KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMAI

PHYTOCHEMICAL SCREENING AND BIOACTIVE PROPERTIES OF THE STEM BARK EXTRACTS OF *ALLANBLACKIA PARVIFLORA* (CHEVALIER) (CLUSIACEAE)

BY

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DECLARATION

I hereby declare that this submission is my own work towards the M. Phil and that, to the best of my knowledge, it contains no material previously published by another person nor material which have been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text

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DEDICATION

This piece of work is dedicated to my dear husband, Daniel Addai-Nsiah and the Amoah family.

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My sincere gratitude goes to the almighty God for the love and tender mercies he has bestowed on me throughout my life. Your grace oh lord has really been sufficient for my weakness from the onset of this piece of work till now.

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ABSTRACT

The phytochemical constituents, antioxidants, antimicrobial and anti-inflammatory activities of the methanolic, aqueous and pet- ether extracts of the stem bark of *Allanblackia parviflora* have been studied in this work with the aim of exploring the medicinal properties of the plant in Ghana. The results obtained from the qualitative phytochemical screening indicated the presence of alkaloids, tanins, flavonoids, cardiac glycosides, reducing sugar, triterpenoids, anthraquinones, saponins and phytosterols and the absence of cyanogenic glycosides. The antimicrobial assay employed Agar-well diffusion and Micro broth dilution methods against 10 microbial strains (i.e. one fungus, five gram-negative and four gram-positive bacteria) using Ciprofloxacin as the reference drug. The methanolic extract exhibited the highest activity against *E. faecalis* with zone of inhibition 20 mm and MIC of 2.5 mg/ml. Pet-ether extract on the other hand was inactive against test microbes. Anti-inflammatory properties of the extracts employed the Carrageenan induced paw model in a week old chicks. The various extracts at 30, 100 and 300 mg/kg exhibited prominent therapeutic anti-inflammatory properties with methanol recording the highest activity at oedema inhibitory percentages of (42.92%, 56.93% and 66.01%) and (49.26%, 51.41% and 59.05%) for therapeutic and prophylatic studies respectively. Followed by aqueous (28.61%, 46.60% and 56.93%), (29.27%, 39.20% and 48.24%) and pet-ether (34.92%, 45.38% and 45.99%), (33.42%, 36.05% and 42.53%) respecitively. Antioxidant assay also employed DPPH scavenging potential, TPC and TAC for the assessment. The results however presented the methanolic extract as most potent with EC_{50} value of 0.03341 mg/ml, TAE of 212.85±0.311 mg/g and AAE of 77.72±0.549 mg/g for

DPPH Scavenging potential, TPC and TAC respectively. These results therefore provide for the first time scientific bases for the folkloric use of *A.parviflora* in managing inflammations, microbial infections and some tropical diseases.

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LIST OF ABBREVIATIONS

ANOVA…… Analysis of variance

ATCC…… American Type Culture Collection

AAE..... Ascorbic Acid Equivalent

CFU…….. Colony forming unit

DMSO……... Dimethyl sulfoxide

DPPH…… 2, 2-diphenyl-1-picrylhydrazyl

MIC…… Minimum inhibitory concentration

TAC…… Total Antioxidant Capacity

TPC…… Total Phenol Content

TAE…… Tannic Acid Equivalent

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Different plant materials have been used by humans since antiquity for the treatment of various diseases and ailments (Linthoingambi and Singh, 2013). The earliest known records of plant-based therapy is from Mesopotamia in 2600 BC and some of plant products employed in these remedies included mandrake which was used as general pain reliever, raw garlic given for circulatory disorders, turmeric for blood clotting properties and the root of endive plant for the treatment of gall bladder disorder. As new diseases evolved and pathogenic organisms became more complex, scientists in the 19th century began an intense search to isolate bioactive compounds to wrestle these diseases. It is out of these investigation that lead to the discovery of potent bioactive compounds such as morphine, quinine, campthothecin, atropine, taxol, podophyllotoxin etc. which are of immense pharmacological importance today.

As civilization and industrial chemicalization advanced, synthetic pharmaceutical products were greatly preferred to those natural products for these same purposes with the rationale that they were more effective than the herbal medicines. In recent times however, majority of the population globally rely on natural product therapies to treat and alleviate the sufferings caused by various ailments as evidenced from the World Health Organization (WHO) report in 2002 which estimated that about 80% of Africans resort to traditional medicine for their health needs. Further to this point, Professor Laud K.N.A. Okine, the Director of the Centre for Scientific Research into Plant Medicine

(CSRPM) in Ghana in 2013 also stipulated that about 65 to 70 percent of the country's population mostly local folks rely on medicinal plant for the management of common ailments including skin infections, malaria, general pains, piles sexual weakness etc. that confronts their everyday lives (http://vibeghana.com/2013/09/04/the-increase-in-the-use-of-herbal-medicine-in-ghana-africa-and-the-world-is-good-king-of-herbal-medicine-naaba-dr-sheikh-amin-bonsu/).

This abrupt shift from synthetic drugs despite its tremendous successes centuries past and present is perhaps due to their cultural acceptance and the recognition that these natural products are readily available, very affordable, non-narcotics, easily biodegradable as well as producing minimum environmental hazards and having minimal adverse effects. (Osei-Djarbeng et al., 2014; Amponsah, 2012; Kuate, 2010). Apart from the numerous reasons stated above, these medicinal plants are also known to contain phytochemicals such as alkaloids, phenols, glycosides, unsaturated long chain fatty acids, steroids and other secondary metabolites which have significant pharmacological applications in treatment therapies. This therefore means that medicinal plants as well as herbal preparations can be potential medicines or front-runners for drug discovery and development

In spite of this great exposé on natural product therapies, research still indicates that just few plants accounting for only 15% of all plant species have been explored for their phytochemistry and only about 5% for one or more biological activities worldwide (Amponsah, 2012). In Ghana however the situation on scientific investigations of these plant products seem pathetic such that in as much as we are endowed with countless number of plants and herbs very few

have been comprehensively studied for their pharmacological properties and among the less or unexploited medicinal plants allanblackia species are no exception though it has promising therapeutic significance in traditional herbal medicine.

Allanblackia species are flowering plants native to Africa precisely the east, west and central regions extending from Tanzania to Sierra Leone (Ofori et al., 2011). They belong to the family Clusiaceae and there are about nine species found in the genus Allanblackia (Rompaey, 2003). The only species in Ghana among the nine known species is *Allanblackia parviflora* (A.Chevalier 1909) (Rompaey, 2003). It is nearly a threatened plant distributed in the rainforest zones in the country specifically western, eastern, Ashanti and central regions (Sefah et al., 2007). Owing to the cosmopolitan nature of Ghana, the plant has different indigenous names including *Sonchi, Osonodokono, Kusieadwe*, *Kusie aduane, Opaa Dufufui*, *Boho, Krupi Atrodua* and *Akosobolo* with different myths surrounding the existence of the names (Kwaku Antwi, 2012). The myth behind the Akan name" *Kusieadwe* or *Kusie aduane"* is that during famine and droughts the fruits and seeds of the plant were the staple food consumed by wild rats and porcupines for survival.

Allanblackia species are said to be versatile in nature because they have found many uses in the traditional herbal medicine, food industries, cosmetics, soap industries as well as wood industries in different countries in Africa.

For instance, in 2002 Unilever realized the potential of Allanblackia oil in the manufacture of cosmetics and food products. It then facilitated the formation of a unique partnership (Public-Private) for the sustainable development of

domestication strategies and supply chain of Allanblackia in Ghana, Nigeria and Tanzania. In Ghana, collaboration was also established with local agencies like Forestry Research Institute of Ghana (FORIG), Novel Development, SNV, IUCN and the International Tree Seed Centre (ITSC) as well as other NGOs for research and development work. The project was undertaken to promote development, poverty alleviation, and biodiversity conservation in the African tropical forest belt (Sefah *et al*., 2007).

Apart from the economic benefits of the oil mentioned above, the Allanblackia species in general have significant usage in traditional medicine. Several researchers over the years have reported remarkable medicinal uses of the whole Allanblackia plant notably in the management of dysentery, skin diseases, rheumatism, impotency, general pains, diarrhea, toothaches and microbial infections (Abiww, 1990; Meshack, 2004; Munjuga et al., 2010; Laird, 1996; Ajbesin et al., 2008). It is against this background the that this research is being undertaken to assess some of the possible therapeutic properties of *Allanblackia parviflora* in Ghana

1.2 Problem Statement

Throughout human history, there has been a constant struggle between pathogens that cause various ailments and humans. This constant struggle seem not to end rather, escalating at tremendous rates with the emergence of new diseases resulting from the persistent modifications and resistance of these microorganisms. For example, hepatitis, human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/ AIDS) as well as Ebola, have impacted quite a substantial proportions of human lives especially African,

causing a considerable rise in morbidity and mortality rates in the past years (Tenover, 2006; Amponsah, 2012)

Although modern synthetic drugs have mitigated this problem in favour of the human race. However, the persistent use of these drugs as chemicals have been associated with innumerable side effects including hypertensive allergic reaction, severe skin rash, anorexia, dizziness, diarrhea, abdominal pains, anaphylaxis, fever, renal toxicity, liver toxicity, eosinophilia (elevated white blood cells), peripheral neuropathy, hemolytic anemia, lethargy etc. depending on the type and class of the antibiotic (www.drug.com/article/antibioticside effects-allergies-reaction.html).

As a result of this and other reasons such as financial constraints and availability, there has been an intense scientific investigations for novel antimicrobial, anti-inflammation, antioxidants etc. compounds devoid of or of minimal side effects but with the same or even better therapeutic activities. In this regard however, plant based natural products are highly favored due to their availability, efficacy and safety successes in the past and present. Classical examples of plant natural products with marked therapeutic activities include vincristine, and vinblastine from *Catharanthus roseus*, podophyllotoxin from *Podophyllum peltatum* and pacilitaxel from *Taxus breviflolia* for cancer management, capsaicin from *Capsicum annum* and ginger root extract from *Zingiber officinalis* for inflammation management etc. owe to this evidences, natural products from plants especially can be feasible alternatives to these synthetic drugs for the treatment of various diseases.

However, in the antimicrobial terrain no major antimicrobial drug has been developed from higher plants, though innumerable studies have generated data showing antimicrobial properties of medicinal plants. This body of results indicates that medicinal plants, even if not fully developed into new mainstream antimicrobial drugs, could indeed be an effective alternative if properly processed and incorporated into primary health care systems. (Amponsah, 2012)

Inflammation in general still remains one of the world's major health problems since it accompanies virtually all kinds of disease especially cardiovascular diseases including heart attack, cancer, and Alzheimer's disease etc. The steroidal and non-steroidal anti-inflammatory drugs still used for managing inflammations have not been successful since they are known to be associated with countless side effects and health problems such as bronchospasm, immunosuppression, renal failure, gastrointestinal damage, hemorrhage, ulceration, kidney dysfunction, cardiovascular failure, hypertension, dyslipidemia etc. after their prolonged usage (Lin et al., 2006; Deepa et al., 2014). Therefore the need for potent, safer and less toxic anti-inflammatory drugs probably from plants to substitute the already existing ones since various studies from plants have shown possible and remarkable anti-inflammatory activities.

The advertent or inadvertent production of free radicals from human metabolic processes and other processes such as inflammation produces injurious effects to vital cellular components as well as vascular tissues. In the inflammation process however, not only free radicals are produced but these prevent or inhibit the healing of wounds. As a result of this and other catastrophic effects of these

reactive species, the immune system has built its own defense system called antioxidants defense system to neutralize or stabilize these oxidants. The antioxidants essentially scavenge and minimize the production or formation of the reactive metabolites. Also the activity of the antioxidants have been report by Amponsah, (2012) to supplement inflammatory processes by promoting the healing of wounds and the rapid repair of tissues as it was evidenced that some plant secondary metabolites that possess anti-inflammatory activities also showed antioxidant activities. However the entire antioxidant defense system in the body is not a perfect or 100% system such that the oxidant-antioxidant balance is drifted more to the reactive oxidants so that oxidative stress on tissues and cellular component increases thereby various posing health concerns. Hence the need for supplementary antioxidant agents or drugs with higher activities possibly from plant sources to balance out the differences (Halliwell, 2001)

One of such plants which still remain untapped as a medicinal plant in Ghana irrespective of its ethnomedicinal uses especially as an antimicrobial, anti-inflammatory, and antioxidant agent elsewhere in Africa is *Allanblackia parviflora*. For instance, in Gabon, Congo, Nigeria, Ghana and Ivory Coast, Allanblackia species are used for managing chickenpox, dysentery, malaria, skin infections, smallpox, toothache, elephantiasis, measles, bronchial infections, asthma, stomach ache, pains, cough etc. These ethnomedicinal applications therefore indicate possible analgesic, antimicrobial, antiviral and anti-inflammatory activities as various scientific reports affirms these. (Fankam et al., 2015; Ajibesin et al., 2008; Nguemfo et al.,2007; Ayoola et al., 2008; Nguemfo et al., 2009; Ymele et al., 2011)

Phytochemicals which are said to be naturally occurring, bioactive chemical compounds in plants that provide color, aroma, flavor and also act as a natural defense system for host plants (Adubofuor et al., 2013) are reported to be contained in these species. These include alkaloids, glycosides, tanins, flavonoids, triterpenes Anthocyanines etc. (Fankam et al., 2015) and hence the pharmacological activities mentioned above.

Apart from the therapeutic activities of the Allanblackia species, it also contain some major and minor minerals needed in the normal functioning of the human system. Examples of which include potassium, phosphorus etc.

In spite of the several uses recorded for *Allanblackia parviflora* at both subsistence and industrial levels as well as its possible therapeutic properties, it has been neglected and underutilized for far too long, also herbalists who use these plants for these therapies have a lot of issues with efficacy safety and effectives since they have limited scientific information on the plants.

Therefore, this research is designed to primarily investigate the phytochemicals, anti-inflammatory, antioxidant and antimicrobial activities of the stem bark *A. parviflora* species in Ghana to ascertain some scientific basis for its medicinal properties as claimed by traditional medicine and possibly to isolate bioactive compounds that may be responsible for these activities.

1.3 Justification of Research

This study does not only seek to explore and capture phytochemicals, antioxidants, anti-inflammation and antimicrobial activities of the stem bark of *A.Parviflora (*A.Chevalier) species in Ghana but provides a comprehensive literature that will provide beneficial information to the food industries,

students, modern herbalist and scholars in their research works. The study could create the awareness of unexploited medicinal plants in Ghana and other countries and also highlight not only their medicinal uses but other uses including economic, culinary and other relevant benefits to exploit their full potential

The study will further emphasize the need for increasing commercial cultivation and production of the allanblackia species through genetic engineering in Ghana since it could be a lucrative business enterprise that would not only improve the living standards of farmers but will also constitute a cost effective means of alleviating the suffering caused by some microbial infection as well as other diseases in Ghana and elsewhere in the world.

1.4 Research Objectives

a) General objectives

The general objective of this study is to assess and explore some pharmacological activities of a neglected and under-utilized plant like *Allanblackia parviflora* in Ghana with pronounced ethnomedicinal applications.

b) Specific Objectives

The specific objectives of this piece of work include;

- To determine the various phytochemicals present in the stem bark of *A. parviflora*
- To investigate the anti-microbial activity of the stem bark of *A. parviflora* using Agar well diffusion and Broth dilution method.
- To investigate the anti-inflammatory activity of the stem bark of *A. parviflora* using paw oedema model induced by carrageenan in chicks.
- To investigate antioxidant activity of the stem bark of *A. parviflora*

1.5 Delimitations of Study

This present study was designed to capture some phytochemicals present in the methanol, petroleum ether and aqueous extracts of the stem bark of *Allanblackia parviflora* species in eastern region of Ghana. The research was to further explore the antioxidant (including DPPH free radical scavenging, total antioxidant and total phenol assays), anti-inflammatory (using paw oedema model induced by carrageenan in chicks) and the antimicrobial activities (employing ten microbes including one fungi and nine bacterial strains) on the various stem bark extracts within a working period of one year. However isolation and characterization of bioactive compounds from the plant (using various chromatographic and spectroscopic methods) which remains the ultimate target of the entire research was not achieved due to time constraints and limited resources.

1.6 Area of study

The area of this study is pharmaceutical chemistry and its said to be multi-disciplinary in nature. This is because it employs scientific ideas and knowledge from medicinal chemistry, pharmacology and microbiology to actually provide valuable and diversify information to scholars and students that will not only add on to the pool of scientific knowledge on medicinal plants but also fill some scientific gabs.

1.7 Research Questions

1. Will *A. parviflora* show antioxidant, anti-inflammatory and antimicrobial activities as compared to its other counterparts in different African countries?
2. Will the slight morphological difference as well as geographical difference affect it activities.
3. Will different extracts have any effects on their activities?

CHAPTER TWO

LITERATURE REVIEW

2.1 Discovery of the Genu Allanblackia and its Species

European botanists in the 19th century extensively explored the tropics for fat and oil containing plants. Through their efforts however, many plant species were scientifically identified and described for the first time.

In 1869, the conscious effort of Prof Daniel Oliver, keeper of the Kew herbarium in London led to the discovery of the genus *Allanblackia* oliv.in the Guttiferae family now Clusiaceae in recent literatures. The first species published in the genus was *Allanblackia floribunda* (Oliv.) found in flora of tropical Africa precisely Cameroon. Engler in 1895 also published a second specie *Allanblackia stuhlmannii* (Engl) from east Tanzania and until then nine species are fully known following the revision of Bamps (Rompaey, 2003).

2.2 The genus allanblackia

Allanblackia is a genus of flowering plants belonging to the Clusiaceae family. These plants have attracted some form of considerations for the past years owing to their enormous oil prospects. They are found throughout the equatorial rainforests of West, East and Central African regions extending from Tanzania to Sierra Leone (Ofori et al., 2011).

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 3.4 and 4.1(Orwa et al., 2009).

There are basically nine (9) species in this genus and are distributed over Africa plus a tenth imperfectly known specie from Fernando Po after a taxonomic revision on the genus in 1969. The report from the revision revealed 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 (Rompaey, 2003)

2.3 Ethnomedicinal 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). Report from Akwa Ibom state of Nigeria shows that decoctions of the leaves stem and root of Allanblackia species are used for managing dysentery,

diarrhea, various skin infections, some microbial diseases etc. (Ajibesin et al., 2008).

The bark is used in Cameroon for the treatment of dysentery, diarrhea and toothache and mostly mixed with *Capsicum frutescens* or *Solanum anguivi* especially for the treatment of cough. It was again added that paste and creams are made from the stem bark of the plant and smeared on the affected area to reliever general body pains as well as toothaches in Gabon and also used as a stomach pain reliever in Cote d'Ivoire (Laird, 1996 and Ajbesin et al 2008). Roots of *A. stuhlmannii* are used to treat rheumatism, impotence and the oil could be smeared on wounds as well as skin rashes (Meshack, 2004; Munjuga et al., 2010).

2.4 Biological and pharmacological activities of allanblackia species

Recent studies of some Allanblackia species have shown diverse biological and pharmacological activities which may require further comprehensive investigations since they can be potential repository of natural products for the treatment of various ailment mentioned above.

2.4.1 Anti-inflammatory Activity

Some Allanblackia species investigated over the years have showed significant anti-inflammatory activities. For example, an in vivo anti-inflammatory study conducted by Nguemfo et al., (2007) on methylene chloride /methanol extract and methanol/methylene chloride fractions of the stem bark of *Allanblackia monticola* using carrageenan, histamine, arachidonic acid dextran and serotonin induced paw oedema method showed positive results on the methylene chloride fractions. The maximum inhibition recorded by carrageenan was (83.33%),

followed by arachidonic acid (64.28%), histamine (42.10%), and dextran (40.29%) with serotonin showing an insignificant modification of the oedema.

Further study by Nguemfo et al., (2009) on some methylene chloride isolates including betulinic acid, lupeol and α-mongostin (5mg/kg and 9.37mg/kg) from the stem bark of *Allanblackia monticola* using the carrageenan-induced model showed significant activities with minimum inhibition of 57.89%, 57.145 and 38.705% respectively

Ymele et al, (2011) also investigated the anti-inflammatory properties of *Allanblackia gabonensis* using carrageen, histamine, or serotonin induced paw oedema method. The aqueous extract of the stem bark showed significant activity against paw oedema induced by carrageenan with a maximum percentage of inhibition reaching 74.01% of the preventive test at a dose of 200mg/kg. It also exhibited a significant reduction of paw oedema induced by both histamine and serotonin with maximal inhibition of 56.94% (200mg/kg) and 40.83 %/(100mg/kg) respectively.

2.4.2 Antioxidant Activity

Boudjeko et al., (2013) employed host antioxidant assays including Ferric Reducing Antioxidant Potential (FRAP), DPPH radical scavenging activity, ABTS Free-Radical-Scavenging and ferrous ion chelating ability to explore the antioxidant potential of four extracts (aqueous, ethanolic, methanolic and 0.1 N HCl) of *A.floribunda and Jatropha curcas*. Generally, extracts of A. floribunda oilseed cake demonstrated greater reducing power, antiradical power and iron-chelating capacity (antioxidant). It was again noticed that the antioxidant

potential of *A. floribunda* oilseed cake in DPPH assay was linearly correlated to its total phenolic compounds.

Studies by Nguemfo et al., (2009), on some methylene chloride isolates including betulnic acid, lupeol and α-mongostin (5mg/kg and 9.37mg/kg) from the stem bark of *Allanblackia monticola* showed concentration-dependent radical scavenging activity by inhibiting 1,1-diphenyl-1-picryl-hydrazyl radical (DPPH) with an IC_{50} valve of 14.60µg/ml with α-mongostin and betulinic acid (500µg/ml) showing weak radical scavenging activity with a maximum inhibition reaching 38,07µg/ml and 26.38µg/ml.

2.4.3 Antimicrobial

Extracts from leaves, roots, fruits and flowers of *Allanblackia gabonensis* has recently been reported to display a broad spectrum activities of about 72% against three bacterial strains with MIC values varying from 16 to 1024ug/ml. The extracts from the fruit of *A. gabonensis* had the best with MIC values below 100ug/ml on 37.9% of the tested bacteria (Fankam et al., 2015)

The ethanol extracts, hexane, ethyl acetate, chloroform and butanol fractions of the leaves, stems bark and root bark of *A. floribunda* were screened for antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa Klebsiella pneumoniae, Candida albicans and Aspergilus flavus* using agar diffusion method. Among the extracts, the ethanol extract of the leaves gave the most significant activity. However no extract showed antifungal activity against C.albicans. The fractions elicited also showed both antibacterial and antifungal activities with leaf extracts having the highest inhibition against *P. aeruginosa* while the ethyl

acetate fraction of the stem bark exhibited the least inhibitory effect against *B. subtilis*. The plant extracts and fractions produced inhibitory zone range between 5 to 35mm (Ajibesin et al 2008).

Kuete et al., (2011) isolated three compounds namely 1,7-dihydroxyxanthone, morelloflavone and 7′-O-glucoside from the root bark of *A. floribunda* and assessed the antimicrobial properties of the isolated compounds using the p-iodonitrotetrazolium chloride colorimetry and Microplate Alamar Blue Assay (MABA). The results from the experiment showed that, compounds 1 to 3 prevented the growth of *Mycobacterium smegmatis*. Both extracts and compound 2 were active against *M. tuberculosis.* The lowest MIC value for the extract (9.76 μg/mL) was recorded against *Enterobacter aerogenes* while the corresponding value for the compounds (4.88 μg/mL) was obtained with compound 2 on *Trichophyton rubrum.*

2.4.4 Anti-hypertensive Activity

Bilanda et al in (2010) examined the anti-hypertensive activity of *A.floribunda*. The study revealed that doses of 200 and 400mg/kg of the extract per day significantly prevented (21.74; 26.65% and 11.71; 24.58% of reduction) the increase in mean blood pressure on Alcohol-induced hypertension (AHR) and Sucrose-induced hypertension (SuHR) respectively. A dose of 400mg/kg of the plant extract also led to the prevention of total cholesterol (42.82%), HDL-cholesterol (36.59%) and triglyceride (9.67%) and a consequential rise in serum lipid in AHR as compared to the untreated AHR. In the same manner the extract appreciably prevented the high concentration of total cholesterol (44.08%) and triglycerides(#33.05%) induced by sucrose as compared to untreated SuHR without affecting that of HDL-cholesterol.

It was further reported that doses of 200 and 400mg/kg also prevented the increase in atherogenic index by 54.45 and 42.94% in AHR and by 23.70% and 44.32% in SuHR. *Allanblackia floribunda* (400mg/kg) prevented the increase in bilirubin urea ALT and AST in AHR and SuHR. Also the plant extract treatment generally prevented the increase in superoxide dismutase (SOD) malondialdehye (MDA) and catalase and the decrease of glutathione concentration in the aorta, heart kidney and liver of AHR and SuHR

Bilanda et al., (2013) evaluated the anti-hypertensive effect of the methanolic stem bark extract *Allanblackia gabonesis* in sucrose-induced hypertensive rats. Doses of (150 and 300mg/kg/) prevented the increase in blood pressure in rats with a significant effect on heart rate

2.4.5 HIV inhibitory Activity

Fuller et al., (1999), investigated the HIV-inhibitory activity in extracts of *Allanblackia stuhlmannii*. The activity was tracked via bioassay-guided fractionation to a new member of the Camboginol Guttiferone, a class of Prenylated benzophenone called Guttiferone F. which may be related to the HIV inhibition.

2.4.6. Anticancer Activity

Recent studies on the stem bark of *A. floribunda* also identified a novel compound called Allanxanthone A which has been shown to have cytoxic activity against KB cell line. Other compounds isolated include benophenones, xanthones and bioflavonoids which are said to exhibit wide a ranging of pharmacological activities (Ayoola et al, 2008; Nkengfack et al, 2002)

2.4.7 Analgesic activity

The anti-nociceptive activity of the methylene chloride fractions were assessed using acetic acid-induced abdominal contraction model as well as formalin and hotplate tests. The anti-nociceptive properties of the plant, that is the methylene chloride fractions caused a dose dependent inhibition on abdominal contractions induced by acetic acid (32.34-77.37%) and significantly inhibited the inflammation pain caused by formalin (40.71-64.78%) (Nguemfo et al., 2007)

The analgesic effect of aqueous extracts of *Allanblackia gabonensis* was also evaluated using acetic acid, formalin, hot plate test, tail immersion and paw pressure tests. It showed significant protective effect against chemical stimuli (acetic acid and formalin) in the mouse. At doses between 100-400mg/kg, the pain induced by acetic acid exhibited a protective effect of at least 69.78% and reduced at the first (67.18% at 200mg/kg) and second (83.87% at 400mg/kg) phases of pain induced per formalin. It also produced a significant increase in the threshold of sensitivity to pressure and hotplate- induced pain in the rats (Ymele et al, 2011)

2.4.8 Anti-malarial Activity

The antisplasmodial activity of *Allanblackia floribunda* from Cameroon was assessed along with the isolation and characterization of some phenolic compounds. Among the compounds characterized, six were subjected to antimalarial screening. The most active compound, maclurxanthone presented a very interesting activity with an IC_{50} of 0.36 and 0.27ug/ml (Azebaze et al 2015)

2.5 Phytochemistry of the allanblackia species

Not much has been done especially in scientific literature on the Phytochemistry of Allanblackia species in general but few authors have published comprehensive papers 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 with the absence of alkaloids, anthraquinones and pthlobotannins in the various plant parts while saponins were abundant in the leaves and absent in the root and stem bark. The extracts of the leaves, stem bark and root also showed varying antimicrobial activities with the leaves recording the highest inhibitory effect and the least being the roots. The plant 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). Phytochemical studies of the stem bark of *Allanblackia gabonensis* resulted in the isolation and characterization of a new xanthone derivative called Allanxanthone D together with some known compounds from *Allanblackia floribunda* and *A. stuhlmanni* including xanthone derivatives Allanxanthone A, 1,5-dihydroxyxanthone, 1.7-dihydroxyxanthone, 1,3,6,7-tetrahydroxy-2-(-3-methylbut-2-enyl)xanthone, Forbexanthone, Guttiferone F, epicathechin, B-sitosterol and campesterol (Azebaze et al, 2008). The structures of some compounds are showed below in figure 2.1.

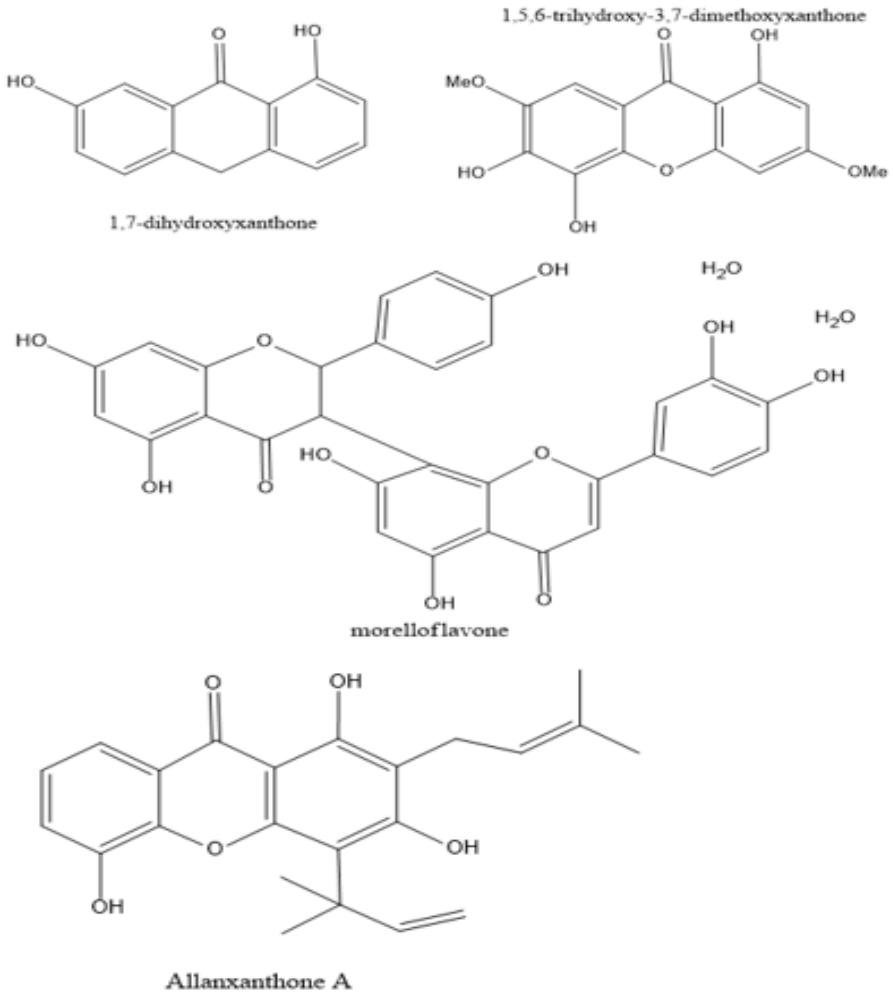

Figure 2.1: Some compounds isolated from various Allanblackia species

2.6 Allanblackia Parviflora

Allanblackia parviflora is an evergreen dioecious tree which remains, the only Allanblackia species in Ghana and is wildly distributed in the rain forest zones in the country stretching from the Ashanti, Western and Eastern regions (Adubofuor et al 2013). It is known by nine (9) indigenous names depending upon the dialect of the area where it is found in the country. Most Akan communities call it *Sonchi, Osonodokono, Kusieadwe, Kusie aduane, Dufufui, or Atrodua, Akosobolo* in the Nzema territories and Krupi or Boho at Atobiase in the Western Region. The myth behind the Akan name" *Kusieadwe* or *Kusie*

aduane" is that during famine and droughts the fruits and seeds of the plant were the staple food consumed by wild rats for survival.

The plant has recently attracted attention by some companies including unilever and other agencies in Ghana due to its enormous oil prospects. As a results, most farmers in Ghana precisely Bogoso, Afosu, Sureso, New Edubease, offinso, sefwi and Twifo praso have started commercial plantation of *A. parviflora* purposely for exploiting its oil prospects for local and foreign markets (Kwaku Antwi, 2012)

Apart from the economic oil prospects of *A. parviflora*, various studies have reported other uses including traditional medicine, seed oil for food and cosmetics, for soap making, source of biodiesel, shade tree for local people and sometimes as a source of timber since it is termite resistant. (Peprah et al., 2009; Adubofour et al., 2013; Ileleji et al., 2015; Munjuga et al., 2010; Rompaey, 2003).

2.6.1 Botanical description and Biology

Allanblackia parviflora is a medium-sized to fairly large tree growing up to about 30 to 40m tall. The bole of tree however has a cylindrical or slightly fluted rarely greater than 50cm diameter at the breast height (DBH) with narrow crown of horizontal branches and large leaves which has shiny surfaces and multiple lateral nerves forked near the margins. The stem bark is reddish brown or gray in color with small rectangular or circular scales over small red pit. (Peprah et al., 2009; Adubofour et al., 2013). The plant also has about 40 to 100 seeds embedded in the gelatinous pulp of the fruit which is also a large ellipsoid berry,

producing a yellow latex, hanging at the end of a short stalk and reported to possess great nutritional as well as medicinal benefits (Orwa et al., 2009).

The tree is said to possess creamy white, red or pink flowers which are either male or female and are thought to be propagated by insect and wind pollination. However in Ghana recent reports indicates that female tree seems to be dominant and takes about 24 to 30 months to germinate (Strom, 2013; Peprah et al., 2009).

Fig 2.2 Whole plant and parts of *A. Parviflora* at the KNUST botanical garden in the Ashanti region of Ghana

2.6.2 Phytochemistry, Biological and pharmaceutical activities of *A. parviflora*

Practically, much studies have not been done especially on the phytochemistry, biological or pharmacological properties of this plant in Ghana and elsewhere but since the plant is in the same genus as other scientifically explored species,

much of their properties are also assumed or claimed by *A. Parviflora* notwithstanding the slight morphological differences and hence this present study.

2.7 In vitro antimicrobial Assays

The susceptibility of microbes to pure compounds and crude extracts leading to the production of a growth response mostly determines the antimicrobial properties of that compounds or extract (Cos et al, 2006). There are several methods employed in antimicrobial assays with different sensitivities, principles as well as limitations. However these assay methods are categorized into four major classes including dilution, diffusion, bio-autographic and conductimetric methods (Cos et al., 2006).

2.7.1 Dilution method

According to Cos et al., (2006) and Amponsah, (2012), dilution methods generally include the assay of crude extracts or pure compounds for determination of Minimum inhibitory concentration and Minimum bactericidal concentration values. The method actually employs redox indicators or turbidimetric endpoints and dose–response effects to enable calculation of inhibitory concentrations (IC_{50} and IC_{90}) at 50 and 90% growth inhibition (Cos et al., 2006).

In the assay, test compounds are mixed with an appropriate medium that has previously been inoculated with the test organism and can be carried out in both liquid and solid media such that microbial growth can be measured in that regard hence, two main subdivision of the dilution method. In the liquid or broth dilution method, turbidity and redox-indicators are mostly used to measure

microbial growth. Turbidity can be estimated visually or obtained more accurately by measuring the optical density at 405 nm (Cos et al., 2006). However, test samples that are not fully soluble may interfere with turbidity readings, emphasizing the need for a negative control or sterility control. Thus the extract should be dissolved in a blank medium without micro-organisms and compared with the results of the test sample.

Redox indicators including 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and Resazurin are also used to quantify bacterial (Eloff, 1998; Gabrielson *et al*., 2002) and fungal growth respectively (Jahn *et al.*, 1995). The liquid dilution method also allows the determination of bactericidal or fungicidal concentration (MBC and MFC) which is determined by plating-out samples of completely inhibited dilution cultures and assessing growth.

In the solid or agar dilution method however, the minimal inhibitory concentration (MIC) is measured. This measurement actually defines the lowest concentration of the extract or pure compound that is able to inhibit microbial growth.

2.7.2 Agar diffusion methods

In the agar diffusion as described by Cos et al., (2006), when an inoculated medium is treated with a sample(at a given concentration) known to possess antimicrobial properties, the diameter of the clear zone around the reservoir (inhibition diameter) is measured after 18-24 hours of incubation. Reservoirs can take the form of filter paper discs, stainless steel cylinders placed on the surface and holes punched in the medium. The hole-punch which remains the

commonest method is the only appropriate diffusion technique for aqueous extracts, because interference by particulate matter is much less than with other types of reservoirs (Cole, 1994). The small sample requirements and the possibility to test up to six extracts per plate against a single micro-organism are specific advantages (Hadacek and Greger, 2000). The diffusion method is somewhat limited to samples that diffuse into agar and hence difficult to run on high capacity platforms.

2.7.3 Bio Autographic Method

Cos et al., (2006) again described that Bio-autographic method as one that limits antimicrobial properties on a chromatographic plate employing different methods such as: (a) direct bio-autographic approach involves growing microbes on the chromatographic plate directly, (b) contact bio-autography approach, involves the transfer of antimicrobial agents from the chromatographic plate to an inoculated agar plate such that the microbes and the antimicrobial agents directly interact and (c) agar overlay bio-autography, also involves the direct application of seeded agar medium onto the chromatographic plate. They further added that despite the high sensitivity of this method, it can only be applied to microbes that thrive on TLC plates.

2.7.4 Conductimetric Method

The conductimetric method is still an evolving test method for antimicrobial assay. The method however, detects microbial growth as a change in the electrical conductivity or impedance of the growth medium caused by microbial metabolism (Cos et al., 2006; Sawai et al, 2002)

2.8 Oxidative Stress

Free radicals are highly reactive chemical species (atoms, ions and molecules) that have one or more unpaired valence electrons. These species are advertently or inadvertently produced in various biochemical and chemical reactions both in natural and anthropogenic processes. For instance, reactive species are generated in the biological system of humans through metabolic processes, in the production of plastics, in the ageing of paints, in fuel combustion and from other environmental pollutants such as pesticides, cigarette smoke etc.

In the biological system of humans, metabolic processes and other processes such as the respirative cycle of oxidative phosphorylation give rise to highly reactive species which produce injurious effects to vital cellular components and macromolecules including, cellular DNA, cell membrane, mitochondria and nucleic acid. These adverse effects may be mutagenic changes, cardiovascular diseases, cell ageing, cancerous tumor growth and eventually cell or tissue dead. Some of these reactive species include hydroxyl (OH^-), nitric oxide ($NO^{\cdot -}$), superoxides ($O_2^{\cdot -}$), (OH**.**)-, peroxyl (ROO-), and peroxinitrite ($\cdot ONOO^-$) (Apak *et al*., 2007).

The effect of free radicals are regulated or neutralized in a way by antioxidants defenses in the body thereby by curtailing oxidative stress on biomolecules. These defense systems which includes enzymes (such as glutathione peroxidase, superoxide dismutase etc.) and non-enzymatic (ascorbic acid, B-carotene, α-tocopherol, minerals notably selenium and zinc) molecules function to deactivate the mechanism of action of the reactive species before it is even initiated (Aqil et al., 2006).

However the defense system is not a perfect one because there exit some kind of imbalance between the excessive formation of oxidants and antioxidant defense system (Sies, 1997). This form of imbalance results from either the decrease levels of antioxidants, (e.g. resulting from permanent alterations of the antioxidant defense enzymes) or increased generation of Free radicals (e.g. by contact with highly reactive toxins) therefore puts some stress on tissues and cellular components thereby posing various over 100 health concerns ranging from hemorrhagic shock nephritis cystic fibrosis arteriosclerosis, diabetes, cataract, Alzheimer's disease, cataracts, acute liver toxicity to carcinogenesis end even AIDS (Abdel-Hameed, 2009; Halliwell, 2001).

2.8.1 Antioxidant Activity

Antioxidants refers to substances which at low concentrations interrupts or ultimately averts the oxidation of other oxidizable substances (Halliwell, 2001). Antioxidants that are produced or formed in vivo are termed as endogenous and those derived from diet are exogenous antioxidants. Endogenous antioxidants constitutes enzymes including catalase, glutathione peroxidase, superoxide dismutase, Coenzyme Q10 ,a-lipoic acid and other specialized enzymes. Superoxide dismutase enzymes (SODs) exert their antioxidant properties by facilitating the conversion of reactive superoxide ($O_2^{\cdot -}$) to form a non-radical hydrogen peroxide H_2O_2 which easily diffuse between and within cells with somewhat useful metabolic functions as shown in the equation below (Halliwell, 2001; Amponsah, 2012)

$$2O_2^{\cdot} + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{Equation 2.1}$$

There are other forms of SODs in human cells having manganese MnSOD (found in the mitochondria), zinc and copper CuZnSOD (found predominantly in the cytosol) as the actives.

Catalase enzymes also accelerate the conversion of peroxides (H_2O_2) to water and oxygen (O_2) this is because peroxides may be less reactive, but can form highly reactive free radical $OH\cdot$ upon chemical interactions with iron and copper ions.

$$H_2O_2 + Fe^{2+} \text{ (or } Cu^{+}) \rightarrow OH\cdot + OH^{-} + Fe^{3+} (Cu^{2+}) \ldots\ldots\ldots\ldots \text{Equation 2.2}$$

As a result, H_2O_2 are efficiently removed from human cells by the action of a selenium based enzyme called glutathione peroxidase. Therefore the overall order reaction involving the removal of reactive free superoxide from human cells is show below.

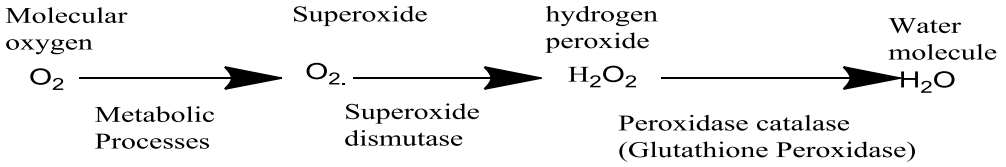

Figure 2.3: Pathway for the removal of reactive free superoxide from human cells

2.8.2 *In vitro* Antioxidant Assays

A comprehensive in vitro antioxidant assessment of plant extracts or drugs require a collaborative investigation of host chemical methods including DPPH radical scavenging, lipid peroxidation, ferric reducing power, total phenol content etc. This is because of the chemical diversity of the reactive species with different reaction mechanisms which renders it quite challenging to distinctively quantify them. Hence different experimental methods.

2.8.3 2, 2-Diphenyl-1-Picrylhydrazyl –Hydrate (DPPH) Method

This method is an electron transfer (ET) method which makes use of two solvents including methanol and ethanol. This is because antioxidants may be soluble in aqueous and lipid media and insoluble or bound to cell wall. The methanol based DPPH method involves electron- transfer leading to the reductive conversion of a purple colored DPPH (methanolic) solution to a yellow colored compound (diphenyl picrylhydrazine) at 517 nm. The ethanol based DPPH method also involves the production of a violet solution in ethanol. Here, the reactive specie which is stable at room temperature is reduced by the antioxidant molecule to yield a colorless ethanol solution. ((Abdel-Hameed, 2009, E.J Garcia et al, 2012). The reduction seen in DPPH methanol or ethanol solution indicated an increase in DPPH radical scavenging activity (Amponsah, 2012) and is mathematically expressed as

$$\%DPPH\ scavenging\ activity = [(Ac - Ae) \div Ac] \times 100 \ldots\ldots \text{Equation 2.3}$$

Where Ac; represents the absorbance of the negative control and

Ae; also denotes the absorbance of the extract and positive control

Generally DPPH assay is a rapid and easy method to assess antioxidant spectrophotometrically.

2.8.4 Lipid Peroxidation method

Lipid peroxidation involves a series of chemical reactions which is triggered the removal of hydrogen and addition of reactive oxygen which ultimately induce an oxidative stress on polyunsaturated fatty acids. In the reaction, hydrogen on the lipid is repeatedly abstracted by HO and RO to form an unstable lipid radical, followed by the addition of a molecular oxygen to alkyl or lipid radicals

resulting in the formation of an unstable lipid peroxyl radical (ROO) which in turn react with another free fatty acid to form lipid peroxide. The chain reaction is terminated by the action of antioxidants (i.e. scavenging the free radicals) generated however ineffective antioxidant action has no effect on the reaction and eventually damages the cell membrane (Repetto et al., 2012; Amponsah, 2012).

2.8.5 Ferric Reducing Power method

The assay method is basically centered on the electron transfer mechanism, such that the antioxidant interacts with potassium ferricyanide, trichloro acetic acid and ferric chloride, to produce a colored compound at an absorbance of 700 nm (Shivaprasad et al., 2005). The, method actually examine the capability of the antioxidant to cause a reduction of Fe^{3+} to Fe^{2+} characterized by an increase in absorbance indicating higher reducing power of the sample (Amponsah, 2012.) This method may be described as robust, simple and easy to perform. However, it is limited in terms of applications because it cannot be employed in species that act by radical quenching as proteins and glutathione (Benzie and Strain, 1999).

2.8.6 Total Antioxidant Capacity (TAC) Assay

Hydrogen atom transfer (HAT) and electron transfer (ET) reactions constitute the broad categories of the entire antioxidant capability. (Apak et al., 2013). HAT-based assays determines the ability of an antioxidant to mop up reactive species (especially reactive peroxyl radicals that are of biological relevance) by donating hydrogen atom. Whereas Spectrophotometric ET-based assay also measures the ability of an antioxidant to facilitate the reductive conversion of an oxidant to a colored complex. The intensity of the colored complex (either

an increase or decrease of absorbance of the probe at a given wavelength) is so much associated with antioxidants concentration present in the test sample (Apak et al., 2013).

2.9 Inflammation

Inflammation has been described as an undesirable health condition since ancient roman times in the era of Aulus Cornelius Celsus. It was characterized by the certain indications including Heat (i.e. the elevated temperature experienced on the skin as a result of the rapid movement of blood through the distended blood vessels delivering warm blood in the affected area), loss of function(i.e. immobility or rigidity in a joints, as a result of the pain and swelling or from injurious tissue), pain (is the direct response of the body to the inflammation itself resulting from stretching of the sensory nerves due swelling), redness (is resultant effect of the hot sensation caused as more blood fill the injured space) and swelling (which results from the movement of body fluids from the distended blood vessels into the affected area) (Neville et al, 2004).

In recent times however, inflammation has taken on a far more complex perspective than the primitive explanations given earlier. According to Amponsah, (2012) inflammation is the body's response to disturbed homeostasis caused by infection, injury or trauma resulting in systemic and local effects. He further expounded that various lesions and abrasions would certainly not heal in the absence of inflammation. Thus, an inflammatory reaction functions or create a physical barrier against the spread of infections and also exerts some therapeutic properties in the management of damaged or injurious tissues. Nevertheless, inflammation that remains unattended to may

possibly lead to various health conditions including rheumatoid arthritis, hay fever etc.

Neville et al, (2004) also viewed inflammation as the general body's response to some infections and tissue injury. They added that inflammation ranges from acute which is associated with skin infections especially from *S. aureus* through to chronic inflammation which results in the debilitating destruction of the joints associated with rheumatoid arthritis, transformation of the arterial and bronchial walls in atherosclerosis and asthma respectively.

2.9.1 Experimental Models of Inflammation

They are various models employed in either acute or chronic anti-inflammatory studies. Among the common models include paw oedema, sponge implantation and air pouch granulomas. Acute inflammatory reaction can be examined by monitoring various reactions including the increase foot volume caused by oedema, the presence of plasma markers in the skin, measurement of inflammatory mediators in plasma exudates, local rise in the temperature of the skin, hyperaemia (an increase in vascular permeability), monocyte infiltration, polymorphonuclear leucocyte and lymphocyte accumulation. These models make use of various agents such as carrageenan, formalin, monosodium urate crystals as well as many other agents to induce inflammation (Amponsah, 2012)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Materials for Analysis

The following drugs, reagents and chemicals of analytical grade were employed in this piece of work; Tannic acid (Fluka U.K), Diphenyl-picryl-hydrazyl (DPPH) (sigma Aldrich USA), Sodium carbonate (BDH chemical Laboratory U.K.), Disodium Hydrogen Phosphate, Ammonium Molybdate, Folin-ciocalteu, Ascorbic Acid, Carrageenan, Tragacanth, Meyer's reagent Dragendorf's reagent, tetrazolium salt, saline, DMSO, Diclofenac powder and other laboratory solvents including methanol, petroleum ether, etc. Muller Hinton agar and nutrient broth were used in the microbial assay.

3.1.2 Sampling and Authentication

The stem bark of *Allanblackia parviflora* was sampled from several farmlands in Afosu (Eastern region of Ghana) and was authenticated by Mr. Osafo Asare at the herbarium in pharmacognosy department of Kwame Nkrumah University of science and technology. The specie was assigned voucher specimen number KNUST/HMI/2015/LO10 for reference purposes and deposited at the department's herbarium.

3.1.3 Sample Preparation

The stem bark of *A. parviflora* were cut into small pieces, washed with water, air-dried under room temperature for 2 weeks and milled into fine particles. 200 g of the powdered sample was serially extracted with methanol, distilled water and petroleum ether using cold maceration for 72 hours. The extracts were filtered and concentrated using a Rotavapor (Type R-210 Buchi, Switzerland)

to yield 7.3%, 2.21% and 6.52%, w/w of methanol, aqueous and pet-ether crude extracts respectively. The crude extract was stored in a refrigerator until required.

3.2 Methods

3.2.1 Phytochemical Screening of *Allanblackia Parviflora*

Qualitative test for the screening and identification of bioactive constituents in the stem bark extracts of *Allanblackia parviflora* were investigated using standard procedures described by Trease and Evans (1989) and Sofowora, (1993) as employed by Ayensu and Quartey, (2015)

3.2.2 General Alkaloids

About 0.2 g of the plant sample was boiled with Amoniacal alcohol (constituting 3ml of ammonia solution in 27 ml of 95% ethanol) for few minutes. The mixture was filtered and evaporated to dryness. The residue was again extracted with dilute sulphuric acid and filtered. Dilute NH_3 solution was added to the mixture followed by CH_3Cl and gently agitated in a separating funnel. The CH_3Cl was again evaporated off from the extract and then dissolved in dilute sulphuric acid. About 1-2 drops of Dragendorf's reagent was added to 1 ml of the resulting mixture, the presence of alkaloids was characterized by an orange brown precipitate. A confirmatory test was performed by acidifying the various extracts followed by the addition of Mayer's reagent to about 2 ml of the acidified extracts. A positive test for alkaloids indicated the absence of a buff white precipitate.

3.2.3 Anthraquinones

Modified Borntrager's test was employed in the qualitative determination of Anthracene glycoside. In the test, exactly 2 ml of dilute H_2SO_4 and 5% of aqueous $FeCl_3$ was used to gently boil about 0.2 g of the plant material for 5 minutes. The resultant mixture was subjected to filtration whiles hot and allowed to cool down. The filtrate was then shaken with chloroform (equal volume as the filtrate). By means of a separating funnel, the filtrate was separated from the chloroformic layer and was again shaken with dilute ammonia solution. The formation of a pink rose coloration showed a positive test for anthraquinones.

3.2.4 Reducing Sugars

0.2 g of the plant sample was subjected to hot extraction with about 5 ml of dilute HCl for 5 minutes. The resultant mixture was subjected to filtration and several drops of 20% NaOH was added to the filtrate. The filtrate was again subjected to gentle heating after 1 ml of Fehling's solution A and 1 ml Fehling solution B were added to the alkalized the filtrate. The formation of a brick red precipitate on heating the filtrate confirms the presence of reducing sugars.

3.2.5 Flavonoids

About 0.2 g of the plant material was subjected to hot extraction with distilled water (5ml) for about 2 minutes and by means of a funnel and cotton wool the mixture was 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 showed a positive test for flavonoids. A confirmatory test was performed by the addition of lead acetate solution to 1ml of the filtrate. The yellow colored precipitate formed indicated the presence of flavonoids.

3.2.6 Glycosides

A quantity of the plant material (about 0.5 g) was extracted with 70% ethanol and the mixture was filtered. A trace amount of ferric chloride solution and about 1 ml glacial acetic acid were added to the filtrate. The mixture was gently swirled to mix and concentrated sulphuric acid was gently dispensed into the mixture. A reddish brown ring at the interface indicated the presence of cardiac glycosides.

3.2.7 Phytosterols

0.2 g of the plant sample was subjected to chloroformic treatment and gently filtered. About 3-4 drops of acetic anhydride was added to the filtrate, and heated for some few minutes. The mixture was then cooled, and 1 ml of Conc. Sulphuric acid was added. A positive test for phytosterols was indicated by the development of brown ring at the junction.

3.2.8 Saponins

10 ml of distilled water was added to dissolve 0.2 g of plant sample. The resultant mixture was stoppered and shaken vigorously for about 2 minutes and observed for the presence of persistent foam.

3.2.9 Tanins

0.5 g of the plant material was subjected to hot extraction with 15 ml of distilled water for a few minutes. The mixture was filtered when cooled and about 1 ml of the filtered sample was diluted to 10 ml with distilled water. A white precipitate was detected when 5 drops of 1 % lead acetate solution was added. A confirmatory test was conducted following the same procedure using 1%

ferric chloride and a dark green precipitate was observed indicating the prevalence of tanins.

3.2.10 Triterpenoids

A chloroformic extract was obtained by shaking 0.2 g of the plant sample with chloroform and filtered. 4 drops of concentrated sulphuric acid was added to 1 ml of the filtrate and gently swirled to mix. The formation of effervescence after the mixture was allowed to stand and a characteristic reddish brown color at the interface indicates the presence of terpenoids

3.2.11 Cyanogenic Glycoside

About 0.2 g of the plant sample was weighed into a conical flask. Few drops of distilled water added to moisten the sample. By means of a cork, a picrate paper stripe was suspended at the neck part of the conical flask and gently heated on a water bath. Changes in the colour of the picrate paper indicates the presence of cyanogenic glycosides.

3.2.12 Cardiac glycosides

Exactly 0.5g of the test samples were subjected to hot extraction using of 70% ethanol. The mixture was allowed to cool and subsequently filtered. The filtrate was diluted with distilled water coupled with the addition of about 5 drops of concentrated lead acetate solution and agitated. The mixture was again filtered and few drops of dilute HCl was added till precipitate disappeared. The resulting mixture was filtered and divided into two and gently agitated with CH_3Cl. 1ml of distilled water was added to the combined chloroformic mixture and again filtered and evaporated to dryness. About 1 to 2 drops of ethanol was added to

the mixture in addition to few drops of 3, 5- dinitobenzoic acid (2% in Ethanol). A purple coloration was observed when dilute NaOH (20%) solution was added.

3.2.13 Antimicrobial assay of extracts

The stem bark extracts of *Allanblackia parviflora* were tested against 10 microbial strains obtained from the Kwame Nkrumah University of Science and Technology precisely the microbiology section at the Faculty of Pharmacy and Pharmaceutical Science. The microorganisms used in the assessment included nine (9) bacterial and one (1) fungal strains of which five were type cultures and others were clinical strains. The bacterial strains also included four Gram-positive bacteria (i.e. *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis ATCC 29212 Streptococcus paratypi A* and *Bacillus subtilis* NCTC 10073), five Gram-negative bacteria (*Salmonella typhi, Neisseria gonorrhoeae, Escherichia coli ATCC 25922, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* ATCC 4853) and one fungal strain (*Candida albicans).*The microbial stains were sub-cultured 24 hours prior to experiment in a nutrient broth at 37ºC

3.2.14 Preparation of Media (Nutrient Agar)

28 g of Mueller-Hinton agar powder was weighed into a beaker and about 500 ml of distilled water was added to completely dissolve it. Enough distilled water was added to make up 1liter. 10 ml portions of the prepared agar was poured into test tubes and sealed firmly with the aid of cotton wool. The various portions were then sterilized in an autoclave (Type; Portable 18L high pressure steam sterilizer, model YX-280A) at a temperature of about 121°C for 15 minutes.

3.2.15 Antimicrobial screening of various extracts

The preliminary antimicrobial assessment employed the Agar Well diffusion method employed by Osei-Djarbeng et al, (2014), and Amponsah, (2012). The whole assay, involved the inoculation of the microbial strains into the nutrient broth followed by incubation (incubator type: Heraeus, Kendro B12) at 37 °C 24 for hours.

In the experiment, 1 ml of the sub-cultured organisms was inoculated into a prepared Mueller-Hinton agar in a sterile petri dish and uniformly smeared on the agar with the aid of a sterile cotton swab. Various wells were then created in the inoculated agar with cork borer (3mm in diameter) and appropriately labeled.

0.2 g of the methanolic, pet-ether extracts were reconstituted in 10 ml each of 2% dimethyl sulfoxide (DMSO) and the same procedure was repeated for the aqueous extract using distilled water as the vehicle. 100µL of 0.02 mg/ml of each extract were carefully dispensed into each well. The prepared petri dishes were allowed to stand for about 30 minutes to enable the extracts diffuse well into the media and was later incubated at 37^{o}C for 24 hours. Positive control used in the experiment was Ciprofloxacin (at the concentration of 0.0001 g/10ml) and the negative control was 2% DMSO.

After the incubation, the effect of the various extracts on the test microbes were shown as clear zones of inhibition. These zones of inhibition were measured using a pair of divider and ruler and the results obtained were reordered. All the experiments were repeated three times to compute the mean zone of inhibition.

3.2.16 Broth Dilution Method (Minimum Inhibitory Concentration (MIC)

Broth dilution Assay was carried out to determine the MIC of the extracts that exhibited considerable antimicrobial activity in preliminary screening. In this method, the test microbes were prepared from a 24 hour nutrient broth culture which was then adjusted to obtain a suspension of 10^8 cfu/ml.

2% concentration of the stem bark extracts were reconstituted in 2% (DMSO) and serially diluted to obtain various concentrations ranging from 0.158 mg/ml to 20 mg/ml.

Sterilized 96-well micro-plates were used in the assay and each well contained 100 µl of double strength nutrient broth, 80 µl of each prepared extract and 20 µl of the various cultures organisms.

The prepared plates were subjected to Incubation for 24 hours at a temperature of 37ºC. The growth of the organisms were estimated by adding 20 µl of 5% tetrazolium salt solution and further incubated for about 15 minute. A dark coloured well infers the presence of microorganisms, since these organisms poses some dehydrogenase enzymes that react with the salt to give a dark colour. Hence the concentration of the dark coloured well represent MIC

Both ciprofloxacin and DMSO were used as positive and negative control respectively and all experiments were performed in triplicates.

3.2.17 *In vitro* Antioxidant Assessment

Three different chemical methods namely DPPH scavenging ability, total antioxidant capacity total phenol content were employed in the in vitro antioxidant assessment.

3.2.18 Total antioxidant capacity

The theory of total antioxidant capacity follows a reaction (reduction) in which a green phosphate-molybdate (Mo^{+5}) complex is formed at an acidic pH due to the reductive conversion of molybdenum, Mo^{+6} to Mo^{+5} (Prieto *et al*., 1999). In the experiment, a mixture containing 1 ml of each extract at various concentrations ranging between 63.5 µg/ml and 500 µg/ml and 3 ml of standard reagent solution (constituting disodium phosphate, sulphuric acid and ammonium molybdate in the concentration 28 mM, 6.0 M and 4 mM respectively) were incubated at 95^{o}C for 90 minutes. The reaction mixtures were subject to cooling at normal room temperature and by means of a UV multi-plate reader (Type: Synergy H1, serial number: 271230) their absorbance were measured at 695 nm.

The same reaction procedure was repeated using ascorbic acid as the positive control with different concentrations ranging from 6.25 µg/ml to 50 µg/ml and a calibration curve of absorbance against concentration was constructed for ascorbic acid. A blank solution was also prepared in the same way excluding the extract or the reference drug and the equivalent of the standard drug (ascorbic acid in mg) per that of the extracts (expressed in g) was used to evaluate total antioxidant capacity

3.2.19 2, 2-Diphenyl-1-Picrylhydrazyl –Hydrate (DPPH) Assay

The DPPH radical scavenging assay follows a principle involving the reduction of purple methanolic DPPH solution to a yellow colored solution resulting from the formation of a stable radical 1, 1-Diphenyl-1-Picrylhydrazyl –Hydrate (DPPH.H) at a maximum absorption of 517 nm.

In the experiment, various concentrations (62.5 µg/ml to 500 µg/ml) of each extract (aqueous, methanolic and pet ether) of the stem bark of *Allanblackia parviflora* were prepared in methanol and that for DPPH was 20 mg/l in methanol.

3.0 ml of DPPH solution and 1.0 ml each of the various extracts constituted the reaction mixture. The reaction mixture was however kept for half an hour in the dark at 25^{o}C and their absorbance were measured using a UV multi-plate reader (Type: Synergy H1, serial number: 271230). The positive and negative controls employed were ascorbic acid (with concentrations ranging between 6.25µg/ml - 50 µg/ml) and methanol respectively. The assays were carried out in triplicates and a graph of concentration against % DPPH scavenging activity was plotted to estimate the concentration needed to reduce the absorbance by a considerable percentage of 50 (EC_{50}). From the experiment, the percentage DPPH scavenging ability was mathematically expressed as;

$$\%DPPH\ \ radical\ scavenging\ activity = [(Ac - Ae) \div Ac]100 \ldots \text{Eqn 3.1}$$

Where Ac; represents the absorbance of the negative control and

Ae; also denotes the absorbance of the extract and positive control.

3.2.20 Total phenol content

This assay employed the method described by Ayensu and Quartey, (2015) with few modification in the procedure. The method basically depends on the reduction of folin -ciocaltau reagent by phenolic compounds in the extracts to form blue oxides at 760 nm. In the experiment 0.1 ml of stem bark extracts in distilled water with different concentration ranging from 62.5µg/ml to 500 µg

/ml was added to test tubes containing 0.1 ml of 0.5 N folin- ciocaltau and subjected to incubation at 25^{o}C for about 15 minutes.

The reaction mixture further received sodium bicarbonate treatment (2.5 ml of 2%) and was incubated for an additional 15 minutes without any physical agitation under the same conditions and their absorbance measured. The same procedure was repeated for the blank (excluding the extract or reference sample) and positive control using tannic acid as the reference sample with various concentrations (3.125µg/ml to 200µg/ml) and a calibration curve of absorbance against concentration was constructed for tannic acid. Therefore the equivalent of the reference sample (tannic acid in mg) per that of the extracts (expressed in g) was used to evaluate total phenol content All experiments were performed in triplicates.

3.2.21 *In Vivo* Anti-inflammatory Assessment

Freshly hatched chicks (n=150) were purchased from Akate Farms (Kumasi in the Ashanti region of Ghana) and accommodated in a stainless steel boxes (34 × 57 × 40 cm). The chicks were divided into 22 groups (6 groups for each extracts for both prophylactic and therapeutic studies, 3 groups for positive control and 1 group for negative control) with about 4 to 5 chicks per box. Chick mash and water were accessible to feed the animals at constant room temperature. Daily care was conducted for 7 days until the chicks were used for the study.

3.2.22 Anti-inflammatory Activity in 7-Days Old Chicks

The carrageenan induced oedema model as employed by Amponsah, (2012) was used for the anti-inflammatory assessment of the various stem bark extracts using Diclofenac as reference drug.

The main focus of the experiment was to assess whether or not the extracts as well the standard drug will exert or have any significant therapeutic as well as prophylaxes effects on oedema 1 hour after carrageenan injection to the 5^{th} hour.

Prior to the experiment, the initial size of the chicks left footpad were measured using a digital caliper (Type: Powerfix IAN56288 Model No: Z22855) after which 10 µl of 2% carrageenan prepared in 0.9% saline was introduced subplanter into the left footpads of the chicks. Subsequent measurements were taken at hourly interval for 5 hours after injection.

For therapeutic analysis, the carrageenan was induced 30 minutes to 1hour before the test samples were administered whiles in the prophylactic assay the test samples were given before carrageenan was induced. Different concentration of the stem bark extracts (30, 100 and 300 mg/kg) and the reference drug i.e. Diclofenac pure powder at 10, 30 and 100 mg/kg were given to the test subjects by oral administration with the control group receiving only the vehicle. The doses for the extracts were prepared by dissolving a known amount of the extracts in 2% Tragacanth (vehicle). However the actual doses of the reference drug and extracts administered were evaluated from the individual weight of the animals. The oedema component was quantitatively expressed as the difference in size of the chicks' footpad at time zero where carrageenan was absent and at the various time intervals after injection.

3.2.23 Statistical Data Analysis

The data obtained from the various analyses precisely anti-inflammatory and antioxidant were subjected to Statistical analysis employing version 5 of Graph Pad Prism as the main tool. The iterative least square method which expresses the concentration that accounts for 50% of the maximal effects (EC_{50}) for the various extracts and reference drugs was employed for the antioxidant studies following the non-linear regression equation below

$$Y = \frac{a+(b-a)}{1+10^{(logEC50-x)}} \ldots\ldots \text{Equation 3.2}$$

Where, *Y* denotes the response and *X* represents the logarithm of the concentration, "a" maximum and "b" minimum represent model parameters of the curve and EC_{50} represents the fitted midpoint of the curve

For the anti-inflammatory studies, the sizes of the chick's footpad were discretely expressed as a percentage of the differences between the individual values at various time points and the values at the zero hour and their respective means were taken. Therefore the total footpad size of the chicks for each treatment was expressed as the area under the curve (AUC) in arbitrary units and the percentage inhibition for each treatment follows the equation below.

$$\% \text{ inhibition} = [(AUCc - AUCt) \div AUCc] \times 100 \ldots\ldots\ldots \text{Equation 3.3}$$

One- way Anova was employed in evaluating the AUCs followed by Dunnett's *post hoc test* for significant difference between treatments. All analyses were performed based on the significance of $P < 0.005$.

CHAPTER FOUR

RESULTS

4.1 Qualitative phytochemical screening

The results obtained from the qualitative phytochemical screening conducted on the various stem bark extracts (aqueous, methanolic and petroleum ether) and pulverized sample of *Allanblackia parviflora* specie found in Ghana is summarized in Table 4.1 below. The outcome revealed the presence and absence of alkaloids, tanins, flavonoid, reducing sugar, cardiac glycosides, anthraquinones, phytosterols and triterpenoids in the various samples. However cynogenetic glycosides was absent in all the samples screened.

Table 4.1: Phytochemicals constituents of the stem bark extracts and pulverized sample of *Allanblackia parviflora*

Test	Pulverized Sample	Methanolic Extract	Aqueous Extract	Petroleum Ether Extract
Tanins	+	+	+	+
Flavonoid	+	+	+	-
Reducing Sugar	+	+	+	+
Saponins	-	+	-	-
Triterpenoids	+	+	+	-
Phytosterols	-	+	-	-
Anthraquinones	+	+	+	-
General Alkaloids	+	-	+	-
Cardiac Glycosides	+	+	+	+
Cyanogenic Glycosides	-	-	-	-

(Key: + indicates present and – indicates absent)

Table: 4.2 provides the summary of results acquired from the preliminary Antimicrobial screening of the stem bark extracts (at the concentration of 0.002 mg/ml) of *Allanblackia parviflora* against 10 microbial strains including four gram positive bacteria *(Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis ATCC 29212, Streptococcus paratypi A* and *Bacillus subtilis* NCTC 10073), five gram negative bacteria (*Salmonella typhi, Neisseria gonorrhoeae, Escherichia coli ATCC 2592 Klebsiella pneumoniae* and *Pseudomonas aeruginosa* ATCC 4853) and a fungus (*Candida albicans)* using ciprofloxacin (at the concentration of 0.0001 g/10ml) and 2% DMSO as positive and negative controls respectively . The results revealed considerable microbial effect (zone of inhibition) of the extracts notably methanolic and aqueous against the test organisms whereas petroleum ether extract was inactive against all the test organisms.

Table 4.2 Antimicrobial activity of the stem bark extracts of *Allanblackia parviflora*

Zones of inhibition (mm)at a conc. of 0.002 mg/ml ±SEM					
Microbial Strains	Methanolic Extract	Aqueous Extract	Pet-Ether Extract	Ciprofloxacin	2% DMSO
E. coli	13.00±0.0	12.50±0.5	NA	15.50±0.5	NA
E. faecalis	20.00±0.0	15.00±0.0	NA	16.00±0.0	NA
S. aureus	17.50±0.5	15.00±0.0	NA	21.50±0.5	NA
C. albicans	17.00±0.0	12.50±0.5	NA	21.00±1.0	Na
B. subtilis	15.00±00	13.00±0.0	NA	16.00±0.0	NA
S. paratypi A	10.00±0.0	10.50±0.5	NA	16.75±0.25	NA
S. typhi	15.50±0.5	14.00±0.0	NA	19.5 ±0.5	NA
N.gonorrhoea	I0.50±0.5	10.00±0.5	NA	15.50±0.5	NA
P. aeruginosa	14.5±0.5	12.50±0.5	NA	19.50±0.5	NA
K. pneumoniae	13.5±0.5	12.50±0.5	NA	15.50±0.5	NA

(Key NA- Not Active|

Table 4.3 also gives the summary of the Minimum Inhibitory Concentrations (MIC) of the extracts that showed considerable microbial effects against the test organisms in the preliminary assay**.** Ciprofloxacin recorded the lowest MIC values followed by methanol and aqueous extracts respectively**.**

Table 4.3 Minimum Inhibitory Concentrations of the stem bark extracts of *Allanblackia parviflora*

Microbial strains	Methanolic extract(mg/ml)	Aqueous extract(mg/ml)	Ciprofloxacin (mg/ml)
E. coli	2.50	5.00	0.025
E. faecalis	2.50	5.00	0.05
B. subtilis	5.00	5.00	0.0125
S. aureus	2.50	2.50	0.05
C. albicans	1.25	5.00	0.0125
S. paratypi A	2.50	5.00	0.025
S. typhi	2.50	5.00	0.05
N. gonorrhoeae	*2.50*	*5.00*	*0.05*
P. aeruginosa	1.25	2.50	0.05
K. pneumoniae	500	5.00	0.05

4.3 *In vivo* Anti-inflammatory Activity

The carrageenan induced paw model has successfully been used to assess the anti-inflammatory properties of the methanolic aqueous and pet-ether stem bark extracts of *Allanblackia parviflora.* The model as described in chapter 3 above employed chicks with different weights between 30 and 64g as test subjects. The various extracts were administered orally at concentrations of 30,100 and 300 mg/kg and 10, 30 and 100 mg/kg for the reference drug. In the prophylactic studies, carrageenan was induced one hour after the extracts have been administered and vice versa for the therapeutic studies. The oedema started developing after one hour period to its maximum peak at the second hour and

was characterized by swelling, pain and redness at the footpad of the chick and this effect rendered the chicks less active as observed in figure 4.1 below.

Figure 4.1 Visible signs that characterize acute inflammation

Beyond the second hour, oedema significantly reduced down to the six hour exhibiting a considerable anti-inflammatory activities for Diclofenac, methanolic, aqueous and pet-ether extracts. However Diclofenac demonstrated the highest activity followed by methanolic, aqueous and pet-ether extracts respectively. The overall effect of each treatment on oedema has been summarized on a time course curve (A) and total oedema response (B) at significance of *** P <0.001,** P<0.01 and **P<0.05 in figures 4.2, 4.3, 4.4, 4.5 4.6 and 4.7 below. The percentage inhibitions for the various treatments are also expressed as the area under the curve and presented in tables 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9 below.

Table 4.4 Percentage Inhibition of oedema by the methanolic stem bark extracts of *Allanblackia parviflora* (prophylactic).

Treatment(mg/kg)		% inhibition
Diclofenac	10	66.03
	30	70.27
	100	77.75
Extract	30	49.26
	100	51.41
	300	59.05

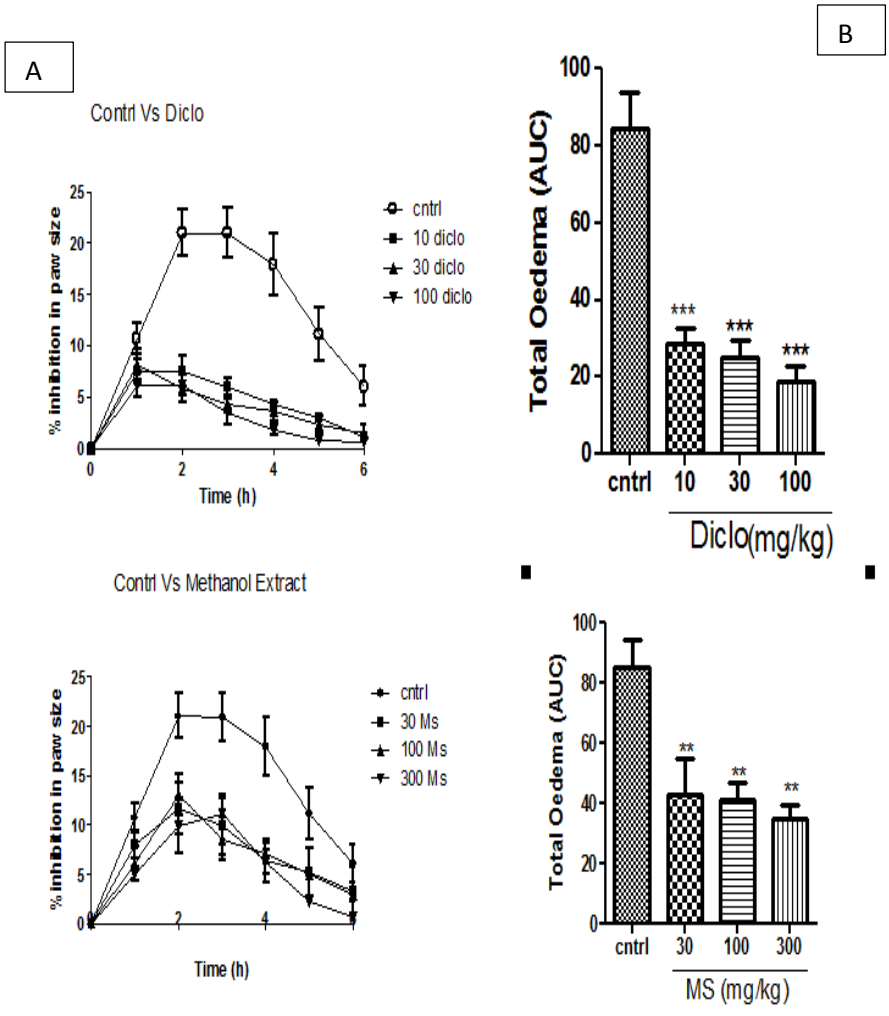

Figure 4.2; Prophylactic effects of the methanolic stem bark extract of *Allanblackia parviflora* and Diclofenac against a control on the footpad oedema induced by carrageenan in chicks expressed by time course curve (A) and total oedema response as AUC (B) at *** P <0.001, P<0.01 and **P<0.05**

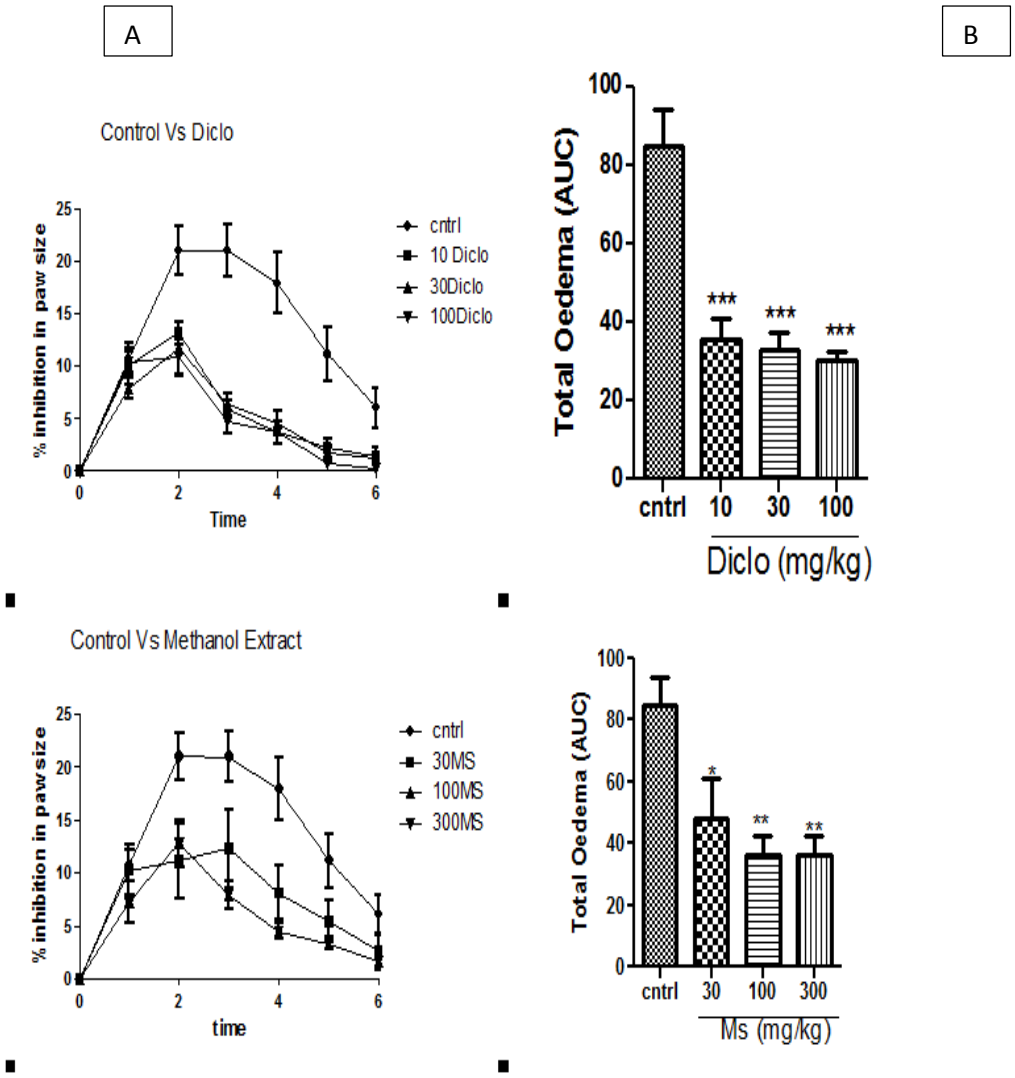

Figure 4.3 Therapeutic effects of the methanolic stem bark extract of *Allanblackia parviflora* and Diclofenac against a control on the footpad oedema induced by carrageenan in chicks expressed by time course curve (A) and total oedema response as AUC (B) at *** P <0.001, P<0.01 and **P<0.05**

Table 4.5 Percentage Inhibition of oedema by the methanolic stem barks of *Allanblackia parviflora* (therapeutic).

Treatment (mg/kg)		% inhibition
Diclofenac	10	58.01
	30	61.17
	100	64.08
Extract	30	42.94
	100	56.93
	300	66.01

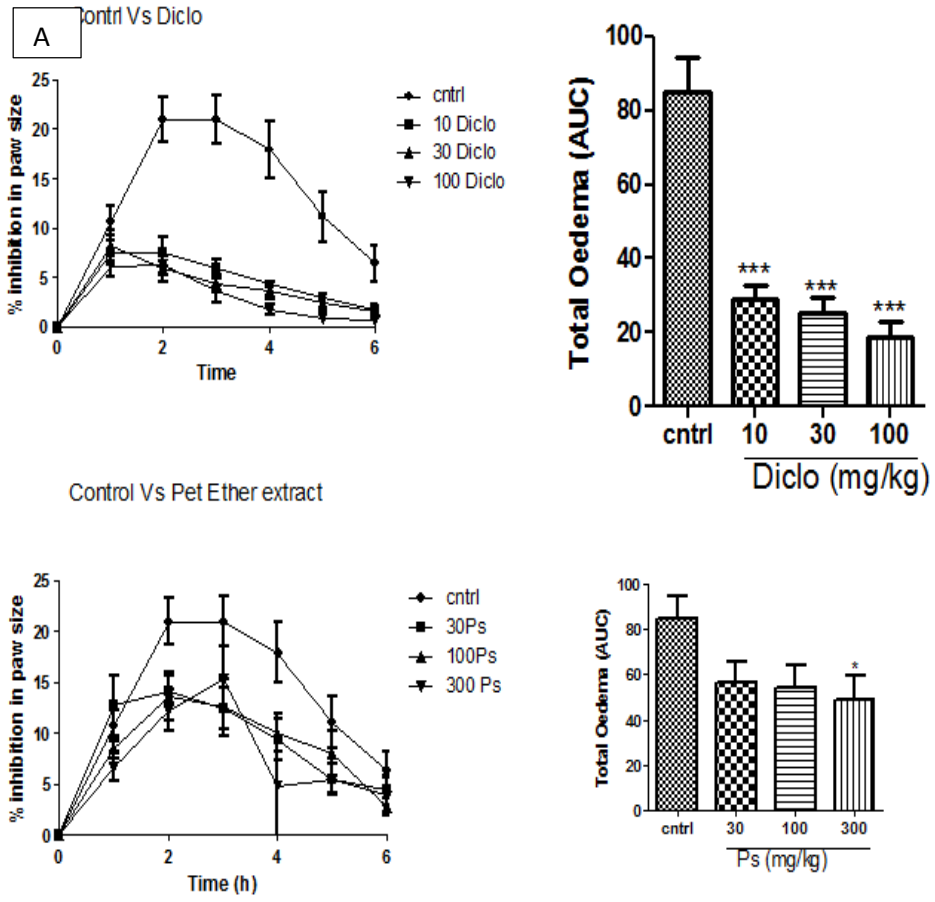

Figure 4.4 Prophylactic effects of the pet-ether stem bark extract of *Allanblackia parviflora* and Diclofenac against a control on the footpad oedema induced by carrageenan in chicks expressed by time course curve (A) and total oedema response as AUC (B) at *** P<0.001, P<0.01 and **P<0.05**

Table 4.6 Percentage inhibition of oedema by the pet-ether stem bark extracts of *Allanblackia parviflora* (prophylactic).

Treatment (mg/kg)		% inhibition
Diclofenac	10	58.01
	30	61.17
	100	64.08
Extract	30	42.94
	100	56.93
	300	66.01

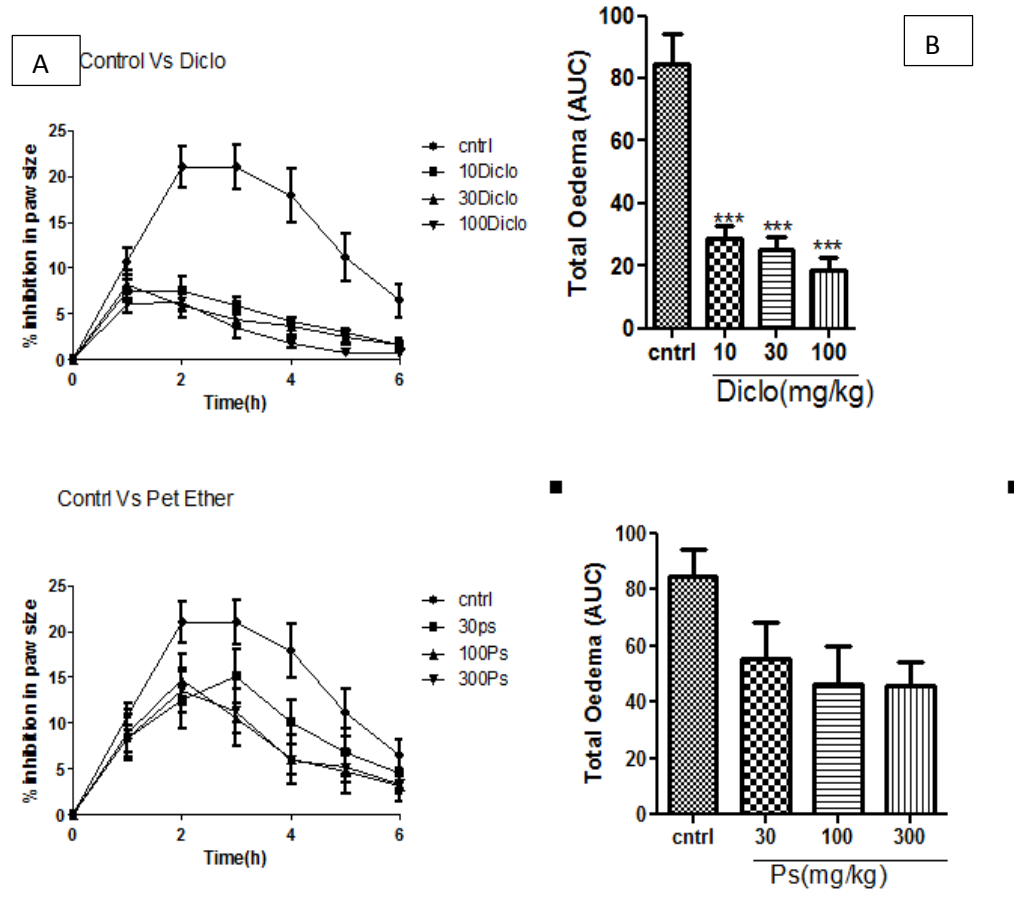

Figure 4.5 Therapeutic effects of the pet-ether stem bark extract of *Allanblackia parviflora* and Diclofenac against a control on the footpad oedema induced by carrageenan in chicks expressed by time course curve (A) and total oedema response as AUC (B) at *** P <0.001, P<0.01 and **P<0.05**

Table 4.7 Percentage inhibition of oedema by the pet-ether stem bark extracts of *Allanblackia parviflora* (therapeutic).

Treatment(mg/kg)		% inhibition
Diclofenac	10	65.81
	30	70.33
	100	77.80
Extract	30	34.92
	100	45.38
	300	45.99

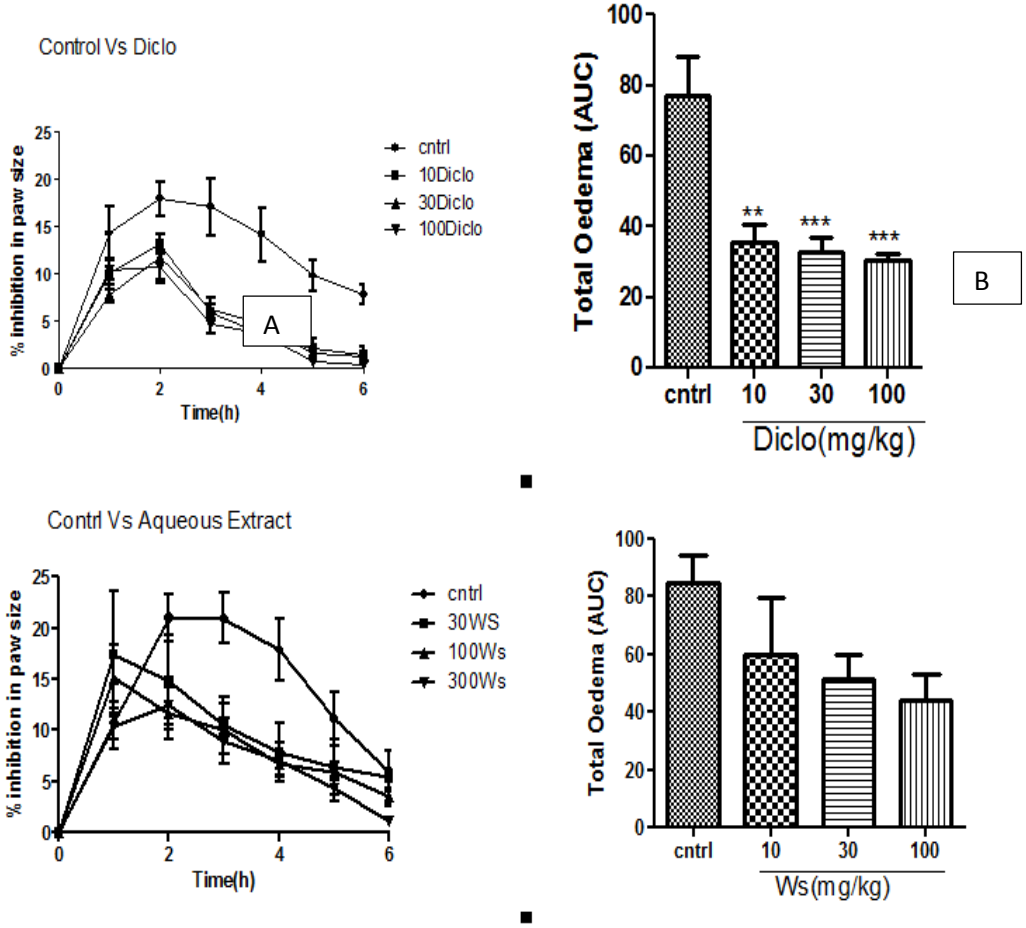

Figure 4.6 Prophylactic effects of the aqueous stem bark extract of *Allanblackia parviflora* and Diclofenac against a control on the footpad oedema induced by carrageenan in chicks expressed by time course curve (A) and total oedema response as AUC (B) at *** P <0.001, P<0.01 and**P<0.05**

Table 4.8 Percentage inhibition of oedema by the aqueous stem bark extracts of *Allanblackia parviflora* (prophylactic).

Treatment(mg/kg)		% inhibition
Diclofenac	10	53.12
	30	67.40
	100	75.60
Extract	30	29.27
	100	39.20
	300	48.24

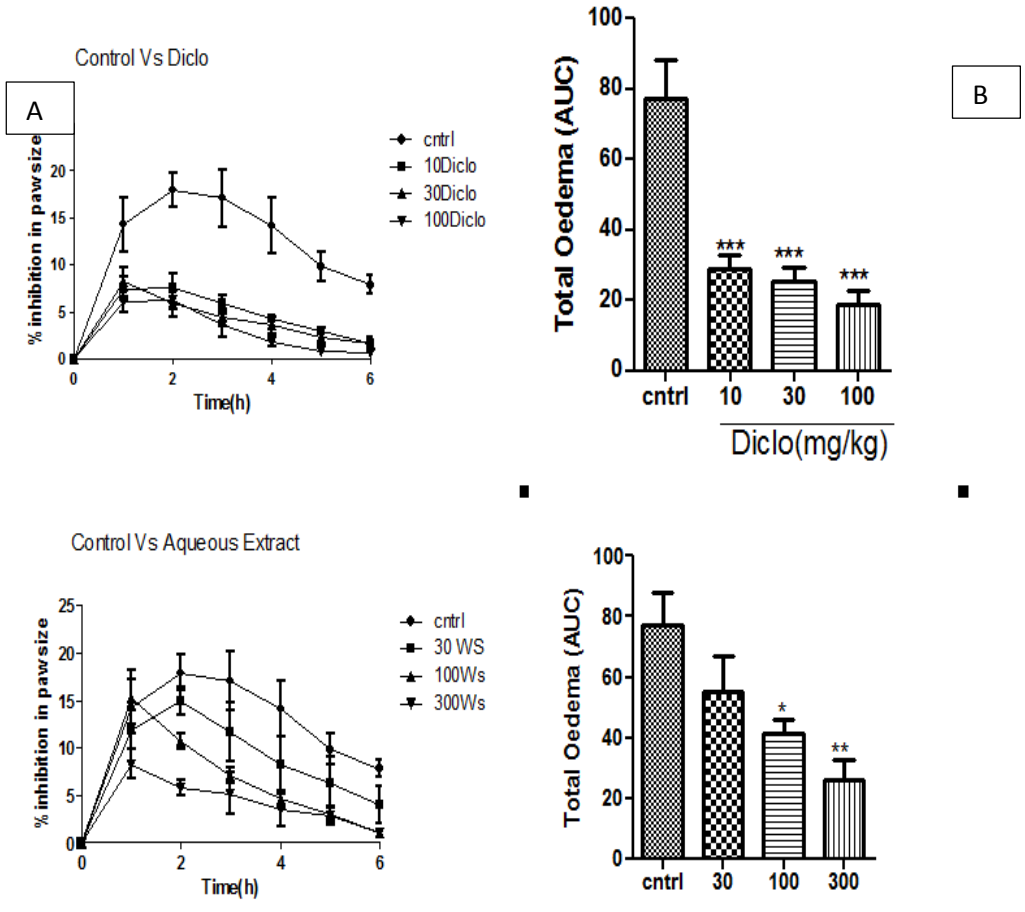

Figure 4.7 Therapeutic effects of the aqueous bark extract of *Allanblackia parviflora* and Diclofenac against a control on the footpad oedema induced by carrageenan in chicks expressed by time course curve (A) and total oedema response as AUC (B) at *** P <0.001, P <0.01 and **P<0.05**

Table 4.9 Percentage inhibition of oedema by the aqueous stem bark extracts of *Allanblackia parviflora* (therapeutic).

Treatment(mg/kg)		% inhibition
Diclofenac	10	53.94
	30	54.27
	100	60.61
Extract	30	28.61
	100	46.60
	300	56.93

4.4 In vitro Antioxidant Assessment

The antioxidant activity also employed a host of chemical methods namely DPPH radical scavenging, total antioxidant capacity (TAC) and total phenol content (TPC) to quantitatively assess the activity.

4.4.1 DPPH Radical Scavenging potential

Figure 4.8 and Table 4.10 compares the % DPPH free radical scavenging ability of the various stem bark extracts to the reference drug. The free radical scavenging power depended so much on the concentrations of the test samples such that % DPPH increased with increasing concentration of extracts as seen in Figure 4.8 below. Ascorbic acid which was used as the reference drug at concentration range of 6.25 µg/ml -500 µg/ml demonstrated a very significant and the highest scavenging potential expressed as EC_{50} (a measure of a drug/extracts potency) of 0.01539 mg/ml among the test samples. In terms of the extracts (at 62.5 µg/ml to 500 µg/ml) methanol exhibited the highest free radical scavenging power as EC_{50} followed by aqueous and pet-ether in that decreasing order as shown Table 4.11

Table 4.10 Depicts the % DPPH scavenging potential of the reference drug and various stem bark extracts of *Allanblackia parviflora* at the highest concentration of 500 µg/ml

Treatment	% DPPH Scavenging power ±SEM
Methanolic	40.74±0.0
Pet-ether	25.96±0.87
Aqueous	32.41±0.0
Ascorbic Acid	75.04 ±0.84

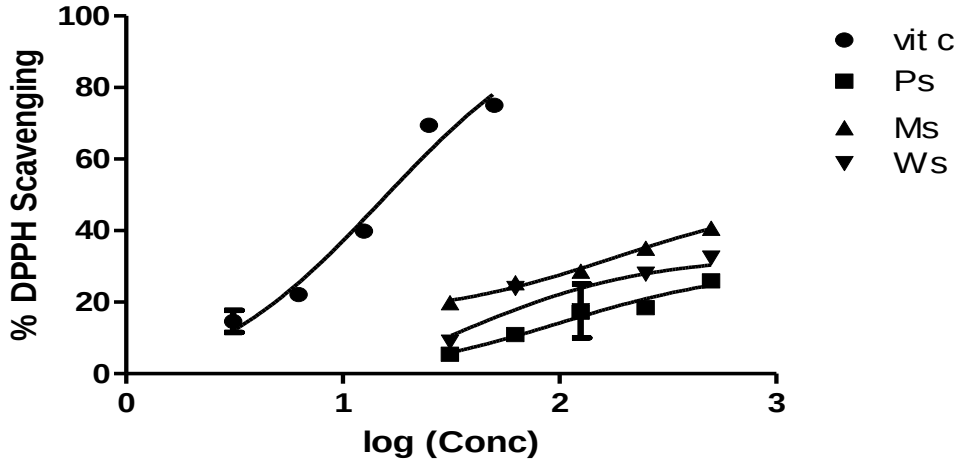

Figure 4.8 Percentage DPPH scavenging potential of (Ascorbic acid) against methanolic Aqueous and pet-ether extracts of *Allanblackia parviflora*

Table 4.11 Depicts EC_{50} values of the reference drug and various stem bark extracts of *Allanblackia parviflora*

Treatment	EC_{50} (mg/ml)
Methanolic	0.03341
Pet-ether	0.1842
Aqueous	0.09991
Ascorbic Acid	0.01539

4.4.2 Total Phenol Content

Table 4.12 summarizes the results obtained from the total phenolic content determination for the methanolic, aqueous and pet-ether extracts using tannic acid as the reference. The total phenolic content of the various test sample were however expressed as TAA, i.e. a proportion of tannic acid (in mg) comparable to each gram of the extracts (phenolic compounds) that triggered the reduction of folin –ciocaltau to form blue oxides at 760 nm. From the results, methanolic extract recorded the highest phenol content (as TAA) followed by aqueous and pet-ether as depicted in figure 4.10 at the highest concentration of 500 µg/ml respectively. The calibration curve for tannic acid shown in figure 4.9 was constructed with linear regression of r^2= 0.9247.

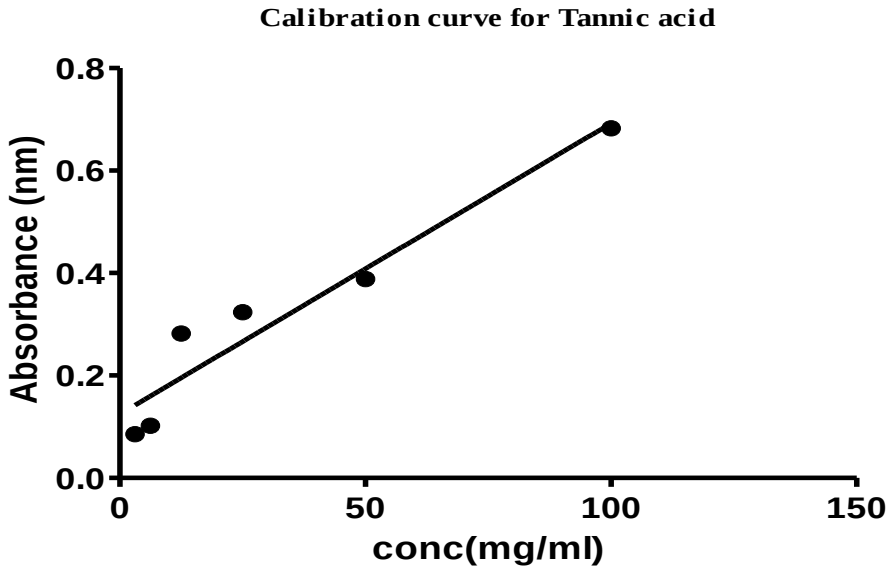

Figure 4.9 calibration curve of Tannic acid

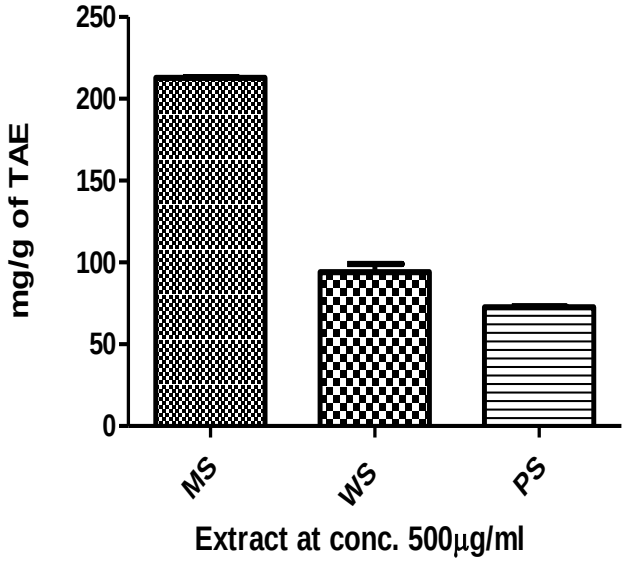

Figure 4.10 Compares the TPC of the extracts using their TAE

Table 4.12; Total Phenol Content of the methanolic, aqueous and pet-ether stem bark extracts of *Allanblackia parviflora* estimated as tannic acid equivalent in mg/g

Treatment	Mg/g equivalent of tannic acid, ±SEM
Methanolic	212.85±0.311
Aqueous	94.05± 5.105
Petroleum-ether	72.68±0.311

4.4.3 Total Antioxidant Capacity

Total antioxidant capacities of the various extract were expressed as AAE which is quantitatively determined as a proportion of ascorbic acid (in mg) equivalent to each gram of the extracts that caused a reductive conversion of molybdenum, (Mo^{+6}) to form a green phosphate-molybdate (Mo^{+5}) at 695 nm The summary of the results depicts a concentration dependent activity exhibited by all the extracts such that the TAC as expressed as AAE increased as the concentration of the extract increases. The effect is seen in table 4.13 which expresses the TAC (as AAE) of the various extracts at the highest concentration of 500 mg/ml. The AAE of the extracts however ranged between 40 and 80 mg/g with pet-ether extracts recording the least activity followed by aqueous and methanol in that increasing order. The calibration curve for ascorbic acid shown in figure 4.11 was constructed with linear regression of r^2= 0.847

Calibration curve for Ascorbic acid

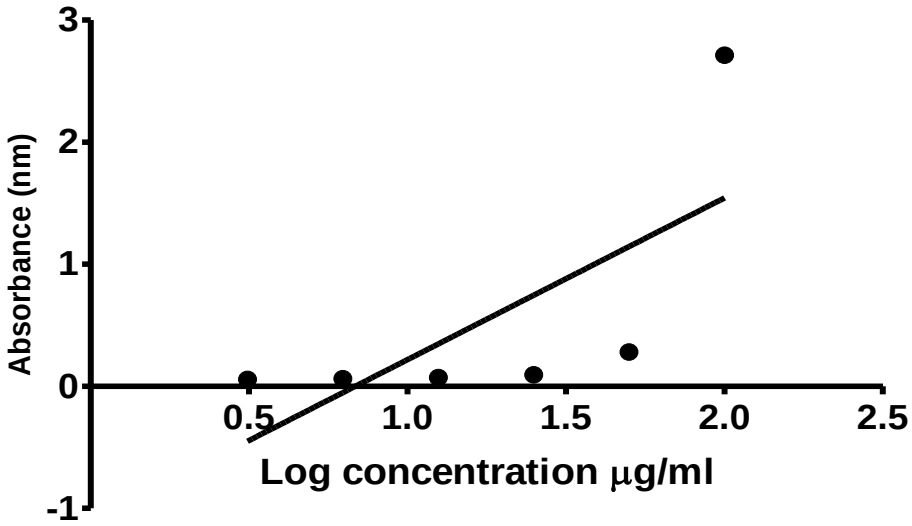

Figure 4.11 calibration curve of Ascorbic acid

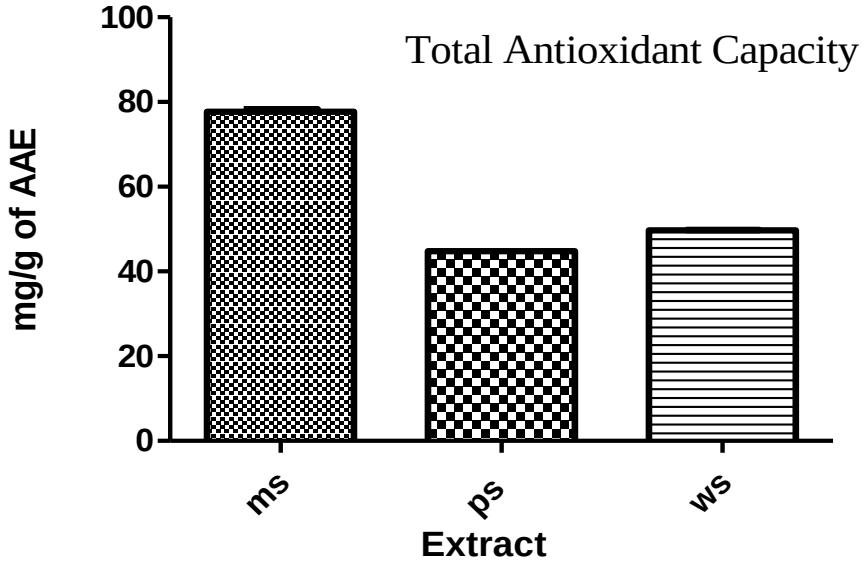

Figure 4.12 compares the TAC of the extracts using their TAA.

Table 4.13 Total Antioxidant Capacities of the methanolic, aqueous and pet-ether stem bark extracts of *Allanblackia parviflora* estimated as Ascorbic acid equivalent in mg/g

Treatment	Mg/g equivalent of Ascorbic acid, ±SEM
Methanolic	*77.72±0.549*
Aqueous	*49.67±0.087*
Petroleum-ether	*44.80±0.052*

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Qualitative Phytochemical Screening

The results obtained from the qualitative phytochemical screening of the stem bark extracts of *Allanblackia parviflora* as shown in Table 4.1 above reveals the presence of major phytochemicals in the various extracts.

Secondary metabolites including alkaloids, tanins, flavonoids, cardiac glycosides, reducing sugar, triterpenoids and anthraquinones were present in the pulverized plant material whereas saponins and phytosterols were absent. Methanolic and aqueous extracts showed the presence of almost all the phytochemicals mentioned above however, general alkaloids as well as phytosterols and saponins were absent in the methanol and aqueous extracts respectively. Petroleum-ether extracts on the other hand showed the presence of only tanins, reducing sugars and cardiac glycosides with the rest being absent. This effect exhibited by the pet- ether extracts may be attributed to the fact that most of the compounds embedded in the plant may be polar in nature hence, insoluble in non-polar solvent like petroleum ether. Also cyanogenic glycosides which accounts for approximately 90% of plant toxins called cyanogens were absent in all the plant samples screened. This therefore suggests to some extent the safety of the *Allanblackia parviflora* plant in general (http://www.food safety.govt.nz/elibrary/industry/Cynogenic_Glycoside_Toxin_which.pdf).

A number of these phytochemicals though not directly involved with major processes including growth, reproduction and metabolism, have shown pronounced pharmacological activities.

For example, most phenolic compounds from plants including flavonoids (Chrysin, Quercetin, and Rutin) and tannins (Ellagitannin) have been proven in scientific literature to have antimicrobial, anthelmintic and antidiarrheal activities. Some plant flavonoids have also shown anticancer, antioxidant, anti-inflammatory as well as anti-allergic activities (Hafidh et al, 2009; Prashant et al, 2011). Other phenolic derivatives such as Aspirin has demonstrated potent anti-inflammatory and analgesic properties (Berman, 2004).

Also phytochemicals including alkaloids have been associated with most anticancer (vincristine and vinblastine), antimalarial (quinine), analgesic (morphine and cocaine), anti-inflammatory (colchicine), agents used in the health systems today. Other alkaloids such as Diterpenoid alkaloids and glycoalkaloid (s. Solamargine) isolated from, Ranunculaceae, or buttercup family and *Solanum khasianum* are reported to possess antimicrobial properties and HIV inhibitory activities respectively. Terpenoids including Artemisin (Sesquiterpenoids) and Taxol have been used in managing malaria and some solid tumors respectively (Osei-Akosah, 2012; Ayensu and Quartey, 2015).

Based on the evidences stated above, it may be stipulated that the presence of these phytochemicals in the stem bark *Allanblackia parviflora* might partially or significantly account for its diverse application in traditional herbal medicine especially in areas where they are mostly prevalent.

5.1.2 Antimicrobial Activity

The study assessed the antimicrobial activities of the stem bark of *Allanblackia parviflora* species in Ghana against ten microbial strains comprising of one fungus, five gram negative bacteria and four gram positive bacteria. The assessment employed the Agar well diffusion method for the preliminary screening and broth micro-dilution method for MIC determination.

The outcome of the assay however revealed that all the test organisms (*S. typhi, E. coli, E. faecalis, S. paratypi A., C. albicans, B. subtilis, K. pneumoniae, N. gonorrhoeae, P. aeruginosa S. aureus)* showed considerable susceptibility to the ciprofloxacin, followed by methanolic and the aqueous extracts with zones of inhibitions ranging between 9.5-21.5 mm. However the pet-ether extract showed virtually no activity against all the 10 microbial strains.

In terms of the individual crude extracts, methanol which comprises the polar constituents of the plant recorded the highest zones of inhibition at 20 mm followed by 17.5 mm against the , *E. faecalis and Staphylococcus aureus* respectively with the same Minimum Inhibitory Concentration of 2.50 mg/ml. This remarkable antimicrobial activity of the crude extract against *E. faecalis, Staphylococcus aureus and P. aeruginosa* therefore justifies the traditional use of the plant in managing tooth infections (*E. faecalis and P. aeruginosa),* skin and bronchial infections(*S. aureus*) since these organisms are the common pathogens in most tooth infection, skin and respiratory related diseases respectively (Leheij et al., 2012; Molander et al., 1998) The lowest antimicrobial activity was exhibited by *S. paratypi A* at 10.00 mm and MIC of 2.50 mg/ml.

The Minimum Inhibitory Concentrations recorded for the methanolic extracts also ranged between 1.25 and 5.0 mg/ml with *C. albicans* and *P. aeruginosa* recording the lowest at 1.25 mg/ml and B. subtilis recording the highest at 5.0 mg/ml. hence all the MIC values were greater than 1000µg/ml.

Substantial antibacterial and antifungal activities was again demonstrated by the aqueous extract with the highest zone of inhibition at 15.00 mm against *Staphylococcus aureus* and *E. faecalis* and the same MIC of 5.00 mg/ml. ***N.*** *gonorrhoeae* again recorded the lowest zone of inhibition value for the aqueous extract at 10.00 mm and MIC of 5.00 mg/ml. Though the activities recorded for this extract was quite considerable, methanolic extracts were much more effective against the microbial strains

Petroleum ether extract which covers the non-polar constituents of plant showed no antimicrobial activity against all the microbial strain. This effect may be as a results of the inability of pet-ether to extract active phytochemical compounds that are responsible for this activity. More to this point, several phytochemical studies indicate that antimicrobial activity are mostly associated with flavonoids and these were absent in the pet-ether extract as shown in Table 4.1 above hence the inactive effect.

Therefore, comparing the antimicrobial activities of the three(3) stem bark extracts of *Allanblackia parviflora,* both methanolic and aqueous extracts gave a broad spectrum activity (i.e. against bacterial and fungal strains,) but methanolic extracts generally inhibited microbial growth more than aqueous and pet-ether in the respective strains.

Ciprofloxacin which was used as the standard drug generally displayed the highest antibacterial and antifungal activities and the lowest mic values. The results however exerted some form of variability for the various microbial strains as seen in that of the extracts. The zones of inhibition ranged between 15.00 and 21.50 mm with *Staphylococcus aureus* demonstrating the highest antimicrobial activity and *N. gonorrhoeae* showing the lowest activity. The MIC values also ranged between 0.0125mg/ml and 0.05mg/l with C. albicans and B. subtilis recording the lowest values thus, exhibiting the highest activity. Ciprofloxacin was preferable to other antibiotics because it has activity against a broad spectrum of microorganisms. It was clearly seen that ciprofloxacin exhibited higher activities and lower MIC values than the extracts because the drug constitute a single compound whiles the crude extracts contains innumerable chemical substances which may interfere with the activity. Nevertheless, further isolation and purification of compounds responsible for such activities may give even better activities and MIC values for the active extracts.

Also considering the types of microbial strains, the active extracts generally showed prominent activities with gram positive bacteria than gram negative bacteria. This effect may be as a results of the difference in the structure of their cell walls. Generally, gram positive bacteria exerts higher susceptibility against chemicals since their cell wall is characterized by its thickness comprising of peptidoglycan which is bonded to different cellular units whiles gram negative bacteria contain some hydrolytic enzymes as well as proteins (porin). These enzymes acts to degenerate various substance that are introduced into the cell from the outer environment and the protein also acts as barrier that prevents

polar substance across the outer layer (Osei-Djarbeng et al, 2014). Hence the effect observed above.

The significant results obtained from this assay, therefore suggests the possible use of the stem bark of the plant for managing bacterial infections especially skin related ones and even fungal infections since *C.albicans* also demonstrated significant activity.

5.1.3 Anti-inflammatory Activity

The aim of the acute anti-inflammatory assessment was to establish a scientific bases for the folkloric use of *Allanblackia parviflora* in managing inflammations and the pain associated with it. Following this background however, inflammation induced by carrageenan in a week old chicks was the model employed for this assessment considering both preventive and curative properties of the stem bark extracts as against a reference drug (Diclofenac). The model basically follows two biochemical stages, the initial stage involves the release of two inflammatory receptors i.e. histamine and serotonin usually within the first hour after injection followed by the second and final stage which involves mediation of prostaglandins. In actual sense, these mediators (histamine causes an increase in the flow of blood to the injured space, thus resulting in the swelling and redness that characterize inflammation) released from injured cells act to generate and prolong the acute inflammatory response (Asongalem *et al.,* 2005).

The results obtained from the assessment revealed a significant ($P<0.001$) reduction in oedema with effect from the second hour as shown in figures 4.2 to 4.7 above. This effect may be attributed to the fact that the extracts/reference

drug might have inhibited the production of some mediators notably prostaglandins and kinins which control acute inflammatory response (Asongalem *et al.,* 2005; Silva *et al*., 2005).

From the results, oedema was generally reduced by the reference drug and the various extracts compared to the negative control as depicted in graph B of figures 4.2 to 4.7 in chapter four above. Diclofenac significantly (*** P <0.001,** P<0.01 and**P<0.05) reduced oedema at percentages ranging between 50 and 80% depending on the concentration. Thus, the highest concentration which is 100 mg/kg therefore recorded the highest percentage inhibition of oedema followed by 30 mg/kg and 10 m/kg with the least inhibition.

Similarly, the various extracts also inhibited oedema at percentages ranging between 27 and 70% with methanolic extracts demonstrating the highest inhibition against oedema for both therapeutic (30mg/kg-42.92%, 100m/kg-56.93% and 300 mg/kg-66.01%) and prophylactic (30mg/kg-49.26%, 100m/kg-51.41% and 300 mg/kg-59.05%) studies. Also Aqueous extracts and pet-ether significantly recorded a reduction in oedema at percentages of (30mg/kg-28.61%, 100m/kg-46.60% and 300 mg/kg-56.93%) , (30mg/kg-29.27%, 100m/kg-39.20% and 300 mg/kg-48.24%) and (30mg/kg-34.92%, 100m/kg-45.38% and 300 mg/kg-45.99%) , (30mg/kg-33.42%, 100m/kg-36.05% and 300 mg/kg-42.53%) for therapeutic and prophylactic studies respectively.

The remarkable inhibitory activity displayed by the stem bark extracts of *Allanblackia parviflora* suggests potent anti-inflammatory activity of the plant. However comparative asssessment of the extracts and the reference drug shows

a clear cut difference in activity. This effect may possibly be due to the presence of impurities and other unwanted compounds that may have in one way or the other interfered with the whole inhibition process. Thus isolation and purification of bioactive compounds responsible for such effect will enhance the anti-inflammatory activity.

Again visual observations of the time course curves which provides a graphical effect of the various treatment within the stipulated time frame as provided in chapter 4 (Figures 4.2-4.7) above reveals that, all the graphs follow a particular trend irrespective of the variations in some of them. The general trend is such that from the zero hour to the first hour, a sharp increase is observed for the control and the increase is steadily sustained between the 2^{nd} and 3^{rd} hours and declined from the 4^{th} to the 6^{th} hour. In the extracts however, the sharp decline is seen at the second hour and decreases down to the 6^{th} hour.

Also considering the bar graphs which represents the total oedema formed, it exhibited some form of trend in terms of oedema reduction at the various concentrations such that at the highest concentration (300 mg/kg for extracts and 100 mg/kg for Diclofenac) oedema is significantly reduced as against the control down to the lowest concentration notwithstanding the variations in the various treatments. This body of results therefore suggests that the stem bark extracts of *Allanblackia parviflora* generally possess anti-inflammatory activities as claimed by traditional medicine but then, therapeutic or curative effects are more prominent than the preventive effect.

5.1.4 Antioxidant Activity

Free radicals which are advertently or inadvertently produced in various biochemical and chemical reactions give rise to highly reactive species which produce injurious effects to vital cellular components and tissues (Apak et al., 2007). The effect of free radicals are however regulated or neutralized in a way by antioxidants which at low concentrations interrupts or ultimately averts the oxidation of these substances to prevent the injurious effects (Halliwell, 2001).The effect of these antioxidants may also be seen in anti-inflammatory responses since antioxidants may perhaps interfere or hinder inflammatory process. This is because, inflammatory injuries associated with tissues are somewhat facilitated by reactive metabolites from phagocytic leukocytes which acts to produce injurious response. Thus, new agents of antioxidant properties are worth researching into.

The study comprehensively assessed the antioxidant activity of the stem bark extracts of the plant employing parameters including total antioxidant capacity, total phenolic content and DPPH free radical scavenging ability. The various assay methods significantly exerted their antioxidant activities as the concentrations of the extracts increased.

In the DPPH scavenging assay, absorbance of DPPH significantly declined as the extracts concentrations were increased coupled with an increase in DPPH scavenging activity (Percentage). This may perhaps be attributed to the fact that as the concentration increases more antioxidant (donating hydrogen atom) present in the extracts mop up more DPPH radical to form DPPH.H thereby reducing the intense absorbance of the Purple DPPH to a yellow colored DPPH.H at 517 nm.

For the various samples assayed, Ascorbic acid significantly scavenged DPPH radical with the highest % DPPH scavenging power of 75.04 ±0.84% (at the highest concentration of 500µg/ml) and the lowest EC_{50} value of 0.01539 mg/ml followed by methanolic extract (EC_{50} of 0.03341mg/ml, at 40.74±0.0), aqueous (0.09991 mg/ml, 32.41±0.0) and pet-ether (0.1842 mg/ml, 25.96±0.87) respectively. Thus, the lower the EC_{50} which depicts the concentration of the extract/drug that delivers ½ maximum activity, the more potent the extract or drug. Therefore considering the results, it can be speculated that all the extracts contain agents that are capable of mopping up reactive species to terminate chain reactions initiated by the free radicals.

Total phenolic content estimates the phenolic compounds in the extracts that are able to significantly reduce folin-ciocaltau to form a blue oxide product at an absorbance of 760 nm. The TPC expressed as TAE revealed methanolic extracts as possessing the highest phenolic content at 212.85±0.311 mg/g. This effect is quite unarguable because methanolic extract generally represent the polar constituents of the plant and phenolic compounds are mostly polar thus, the effect observed. However, antioxidant activity in general is not only associated with phenolic compounds others may include phytochemicals such as essential oils, carotenoids and even vitamins (Amponsah, 2012). Aqueous extract followed with TAE of 94.05± 5.105 mg/g and pet-ether (72.68±0.311 mg/g) respectively. Hence the stem bark of *Allanblackia parviflora* may contain some phenolic compounds that have antioxidant activity as affirmed by Ozgova et al., (2003) that polyphenolic compounds are potent antioxidants.

Total Antioxidant Capacity of the various extracts also showed significant activities with their TAC expressed as AAE (77.72±0.549, 49.67±0.087 and

44.80±0.052) mg/g for methanol, aqueous and pet-ether extracts in a decreasing order. Also the linear regression values obtained for both TPC (r^2= 0.9247) and TAC (r^2= 0.847) expresses a good correlation between phenolic compounds present in the extracts and the total antioxidant capacity Thus, various phenolic compounds as well as non-phenols present in the extracts possibly interact synergistically to account for the observed antioxidant activity.

5.2 Conclusion

This study has shown from the results that the stem bark extracts of *Allanblackia parviflora* contain major phytochemicals including alkaloids, tanins, flavonoids, cardiac glycosides, reducing sugar, saponins, phytosterols, triterpenoids and anthraquinones which work synergistically to produce marked anti-inflammatory, antioxidants and antimicrobial activities in the various extracts. Comparatively, the methanolic extracts which present the polar constituents of the plant (stem bark) generally exhibited the highest anti-inflammatory, antioxidants and antimicrobial activities followed by aqueous and pet-ether stem bark extract which comprises of the non- polar part of the plant (stem bark). Thus, it can be speculated that the polar extracts contributed significantly to the observed activities.

In the anti-inflammatory study, therapeutic properties were more prominent with the various extracts than the prophylactic properties and hence, affirms the mode of treatment (therapeutic) employed by traditional folks. The microbial studies also revealed antibacterial and antifungal activities of the various extracts inferring broad spectrum antimicrobial activity of the plant (stem-bark). Also all the results obtained in this study was comparable to that obtained from other species including *A. floribunda, A stuhlmanni, A gabonensis* etc. as

reviewed in chapter two above. Thus, provides some answers to the research questions above.

As part of emphasizing the need for commercialization, findings from this work will be given to CRIG Afosu branch to educate famers on both medicinal and economic benefits

On the whole, it can be confidently stipulated that the study has established some scientific bases for the folkloric use of the stem bark of *Allanblackia parviflora* in managing acute inflammation, microbial infections and many other tropical diseases.

5.3 Recommendation

Further studies will consider the isolation and characterization of bioactive compounds from the various parts of *Allanblackia parviflora* in Ghana and probably try the effects of the isolated compounds on these and other pharmacological activities (such as anticancer, analgesic, antidiarrheal).

REFERENCES

A.M.G.A Leheij, J.O. Kistler, G.N. Belibasakis, H. Valimaa and J.J. Soet, (2012). Healthcare-Associated Viral and Bacterial Infections in Dentistry. Journal of Oral Microbiology Vol (4) 17659

Abdel-Hameed, E.S., (2009). Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. Food chemistry, 144: 1271–1277.

Adubofuor Joseph, Sefah Wilfred and Oldham James Henry, (2013) Nutrient composition of *Allanblackia parviflora* seed kernels and oil compared with some plant fats and oils and application of the oil in soap preparation. Journal of Cereals and Oil seeds Vol. 4(1), pp. 1-9,

Ajibesin K.K., Rene N., Bala D. N. and Essiett U.A., (2008). Antimicrobial Activities of extracts and fractions of *Allanblackia floribunda*. Biotechnology 7(1):129-133,

Alvum-Toll Kajsa, (2013). *Allanblackia stuhlmannii* – a tree under current domestication: what are the soil requirements? SLU, Swedish University of Agricultural Sciences Faculty of Natural Resources and Agricultural Sciences Department of Soil and Environment http://stud.epsilon.slu.se. Downloaded; 21, July 2015

Amponsah I. K., (2012). Chemical Constituents, Anti-inflammatory, Anti-oxidant and Antimicrobial Activities of the Stem Bark and Leaves of *Ficus Exasperata* (Vahl). PhD. Thesis, Kwame Nkrumah University of Science and technology, Kumasi – Ghana.

Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S. E., Bektaşoğlu, B., Berker, K. I. and Özyurt, D., (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC. *Molecules*, 12: 1496-1547.

Asongalem, E. A., Martins, F. R. and Matheus, M. E., (2005). Investigation of anti-inflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. Journal of Ethnopharmacology, 95: 63-68

Ayensu I. and Quartey A. K., (2015). Phytochemical screening and in vitro antioxidant properties of the stem bark of *Trichilia tessemannii (*harms) (Meliacease). World Journal of Pharmacy and Pharmaceutical sciences. Vol 4, issues3, 2015.

Ayoola G.A, Ipav S.S., Sofidiya M.O., Adepoju-Bello A.A., Coker H.A.B. and Odugbemi T.O., (2008) Phytochemical screening and free radical scavenging activity of fruit and leaves *Allanblackia floribunda*. International Journal of Health Research, June 2008; 1(2): 87-93l

Azebaze G. A, Ouahouo B.M.W., Vardamides J.C., Valentine A., Kuete V., Acebey L., Beng V.P., Nkengfack A.E and Meyer M., (2008). Antimicrobial and antileishmaial xanthones from the stem bark of *Allanblackia gabonensis*. Chemistry of natural compounds. vol. 44 issue 5 pp. 582-587.

Berman B., (2004): Willow Bark. Baltimore: University of Maryland Medical Center. (http://www.umm.edu/altmed/ConsHerbs/WillowBarkch.html).

Bilanda Daniella Claude, Andomo Mewo Yannic Desire, Dzeufiet Djameni Paul Desire, Azebaze Anatole Guy Blaise, Kamtchouing Pierre and Dimo Theophile, (2013). Methanol extract of *Allanblackia gabonesis* (Guttiferae). Prevents hypertension and oxidative stress induced by chronic sucrose consumption in rats. Pharmacologyonline. Vol 194-200.

Bilanda Daniella Claude, Dimo Theophile, Dzeufiet Djameni Paul Desire, Ndzana Martin Thierrry Bella, Oumarou Bibi Farouck Aboubakar, Telesphore Benoit Nguelefack, Tan Paul V. and Kamtchouing Pierre, (2010). Antihypertensive and antioxidant effect of *Allanblackia floribunda* oliv (Clusiaceae) aqueous extract in alcohol and sucrose-induced hypertensive rats. Journal of ethnopharmacology. Vol 128(3)634-640

Bilanda DN, Dimo T, Dzeufiet Djomenia PD, Ndzana M, Bella T, Aboubakar OBF, Nguelefack TB, Tan PV, Kamtchouing P., (2010). Antihypertensive and antioxidant effects of *Allanblackia floribunda*

Oliv. (Clusiaceae) aqueous extract in alcohol- and sucrose-induced hypertensive rats. Journal of Ethnopharmacology, 128:634–640.

Boudjeko Thaddée, Judith Emery Kanemoto Ngomoyogoli1, Alice Louise Woguia2 and Nicolas Njintang Yanou, (2013). Partial characterization, antioxidative properties and hypolipidemic effects of oilseed cake of *Allanblackia floribunda* and Jatropha curcas. BMC Complementary and Alternative Medicine.13:352 .http://www.biomedcentral.com/1472-6882/13/352.

Cos, P., Vlietinck, A. J., Berghe, D. V. and Maesa, L., (2006). Anti-infective potential of natural products: How to develop a stronger *in vitro* „proof-of-concept". Journal of Ethnopharmacology, 106: 290–302.

Evans, W.C. in Trease and Evans Pharmacognosy. 13th Edition, Ballier Tindall. London. 1989.

Fankam G. Aime, Kuiate R. Jules and Kuete Victor, (2015). Antibacterial and antibiotic resistance modifying activity of the extracts from *Allanblackia gabonensis, Combretum molle and Gladiolus quaetiniaus* against gram-negative bacteria including multi-drug resistant phenotypes. BMC Complementary and alternative medicine. 15:206.

Fobane Jean Louis, Ndam Elvis Nsoh and Mbolo Marie, (2014). Population structure ad regeneration of *Allanblackia floribunda* Oliv. (Clusiaceae) in a forest concession of eats Cameroon. Journal of biodiversity and environmental sciences (JBES) Vol.4 No.2, p. 403-410.

Fuller R.W., Blunt J.W., Boswell JL, Cardelina J.H. and Boyd M.R., (1999). Guttiferone F, the first prenylated benzophenone from *Allanblackia stuhlmannii.* J.Nat.Prod. 62: 130-132, 1999.

Halliwell Barry, (2001). Free radicals and other reactive species in disease. National University of Singapore, Singapore. Encyclopedia of Life Sciences / & 2001 Nature Publishing Group / www.els.net

Halliwell, B. and Gutteridge, J. M. (1999). Free radicals in biology and medicine. 3rd ed. Oxford University Press/Clarendon Press

http://vibeghana.com/2013/09/04/the-increase-in-the-use-of-herbal-medicine-in-ghana-africa-and-the-world-is-good-king-of-herbal-medicine-naaba-dr-sheikh-amin-bonsu/

Ileleji Faith, Hamadina Elsie I. and Orluchukwu Joseph A., (2015). Germination of *Allanblackia floribunda* Seeds: The Effect of Soak Duration in Fluridone on Germination and Seedling Growth. Agriculture, Forestry and Fisheries. Vol. 4, No. 3, pp. 142-147.

Kuete V., Azebaze Anatole G.B., Mbaveng Armelle T., Nguemfo Edwige L., Tshikalange Emmanuel T., Pierre Chalard and Nkengfack A. E., (2011). Antioxidant, antitumor and antimicrobial activities of the crude extract and compounds of the root bark of *Allanblackia floribunda*, Pharmaceutical Biology, 49:1, 57-65.

Kwaku Antwi, (2012). The variability between the strength and some physical properties of *Allanblackia parviflora* for furniture production. Thesis submitted to the faculty of renewable natural resources, in partial fulfilment of the requirement for the award of the Master of Science (M.Sc.) degree in wood technology and management degree. Kwame Nkrumah University of science and technology, pp. 19-21.

Lin, C. R., Amaya, F., Barrett. L., Wang, H., Takada, J., Samad, T. A and Woolf, C. J. (2006). Prostaglandin E2 receptor EP4 contributes to inflammatory pain hypersensitivity, Journal of Pharmacology and Experimental Therapeutics, 319: 1096- 1103

Linthoingambi W. and Mutum S. Singh, (2013). Antimicrobial activities of different solvent extracts of *Tithonia diversifolia* (Hemsely) A. Gray Department of Life Sciences, Manipur University, Canchipur, Manipur, India, Asian Journal of Plant Science and Research, 3(5):50-54.

Meshack, C., (2004). Indigenous knowledge of *Allanblackia stuhlmannii* in the East Usambara Mountains, Tanzania.

Molander A., Reit C., Dahlen G., Kvist T., (1998). Microbiology status of root-filled with apical periodontitis. International Endodontic Journal. 31 (1): 1-7

Munjuga, M., Mwaura, L., & Schmidt, L. H., (2010). *Allanblackia stuhlmannii* Engl. Seed Leaflet,

Aqil F., Ahmad I. and Mehmood Z. (2006). Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants. Turkish Journal of Biology, 30: 177-183

Nguemfo E.L., Dimo T., Dongmo A.B., Azebaze A.G.B., Alaoui A.E., Asongalem K., Cherrah Y. and Kamtchouing P., (2007) . Anti-inflammatory and Anti-nociceptive activities of the stem bark extracts from *Allanblackia monticola* Staner L.C (Guttiferae). Journal of Ethnopharmacology Volume 114(3), pp. 417-424

Shivaprasad H. N., S. Mohan, M. D. Kharya, Mahendra R. and Lakshman K. (2005). In vitro models for antioxidant activity evaluation: A review. Pharmaceutical reviews, 3(4).

Nguemfo E.L., Dimo T., Dongmo A.B., Azebaze A.G.B., Alaoui K., Asongalem A.E., Cherrah Y. and Kamtchouing P., (2009). Antioxidants and Anti-inflammatory Activities of some isolated constituents from the stem bark of *Allanblackia monticola* Staner L.C (Guttiferae). Journal of Ethnopharmacology Volume 17.issue 1, pp. 37-41

Nkengfack A.E., Azebaze G.A., Vardamides J.C., Fomum Z.T., Van Heerden F.R., (2002). A prenylated xanthone from *Allanblackia floribunda*. Phytochemistry 60, 381-384.

Ofori, D. A. and Peprah, T., (2011). Domestication of *Allanblackia parviflora* in Ghana. Report to ICRAF – January 2010-March 2011, Pp10.

Ofori, D.A., Peprah. T., lIenneh, S., Bosompem, S.A., (2008). Allanblackia Domestication Project; Report on training workshop on vegetative propagation of *Allanblackia parviflora*, CSIR-Forestry Research Institute of Ghana, Kumasi 25th to 29th February, 2008.

Orwa C, Mutua A, Kindt R, Jamnadass R and Simons A., (2009). Agroforest tree Database: a tree reference and selection guide version (http://www.worldagroforestry.org/af/treedb/)

Osei-Akosah Emmanuel, (2010) Antimicrobial activity profile of the constituents of four Ghanaian aromatic medicinal plants. Thesis submitted to the department of chemistry in partial fulfilment of the requirement for the award of the Master of Science (M.Sc.) degree in organic chemistry. Kwame Nkrumah University of Science and Technology, pp. 13-15

Osei-Djarbeng Samuel N., Amonoo-Neizer Jacob, Boadi Portia, Opoku Priscilla N.A. and Osei-Asante Samuel, (2014). Comparative antimicrobial activities of different solvent extracts and a refreshing drink (*Sobolo*) made from *Hibiscus sabdariffa* Linn. International Journal of Herbal Medicine 2014; 2 (3): 01-04.

Ozgova S. Hermanek J. Gut I., (2003). Different Antioxidant effects of polyphenols on lipid peroxidation and hydroxyl radicals in the NADPH-, Fe-ascorbate and Fe-microsomal systems. Biochemical Pharmacology, 66:1127-1137.

Peprah T., Ofori D. A., Siaw D. E. K. A., Addo-Danso S. D., Cobbinah J. R., Simons A. J. and Jamnadass R., (2009). Reproductive biology and characterization of *Allanblackia parviflora* A. Chev. in Ghana. Genet Resour Crop Evol (2009) 56:1037–1044

Phipps S. M., Sharaf M. H. M. and Butterweck V., (2007). Assessing Antioxidant Activity in Botanicals and Other Dietary Supplements. Pharmacopeial Forum, 33(4): 810-814.

Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur, (2011). Phytochemical screening and Extraction: A Review, radical scavenging activity by DPPH. Bulg. J. Agric. Sci., 17: 11-24.

Ravi V., Saleem T.S.M, Patel S.S., Raamamurthy J. and Gauthaman K., (2009). Anti-Inflammatory Effect of Methanolic Extract of *Solanum nigrum* Linn Berries. International Journal of Applied Research in Natural Products Vol. 2(2), pp. 33-36.

Rompaey Renaat Van, (2003) distribution and ecology of *Allanblackia spp*. (Clusiaceae) in African rain forest. Report to unilever research laboratories, Vlaardingen.

Sefah W., (2006) Extraction and characterization of vegetable fat from *Allanblackia floribunda.* Thesis submitted to the department of Biochemistry and Biotechnology in partial fulfilment of the requirement for the award of the Master of Science (M.Sc.) degree in food science and technology. Kwame Nkrumah University of Science and Technology, pp. 146.

Sefah W., Adubofour, J. and Oldham, J. H., (2007). Extraction and Characterization of vegetable fat from *Allanblackia floribunda*, MSc. Thesis, Kwame Nkrumah University of Science and technology, Kumasi – Ghana.

Silva, G. N., Martins, F. R. and Matheus, M. E., (2005). Investigation of anti-inflammatory and antinociceptive activities of *Lantana trifolia*. Journal of Ethnopharmacolog, 100: 254-259.

Ström Helena, (2013) Examination of mycorrhizal associations of *Allanblackia stuhlmannii* – a tree under current domestication Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology http://stud.epsilon.slu.se

Tenover, F. C. (2006). Mechanisms of Antimicrobial Resistance in Bacteria. The American Journal of Medicine, 119 (6A): 3–10

Traditional medicine strategy lunched, (WHO News) 2002, 80, 610.

Verpoorte, R., (2000). Pharmacognosy in the new millennium: lead finding and biotechnology. Journal of Pharmacy and Pharmacology, 52: 253-262.

Ymele Edwige V., Dongmo A. Bertrand and Dimo Theophile, (2013). Analgesic antiinflammatory effect of aqueous extract of the stem bark of *Allanblackia gabonensis* (Guttiferae). Inflammophamacology. vol. 21 issue 1 pp. 21-30.

APPENDICES

Appendix 1; results from Antioxidant assay

1A Results from Total Antioxidant Assay (mg/ml)

MS(mg/ml)			PS(mg/ml)			WS(mg/ml)			Vit. C(mg/ml)		
0.095	0.094	0.098	0.058	0.056	0.057	0.061	0.062	0.061	0.058	0.055	0.056
0.113	0.112	0.113	0.085	0.087	0.084	0.062	0.065	0.061	0.064	0.062	0.061
0.143	0.144	0.145	0.097	0.095	0.095	0.071	0.07	0.073	0.072	0.073	0.070
0.184	0.181	0.182	0.128	0.123	0.121	0.088	0.089	0.08	0.101	0.095	0.086
0.562	0.587	0.572	0.139	0.139	0.138	0.205	0.201	0.203	0.272	0.288	0.284
									2.723	2.729	2.686
Control											
0.067	0.061	0.067									
0.057	0.055	0.058									

1B Results from Total Phenolic Content (mg/ml)

MS(mg/ml)			PS(mg/ml)			WS(mg/ml)			Tannic acid (mg/ml)		
0.107	0.108	0.109	0.098	0.097	0.099	0.106	0.107	0.109	0.084	0.089	0.084
0.13	0.132	0.133	0.108	0.109	0.106	0.125	0.127	0.128	0.102	0.102	0.102
0.226	0.241	0.256	0.112	0.113	0.113	0.17	0.191	0.273	0.283	0.281	0.282
0.301	0.307	0.313	0.125	0.126	0.124	0.2	0.201	0.222	0.32	0.328	0.323
0.708	0.71	0.711	0.31	0.313	0.312	0.346	0.375	0.396	0.398	0.398	0.369
									0.682	0.683	0.682

1C Results from DPPH Scavenging potential (mg/ml)

MS(mg/ml)			PS(mg/ml)			WS(mg/ml)			Ascorbic acid (mg/ml)		
1.054	0.949	1.026	0.712	0.719	0.713	0.448	0.546	0.594	0.606	0.643	0.687
0.658	0.663	0.699	0.658	0.663	0.699	0.597	0.576	0.539	0.588	0.589	0.590
0.627	0.623	0.627	0.627	0.623	0.627	0.509	0.541	0.558	0.456	0.456	0.452
0.613	0.618	0.619	0.613	0.618	0.619	0.494	0.495	0.483	0.245	0.205	0.243
0.552	0.555	0.573	0.552	0.555	0.573	0.606	0.664	0.606	0.188	0.2	0.178
Control(DPPH+Methanol)											
0.8	0.778	0.764	0.674	0.832							
0.637	0.712	0.61	0.715	0.569							

Appendix 2 Results from the anti-inflammatory assessment.

2A Anti-inflammatory results for methanolic stem bark extract.

Prophylactic studies							
G1(300mg/kg)	0h	1h	2h	3h	4h	5h	6h
1	6.34	6.84	7.66	7.40	6.65	6.41	6.35
2	6.16	6.43	6.50	6.79	6.68	6.25	6.17
3	5.87	6.10	6.35	6.42	6.08	6.02	5.87
4	6.36	6.64	6.95	7.08	6.96	6.55	6.38
5	5.87	6.12	6.20	6.34	6.15	6.04	6.00
average	6.12	6.43	6.73	6.81	6.50	6.25	6.15
G2(100mg/kg)							
1	5.76	6.08	6.36	6.09	6.08	6.04	5.97
2	5.70	5.86	6.43	6.15	6.13	6.06	5.88
3	5.93	6.30	6.41	6.20	6.19	6.17	5.96
4	6.07	6.27	7.32	7.09	6.70	6.34	6.04
5	5.74	6.46	6.50	6.19	6.17	6.03	5.91
average	5.84	6.19	6.60	6.34	6.25	6.13	5.95
G3(30mg/kg)							
1	5.80	6.36	6.41	6.26	6.12	5.92	5.83
2	5.74	6.36	6.51	6.07	6.00	5.93	5.80
3	5.50	6.06	6.65	6.62	6.31	6.32	6.21
4	5.96	6.33	6.35	6.13	6.07	6.06	6.02
5	6.27	6.48	6.71	7.09	6.60	6.53	6.34
average	5.85	6.32	6.53	6.43	6.22	6.15	6.04
negative control							
G16							
1	6.03	6.6	7.44	7.67	7.19	6.65	6.54
2	6.15	6.65	7.46	7.26	7.16	6.634	6.37
3	5.59	6.36	6.39	6.47	6.25	6.59	6.28
4	5.86	6.3	6.93	6.87	6.65	6.09	5.97
5	5.67	6.5	7.24	7.18	7.29	6.56	5.89

Therapeutic Studies							
G4(300mg/kg)							
1	5.91	6.40	6.28	6.09	5.98	5.95	5.92
2	5.67	6.55	6.62	6.03	6.23	6.01	5.79
3	5.65	6.41	6.90	7.02	6.60	6.32	6.19
4	6.42	6.54	6.87	7.40	6.83	6.83	6.45

5	5.99	6.70	6.21	6.73	6.36	6.10	6.02
average	5.93	6.52	6.58	6.65	6.40	6.24	6.07
G5(100mg/kg)							
1	6.09	6.53	6.20	6.16	6.11	6.10	6.09
2	5.81	6.70	6.63	6.21	6.17	6.13	5.99
3	5.80	6.10	6.20	6.25	5.99	5.85	5.83
4	5.81	6.65	6.47	6.05	6.02	5.89	5.85
5	5.99	6.36	6.24	6.31	6.14	6.03	6.00
average	5.90	6.47	6.35	6.20	6.09	6.00	5.95
G6(30mg/kg)							
1	6.21	6.64	7.23	6.82	6.55	6.47	6.25
2	6.02	6.25	6.32	6.19	6.14	6.10	6.07
3	5.80	5.97	6.40	6.31	6.13	6.06	5.94
4	6.10	6.64	7.02	6.68	6.31	6.26	6.22
5	5.88	6.68	6.88	6.39	6.22	6.11	6.00
average	6.00	6.44	6.77	6.48	6.27	6.20	6.10
negative control							
G16							
1	6.03	6.6	7.44	7.67	7.19	6.65	6.54
2	6.15	6.65	7.46	7.26	7.16	6.634	6.37
3	5.59	6.36	6.39	6.47	6.25	6.59	6.28
4	5.86	6.3	6.93	6.87	6.65	6.09	5.97
5	5.67	6.5	7.24	7.18	7.29	6.56	5.89
positive control (Diclofenac)							
Gp13 100mg/kg							
1	5.29	5.79	5.60	5.50	5.43	5.33	5.30
2	5.71	5.83	6.05	6.01	6.00	5.91	5.82
3	5.68	6.32	6.25	6.06	6.01	5.96	5.91
4	6.24	6.86	6.34	6.34	6.29	6.26	6.25
5	5.73	6.20	6.06	5.98	5.93	5.87	5.82
Gp14 30mg/kg							
1	6.05	6.36	6.37	6.21	6.10	6.06	6.06
2	6.12	6.48	6.62	6.27	6.25	6.18	6.16
3	5.72	6.31	6.20	6.19	5.92	5.82	5.82
4	6.34	6.55	6.53	6.40	6.38	6.36	6.35
5	6.06	6.43	6.43	6.27	6.16	6.11	6.10
Gp15 10mg/kg							
1	5.44	5.96	6.09	5.84	5.68	5.57	5.50
2	5.83	6.06	6.08	6.08	6.05	6.00	5.97
3	5.68	6.27	6.23	6.15	6.00	5.91	5.75
4	5.59	5.93	5.82	5.81	5.77	5.73	5.59
5	5.64	6.06	6.06	5.97	5.88	5.80	5.70

2B Anti-inflammatory results for pet-ether extract.

pet-ether stem(Prophylactic)							
G7(300mg/kg)	0h	1h	2h	3h	4h	5h	6h
1	6.1	6.56	7.18	7.31	6.54	6.51	6.39
2	6.37	6.48	6.73	6.58	5.52	6.5	6.39
3	6.25	6.62	6.98	7.28	6.6	6.42	6.26
4	5.44	5.92	6.26	6.63	6.25	6.01	5.98
5	5.92	6.49	6.6	6.83	6.47	6.24	6.17
Gp8 100mg/kg							
1	5.69	6.29	6.38	6.43	6.28	6.21	5.81
2	5.89	6.5	7.13	7.19	6.86	6.83	6.2
3	5.91	6.5	6.5	6.31	6.26	6.28	6.18
4	6.28	6.64	6.78	6.74	6.72	6.47	6.34
5	5.84	6.21	6.84	6.63	6.46	6.18	5.9
Gp9 30mg/kg							
1	5.81	6.51	6.60	6.82	6.55	6.27	6.20
2	6.19	6.65	6.86	6.55	6.33	6.29	6.21
3	5.94	6.87	6.89	6.61	6.45	6.06	6.03
4	5.85	6.19	6.55	6.54	6.41	6.34	6.25
5	5.62	6.89	6.67	6.52	6.39	6.06	6.00
therapeutic							
Gp10 300mg/kg							
1.00	6.09	6.38	6.92	6.67	6.31	6.29	6.19
2.00	6.01	6.36	6.65	6.68	6.62	6.58	6.32
3.00	6.12	6.70	6.51	6.35	6.26	6.19	6.18
4.00	5.83	6.59	7.04	6.94	6.40	6.33	6.24
5.00	5.88	6.37	6.83	6.64	6.11	6.07	6.00
average	5.99	6.48	6.79	6.66	6.34	6.29	6.19
Gp11 100mg/kg							
1.00	6.09	6.31	6.34	6.20	6.13	6.11	6.09
2.00	6.18	6.47	7.11	7.12	6.30	6.29	6.21
3.00	5.52	6.40	6.59	6.31	6.22	6.06	5.95
4.00	6.24	6.58	7.27	6.49	6.37	6.28	6.24
5.00	5.59	6.40	6.62	6.57	6.30	6.19	6.02
Gp12 30mg/kg							
1	6.09	6.37	6.45	6.72	6.35	6.21	6.14
2	6.11	6.23	6.51	6.93	6.71	6.2	6.18
3	5.32	6.2	6.34	6.74	6.3	6.09	5.83
4	5.84	6.39	7.05	6.61	6.48	6.46	6.29
5	5.61	6.13	6.19	6.29	6	5.92	5.82

negative control							
G16							
1	6.03	6.6	7.44	7.67	7.19	6.65	6.54
2	6.15	6.65	7.46	7.26	7.16	6.634	6.37
3	5.59	6.36	6.39	6.47	6.25	6.59	6.28
4	5.86	6.3	6.93	6.87	6.65	6.09	5.97
5	5.67	6.5	7.24	7.18	7.29	6.56	5.89
positive control (Diclofenac)							
Gp13 100mg/kg							
1	5.29	5.79	5.6	5.5	5.43	5.33	5.3
2	5.71	5.83	6.05	6.01	6	5.91	5.82
3	5.68	6.32	6.25	6.06	6.01	5.96	5.91
4	6.24	6.86	6.34	6.34	6.29	6.26	6.25
5	5.73	6.20	6.06	5.98	5.93	5.87	5.82
Gp14 30mg/kg							
1	6.05	6.36	6.37	6.21	6.10	6.06	6.06
2	6.12	6.48	6.62	6.27	6.25	6.18	6.16
3	5.72	6.31	6.20	6.19	5.92	5.82	5.82
4	6.34	6.55	6.53	6.40	6.38	6.36	6.35
5	6.06	6.43	6.43	6.27	6.16	6.11	6.10
Gp15 10mg/kg							
1	5.44	5.96	6.09	5.84	5.68	5.57	5.50
2	5.83	6.06	6.08	6.08	6.05	6.00	5.97
3	5.68	6.27	6.23	6.15	6.00	5.91	5.75
4	5.59	5.93	5.82	5.81	5.77	5.73	5.70
5	5.64	6.06	6.06	5.97	5.88	5.80	5.73

2C Anti-inflammatory Results for Aqueous extract.

water stem prophylactic							
G7 300mg/kg	0h	1h	2h	3h	4h	5h	6h
1	5.21	7.36	6.87	6.26	6.18	6.09	6.00
2	6.01	6.38	6.51	6.39	6.28	6.21	6.14
3	6.00	6.69	6.53	6.27	6.16	6.07	6.02
4	5.96	6.78	6.69	6.64	6.28	6.19	6.17
5	5.69	6.52	6.45	6.26	6.16	6.09	6.03
average	5.77	6.75	6.61	6.36	6.21	6.13	6.07
G8 100mg/kg							
1	5.37	6.47	6.44	6.42	5.89	5.80	5.51
2	5.89	6.44	6.56	6.35	6.30	6.27	6.27
3	6.03	6.37	6.27	6.44	6.30	6.28	6.10
4	5.88	6.86	6.53	6.50	6.39	6.32	6.10
5	5.89	7.27	6.61	6.25	6.13	6.12	6.10
G9 30mg/kg							
1	5.37	6.12	6.12	5.97	5.86	5.82	5.42

2	6.02	6.51	6.58	6.31	6.28	6.19	6.13
3	5.83	6.01	6.18	6.00	5.96	5.92	5.88
4	5.96	6.93	7.18	6.88	6.58	6.22	5.99
5	5.43	6.01	6.12	5.99	5.95	5.67	5.51
negative control							
G16							
1	6.03	6.6	7.44	7.67	7.19	6.65	6.54
2	6.15	6.65	7.46	7.26	7.16	6.634	6.37
3	5.59	6.36	6.39	6.47	6.25	6.59	6.28
4	5.86	6.3	6.93	6.87	6.65	6.09	5.97
5	5.67	6.5	7.24	7.18	7.29	6.56	5.89
therapeutic							
G10 300mg/kg							
1	6.05	6.82	7.16	6.59	6.26	6.11	6.09
2	5.60	6.64	6.47	6.85	6.63	6.32	6.23
3	5.66	6.02	6.30	6.09	5.94	5.80	5.78
4	5.81	6.55	6.85	6.65	6.49	6.55	6.11
5	5.91	6.45	6.61	6.21	6.06	6.06	5.98
average	5.81	6.50	6.68	6.48	6.28	6.17	6.04
G11 100mg/kg							
1	5.78	6.49	6.24	6.24	6.10	6.04	5.93
2	6.09	7.48	6.80	6.58	6.38	6.24	6.14
3	5.93	6.97	6.66	6.42	6.19	6.13	5.96
4	5.90	6.25	6.60	6.12	5.99	5.92	5.92
5	6.18	7.29	6.76	6.64	6.60	6.47	6.25
average	5.98	6.90	6.61	6.40	6.25	6.16	6.04
G12 30mg/kg							
1	5.48	5.80	5.78	5.63	5.54	5.69	5.55
2	6.06	6.40	6.29	6.20	6.17	6.18	6.10
3	6.09	6.93	6.42	6.31	6.20	6.15	6.14
4	6.18	6.57	6.52	6.39	6.33	6.25	6.21
5	6.06	6.67	6.60	6.91	6.69	6.44	6.19
average	5.97	6.47	6.32	6.29	6.19	6.14	6.04
Negative control							
G16							
1	5.99	6.37	6.75	6.32	6.31	6.34	6.33
2	5.57	6.88	6.87	6.60	6.87	6.36	6.01
3	5.61	6.55	6.50	6.65	6.30	6.25	6.18
4	5.87	6.68	7.09	7.28	6.71	6.23	6.20
5	5.56	6.18	6.51	6.61	6.42	6.20	6.11