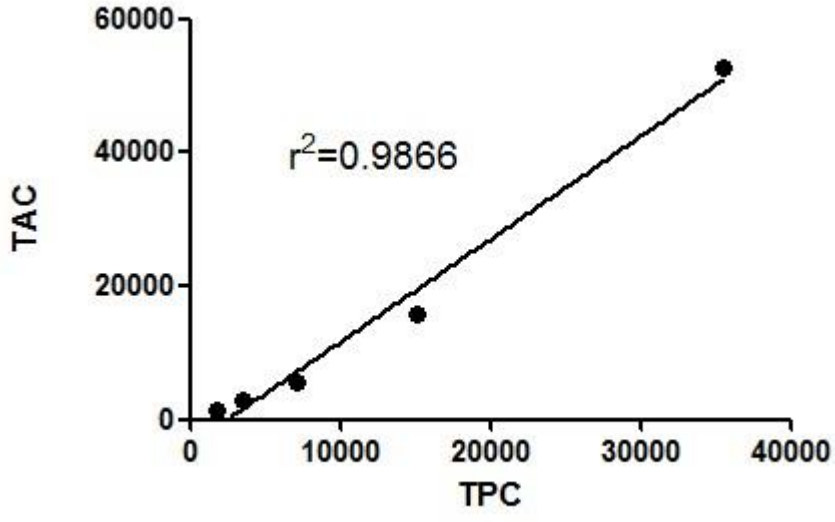

Figure 4.12: Correlation graph for TAC and TPC (methanol extract)

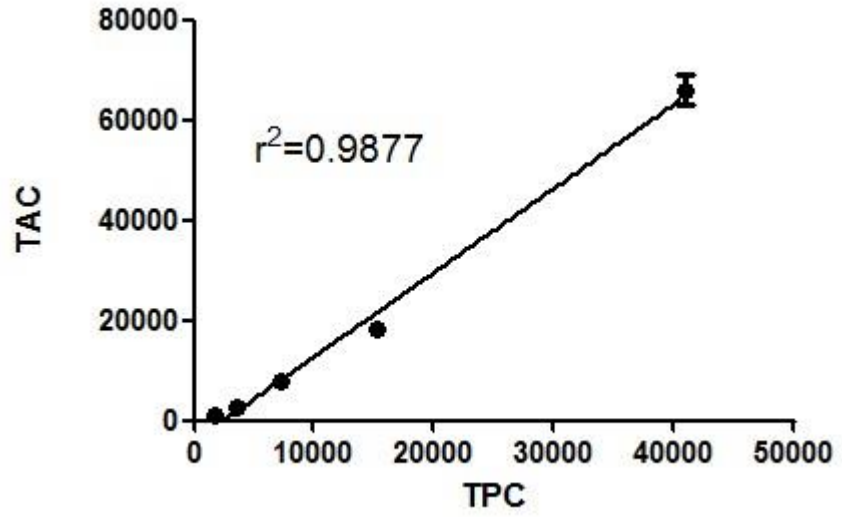

Figure 4.13: Correlation graph for TAC and TPC (Pet ether extract)

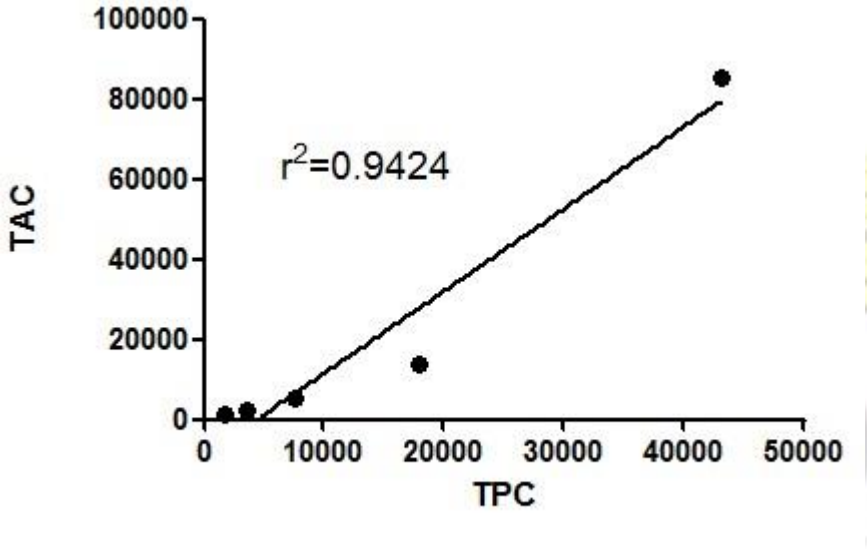

Figure 4.124: Correlation graph for TAC and TPC (Aqueous extract)

CHAPTER FIVE

DISICUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The research investigated the phytochemical constituents, antimicrobial, antiinflammatory and antioxidant activities of the leaves of *Allanblackia parviflora*. The following discussion therefore takes a look at the phytochemical screening, antimicrobial, anti-inflammatory and antioxidant assay results of the leaves of *Allanblackia parviflora*.

5.1.1 Phytochemical Screening

The plant materials including the pulverized and the extracts were taken through various phytochemical tests to identify the phytochemicals present. For pulverized sample, the phytochemical screening revealed the presence of flavonoids, tannins, reducing sugars, alkaloids glycosides, terpenoids, steroids anthraquinones and cardiac glycoside. There were absence of saponins and cyanogenic glycosides. The methanolic extract showed the presence of flavonoids, tannins, reducing sugars, glycosides, terpenoids, steroids, anthranquinones, and cardiac glycoside. There were absence of saponins, alkaloids and cyanogenic glycosides. Pet ether extract showed the presence of reducing sugars, terpenoids and cardiac glycoside. There were absence of flavonoids, tannins, alkaloids, steroids, anthraquinones and cyanogenic glycosides. Aqueous extract also revealed the presence of flavonoids, tannins, alkaloids, reducing sugars, glycosides, terpenoids, anthraquinones and cardiac glycosides. There were absence of saponins, steroids and cyanogenic glycosides. The absence of saponins and cyanogenic glycosides in the extracts

is a reflection of the absence of these phytochemical constituents in the pulverized sample.

These phytochemical constituents of which many have been isolated and characterised are believed to have physiological and therapeutic effects on humans (Newman, 2008).For instance, some flavonoids have been shown to possess antioxidants, anti-inflammatory, anti-cancer, anti-viral and antiallergic activities (Abdel-Hadi, 2014; Lotito *et al*., 2011).

Alkaloids are groups of secondary metabolites proven to have good therapeutic effects and used to improve human health. Some of the isolated compounds that are useful for medicinal purposes are cocaine and morphine which work on the nervous system (Charbogne *et al*., 2014), vinblastine and vincristine for the treatment of some cancer (Sun *et al*., 2014). A group of some terpenoids are used for tumor treatment. Quinine is also used for malarial treatment (Kessler *et al*., 2014). In addition, some steroids have been proven to have antimicrobial and anti-inflammatory activities. Tannins are also reported to have some interactions with protein to give an effect which makes it important for management of inflamed or ulcerated cells.

Phytochemical constituents present in each of the extracts and the quantity of these constituents may be responsible for the differences in the results obtained for antimicrobial, anti-inflammatory and antioxidant activities of various extracts. Furthermore, the phytochemical constituents' present in the plant may also be responsible for the acclaimed medicinal uses by local people.

5.1.2 Antimicrobial

For the antimicrobial investigation, two bioassay methods were used to assess the antimicrobial activities of the leaves extract.

Agar well diffusion method was used to preliminary investigate possible antimicrobial activity of the extracts. Methanolic and aqueous extracts showed zones of inhibition against nine selected bacteria (Four gram positive bacteria, five gram negative bacteria) and one fungi (Table 4.1). The zones of inhibition varied from one microorganism to the other. The best result obtained was the aqueous extract with candida albicans having the largest zone of inhibition of 15.00mm and streptococcus having the list zone of inhibition of 10.25mm. For the methanolic extract, the zone of inhibition for candida albicans was observed to be lower than that of the aqueous at 9.00mm. The highest zone of inhibition for methanolic extract was staph aureus with a zone of inhibition of 12.00mm and the lowest was Neisseria *gonorrhoeae* with zone of inhibition of 8.00mm. The differences in zones of inhibition could be as a results of differences in phytochemical constituent present in each extract, since most of these constituent possess different therapeutic effects.

Pet ether extract did not show any zone of inhibition for any of the microorganisms. This may be due to the absence of flavonoids, alkaloids, and tannins in the extracts of which flavonoids and tannins are present in both methanolic and aqueous extracts. Alkaloids were however present in aqueous extract. Since these have shown to possess antimicrobial activities, it would imply that the activities of the extracts against these microorganisms may be due to the presence of the aforementioned three phytochemical constituents.

The difference in inhibition in the methanolic and aqueous extracts may be due to the alkaloids present in the aqueous extracts and absence in methanolic extract. However this could also be due to the quantity of these secondary metabolites in both extracts. The positive control showed higher zones of inhibition, implying that a high state of purity is desirable compared to the crude extracts.

After this, the agar well diffusion method was carried out on the extracts. Those that showed zones of inhibition were then taken through both diffusion method to determine minimum inhibitory concentration of those extracts.

The microbial assay performed to determine MIC showed varying inhibitory effect against the 10 selected microorganisms (Four gram positive bacteria, five gram negative bacteria) and one fungi of the extracts (table 4.3). Methanolic extract gave the best MIC results as compared to the aqueous extracts .The MIC results for methanol extract that were obtained were 2.5mg/ml to 5mg/ml (table 4.2.2). The highest activity observed with the use of methanolic extract was against B. *subtilis, Pseudomonas,* and E. *feacalis* with a MIC of 2.5mg/ml. The rest of the microorganisms (E. *coli,* Staph *aureus,* Candida *albicans,* S. *Typh* and N. *gonorrhoeae,*) gave MIC of
5.0mg/ml.

Aqueous extract assay result showed an MIC of 5mg/ml to 20mg/ml with the best MIC of 5mg/ml against E. *feacalis,* the second highest MIC was 10mg/ml for *Pseudomonas,* Neisseria *gonorrhoeae,* Candida *albican*s, B. *subtilis, Strep* and E. c*oli*. The lowest were 20mg/ml for K. *pneumoniae,* Staph *aureus* and S. *Typh.*

Comparing the two extracts, methanolic extract showed better MIC than that of aqueous extract with the highest MIC of 2.5mg/ml methanol extract and 5.00mg/ml being for Aqueous extract. The reasons for this could be that, the phytochemical constituent present in them may vary in their quantity or some phytochemical constituent that are present in the methanol extract and not in the aqueous extract which have not been tested for. It is suggested that these phytochemical constituents play major roles in antimicrobial activity (Wallace *et al.*, 2004).

Evaluating the antimicrobial activities of the extracts with that of the standard drug, it was observed that the standard drug gave a better MIC than that of the extracts. This could mean that, because of the high state of purity of the standard drug with respect to the crude extract, more potency is observed.

5.1.3 Antioxidant Assay

Assessment of antioxidant activity on the crude extracts of each solvent extract using DPPH scavenging assay, Total antioxidant capacity and Total phenol content were carried out.

The DPPH scavenging activity assay showed an increase in percentage DPPH scavenging activity as concentration dependant. This means that when there is an increase in concentration of the extract, there is a corresponding increase in percentage DPPH scavenging activity. However increase in concentration causes a decrease in absorbance of the DPPH as a result of mopping up of the free radicals by the test samples. The IC_{50} values that were obtained for the extracts were 48.97µg/ml for methanol extract, 158µg/ml for aqueous extract

and1479µg/ml for pet ether extract. For the standard (ascorbic acid), it was 29.91µg/ml (table 4.5).

The result obtained for the extracts means that the extract may contain some phytochemical constituent which could have the ability to remove free radicals or prevent them from occurring by donating hydrogen atoms or elements to stabilize free radicals, thus reducing the DPPH to form DPPH.H complex which gives a yellow colour (Aliyu *et al*., 2010). The donation of electrons to free radicals terminate the chain reaction that are initiated by these free radicals.

The extracts results show methanol extract having the highest IC_{50} of 48.97µg/ml, as compare to aqueous and pet ether 158µg/ml and 1479µg/ml respectively (table 4.5). The variations in the IC_{50} of the various extracts may be due to the difference in the phytochemical constituent present in these extracts. For instance, it has been proven that some phytochemical constituent such as phenols, polyphenolics, flavonoid and terpenoids have shown to possess antioxidant capacity of which some of the extracts studied do not have when screened for phytochemical constituents (Litto *et al*., 2011). The low in DPPH scavenging activity of pet ether extract may be as a result of absence of flavonoid in the extract which is one of the major sources of antioxidant in plants.

The standard drug (ascorbic acid) gave an IC_{50} of 29.9µg/ml making it the highest when compare it with the extracts, which implies that ascorbic acid is more potent than the extracts when it comes to antioxidant activity. The reason could be because of the extent of purity of the standard drug as compare to the crude extract.

Total antioxidant capacity assay involves the reduction of Mo^{+6} to Mo^{+5} by the extracts which will lead to the formation of green phosphomolybdenum (V) complex at acidic pH (Das *et al*., 2014). The complex formation is measured at an absorbance of 695nm using micro-plate reader. Total antioxidant capacity assay values obtained were 14.7953mg/g, 13.7681mg/g and 12.6185mg/g for methanol extract, Pet ether extract and aqueous extract respectively. This means that for any 1g of each extract taken, only 14.79%, 13.68% and 12.619% of methanol extract, Pet ether extract and Aqueous extract respectively act like ascorbic acid (standard drug) (table 4.6). The result also means that the extract possess some antioxidant activity and can be used in managing disease conditions involving oxidative stress.

Total phenol content assay is based on the reduction of folin-ciocalteu reagent by phenols in the extracts. The results obtained for total phenol content of the various extract were 21.515mg/g for methanol, 19.1836mg/g for Pet ether and 15.66mg/g for Aqueous of tannic acid respectively. This implies that for every 1g of the extract taken, only 21.52%, 19.184% and 15.66% of the respective extracts behave like tannic acid (table 4.7).

The correlation between total antioxidant capacity and total phenol content was assessed and the result obtained were 98.66%, 98.77% and 94.24% for methanol extract, Pet ether extract and Aqueous extract respectively (figure 4.10). The results therefore means that about 98.66% total antioxidant capacity obtained for methanol extract is as a result of phenols present in the extract, 98.77% for Pet ether extract and 94.24 for Aqueous extract respectively.

The result obtained from the antioxidant assay implies the plant extracts can be used in the management of oxidative stress related disease, since the plant

contain some of the phytochemical constituent reported to have antioxidant properties such as phenols, terpenoids, porlyphenolic, flavonoids and coumarins (Sie, 2000; Ozgavia *et al*., 2003).

5.1.4 Anti-Inflammatory Assay

An *in vivo* carrageenan induced paw oedema method was used to assess antiinflammatory activity of the extracts in chicks. The assay involves the inoculation of carrageenan into the paw of the chicks causing an inflammation by the release of some mediators such as prostaglandins. This leads to the release of fluids into the vessels of the cell which causes the formation of oedema. The extracts were tested for their ability to prevent or inhibit the release of these mediators which lead to oedema formation. The extracts were tested for their therapeutic (curative) and prophylactic (preventive) activity.

For the therapeutic analysis, carrageenan was induced in the chicks one hour before the extracts were administered. For prophylactic analysis the extract were given to the chicks one hour before the carrageenan was induced. The reason for the analysis was to determine whether the extracts are curative or preventive. The standard drug used was Diclofenac with carrageenan (10µl or 0.1ml of a 2% suspension in 0.9% saline) as the control. Data such as the mean ± SEM of 5 chicks and significant difference of the various extract from control were obtained using One- Way ANOVA followed by Dunnet's post-

test with p values for all extracts and standard drug.

From the results obtained, methanol extract gave better results as compare to pet ether extract and Aqueous extract for both therapeutic and prophylactic analysis.

Therapeutic analysis result gave methanol extract a significant of $P<0.001$ for 300mg/kg, $P<0.001$ for 100mg/kg and $P<0.01$ for 30mg/kg with percentage inhibition of 57.2%, 54.19% and 53.16% respectively when compare with control (figure 4.1 and table 4.3). The differences in percentage inhibition and the P values is as a result of concentration dependant effect when compared with the control. This means that the higher the concentration of the extract the higher the percentage inhibition.

Prophylactic result for methanol extract also gave $P<0.01$ for 300mg/kg, $P<0.05$ for 100mg/kg but did not show any significant differences for 30mg/kg when compare with control (figure 4.2). This implies that when 30mg/kg of the extract was given to the chicks the results obtained compared to that of the control, it did not show any significant difference between the two. Prophylactic effect of the extract gave percentage inhibition of 54.60%, 51.20% and 37.52% of various concentrations respectively (table 4.4). Evaluating the methanol therapeutic analysis result with respect to the prophylactic analysis result, the therapeutic analysis gave a better result than prophylactic suggesting that the extract is better curative than preventive.

Pet ether extract gave a result of $P<0.05$ for 300mg/kg with no significant difference in 100mg/kg and 30mg/kg when compare to control for prophylactic analysis (figure 4.3). For therapeutic, significant values were $P<0.05$ for 300mg/kg and $P<0.05$ for 100mg/kg but did not show any significant for 30mg/kg when compare with control (figure 4.4). The two analysis resulted in better therapeutic effect than prophylactic effect. The percentage inhibition for various concentrations are as follows 52.30%, 30.00% and 25.43% for prophylactic (table 4.4) and 50.12%, 47.00% and

25.00% for therapeutic respectively (table 4.3).

Therapeutic analysis for Aqueous extract gave significant values of P<0.05 for 300mg/kg but no significant for 100mg/kg and 30mg/kg (figure 4.5). Prophylactic analysis did not show any significant difference for the various concentration when compare with control (figure 4.6), but gave a percentage inhibition for the extract as 50.90%, 46.29% and 45.41% for therapeutic (table 4.3) and 45.41%, 35.96% and 23.00% for prophylactic (table 4.4) of the various concentration respectively.

From the result obtained, the extracts showed more curative than preventive effects, making the plant suitable as an anti-inflammatory medicinal plant. The variation in the results obtained from the extracts may be as a result of differences in the phytochemical constituent in each solvent extracts, since it has been proven that some of the phytochemical constituent in the plant possess anti-inflammatory properties.

The standard drug (Diclofenac) used gave significant values of P<0.001, P<0.01 and P<0.05 with percentage inhibitions for therapeutic as 81.30%, 75.10% and 71.50%respectively, and for prophylactic, 77.80%, 70.31% and 65.8% respectively (table 4.3 and 4.4, figure 4.1 and 4.2) when compared with control and particular extract. Standard results from Diclofenac were better than some of the extract and the reason may be because it is a pure drug as compare to the crude extract.

5.2 Conclusion

The study was to investigate the phytochemical constituents, antimicrobial, ant-inflammatory and antioxidant activities of the plant *Allanblackia parviflora*

leaves extracts using methanol, pet ether and water. The study screened for phytochemical constituent in the pulverized plant sample, as well as solvent extracts. The analysis showed the presence of some phytochemical constituent including alkaloids, flavonoids, terpenoids, tannins, steroids, reducing sugars and glycosides. Saponins was therefore absent in the pulverized sample and the solvent extract.

The study also discovers that methanol extract and aqueous extract have some antimicrobial activities against some selected microorganisms with methanol extract having the highest minimum inhibitory concentration (MIC). Pet ether extract did not show any inhibition against the selected microorganisms. It can also be reported from the study that, the extracts show some good antiinflammatory and antioxidant properties which makes the plant worth investigating into. All activities of the various extracts are due to some phytochemical constituent present in the plant.

5.3 Recommendation

From the study, it is recommended that fractionation of the extract should be done and retested for it antimicrobial, antioxidant and anti-inflammatory activities. Chromatographic analysis, elementary composition analysis, isolation and characterization is also recommended.

It is also recommended that different non-polar solvent should be used for the extraction instead of pet ether.

Since plants are believed to have numerous benefit to mankind in terms of it medicinal aspect, it is therefore recommended that other possible activities of the plant should be investigated.

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