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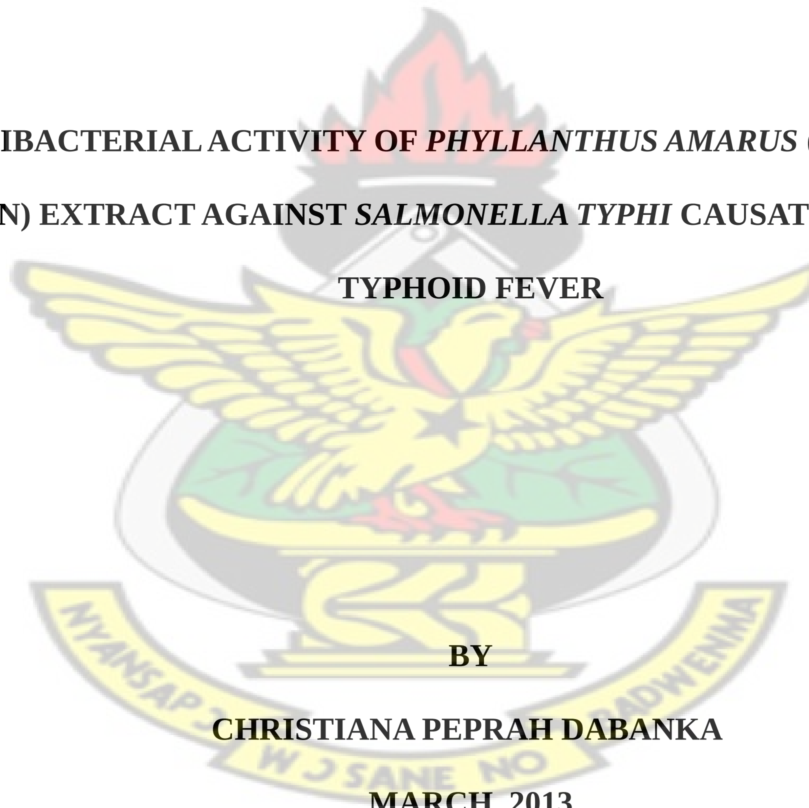

ANTIBACTERIAL ACTIVITY OF *PHYLLANTHUS AMARUS* (*SCHUM* AND THONN) EXTRACT AGAINST *SALMONELLA TYPHI* CAUSATIVE AGENT OF TYPHOID FEVER

BY

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MARCH, 2013

ANTIBACTERIAL ACTIVITY OF *PHYLLANTHUS AMARUS* (*SCHUM* AND THONN) EXTRACT AGAINST *SALM*[illegible] *A TYPHI* CAUSATIVE AGENT OF TYPH[illegible] ER

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A THESIS SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ENVIROMENTAL SCIENCE

BY

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MARCH, 2013.

DECLARATION

I hereby declare that, except for specific references which have been duly acknowledged, this project is the result of my own research and it has not been submitted either in part or whole for any other degree elsewhere.

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DEDICATION

To the glory of God Almighty

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ACKNOWLEDGEMENTS

I am heartily thankful to my supervisor, Mr W. Gariba Akanwariwiak whose guidance and support from the initial to the final level enabled me to develop an understanding of the subject.

Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project

ABSTRACT

The study was conducted to assess the antibacterial activity of *Phyllanthus amarus (*Schum and Thonn) extract against *Salmonella typhi* causative agent of typhoid fever at the laboratories of the Departments of Chemistry and Theoretical and Applied Biology of the College of Science, Kwame Nkrumah University of Science and Technology, Kumasi. The objectives were to determine the highest yield of crude extract of *P. amarus* using different proportions of water to ethanol and to determine the sensitivity of *Salmonella typhi* to these. Three different extraction procedures were carried out. In the first procedure, seven extraction setups each containing different proportions of the two extract (water and ethanol) were used with 10g of the plant sample. In the second procedure, eight setups were used for the two solvents. Ten grams of both fresh and dry plant sample were extracted in two different 200ml of water and in another two different 200ml of water; 20g of both fresh and dry plant sample were again extracted. The same procedure was repeated using ethanol as the solvent. In the third procedure, 10g each of fresh plant sample were boiled in 100ml and 200ml of water for 30 minutes. A sensitivity test to determine the zones of inhibition for the various plant extracts was done on *Salmonella typhi* isolated from human. Results from the crude yield of *P. amarus* using water only had the highest crude yield of 2.57g, followed by ethanol only which was 2.52g. The sensitivity studies conducted on the fresh *P. amarus* indicated that aqueous extract of *P. amarus* inhibited *S. typhi* to a zone of 5.00mm in 10g/200ml and 7.17mm in 20g/200ml. Ethanol extract also recorded an inhibition zone of 2.67mm and 5.33mm in 10g/200ml and 20g/200ml respectively. Again, sensitivity studies using dry *P. amarus* samples showed that the aqueous extracts recorded a zone of inhibition of 7.33mm in 10g/200ml and 13.50mm in 20g/200ml. Also ethanol extracts also recorded an inhibition zone of 6.83mm in 10g/200ml and 10.50mm in 20g/200ml. Significant differences were observed among the extracts and the control in both 10g/200ml and 20g/200ml concentrations ($P<0.05$). Aqueous and ethanol extracts of *P. amarus* proved inhibitory to *S. typhi*. .

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CHAPTER ONE

INTRODUCTION

Medicinal plants have been used for the treatment of several human diseases over the century and have been very important in the health care delivery of every nation at one stage or the other (Oluma,et al., 2004). Recent research has focused on natural plant product as alternatives to the existing drugs for disease remedy in developing countries (Aiyegoro *et al*., 2007). Plant derived medicines have been part of traditional health care in most parts of the world for ages and there is increasing interest in them as sources of agents to fight microbial diseases (Mohana *et al*., 2008: Ghaleb *et al*., 2009; Ajayi and Akintola, 2010).

The development of multiple antibiotic resistant organisms has constituted a global problem as far as treatment of some infectious diseases is concerned. Typhoid fever caused by *Salmonella typhi* has since 1989 developed simultaneously, resistance to conventional antibiotics of choice in several endemic areas (Greenwood *et al*., 2009). The vehicles of transmission of this etiologic agent are mainly food and water. Several other disease-causing organisms of medical importance have also developed resistance to these conventional antibiotics.

Infectious diseases still remain an important cause of morbidity and mortality in man, especially in developing countries. Today, in Africa, many resort to the use of locally made herbal medicines prepared as infusions in hot water, decoction in cold water, concoction with food and as tinctures with alcohol as an alternative therapy for

bacterial infections (Oluduro and Omoboye, 2010). Plant parts such as the roots, leaves, shoots, barks, fruit peels, immature and unripe fruits have been used in most herbal preparations.

According to George and Pamplona-Roger (1998), the therapeutic value of some common plants such as *Anthocleista vogelii* Planch, *Morinda lucida*, *Triplochiton scleroxylon*, *Alchornea cordifolia*, *Cassia sieberiana*, *Mangifera indica*, *Anacardium occidentale*, *Nauclea latifolia*, *Daniela oliveri*,*Citrus paradise*, *Ananas sativus* and *Carica papaya* have been used in the treatment of various ailments including enteric fever, diarrhoea, dysentery, malaria, common cold, convulsion, yellow fever, jaundice, dental caries, intestinal parasites, gastroenteritis, bacterial, viral and protozoan diseases. Antiseptic, diuretic, antibacterial and anti-inflammatory properties have equally been reported (Alanis *et al*., 2005).

Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potential for introducing new templates into modern medicine (Akinyemi *et al*., 2005). In many parts of the world, including Ghana, herbal medicine practitioners are still consulted as a first choice in the treatment of ailments, due to the fact that traditional medicine blends readily with the socio-cultural life of the people, and the fact that orthodox medicine are more expensive to procure and some orthodox pharmaceutical preparations are many times faked (Amuse *et al*., 2011). There is a vast array of medicinal plants used singly or in combination with other medicinal plants that confer synergistic effect in the treatments of various ailments.

These medicinal plants or their extracts are administered orally, topically, by inhalation of vapours or by steam bathing.

Phyllanthus amarus is reported to have healing properties and not toxic to either the kidney or liver. The plant also contains several phytochemical elements including glycosides, flavonoids, alkaloids, phenylpropanoids, sterols, saponins, limonine among others. *P. amarus* is used for the treatment of several medical conditions including liver, kidney and bladder problems, diabetes, intestinal parasites, inflammation, prostate, influenza, dropsy and jaundice problems (Heyde, 1990; Foo, 1993).

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OBJECTIVES OF THE STUDY

The objectives of the study are therefore to:

1. Determine the yield of the crude extract of *Phyllanthus amarus* using different proportions of water: ethanol
2. Determine the sensitivity of *Salmonella typhi* to the various concentrations of the crude extract of *P. amarus*

1.1 PROBLEM STATEMENT

The use of plant extracts in the food, cosmetics and pharmaceutical industries is increasing. A systematic study of medicinal plants is very important. Findings from researches conducted suggest that non polar solvents are best for extraction of active substances from medicinal plants (Junaid *et al*., 2006). Others have suggested that alcohol is the best polar solvent for the extraction of active substances from medicinal plants (Parekh *et al*., 2006). In traditional pharmacognosy, the solvents

normally used for extraction include water, ethanol and palm kernel oil, however, water is the main extractive solvent mostly used (Musa *et al*., 2011). The acceptance now of traditional medicine as alternative form of healthcare and the development of resistance to the available antibiotics have led to widespread investigation into the antimicrobial activity of medicinal plants (Bisignano *et al*., 1996; Hammer *et al*., 1999). There is therefore a need to study the synergistic antibacterial activity of the aqueous and ethanoic extracts of *P. amarus* used in Ghana for the traditional treatment of typhoid fever.

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1.2 JUSTIFICATION

Phyllantus amarus is a medicinal herb considered efficient for the cure of various ailments. In Ghana the plant is known mostly for the traditional use in curing and treating diseases such as jaundice, diarrhoea, dysentery, intermittent fevers, typhoid fever, scabies, ulcers, wounds and diseases of the urino-genital system. It is also used in treating liver ailments and kidney stones. It is noted that evaluation of this herb and herbal products in general faces major problems. First, is the use of mixed extracts (concoctions) and variations in methods of harvesting, preparing, and extracting the herb, which can result in dramatically different levels of certain alkaloids. Secondly, the lack of controlled clinical studies such as empiricism and a holistic philosophy and controlled studies which Traditional Eastern Medicine relies on are considered unnecessary. These are views shared by many Western supporters of alternative medicine. Related to these issues are concerns about the safety of herbal remedies. Numerous reports of toxic effects contradict the popular view that herbals are natural and therefore harmless. A survey of the National Poison

Information Service for the year 1991-1995 documented 785 cases of possible or confirmed adverse reactions to herbal drugs, among which hepatotoxicity was the most frequent. This study has become necessary to find out if only the extracts of *Phyllanthus amarus* can effectively cure typhoid fever and also find out the best extraction method to get the maximum plant crude extract.

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CHAPTER TWO

LITERTURE REVIEW

2.1 DESCRIPTION OF *PHYLLANTUS AMARUS*

Phyllantus amarus is a small erect tropical herb that grows to a height of 10-60cm. It is an annual and widespread throughout the tropics and subtropics. It is found in sandy regions as a weed in cultivated and wastelands. The plant is a common tropical weed that grows well in moist, shady and sunny places (Nanden, 1998). Some common names of *Phyllanthus amarus* in North, Central and South America are black catnip, carry-me seed, egg woman, hurricane weed, quinine creole, quinine weed, seed-under-leaf, stone breaker among others (Morton, 1981).The name 'Phyllanthus' means "leaf and flower" since both the flower as well as the fruit seems to come one with the leaf (Cabieses, 1993).

P. amarus is a member of the Euphorbiaceae family (Spurge family), with over 6500 species in 300 genera. Euphorbiaceae family consist of upright or prostrate herbs or shrubs, often with milky acrid juice (Lewis and Elvin-Lewis, 1977) and is mainly a pan-tropical family with some species either more or less temperate. Numerous species of this family are native to North, Central and South America (Unander, 1995). The plants are monoecious or monogamous. It has smooth cylindrical stem (1.5-2mm) thick and deciduous horizontal branchlets (4-12cm) long and 0.5cm thick, with 15 to 30 leaves. The leaves are simple, alternate or opposite and leathery, and borne on petioles 0.3 to 0.5 mm long. The flowers are very small and diclinous, often in clusters borne in greenish cup-shaped structures with glands. The fruit is a

three-lobed capsule containing six seeds and extends from the cup with a long stalk pendant about 1-2mm(Lewis and Elvin-Lewis, 1977). The seeds are triangular (like an orange segment), light brown, 1mm long, with 5-6 ribs on the back and the seeds are hurled away when the fruits burst open (Morton, 1981).

Plate 2.1: *Phyllantus amarus*

2.1.1Origin and Distribution of *Phyllanthus amarus*

The plant is indigenous to tropical Americas, the Philippines and India (Cabieses, 1993; Tirimana, 1987; Chevallier, 2000). Plants in the genus *Phyllanthus* are found around all tropical regions of the world; from Africa to Asia, South America and the West Indies. The genus contains about 550-750 species in 10-11 subgenera (Unander, 1995).Some related species with medicinal importance include. *epiphyllanthus*, *P. niruri P. urinaria*, *P. acuminatus* and *P. emblica* (Tirimana, 1987). The plant can be found along roads, valleys, on riverbanks and near lakes. *P. amarus* is sometimes mistaken and wrongly identified with the closely related *P. niruri*L. in appearance, phytochemical structure and history of use. *P. niruri* also reaches a height of 60 cm with larger fruits and dark brown and warty seeds than that of *P. amarus* (Morton, 1981).

2.2 TRADITIONAL USE OF *PHYLLANTHUS AMARUS*

Phyllanthus plants are used in folk remedies in many countries. It has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites (Foo, 1993). *P. amarus*, *P. nururi* and *P. urinaria* have also been used in the treatment of kidney related problems, gallstones, appendix inflammation and prostate problems (Heyde, 1990).

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Phyllanthus amarus has also shown to work as an antifungal, antibacterial and antiviral agent (Houghton *et al*., 1996).Adeneye *et al*., (2006a) reported that *P. amarus* was used in traditional medicine for its hepatoprotective, anti-diabetic, anti-hypertensive, analgesic, anti-inflammatory and antimicrobial properties. Joy and Kuttan (1998) also reported the anti-nociceptive, anti-lipidemic, anti-diabetic, anti-inflammatory, anti-lithic and anti-bacterial properties of the plant.

Traditionally, it is used in the treatment of liver ailments and kidney stones. Base on its Spanish name 'chanca piedra' meaning "stone breaker or shatter stone. "The plant is a popular medicinal herb used as a remedy around the world for influenza, dropsy, diabetes and jaundice (Foo, 1993).Whole plant is employed in some genitourinary infections. The young tender shoots are used in chronic dysentery and the juice of the stem is mixed with oil in ophthalmia for eye treatments. According to Foo and Wong (1992), the aerial part of *Phyllanthus amarus* is highly valued in traditional medicine for its healing properties. Fresh leaf juice of the plant can be applied externally for the treatment of cuts and bruises. It is also good for treating Arthritis and Asthma in patients (Adebisi, 1999).

In South America, the plant has been used to eliminate gall bladder and kidney stones, and in treating gall bladder infections. In India, this plant has been used in traditional medicine to treat liver diseases such as asthma and bronchial infections (Foo and Wong, 1992). Chevallier (2000) reported that *P. amarus* has been used traditionally in India to treat cardiovascular problems. In Suriname, *P. amarus* is always sold as fresh and dry plant material in the herb markets. *P. amarus* is a restoration herb used as a tonic in Suriname where decoctions are used in herbal baths and in postnatal care (Titjari, 1985; Sedoc, 1992).

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The plant is also used in traditional medicine as herbal decoction in treating bladder and kidney disorders, cramps and uterus complaints (with other herbs) and also works as an appetizer (Sedoc, 1992).Heyde (1990) reported that plant extracts of *P. amarus* can be used as blood purifiers, for light malaria fevers, anaemia, colic and also helps the release of phlegm.

Nanden (1998) also reported that the herb is used to combat fever, flu and asthma in combination with other herbs. The plant when boiled with the leaves is considered to be a diuretic and can be used in treating diabetes, dysentery, hepatitis, menstrual disorders, and skin disorders. The herb can also be used for constipation. The extracts from the roots can be used to treat jaundice (Heyde, 1990).

In recent years, the plant has been used successfully as a liver-protectant/detoxifier for conditions such as jaundice and hepatitis B and can rapidly restore full functioning of a damaged liver. Meixa *et al*. (1995) confirm the anti-viral activity of

P. amarus against hepatitis B virus. *P. amarus* which is otherwise called Eyin-olobe in South-Western Nigeria has healing effects on hypertensive patients. It was equally found efficacious for treating malaria, diabetes, kidney stones and jaundice. Chaudhury (2007) and Odugbemi (2008) reported that the plant is effective for treating gonorrhoea, genito-urinary diseases, asthma, diabetes, typhoid fever, jaundice, stomach-ache, dysentery, ringworm, and hypertension. Kokwaro (1976) confirmed the use of the plant in the treatment of stomach disorders, skin diseases and cold.

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P. amarus can be taken for weight loose and help to increase male fertility. It has widely been reported to offer good treatment for leprosy, hiccup, and peptic ulcer. It is anti-spasmodic, good laxative, blood tonic, treatment of itch, flu, fever, dyspepsia, blennorrhagia, tenesmus, gonorrhoea, malaria, uterus complaints, constipation, anorexia, carminative, tumour, colic; it has HIV inhibitory activity, good anti – inflammation of appendix, bladder disorder (Obianime and Uche, 2009).

2.3 PHYTOCHEMICAL PROPERTIES OF *PHYLLANTHUS AMARUS*

Hydro-alcoholic extract of *Phyllanthus amarus* showed the presence of various phyto-constituent such as alkaloids, flavonoids, saponins and tannins (Umbare *et al*., 2009). Experiments conducted into the phytochemicals present in *Phyllanthus amarus* using UV, IR, Mass and NMR spectroscopy revealed that alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols were present in the plant (Foo and Wong(1992); Foo (1993); Foo (1995). Houghton *et al*. (1996) isolated

securing type alkaloids by Column Chromatography (CC) and preparative Thin Layer Chromatography (TLC).

Previous studies have reported some of these phyto components to elicit a wide range of biological activities, which include hypolipidemic, hypoglycemic, hypoazotemia among others (Olapade *et al*., 1995). Saponin is known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile acids (Topping *et al*., 1980). In the process, the conversion of cholesterol to bile acid is enhanced in the liver resulting in concomitant hypocholesterolemia (Kritchevsky, 1977).

The lignan constituents in the plant consist of phyllanthin and hypophyllathin, amarinic acid, nynphyllin, phyllarurin and neolynan. Phyllanthin and hypophyllanthin are chemicals that help in carrying out liver protecting activities. Geranin a phytochemical in the plant has hypertensive effects and account for it's used in hypertension conditions. This chemical can inhibit several neurotransmitter processes that relay and receive pain signals in the brain. Geranin also has anti-ulcerous properties (Chaudhury, 2007).

Alkaloids and tannins contain in the plant contribute to the plants effects as anti-malaria, anti-diarrhoea and analgesic agents. The major lignans, Phyllanthin and Hypophyllanthin have been reported to exhibit anti-hepatotoxic activity.

2.4 PHARMACOLOGY AND CLINICAL STUDIES OF *PHYLLANTHUS AMARUS*

Mehrotra *et al*. (1991), in their *in vitro* studies of the anti-viral activity of *P. amarus* on hepatitis B virus reported that extracts of *P. amarus* were effective against HBV antigens with butanol extract being the most potent. Enhanced activity was seen in chromatographic fractions where the active fractions inhibited the interaction between HBsAg/HBeAg and their corresponding antibodies suggesting anti-HBs, anti-HBe-like activity and also an effect on HBV-DNA.

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In a clinical trial conducted on the anti-hyper glycemic activity of *P. amarus*, nine mild hypertensive patients (four of them suffering from Diabetes Mellitus) were treated with a preparation of the whole plant of *P. amarus* for 10 days. The observations indicated that *P. amarus* as a potential diuretic hypotensive and hypoglycaemic drug for human. Also blood glucose was significantly reduced in the treated group (Srivadya and Periwal 1995.). In another clinical trial, 25 patients in the age group of 35-55 years with moderate and severe diabetic blood sugar level (250-400mg/100ml) showed significant lowering of blood sugar levels at a dose of 1gm thrice daily for a period of three months (Sivaprakasam *et at*., 1995).

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In an anti-hepatotoxic activity test conducted on albino rats, administration of whole plant powder for 7 days at dosage of 35mg and 70mg per kg body weight helped in restoring the levels of biochemical parameters to normal within next 48hours in calves. AST, ALT, bilirubin and icteric were elevated within 24 hours indicating liver parenchymal damage. The butanol fraction of the whole plant also exhibited anti-hepatotoxic activity. In another study, whole plant powder administered at a

dosage of 0.66g/kg in rat showed hepatoprotective activity against CCl_4 induced liver damage (Sane *et al*., 1995).

2.5 ANTIMICROBIAL PROPERTIES OF MEDICINAL PLANTS

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Parekh *et al*., 2005). The screening of plants usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study.

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare (Joshi, 2011). Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. In vitro studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak (Zaika, 1998). Medicinal plants like Tulsi, Neem, Datiwan and Clove are being used traditionally for the treatment of inflammation, wound healing, carminative, cough, toothache, antiseptics expectorant, stomatitis and some fungal infection like

candidiasis. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. For instance, phytochemical analysis of *A. indica* revealed the presence of terpenes and glycosides (Kraus *et al*., 1981; Joshi, 2011).Glycoside is a major bioactive component that offers anti secretory and antiulcer effects (Bandyopadhyay *et al*., 2002). Plant glycosides, which are not normally toxic when ingested orally, are known to inhibit chloride transport in the stomach (Machen and Forte, 1979). Also neem oil is believed to have medicinal properties, such as antibacterial (Singh and Sastri, 1981) antifungal (Kher and Chaurasia, 1977) and anti-diabetic (Kraus *et al*., 1981).

An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Sikkema *et al*., 1994). The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. MBC is the lowest concentration of antibacterial substance required to produce a sterile culture (Cheesbrough, 1991).

Most plant extracts are most effective against gram positive bacteria, *S. aureus*, a pyrogenic bacterium known to play a significant role in invasive skin diseases including superficial and deep follicular lesion and food poisoning. *Salmonella spp*. infects a number of animal species (Furowicz and Terzolo, 1975) and *S. typhi,* which causes typhoid fever in humans, has also been tested against different plant extract and found to be effective. Intensive use of antibiotics often resulted in the

development of resistant strains (Sydney *et al*., 1980) these create a problem in treatment of infectious diseases. Furthermore side effects associated with antibiotics are often fewer when using medicinal plants (Cunha, 2001). Medicinal plants have some advantages over antibiotics such that there is better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermani and Garg, 2002).

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2.6 SALMONELLA TYPHI

2.6.1 Description and Significance of *Salmonella typhi*

There are over 2,000 various groupings (serovars) that comprise *Salmonella enterica*, each very closely related to each other making *Salmonella typhi* a prime example of a serovar. *S. typhi* is a gram negative bacterium that causes systemic infections and typhoid fever in humans. This rod-shaped, flagellated organism's sole reservoir is humans. It has caused many deaths in developing countries where sanitation is poor and is spread through contaminated water and undercooked food. Eradication seems highly unlikely due to recent emergence of multi drug resistance strains. *S. Typhi* strain Ct18 was originally isolated from a patient in a hospital in Vietnam. The chromosome sequence is 4,809,037 bp in length with a G+C content of 52.09%. The chromosome was sequenced though the method of shotgun sequencing with 97,000 shotguns reads. Since then, *S. typhi* has undergone evolutionary change and has become resistant to antibiotics (Deng *et al*., 2003).

2.6.2 Genome Structure of *Salmonella typhi*

The genome for *Salmonella typhi* has been completely sequenced. There are about 204 pseudogenes encoded in *S. typhi*. A majority of these genes have been inactivated by a stop codon, which shows that the genes were recently modified due to evolutionary changes. Of the 204 genes, twenty seven (27) are remnants of insert sequences and genes of bacteriophage origin. Seventy five (75) are involved in housekeeping functions and 46 of the gene mutations have to do with host interaction. There are two commonly used strains of *S. typhi*, CT18 and Ty2. *S. typhi* CT18 has a large circular chromosome consisting of 4.8 Mb and two plasmids, pHCM1 and pHCM2, one of which has multiple drug resistance (pHCM1). *S. typhi* Ty2 has one large chromosome that is 4.7 Mb and unlike CT18, it does not have plasmids and can be affected by antibiotics. In fact, the current vaccine was developed using S. typhi Ty2. Out of the 204 pseudogenes in Salmonella, 195 genes are the same in both strains CT18 and Ty2, making them 98% identical (Deng *et al*., 2003; Parkhill *et al*., 2001)

2.6.3 Cell Structure and Metabolism of *Salmonella typhi*

Salmonella typhi is a rod-shaped, gram negative bacteria that contain features that separates itself from other types of bacteria which include: having 2 membranes (an outer and an inner), periplasm, and a Lipopollysaccharide chain that consists of α-d-galactosyl-(1 → 2)-α-d-mannosyl-(1 → 4)-l-rhamnosyl-(1 → 3)-repeating units, and has short branches of single 3,6-dideoxyhexose residues (Kita and Nikaido, 1973).*S. typhi* has a complex regulatory system, which mediates its response to the changes in its external environment. Sigma factors, which are global regulators that

alter the specificity of RNA polymerase, are examples of such regulation. Some sigma factors direct transcription to produce stress proteins, which increases the chances of the bacteria surviving environmental changes. RNA polymerase S is produced in response to starvation and changes in pH and temperature. It also regulates the expression of up to 50 other proteins and is also involved in the regulation of virulence plasmids. In order to survive in the intestinal organs of its hosts where there are low levels of oxygen, *S. typhi* has to be able to learn to use other sources other than oxygen as an electron acceptor. Therefore, Salmonella has adapted to grow under both an aerobic and anaerobic conditions. Salmonella's most common source of electron acceptors is nitrogen. Examples of other electron acceptors are: nitrate, nitrite, fumarate and dimethlysulphoxide. Global and specific regulatory systems of anaerobic gene expression, like the ones mentioned above, are implemented to make sure that the most energetically favourable metabolic process is used. Evidence shows that the availability of oxygen is an environmental signal that controls Salmonella's virulence (Contreras *et al.*, 1997).

2.6.4 Ecology of *Salmonella typhi*

Salmonella typhi is a food borne pathogen and that is increasingly difficult to control. Salmonella's ability to change its phenotype and genotype in response to environmental changes make it almost impossible to eradicate from the food chain. When a culture of Salmonella was transferred to higher temperatures (60°C), it took 60 minutes to maximize heat resistance. When the pH was lowered, acid resistance increased. The time taken to kill 90% was 4-14 minutes. Salmonella cells experience gradual changes which is why Salmonella thrives in undercooked meat. It is able to

adapt to survive the cooking process and also has the ability to cross the gastric acid barrier (this is how they enter the human intestine). A high-fat matrix protects Salmonella against these stressful environments (Humphrey, 2004).

2.6.5 Pathology of *Salmonella typhi*

Salmonella typhi has killed over 600,000 people annually all over the world. It is a deadly bacterial disease that causes typhoid fever and is transmitted through food and water. It has become an epidemic in South Asian countries where sanitation is lacking. *S. typhi* usually invades the surface of the intestine in humans, but have developed and adapted to grow into the deeper tissues of the spleen, liver, and the bone marrow. Symptoms most characterized by this disease often include a sudden onset of a high fever, a headache, and nausea. Other common symptoms include loss of appetite, diarrhoea, and enlargement of the spleen (depending on where it is located). *S. typhi* involves colonization of the reticuloendothelial system. Some individuals who are infected with *S. typhi* become life-long carriers that serve as the reservoir for these pathogens. *S typhi* has an endotoxin (which is typical of Gram negative organisms), as well as the VI antigen, which increases virulence. It also produces a protein called invasion that allows non-phagocytic cells to take up the bacterium and allows it to live intra cellular. *S. typhi* is a strong pathogen for humans due to its resistance to the innate immune response system (Falkow *et al*., 2004).

Recently, strains of MDR (multi-drug resistant) Salmonella have been identified and grouped together in a single haplotype named H58. It has been found that these strains are now resistant to nalidixic acid and have reduced susceptibility to

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fluoroquinolones. This strain has been recently found in Morocco, which shows that the MDR strain has reached as far as Africa (Ojcius, 2007).

2.6.6 Current Research on *Salmonella typhi*

Much research is going on since the global emergence of multi drug resistant strains. In India, samples of 21 *Salmonella typhi* strains were tested for their vulnerability to antimicrobial agents. Three different antibiotics were tested; chloramphenicol (256mg/l), trimethoprim (64mg/l), and amoxicillin (>128mg/l). Eleven of the *S. typhi* strains were resistance to chloramphenicol, trimethoprim and amoxicillin. Four of the isolates were resistant to all of them except for amoxicillin. Six of the isolates were completely sensitive to all of the antimicrobial agents tested. All the *S. typhi* isolates were susceptible to cephalosporin agents, gentamicin, amoxicillin plus clavulanic acid, and imipenem. The genes responsible for the resistance of the antibiotics included chloramphenicol acetyltransferase type I, dihydrofolate reductase type VII, and TEM-1 β-lactamase. Pulsed-field gel electrophoresis analysis of XbaI-generated genomic restriction fragments identified a single distinct profile (18 DNA fragments) for all of the resistant isolates. After comparing this, six different profiles were recognized among the sensitive isolates. It was found that a single strain containing a plasmid having multi drug-resistance has emerged in the *S. typhi* population and has been able to adapt and survive the antibiotics as they are introduced into clinical medicine (Philippa *et al*., 1998).

With the current spread of Salmonella, researchers are looking for easier ways to detect typhoid fever in order to better treat patients. Another project has to do with

dipstick assay which detects antibodies and analyses the effect of typhoid fever in patients. It found specific IgM antibodies on patients in 43.5%, 92.9%, and 100% for samples collected 4-6 days, 6-9 days, and greater than 9 days after the onset of fever, respectively, the number of antibodies increasing during the length of the duration. Testing of serum samples from culture negative patients with a clinical diagnosis of typhoid fever resulted in staining of the dipstick in 4.3% of the samples collected on the day of admission and in 76.6% one week later. This shows the late development of antibodies in the blood for a large number of patients. The advantages of the dipstick assay are that the result can be obtained on the same day allowing a prompt treatment. No special laboratory equipment is really needed to perform the assay and one would only need a small amount of serum. What makes it even better is that the simplicity of the assay would allow it to be used in places that lack laboratory facilities, such as third world countries that lack modern facilities and where disease is running high(Halta *et al*., 2002).

More people have taken a greater interest in *Salmonella typhi* since the decreasing effects of antibiotics. In 2006, more research was done in order to find the global gene expression by *Salmonella typhi* during infection. Global expression profiles of *S. typhi* grown *in vitro* and within macrophages at different time points were obtained and studied. Virulence factors, such as the SPI-1- and SPI-2-encoded type III secretion systems, were found as expected during infection by Salmonella. The results concluded that *S. typhi* had an increased expression of genes encoding resistance to antimicrobial peptides, which used the glyoxylate bypass for fatty acid utilization, and did not induce the SOS response or the oxidative stress response. It

was also found that genes coding for the flagella apparatus, chemo taxis, and iron transport systems were down-regulated in vivo. This experiment allowed a better understanding of Salmonella and found a safer and more effective way to determine the bacterial transcriptome *in vivo*. This could possibly lead to the investigation of transcriptional profiles of other bacterial pathogens without the need to recover much bacterial mRNA from the host (Faucher *et al*., 2006).

2.7 DESCRIPTION OF TYPHOID FEVER

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Typhoid fever is an acute illness associated with fever that is most often caused by the *Salmonella typhi* bacteria. It can also be caused by *Salmonella paratyphi*, a related bacterium that usually leads to a less severe illness. The bacteria are deposited in water or food by a human carrier and are then spread to other people in the area. Typhoid fever is rare in industrial countries but continues to be a significant public-health issue in developing countries (Brusch, 2011; Balentine, 2011).

The incidence of typhoid fever in the United States has markedly decreased since the early 1900s. Today, approximately 400 cases are reported annually in the United States, mostly in people who recently have travelled to endemic areas. This is in comparison to the 1920s, when over 35,000 cases were reported in the U.S. This improvement is the result of improved environmental sanitation. Mexico and South America are the most common areas for U.S. citizens to contract typhoid fever. India, Pakistan, and Egypt are also known high-risk areas for developing this disease. Typhoid fever affects more than 13 million people worldwide, annually, with over 500,000 patients dying of the disease (Balentine, 2011).

Ideal antibiotic therapy is based on determination of the aetiological agent and its relevant antibiotic sensitivity. Empiric treatment is often started before laboratory microbiological reports are available when treatment should not be delayed due to the seriousness of the disease. The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection, and the ability of the bacteria to resist or inactivate the antibiotic. Some antibiotics actually kill the bacteria i.e. bactericidal, whereas others merely prevent the bacteria from multiplying i.e. bacteriostatic so that the host's immune system can overcome them (Lima, 2009).

2.7.1 Antibiotic Sensitivity Testing For Typhoid Fever

Antibiotic sensitivity is a term used to describe the susceptibility of bacteria to antibiotics. Antibiotic Susceptibility Testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. In this method, small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth. Other methods to test antimicrobial susceptibility include the Stokes Method, E-test (also based on antibiotic diffusion), Agar and Broth Dilution Methods for Minimum Inhibitory Concentration determination (MIC). Muller Hinton agar is most frequently used in antibiotic susceptibility test (Lalitha, 2004).

CHAPTER THREE

MATERIALS AND METHODS

The study was conducted at the laboratories of the Departments of Chemistry and Theoretical and Applied Biology, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi.

3.1 SOURCE OF PLANT MATERIALS

The plant used for the study was *Phyllanthus amarus*. Plant samples were collected from Kwame Nkrumah University of Science and Technology, Kumasi campus and Saint Louis College of Education campus, all in the Kumasi Metropolis. It was observed that the plant was shade loving and grew best in moist areas and sandy soils, especially at the edges of concrete floors. Identification of plant sample collected was done at the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi.

3.2 PREPARATION OF PLANT SAMPLES

Plant materials collected were washed under running tap water and were allowed to drain before air drying under shade for two weeks. The roots were separated from the leaves and the stem, because that is what is traditionally done. The leaves together with the stem and the small branches were then grinded mechanically with mortar and pestle

3.4 EXTRACTION PROCEDURE

Three different extraction procedures were carried out. The first extraction process consisted of using different proportions of the two main extraction solvents namely water and ethanol. The ratios of water to ethanol used for the proportions are presented in Table 3.1.

Table 3.1: Proportions of water to ethanol used in extraction

Label	Ratio	Description
W_9E_1	9:1	90% water : 10% ethanol (180ml of water to 20ml of ethanol)
W_4E_1	4:1	80% water : 20% ethanol (160ml of water to 40ml of ethanol)
W_7E_3	7:3	70% water : 30% ethanol (140ml of water to 60ml of ethanol)
W_3E_2	3:2	60% water : 40% ethanol (120ml of water to 80ml of ethanol)
W_1E_1	1:1	50% water : 50% ethanol (100ml of water to 100ml of ethanol)
W_1	1	100% water (200ml of water)
E_1	1	100% ethanol (200ml of ethanol)

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Ten grams (10g) of the *P. amarus* plant samples was placed into each of the seven flasks containing the different proportions of the two extraction solvents. The flasks were then connected to a setup consisting of Soxhlet Extractor Apparatus and a condenser. Power was applied for the extraction process to begin.

In the second extraction process, eight extraction setup were used for the two solvent (water and ethanol), four for each solvent. For water extraction, 10g of both fresh and dry *P. amarus* plant samples were extracted in 200ml of water and another 20g

of both fresh and dry P. amarus plant sample was also extracted in 200ml of water. The same procedure was repeated for ethanol extraction.

In the third extraction process, 10g of fresh *P. amarus* plant sample was boiled in 100ml and 200ml of water for 30minutes.

3.5 ISOLATION OF SALMONELLA FROM HUMAN FAECES

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3.5.1 Preparation of Buffered Peptone Water

Twenty grams (20g) of powdered peptone water was weighed and dissolved in 1 litre of distilled water in an Erlenmeyer flask. The solution was thoroughly mixed before distributing into universal bottles. The universal bottles with their contents were sterilized by autoclaving at 121 ℃ for 15 minutes.

3.5.2 Preparation of Non-Selective Pre-Enrichment Medium

Twenty-five grams (25g) of faeces was weighed and placed into an Erlenmeyer flask. 225ml of buffered peptone water was added to the Erlenmeyer flask to obtain 1 part sample and 9 parts buffered peptone water. This was thoroughly mixed and incubated at 37°C overnight (16-20 hours).

3.5.3 Preparation of Selective Enrichment Medium

3.5.3.1 Selenite broth preparation

Twenty-three grams (23g) of selenite broth powder was suspended in 1 litre of distilled water. The suspension was thoroughly mixed before heating to boiling point. The resulting solution was then distributed into universal bottles using syringe.1ml of the non-selective pre-enrichment medium was transferred with a pipette into 10ml of the selenite broth and incubated at 44°C for 48 hours.

3.5.3.2 SS agar preparation

Fifty-two grams (52g) of SS agar powder were suspended into one litre of distilled water. The solution was boiled until completely dissolve. 10ml of the resulting solution was poured unto sterile petri dishes (plates) and allowed to solidify.

3.5.4 Inoculation of Salmonella on Selective Agar Plates

Using an inoculating loop, streaks from the selenite broth were made on the solidified SS agar on the plate. The inoculated plates were then incubated at 37°C overnight (18-24 hours). Black colonies on the SS agar were seen after 48 hours confirming the presences of *Salmonella typhi*.

3.5.4.1 Storing of *Salmonella typhi*

Black colony of *Salmonella typhi* identified was washed with 10ml distilled water before adding to a selective SS agar medium in a universal bottle. This was incubated at 37°C for 48hours before storing in a refrigerator for future use.

3.6 EXPERIMENTAL DESIGN

A Completely Randomised Design with three replications was used. Three sets of experiment were carried out. In the first experimental setup, different ratios of the extraction solvent were used to determine which combination gives the highest plant extract. In the second experimental setup, sensitivity of *S. typhi* to fresh plant extract was investigated. The treatments used consisted of (i) boiled plant sample in water, (ii) crude extract of plant with water, (iii) crude extract of plant with ethanol and (iv) a control treatment consisting of a standard antibiotic (Chloramphenicol tablet).

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In the third experiment, sensitivity of *S. typhi* to dry plant sample was investigated. The treatments used consisted of (i) crude extract of plant with water, (ii) crude extract of plant with ethanol and (iii) a control treatment consisting of a standard antibiotic (Chloramphenicol tablet).

3.7 SENSITIVITY TEST

To determine the effect of the plant extracts and chloramphenicol on the test organisms, a disc diffusion technique using the Kirby- Bauer method was applied in testing pure cultures of the test organism for their antimicrobial sensitivities based on zones of inhibition on agar plates (Prescott *et al.*, 2005). In this method, circular discs from filter paper were sterilized in a hot air oven for 1hour. The discs were then impregnated by soaking with each plant extract and air-dried for a few minutes. 1ml of *Salmonella typhi* culture suspension was placed onto solidified SS agar plates using a sterile micropipette. A glass spreader was used to evenly distribute the test suspension on the SS agar. A sensitivity disc of each plant extract and the standard chloramphenicol disc of 10μg potency were aseptically transferred onto the SS agar

plates using a sterilized forceps. The plates were labelled accordingly. The plates were then incubated at 37°C for 24-48 hours. The plates were later observed for zones of inhibition.

3.8 STATISTICAL ANALYSIS

The data collected were subjected to analysis of variance (ANOVA) using Statistic 9 statistical package. Differences in means were done using the least significant difference (LSD) at P=0.05. The results were expressed as mean ± standard error of mean (S.E.M.).

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CHAPTER FOUR

RESULTS

4.1 YIELD OF CRUDE EXTRACT OF *PHYLLANTHUS AMARUS*

Table 4.1 indicates the yield of crude plant extract from different fractions of extraction solvent. Using water as the sole solvent yielded the highest crude extract of 2.57g. Ethanol only also yielded the second highest crude extract of 2.52g. A fraction of water/ethanol (3:2) yielded 2.37g of crude extract. Also a fraction of water/ethanol (7:3) yielded 2.21g of crude extract. 2.14g of crude extract was recorded by a fraction of water/ethanol (9:1) while a fraction of water/ethanol (4:1) yielded 2.01g of crude extract. However, a fraction of water/ethanol (1:1) yielded the lowest crude extract of 1.05g.

Table 4.1: Yield of crude plant extract from different fractions of extraction solvent

Proportion of extraction solvent	Ratio	Yield of plant extract (g)
Water/Ethanol (90:10)	9:1	2.14
Water/Ethanol (80:20)	4:1	2.01
Water/Ethanol (70:30)	7:3	2.21
Water/Ethanol (60:40)	3:2	2.37
Water/Ethanol (50:50)	1:1	1.05
Water only	1	2.57
Ethanol only	1	2.52

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4.2 SENSITIVITY OF FRESH *PHYLLANTHUS AMARUS* TO SALMONELLA TYPHI

Sensitivity of *S. typhi* to fresh *P. amarus* sample in 10g/200ml is presented in Table 4.2. The results showed that water extract of fresh *P. amarus* was able to suppress activities of *S. typhi* and recorded the largest zone of inhibition of 5.00mm. This was followed by ethanol extract of fresh *P. amarus* which recorded an inhibition zone of 2.67mm. However, boiling of plant sample in water and the standard antibiotic, chloramphenicol recorded the smallest zone of inhibition of 1.83mm each. Statistically, there were significant differences observed among the extraction methods and the standard antibiotic ($P<0.05$).

Table 4.2: Sensitivity of *S. typhi* to fresh *P. amarus* sample in 10g/200ml

Extraction method	Zone of inhibition (mm)
Boiling with water	1.83 ± 0.29
Water extract	5.00 ± 1.00
Ethanol extract	2.67 ± 0.58
Chloramphenicol (control)	1.83 ± 0.29
Lsd $_{(P=0.05)}$	1.15
P-value	0.001

Table 4.3 shows the sensitivity of *S. typhi* to fresh *P. amarus* sample in 20g/200ml. The results of the experiment showed that *S. typhi* was very sensitive to the water extract of P. amarus with the largest inhibition zone of 7.17mm. The ethanol extracts recorded the second largest zone of inhibition of 5.33mm. The standard antibiotic, chloramphenicol had an inhibition zone of 5.00mm. However, boiling of fresh *P. amarus* in water recorded the smallest zone of inhibition of 3.33mm. There were significant differences observed among the different extraction methods and the standard antibiotic (P<0.05).

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Table 4.3: Sensitivity of *S. typhi* to fresh *P. amarus* extract in 20g/200ml

Extraction method	Zone of inhibition (mm)
Boiling with water	3.33 ± 0.76
Water extract	7.17 ± 1.00
Ethanol extract	5.33 ± 1.00
Chloramphenicol (control)	5.00 ± 1.53
Lsd $_{(P=0.05)}$	1.88
P-value	0.008

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4.3 SENSITIVITY OF DRY *PHYLLANTHUS AMARUS* TO SALMONELLA TYPHI

Sensitivity of *S.typhi* to dry *P. amarus* in 10g/200ml is presented in Table 4.4. The results showed that activities of *S. typhi* were inhibited by water extract of dry *P. amarus* with the largest zone of inhibition of 7.33mm. Ethanol extract of dry *P.*

amarus was able to suppress activities of *S. typhi* to a zone of 6.83mm. The control treatment, chloramphenicol however, recorded the smallest zone of inhibition of 1.00mm. There were significant differences observed among the extraction methods and the control (P<0.05).

Table 4.4: Sensitivity of *S. typhi* to dry *P. amarus* extract in 10g/200ml

Extraction method	Zone of inhibition (mm)
Water extract	7.33 ± 0.17
Ethanol extract	6.83 ± 0.17
Chloramphenicol (control)	1.00 ± 0.00
Lsd $_{(P=0.05)}$	0.47
P-value	0.000

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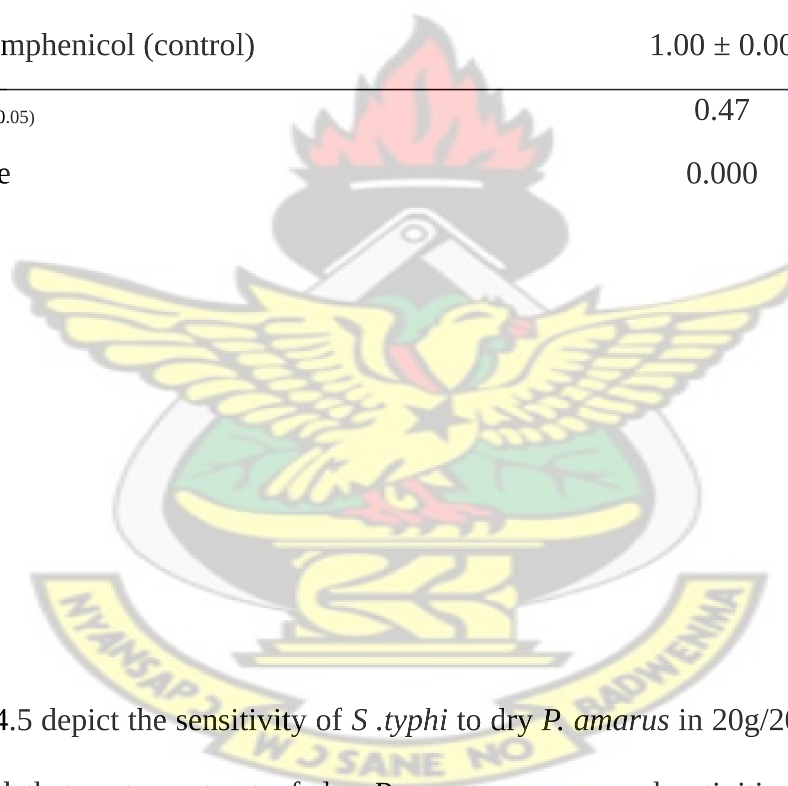

Table 4.5 depict the sensitivity of *S .typhi* to dry *P. amarus* in 20g/200ml. The results showed that water extract of dry *P. amarus* suppressed activities of *S. typhi* and recorded the largest zone of inhibition of 13.50mm. Again, activities of *S. typhi* were inhibited by ethanol extract of dry *P. amarus* and recorded an inhibition zone of 10.50mm. However, chloramphenicol recorded the small zone of inhibition of 6.33mm. There were significant differences observed among the extraction methods and the control (P<0.05).

Table 4.5: Sensitivity of *S. typhi* to dry *P. amarus* extract in 20g/200ml

Extraction method	Zone of inhibition (mm)
Water extract	13.50 ± 0.87
Ethanol extract	10.50 ± 0.87
Chloramphenicol (control)	6.33 ± 1.20
Lsd $_{(P=0.05)}$	3.43
P-value	0.006

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CHAPTER FIVE

DISCUSSION

5.1 SENSITIVITY OF PHYLLANTHUS AMARUS TO *SALMONELLA TYPHI*

The sensitivity tests of *Phyllanthus amarus* to *Salmonella typhi* showed that both aqueous and ethanolic extracts of the plant material showed antimicrobial activity on the test organism. The test pathogen, *S. typhi* was more susceptible to the aqueous extract of *P. amarus* than the ethanol extract at all concentrations. The highest susceptibility was recorded with the dry plant aqueous extract at 20g/200ml, followed by the ethanol extract at 20g/200ml. The susceptibility of the test inoculum to the extract of the fresh and dry plant material increased with increasing concentration of the extract. The antibacterial efficacy of the plant extracts were very high compared to the low activities recorded with chloramphenicol. Chlorampenicol showed different sensitivity to the test organism, this indicates that it still has the potential of curing typhoid fever. But it was realized that, the sensitivity increased with increasing concentration. According to Oluduro and Omoboye (2010) the antibacterial activities of most plant extracts are concentration dependent as zone of growth inhibition increased with increasing concentration of the extracts. Ekwenye and Elegalam (2005) and Azu and Onyeagha (2007) reported that the efficacy of most plant extracts is concentration dependent.

Umbare *et al*. (2009) assessing the quality of *Phyllanthus amarus* leaves extract for its hypolipidemic activity found the presence of four phyto-constituent namely alkaloids, flavonoids, saponins and tannins in the plant sample. Flavonoids, tannins,

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alkaloids, steroids, terpenoids, saponins and glycosides were also obtained by Obianime and Uche (2009) in their comparative study of the methanol extract of P. amarus leaves. Oluduro and Omoboye (2010) indicated that the presence of phytochemicals in plant extracts is a function of their antimicrobial activities against the test pathogen as they play important roles in bioactivity of medicinal plants. They further explained that phytochemicals exert antimicrobial activity through different mechanisms. Chonoko and Rufai (2011) also indicated that there was a link between the antibacterial activity exhibited by the plant extracts to the presence of steroids flavonoids, tannins, alkaloids and saponins.

Tannins, for example, act by iron deprivation, hydrogen binding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991; Akinpelu *et al*., 2008). Herbs that have tannins as their component are astringent in nature and are used for the treatment of gastrointestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003; Asguith and Butler, 1986). Saponinis believed to be responsible for numerous pharmacological properties (Estrada *et al*., 2000) and has been shown to have immense significance as anti hypercholesterol, hypotensive and cardiac depressant properties (Price, 1987). This perhaps justifies the already locally established function of the plant in the treatment and management of hypertensions. Alkaloids on the other hand are detoxifying and have antihypertensive properties (Trease and Evans, 1978; Zee-cheng, 1997). It's toxicity against cells of foreign organisms has been reported by Akinpelu *et al*. (2008).Waterman (1992) reported that alkaloids and flavonoids were useful as antimicrobial, anti-inflammatory and anti-oxidant agents. Okwu and Josiah (2006) indicated that

flavonoids are antioxidants; hence, Adeneye *et al.* (2006b) reported that the flavonoids contributed to the antioxidant activity of the *P. amarus* plant.

The choice of antimicrobial drugs in the absence of susceptibility information is often influenced by the signs and symptoms of disease, site of infection, and history of illness including patient's age. In many medical set-ups in the developing nations where laboratory facilities are inadequate, broad spectrum antibiotics are often used for suspected bacterial infections. These practices are not without danger which could worsen the disease prognosis. Intensive use of antibiotics often results in the development of resistant strains creating a problem in the treatment of infectious diseases (Sydney *et al*., 1980).

Harvey *et al*. (2006) reported chloramphenicol as the drug of choice in the management of typhoid fever although its toxicity is well known. According to them , higher doses of chloramphenicol may not be favourable to the host because its toxicity is known to precipitate some serious adverse reactions. Cunha (2001) reiterated that intake of antibiotics are associated with side effects. The increasing emergence of antibiotic-resistant strains to traditional antimicrobials makes it necessary that sensitivity tests should be carried out prior to initiation of antibiotic therapy (Madukosiri *et al*., 2009). Disk diffusion and subsequent Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests have remain the most commonly used methods to test organisms' susceptibility to antibiotics although they were outside the scope of the present study (Nester *et al*., 2004).

The present work has provided the basis for selecting plant extracts that are likely to be effective against *Salmonella typhi*. The problem of increasing emergence of resistance to antibacterial drugs makes these studies unavoidable, and equally highlights the need for periodic review of antimicrobial activities of common antibiotic drugs available on the market since chloramphenicol was found to be less effective at a lower concentration or dosage than at a higher one. Moreover, medicinal plants often have fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermani and Garg, 2002).

Generally, the present study has shown that all the different concentrations of the plant extract tested possess a measure of antimicrobial properties and is concentration dependent. The plant material examined in the present study have been in used in Ghana in the preparation of decoction and concoction for the treatment of typhoid fever caused by the test pathogen particularly, when modern drugs of choice failed in achieving the therapy.

This study has confirmed the antibacterial potentials of *P. amarus*, thus supporting their folklore application as a medical remedy for typhoid fever. Hence, supports the usefulness of the plant in the treatment of other ailments caused by microorganisms.

5.2 YIELD OF CRUDE EXTRACT OF *PHYLLANTHUS AMARUS*

The use of water as the sole solvent yielded the highest crude extract of 2.57g. Ethanol only also yielded the second highest crude extract of 2.52g. A fraction of water/ethanol (3:2) yielded 2.37g of crude extract. Also a fraction of water/ethanol (7:3) yielded 2.21g of crude extract. 2.14g of crude extract was recorded by a fraction of water/ethanol (9:1) while a fraction of water/ethanol (4:1) yielded 2.01g of crude extract. However, a fraction of water/ethanol (1:1) yielded the lowest crude extract of 1.05g. Comparing the use of the different fractions of water to ethanol, it was identified that there was no relation between the ratio of water to ethanol used and the yield of the crude plant extract. Factors like age of the plant and the polarity of the solvent used may affect the yield. Water and ethanol seems to be a good solvent for this plant which supports the use of water and alcohol as traditional solvent.

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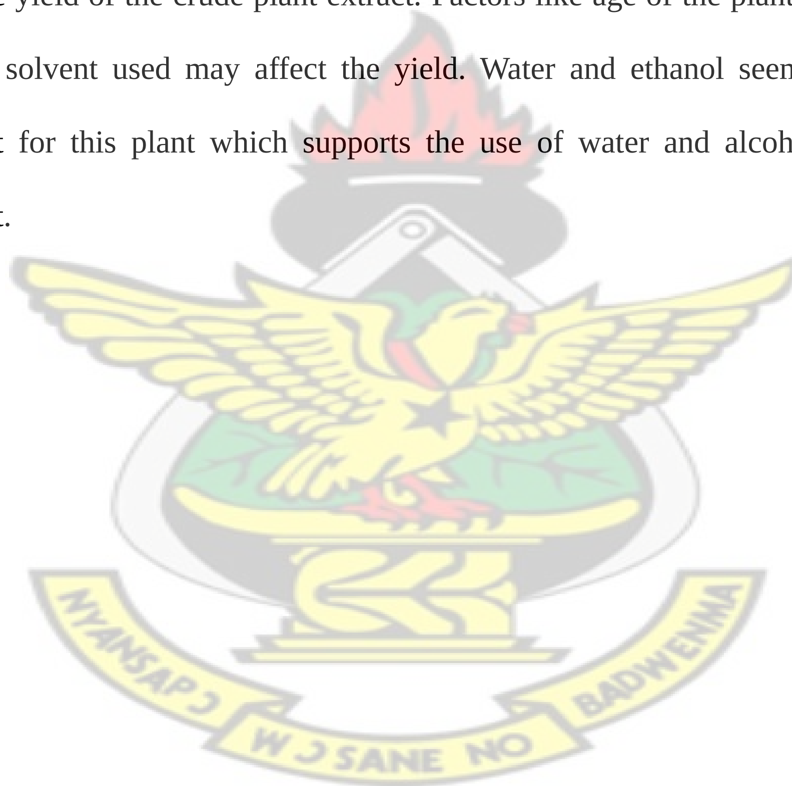

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSIONS

Generally water was the best solvent used for the crude extraction of *Phyllanthus amarus* since it gave the highest crude extract. Ethanol gave the second highest yield of crude extract of *P. amarus*. The different proportions of water to ethanol gave different yields of the crude plant which has no relation. The proportion of 1:1 of water to ethanol gave the least yield.

The water extract of fresh *P. amarus* showed the largest and significant zone of inhibition of 5.00mm and 7.17mm in 10g/200ml and 20g/200ml concentration respectively compared to the other extracts antibacterial activity on *S. typhi* investigated in the study.

The ability of the dry *P. amarus* extract showing sensitivity to *S. typhi* was observed in the water extract which also showed the largest and significant zone of inhibition of 7.33mm and 13.50mm in both 10g/200ml and 20g/200ml concentration respectively.

However, the ethanolic extracts of both the fresh and dry *P. amarus* in 10g/200ml and 20g/200ml concentration also showed significant sensitivity on the test organism. Chloramphenicol which was the control showed different sensitivity on the test organism.

The present study showed that fresh and dry extracts of *Phyllanthus amaru s*howed antimicrobial activity on *S. typhi*, hence this provides a scientific basis that reflects the idea of traditional healers for using this plant for curing of typhoid fever as well as other ailments.

In conclusion, the results of the present study support the folkloric usage of the plant and suggest that both the water and ethanolic plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of the plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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6.2 RECOMMENDATION

Based on the findings from this study, it is recommended that:

1. Further studies should be conducted to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Phyllanthus amarus a*gainst *Salmonella typhi* because of the antibacterial activity exhibited by the fresh and dry plant extracts.
2. Evaluation of the phytochemical properties and toxicological studies of *Phyllanthus amarus* must be carried out to ascertain its relative safety as a possible antimicrobial agent.
3. The extracts of *Phyllanthus amarus* should also be tested on other pathogenic microorganisms that cause serious human infections.

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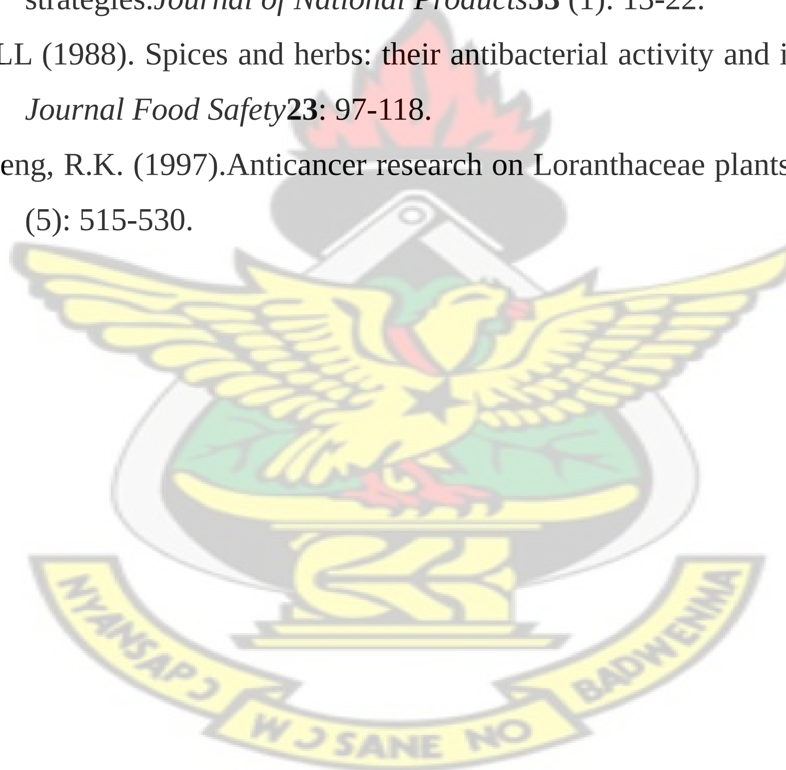

APPENDIX

Complete Randomized AOV for fresh plant sample in 10g/200ml

Source	DF	SS	MS	F	P
Treatments	3	51.1667	17.0556	45.48	0.0000
Error	8	3.0000	0.3750		
Total	11	54.1667			

Grand Mean 3.6667 CV 16.70

Complete Randomized AOV for fresh plant sample in 20g/200ml

Source	DF	SS	MS	F	P
Treatments	3	22.0000	7.33333	6.40	0.0161
Error	8	9.1667	1.14583		
Total	11	31.1667			

Grand Mean 4.3333 CV 24.70

Complete Randomized AOV for dry plant sample in 10g/200ml

Source	DF	SS	MS	F	P
Treatment	2	74.3889	37.1944	669.50	0.0000
Error	6	0.3333	0.0556		
Total	8	74.7222			

Grand Mean 5.0556 CV 4.66

Complete Randomized AOV for dry plant sample in 20g/200ml

Source	DF	SS	MS	F	P
Treatment	2	77.7222	38.8611	13.20	0.0064
Error	6	17.6667	2.9444		
Total	8	95.3889			

Grand Mean 10.111 CV 16.97