

**SCHOOL OF PUBLIC HEALTH  
COLLEGE OF HEALTH SCIENCES**



**ANTICANCER AND ANTIOXIDANT PROPERTIES OF VERNONIA  
AMYGDALINA**

**SUBMITTED BY**

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DECLARATION

I declare that this thesis which resulted from a research was done personally under supervision. This work has not been submitted in any form for a degree or diploma at any University or any tertiary institution. Authors and publishers whose works were used in the study have been acknowledged in the text and listed in the references.

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## **DEDICATION**

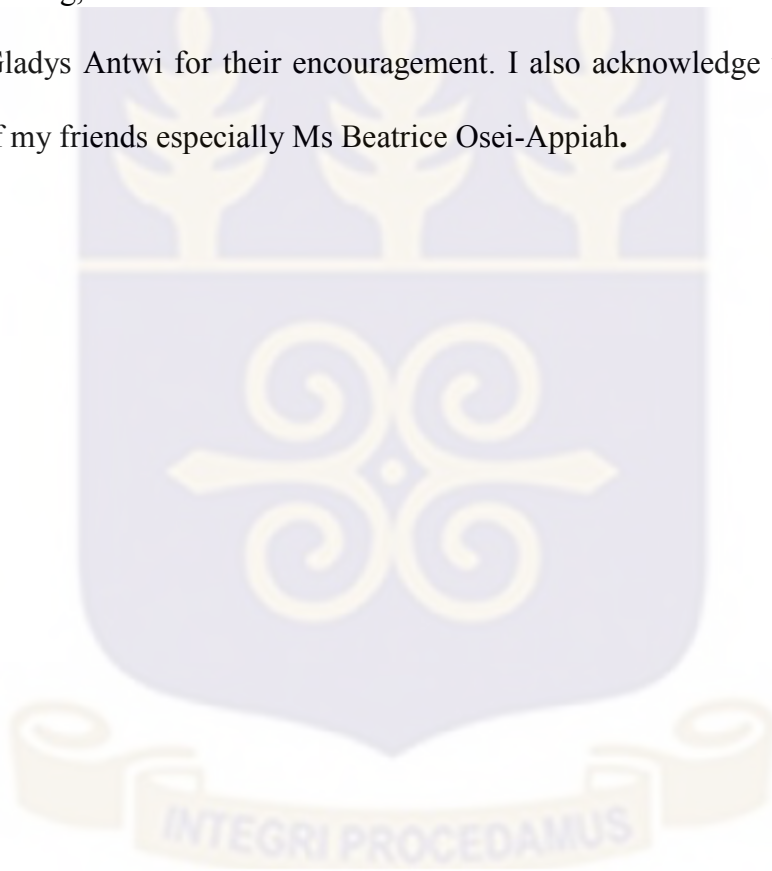
This work is dedicated to God Almighty whose faithfulness has brought me this far. Also to the Head of Clinical Pathology Department of Noguchi Memorial Institute for Medical Research, Dr (Mrs.) Regina Appiah-Opong.



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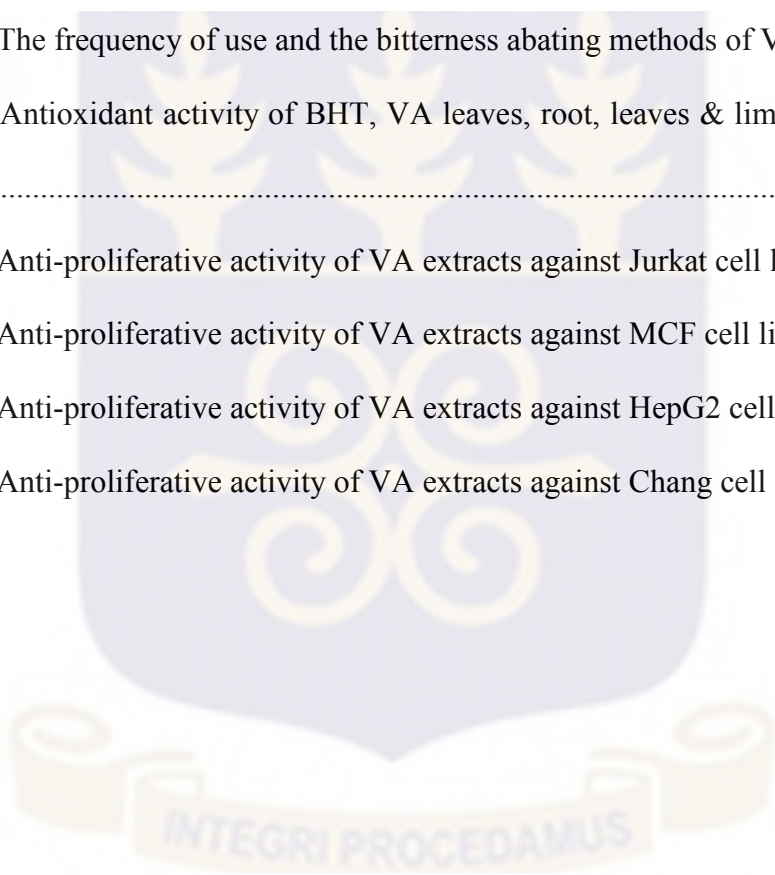
## ABBREVIATIONS

VA.....	Vernonia amygdalina
MTT.....	3,4,5-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DPPH.....	2,2, diphenyl-1-picrylhydrazyl
CAM.....	Complementary and alternative medicine
ACTs.....	Alternative cancer treatments
Jurcat.....	Leukemia cell lines
MCF-7.....	Breast cancer cell lines
HepG2.....	Liver cancer cell lines
DPPH.....	2,2 diphenyl-2-picrylhydrazyl
DMEM.....	Dubelcco's modified eagle medium
FBS.....	Fetal bovine serum
SD.....	Standard deviation
IC <sub>50</sub> .....	50% inhibitory concentration



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## ABSTRACT

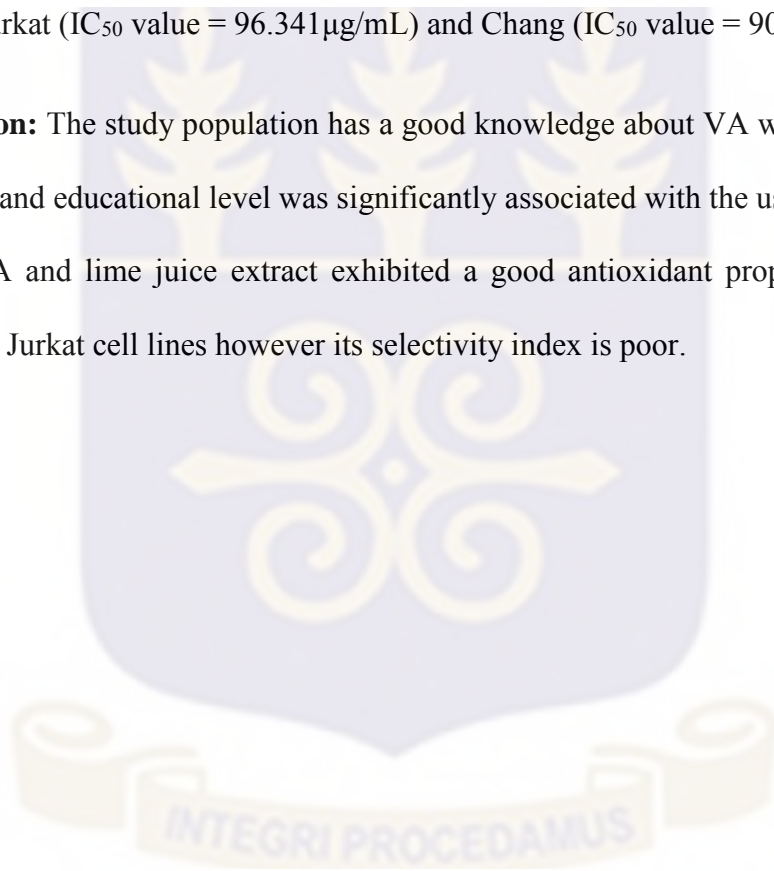
**Introduction:** Medicinal properties of plants have been investigated throughout the world due to their potent pharmacological activities, no side effects and economic viability. Although the documentation of therapeutic potential of plant material is limited, traditional medicine has been integrated into the National health delivery system in Ghana. *Vernonia amygdalina* possesses phytochemicals that exhibit anticancer properties. This led to the determination of the prevalence of use, antioxidant and anticancer properties of *Vernonia amygdalina*.

**Methods:** This study was conducted at Lekma Hospital, Dodowa District Hospital and Noguchi Memorial Institute for Medical Research (NMIMR). Data was collected by administering semi-structured questionnaires to 359 patients and Health staffs using systematic random sampling method. Antioxidant activity of VA and Cytotoxic effect of VA extracts against Jurkat, MCF-7, HepG2 and Chang liver cells were determined using 2,2, diphenyl-1-picrylhydrazyl (DPPH) and 3,4,5-(dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay respectively. Data was analysed using Microsoft excel and Stata version 13.0. The correlation between % antioxidant activity and concentration was determined using linear regression. P-values were determined by chi-square and student t-test.

**Results:** A total of 359 Patients and Health workers participated in the study with a mean age of  $39 \pm 1.33$  years, made up of 73.4% females. Seventy-six percent of the study participants accept the usefulness of traditional medicine and the prevalence of the use of VA was 53.1%. Majority of the respondents were Christians (95.5%) while the others were Islamic and Traditionalist from five ethnic groups in Ghana. The use of VA was significantly associated with ethnicity and educational level with p values of 0.008, and 0.004, respectively.

The laboratory analysis focused on the root, leaves and a mixture of the root, leaves and lime juice extracts of VA which is mostly used by the Participants in the treatment of cancer. The mixture of the leaves and the lime juice extract exhibited the best free radical scavenging activity with an  $EC_{50}$  value of  $2.14 \pm 0.06$ mg/ml. All extracts were cytotoxic towards MCF, HepG2, Jurkat and Chang cell lines in a dose dependent manner (0-1000 $\mu$ g/mL). The mixture of the leaves and the lime extract was active against all the tested cell lines with its strongest inhibition against Jurkat ( $IC_{50}$  value = 96.341 $\mu$ g/mL) and Chang ( $IC_{50}$  value = 90.97  $\mu$ g/mL).

**Conclusion:** The study population has a good knowledge about VA with high prevalence of use. Ethnicity and educational level was significantly associated with the use of VA. The combination of the VA and lime juice extract exhibited a good antioxidant property and it is effective in inhibiting Jurkat cell lines however its selectivity index is poor.



## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background

Cancer is an” abnormal cell growth, that occurs as a result of cumulative mutations in genes that regulates cell growth, cell death, and express other genes (Harlan et al., 1991) . Globally, cancer is the second-leading cause of death after cardiovascular disease, and the mortality rate around the world has increased from 12% in 1990 to 15% in 2013 (Naghavi et al., 2015). This represents 14.9 million new cancer cases and 8.2 million cancer deaths. As new cases increase, the death toll from cancer is also changing. The common ones in terms of the burden of disease worldwide are cancers of the breast, prostate, liver and leukemia (World Health Organisation, 2010).

Breast cancer caused 13.1 million Disability Adjusted Life Years (DALYs) in 2013 with 63% occurring in developing countries and 37% occurring in developed countries (GBD, 2013). According to Global Burden of Disease (GBD) estimates, one in 18 women developed breast cancer between birth and age 79. In 2013, there were 1.4 million, 792,000 and 414,000 incident cases and 293,000, 818,000 and 265,000 deaths of prostate cancer, liver cancer and leukemia, respectively. Prostate cancer caused 4.8 million DALYs globally in 2013, with 57% occurring in developed countries, and 43% occurring in developing countries. Liver cancer caused 20.9 million DALYs in 2013, with 86% occurring in developing countries and 14% occurring in developed countries (Ferlay et al., 2013). It is more common in men, with 1 in 45 men being diagnosed before age 79 years compared with 1 in 121 women. Leukemia caused 9.3 million DALYs globally, with 78% occurring in developing countries and 22% occurring

in developed countries. One in 127 men and 1 in 203 women developed leukemia between birth and age 79 years. Globally, liver cancer and leukemia ranked sixth and tenth for cancer incidence, third and ninth for cancer death in 2013, respectively (Kepenekian et al., 2016). For developed countries, both ranked eleventh and twelfth for incidence, seventh and eighth for mortality. In developing countries, they ranked fifth and tenth for incidence, second and eighth for cancer deaths (Akinde et al., 2015).

There are more than 100 types of cancers that are named after the organs or the tissue where they are formed (Zhao, et al., 2010). The type of cell that forms them such as squamous cell or epithelial cell may also be used to describe cancer. Some categories of cancers that begin in specific cell types include carcinomas, sarcomas, leukemia, Lymphomas, multiple myeloma, carcinoid tumors, neuroendocrine tumors, germ cell tumours melanoma, brain and spinal cord tumors (Welch et al., 2010). Unlike normal cells, cancer cells are not limited in the number of times they can divide. They invade nearby tissues and spread to distant parts of the body (Rogers et al., 2010). Cancer cells do not respond to signals to stop dividing but have the ability to promote the growth of blood vessels to provide nutrients for tumor growth (Schernhammer et al., 2001).

In Ghana, the commonest cause of cancer deaths are cancers of the liver, prostate, lung, breast, ovary and leukemia. The estimated cancer incidence Age Adjusted Standardized Rates for males and female in 2012 were 10.9 and 22.4 per 100, 000, respectively (Zelle et al. 2012). There are many risk factors and they vary by the type of cancer. Lung, esophageal and kidney cancers are associated with tobacco use which is responsible for 1.8 million cancer deaths per year, 60% of these occur in low and middle income Countries (Masterson et al. 2016). There are also numerous organisms, environmental and occupational carcinogens such

as hepatitis B virus, schistosoma, asbestos, dioxins, benzene, aflatoxin B<sub>1</sub>, gamma rays and alpha particle that are associated with cancer. Annually, 274,000 cancer deaths occur due to being overweight, obese or physical inactivity. “Women with diets low in fruits and vegetables may be at increased risk for cervical cancer” (Williams 2014).

The ever-increasing understanding of genes and molecular pathways disruption in cancer has yielded a cornucopia of new therapies based on drugs that targets these alterations. This has led to the integration of molecular targeted therapies into cancer patient care, alongside surgical techniques, radiation therapy, developed chemotherapy and adjuvant therapy (Sasaki et al., 2010). Globally, the development of these modern, evidence-based treatments has reduced the mortality rate of cancer patients from 90% to 34% within five years (Teicher et al., 2010).

Given the drawbacks involved in current treatment methods that compromise cancer therapy, the search for new and effective drugs to treat cancer is a major priority. According to WHO report in 2001, Malaysia spent about US\$500 million annually on traditional medicine and spends about US\$ 300 million on orthodox medicine (Zelle et al., 2012) . In working steadily towards the goal of enhancing the effectiveness of traditional medicine, even the highly literate consult to traditional medicine before visiting orthodox health institutions in Sri Lanka ((Paskins, 2001). The percentage of the population that uses traditional medicine ranges from 70% in Benin, Cote d’Ivoire, Ghana and Mali to 80% in Burkina Faso and 90% in Burundi and Ethiopia (Grigorescu et al., 2015). A study published by cancer research, in the United Kingdom shows that about 60% of cancer patients use traditional medicine to manage side effects and symptoms such as nausea, insomnia, fatigue, pain, and vomiting, and (Akinde et al., 2015). Natural products such as Paclitaxel (TAX), vinca alkaloids, vinblastine, vincristine,

etoposide and teniposide have been used as chemopreventive and chemotherapeutic agents against cancers (Vaghasiya et al., 2011).

In 2009, an evidence-based clinical practice guideline was issued by the Society for Integrative Oncology “for health care providers to consider when” to incorporate complementary health approaches in the care of cancer patients. This guideline pointed out that, CAM help to control symptoms and enhance patients’ well-being when used in addition to conventional therapies (WHO 2007). The guidelines warn, however, that unapproved methods should not be used in place of conventional treatment because delayed treatment of cancer reduces the likelihood of a remission or cure.

Cancer is a major public health concern and it represents non-communicable diseases responsible for 63% of deaths worldwide (Choi et al., 2010). According to a survey by the National Center for Complementary and Integrative Health on complementary and alternative medicine (CAM), CAM increases the quality of life of cancer patients. The use of CAM is a chemopreventive approach based on plant products, herbs, vegetables, and spices used in folk and traditional medicine (WHO, 2001). Unfortunately, to date, CAM has not been totally accepted by physicians and other caregivers (Schiavone et al., 2008).

In recent scientific developments, medicinal properties of plants have been investigated throughout the world due to their potent pharmacological activities, limited side effects and economic viability (Udochukwu et al., 2015). Although scientific investigation and information of the therapeutic potential of plant material is limited, traditional medicine has been integrated into the National health delivery system (Kepp et al., 2012). The National strategy for cancer control for 2012-2016 drew a national cancer plan, an excellent tool to

combat cancer in Ghana. Hence, the Ministry of Health invited its partners and all stakeholders including the local medicine practitioners to cooperate in order to achieve a common purpose (Zhao et al., 2010). This calls for the evaluation of CAM therapies with long and careful research process to identify treatment, which is less expensive, causes fewer side effects and increases the rate of survival.

### **1.2 Problem Statement**

In recent studies, statistics have showed that attendants reporting to some Ghanaian hospitals come with their sicknesses in its deteriorating state (Kretchy et al., 2014b). The subjective data collected from these patients indicated that patients resort to traditional medicine for the treatment of their sickness in the early stages and seek conventional treatment in the hospital if their sicknesses are not cured (Kar, 2003). Unfortunately, many alternative cancer treatments are unapproved, some are considered dangerous and interfere with cancer treatments by rendering cancer drugs less effective. Literature has shown that several herbs exhibit anticancer activities but the scientific evidence is limited, and many clinical trials have not been well-designed. Use of herbs for managing symptoms also raises concerns about potential negative interactions with conventional cancer treatments including tissue damage (Vaghasiya et al., 2011). Herbal supplements may be harmful when taken by themselves, with other substances, or in large doses (WHO, 2005). Some CAM therapies have been evaluated and have been found to be safe and effective (Ventola, 2010). However, others have been found to be ineffective or possibly harmful (Vattem et al., 2005) (Tabish, 2008). Less is known about many CAM therapies, and research has been slower due to regulatory and funding issues (Vermerris et al., 2006). Alternative cancer treatments (ACTs) are usually

promoted as replacements to conventional cancer treatments. Some of the most popular alternative cancer treatments comprise dietary therapies, antioxidants, high dose vitamins and herbal therapies. Among the dietary and herbal therapies in some part of West Africa especially in Nigeria, *Vernonia amygdalina* is one of the plants known to have exhibited anticancer properties (Udochukwu et al., 2015). Significant proportion of studies on *Vernonia amygdalina* has been haphazard and peripheral. Research is needed to elucidate the specific mechanism of actions systematically, in order to provide crucial insights about the treatment of cancer (Chanda et al., 2013). It is important to take into account the different cancer subtypes and recognize the differential responses when screening for cancer therapies (Schernhammer et al., 2001).

### **1.3 Justification**

According to previous work done in Nigeria, *Vernonia amygdalina* contains flavonoids that possess anticancer and antioxidant properties (Gadow et al., 1997). The leaves may be consumed either as a vegetable (macerated leaves in soups) or as aqueous extracts in addition to lime juice as tonics for the treatment of various illnesses including cancers (Nwauzoma et al., 2013). Modern, evidence-based treatments have reduced the mortality rate of cancer patients yet there is uncertainty about the effectiveness and the cost of cancer treatment accompanied with severe toxicity, multidrug resistance together with side effects (Walker et al., 2009). The conventional forms of cancer treatment prolong life or cure cancer permanently but have side effects ranging from unpleasant to fatal such as infection, fatigue, stroke, thrombosis, slow heart rate, seizure, increased blood pressure, nausea, emesis and

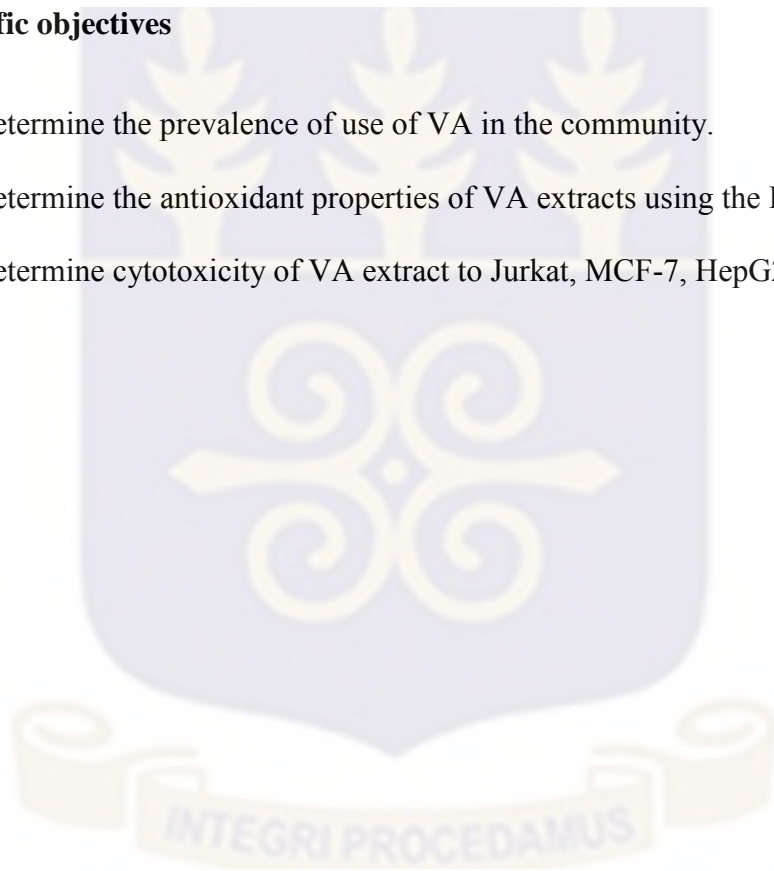
severe pain (Vergote et al., 2010). However, these uncertainties have made CAM appealing to many patients with cancer.

#### **1.4 General Objective**

The general objective of this study is to determine the prevalence of use, the antioxidant activity and anti-cancer properties of VA extract.

#### **1.5 Specific objectives**

1. Determine the prevalence of use of VA in the community.
2. Determine the antioxidant properties of VA extracts using the DPPH assay.
3. Determine cytotoxicity of VA extract to Jurkat, MCF-7, HepG2 and Chang liver cells.



## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 *Vernonia amygdalina* and Cancer

There has been growing interest in *Vernonia amygdalina* (VA), as a source for anti-cancer drugs due to its diverse medicinal uses in traditional folk medicines in Kenya, Nigeria, Malaysia and South Africa (Mishra et al., 2011). *Vernonia amygdalina* possess diverse therapeutic effects such as anti-malarial, anti-microbial, anti-fungal, anti-plasmodial, anti-diabetic and anti-cancer effects (Ibrahim, 2001). The anti-cancer effect of VA was first shown in the treatment of human carcinoma of nasopharynx and later in leukemia cells P-388 and L-1210 using the chloroform extract of VA (Khalafalla et al., 2009). Different extracts of VA have thus been used in scientific research to reveal the therapeutic properties of this plant. Current research conducted on the anti-cancer effect of VA has focused exclusively on human breast tumour cells (MCF-7) (Mishra et al., 2011). Moreover, there are subtypes of breast cancer cells, which are known to be the most aggressive with poor prognosis. The assessment of cytotoxic effect of *Vernonia amygdalina* has showed that the leaf extracts are cytotoxic to cultured human breast tumour cells (MCF-7) at higher doses (0, 0.5 and 2mg/ml) of exposure (Farombi et al., 2011). The leaves that serve as vegetables for indigenes of most West Africa Countries may be relatively toxic when consumed in large quantities. To extrapolate, an average body weight of 70kg requires consumption of 28-56g of *Vernonia amygdalina* for treatment. Low concentrations of water-soluble leaf extract is known to have anti-proliferative activity on estrogen receptor positive human breast cancer (MCF-7) cells *in vitro* in dose dependent manner. Phases I and 2-gene expression in MCF-7 cells are modulated by VA in dose and time dependent fashion (Ong et al., 2011).

## 2.2 *Vernonia amygdalina*

*Vernonia amygdalina* is a small shrub that belongs to the kingdom Plantae. It is an angiosperm, of the order Asterales, of the family Asteraceae, genus *Vernonia*, and species VA. It grows in tropical Africa with petiolate leaf of about 6 mm diameter and elliptic shape (Yeap et al., 2010). It is usually known as “bitter leaf” because of its bitter taste. The bitterness can be abated by boiling or by soaking the leaves in several changes of water (Ibrahim et al., 2001). In Ghana, the bitterness is sometimes abated by the addition of lime juice to the VA extract. The bitter taste is due to bioactive constituents such as alkaloids, saponins, tannins, and glycosides (Nwanjo 2005). In Nigeria, it is called “Onugbu” in Igbo language, “Ewuro” in Yoruba language, “Oriwo” in Bini language, and “Chusar doki or fatefate” in Hausa (Erasto et al., 2006). In Ghana, it is known as ‘egbo’ in Ewe language. The leaves are used as green leafy vegetable and may be consumed either as a vegetable or aqueous extracts used as tonics for the treatment of various illnesses (Gyang et al., 2004). Many herbalists and native doctors in Africa recommend the use of its aqueous extracts to patients as treatment for various ailments including diabetes, loss of appetite, emesis, nausea, dysentery, other gastrointestinal tract problems and sexually transmitted diseases. In the wild, chimpanzees have been observed to ingest the leaves when suffering from parasitic infections (Abosi et al., 2003).

*Vernonia amygdalina* is used in traditional medicine practice as therapeutic agents and it plays a vital role in the health of individuals and the communities (Ojiako et al., 2006). Its medicinal value lies in phytochemicals that produce definite physiological actions in the human body. These plant chemicals comprise alkaloids, tannins, flavonoids and phenolic compounds. Every part of *Vernonia amygdalina* contains complex active components such as

anthraquinones, steroids and cardiac glycosides that are useful pharmacologically (Georgewill et al., 2010). The leaves are green with a characteristic odor and bitter taste. In ethno medicine, the roots and the leaves can be used as antibacterial, active cancer, anti-parasitic anti-malarial agent and are used to treat fever, hiccups, kidney problems, vomiting, intestinal illness and stomach discomfort (Fatima et al., 2010). The root and the stems are used as chewing stick in many West African Countries such as Cameroon, Ghana and Nigeria. Previous studies have shown that *Vernonia amygdalina* exhibit hypoglycaemic and hypolipidaemic activity in experimental animals (Ebong et al., 2011).

### **2.2.1 Knowledge and acceptability of *Vernonia amygdalina***

The acceptance and use of *Vernonia amygdalina* is on the increase and it forms an important part of healthcare in Nigeria (Nwauzoma et al., 2013). Although no clinical trials have been conducted on the safety and efficacy of *Vernonia amygdalina*, the noted increased use of the plant is as a result of the anecdotal therapeutic evidence of the herbal remedies (Chikezie et al., 2015). According to World Health Organization, about 60% of the world's people use herbal medicine for treating their sicknesses (Njan 2004). In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicine at home (Orimadegun et al., 2015). A previous survey has shown several plants such as *Carica papaya*, *Moringa oleifera*, *Azadirachta indica*, *Chromolaena odorata*, *Mangifera indica*, *Anana comosus*, *Acacia nilotica* including *Vernonia amygdalina* as the commonest medicinal plants used in Ghana for the treatment of malaria (Abuaku et al., 2004). In spite of poor knowledge on most of the plants, the Study Participants pre-dominantly exhibited interest in herbal medicine and high level of personal use.

The dependence of people on indigenous herbal medicine are influenced by factors such as acceptability, less communication means, poverty, cost-effectiveness, ignorance, accessibility unavailability of modern health facilities and the belief that natural products pose no risk (Mohamed et al., 2017). The rich history of African cultures and their innovative utilisation of plants as sources of remedies have been passed down through generations largely by oral tradition ( Oshikoya et al., 2008). This knowledge is gradually being lost as the custodians often die before passing on information to the younger generations. Beside the gradual loss of ethnobotanical knowledge due to lack of documentation, overharvesting of medicinal materials from their natural habitat has been one of the major threats to traditional medicine (Reyes-García et al., 2013). In order to conserve wild plant species, there is need for reliable data on their distribution and level of use (Meragiaw et al., 2016). The identification of local names and indigenous uses of plants not only preserves indigenous knowledge but also facilitates future research on safety and efficacy of medicinal plants in treatment of various ailments (Vandebroek et al., 2012).

### **2.3 Classification of Phytochemicals**

Phytochemicals are biologically active chemical compounds that occur naturally. They are found in plants and provide health benefits for human and animals when their dietary intake is significant (Nwanjo, 2005). Over 4000 phytochemicals are classified as primary and secondary constituents depending on their role in plant metabolism, protective function, physical characteristics and chemical characteristics (Asase et al., 2010). The primary constituents are amino acids, chlorophyll, common purines, pyrimidines and sugars of nucleic acids. The secondary constituents are alkaloids, curcumines, saponins, glucosides, terpenes, flavonoids, lignans, phenolics, and plant steroids (Kretchy et al., 2014). Phytochemicals

accumulate in the fruits, seeds, roots, stems, leaves or flowers of the plant depending upon the stage of growing and processing (Gyang et al., 2004).

The dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices. The common sources are garden eggs, okro, carrots, cabbage, bread, onions, garlic, whole wheat, grapes tomatoes, grapes, cherries, strawberries, raspberries, beans, soy foods, legumes and VA. (Moshi et al., 2010). Previous works have shown that the most numerous and structurally diverse plants constituents are the phenolic (Simbo, 2010).

#### **2.4 Phenolic**

The most widely distributed and the largest categories of phytochemicals in plant kingdom are the phenolics. They are hydroxyl group (-OH) containing aromatic hydrocarbon group. The flavonoids, phenolic acids and polyphenols are the three most important dietary phenolic components (Ong et al., 2011). The largest group of Phenols are flavonoids. The Phenolic acids form a diverse group that includes hydroxybenzoic and hydroxycinnamic acids. Phenolic polymers such as tannins are high molecular weight compounds that are grouped as condensed and hydrolysable proanthocyanidins or tannins. The phenolics exhibit a wide range of antioxidant properties that defines acts as protective agents against free radical-mediated disease processes (Fatima et al., 2010).

Phenolic Acids possess one carboxylic acid functional group with hydroxylated aromatic rings. Phenolic acids are biologically available through an indirect deriving by gastric, hepatic, intestinal metabolism and direct intake through food consumption.(Ganga et al., 2011). Naturally, occurring phenolic acids contain hydroxycinnamic and hydroxybenzoic structures. Hydroxycinnamic compounds are simple esters with hydroxy carboxylic acids or

glucose. Phenolic acids are characterized by diverse biological activities including antioxidant, anti-ulcer, anti-inflammatory, anti-tumour, anti-spasmodic, anti-depressant and cytotoxic activities (Natalia et al., 2011). These compounds protect against oxidative damage leading to various degenerative diseases including cardiovascular diseases, inflammation and cancer, which are sensitive to oxidative stress due to their higher levels of reactive oxygen species (Kurmukov, 2013).

## **2.5 Flavonoids**

Flavonoids are polyphenolic compounds that occur as aglycones, glucosides and methylated derivatives among vascular plants. They are classified into two as flavone and isoflavone by the position of the benzenoid substituent. More than 4000 flavonoids occur in fruits, vegetables and beverages like coffee, fruit drinks and tea (Lee et al., 2005). Currently, 650 flavones and 1030 flavonols are known and small amounts of flavonoids without attached sugar (aglycones) which represent a considerably important proportion of the total flavonoids structure in the plant. The naturally occurring flavonoids are associated with sugar in conjugated form and may be identified as monoglycosidic, dyglycosidic and polyglycosidic (Mercier et al., 1993). Several studies have reported that flavonoids exert multiple biological properties including anti-inflammatory, antimicrobial, anti-allergic, vascular, cytotoxicity, antitumour, enzyme inhibition, oestrogenic, and powerful antioxidant activities that protect the human body from oxidative stress (Donovan et al., 1998). The antioxidant and the free radical scavenging activities of flavonoids is determined by the position of their hydroxyl groups and other features of their chemical structure like catechins and luteolin are better antioxidants than the nutrient antioxidants; vitamin C, vitamin E and  $\beta$ -carotene (Ritter et al., 1992).

## 2.6 Antioxidants

Antioxidants are nutrients found naturally in both human and plant that may prevent or delay some types of cell damage by neutralizing free radicals. The body produces antioxidants called endogenous antioxidants (Morandi, 1996). However, the body relies on external (exogenous) sources such as grains, fruits and vegetables. The exogenous antioxidants are commonly known as dietary antioxidant and they are available as dietary supplements. Examples of exogenous antioxidants include beta-carotene, lutein lycopene, selenium, vitamin A, vitamin C and vitamin E (Blum et al., 1999). Endogenous antioxidants include enzyme systems like glutathione peroxidases, superoxide dismutases, and alpha-tocopherol.

Free radicals are atoms or molecules that have unpaired electrons, usually unstable, highly reactive and have the potential to harm cells. The two forms of free radicals in biological systems are; reactive nitrogen species (RNS) and reactive oxygen species (ROS) and are obtained from alcohol, cigarette smoke, ionizing radiation and air pollutant among others (Gutfinger, 1981). They can initiate lipid peroxidation, cause DNA strand breaks and indiscriminately oxidize virtually all molecules in biological membranes and tissues, resulting in injury. Free radicals are formed naturally in the body and play an important role in many normal cellular processes (von Gadow et al., 1997). At high concentrations, however, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and other health conditions (Leedj r v et al., 2006). The most frequently targeted organ in terms of drug toxicity is the liver. In an early event of drug hepatotoxicity, the production of radical species, specifically ROS and RNS is an indicator of hepatotoxic potential (Meyer et al., 1997).

It has been discovered that anticancer drugs induce oxidative stress including increase of cellular oxidants, lipid peroxidation and depletion of antioxidants in the liver. The use of natural and synthetic antioxidants as an anti-oxidative therapy represents a reasonable therapeutic approach for the prevention and treatment of “diseases such as cancer due to the role of oxidative stress in contributing to initiation and progression of cellular damage (Wolfe et al., 2007)”.

## **2.7 Alkaloids**

Alkaloids are organic nitrogen-containing bases that occur naturally. They exhibit diverse and important physiological effects on humans and other animals. The name alkaloid was originally applied because they react with acids to form salts. In their pure state, most alkaloids are colourless, nonvolatile, crystalline solids and they tend to have a bitter taste (Del Signore et al., 1997). They are found primarily in plants and more than 3,000 different types of alkaloids have been identified in 4, 000 plant species. A few alkaloids have been found in animal species such as the new world beaver (castor Canadensis) and poison-dart frogs (Phyllobates). Ergots and few other fungi also produce them (Walker et al., 2009). Alkaloids are often classified based on their chemical structure. This dual classification system is important because there is a rough correlation between the chemical types of alkaloids and their biological distribution.

Well known alkaloids such as morphine, strychnine, quinine, ephedrine, and nicotine exhibit medicinal properties. Ergonovine and ephedrine act as blood-vessel constrictors ( Klabunde et al., 2011). Ergonovine is used to reduce uterine hemorrhage after childbirth, and ephedrine relieves the discomfort of common colds, sinusitis, hay fever, and bronchial asthma (Babu et al., 2008). Quinine (from Cinchona species) is a powerful anti-malarial agent that was

formerly the drug of choice for treating that disease, though it has been largely replaced by less toxic and more effective drugs (Pelletier et al., 2011.). The alkaloid tubocurarine is the active ingredient in the South American arrow poison, curare (obtained from *Chondrodendron tomentosum*), and is used as a muscle relaxant in surgery (Ballantyne et al., 1997). Two alkaloids, vincristine and vinblastine from *Vinca rosea*, are widely used as chemotherapeutic agents in the treatment of many types of cancer (Lucas et al., 2010).

## **2.8 Tannins**

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Barros et al., 2009). Therefore, foods rich in tannins are considered to be of low nutritional value. However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances (Bhattacharya et al., 2010). Incidences of certain cancers, such as esophageal cancer, have been reported to be related to consumption of tannins-rich foods such as betel nuts and herbal teas, suggesting that tannins might be carcinogenic (Hyršl et al., 2007). However, other reports indicated that the carcinogenic activity of tannins might be related to components associated with tannins rather than tannins themselves (Chung et al., 1998). Interestingly, many reports indicated negative association between tea consumption and incidences of cancers (Zheng et al., 1996)(Groessl et al., 2016). Tea polyphenols and many tannin components were suggested to be anti-carcinogenic (Uyama et al., 2001). Many tannin molecules have also been shown to reduce

the mutagenic activity of a number of mutagens (Gülçin et al., 2010). Many carcinogens and/or mutagens produce oxygen-free radicals for interaction with cellular macromolecules (Fukuhara, et al.,1981). The anti-carcinogenic and anti-mutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation (Cilliers et al., 1989).

## **2.9 Saponins**

Saponins are phytochemicals, which can be found in most herbs, beans and vegetables. Saponins also known as sapotoxin, are toxic and have a bitter taste (Smejkal et al., 2007). The best-known sources of saponins are peas, soybeans, and some herbs with names indicating foaming properties such as soapwort, soapbark, taproot, and soapberry. Commercial saponins are extracted mainly from *Quillaja saponaria* and *Yucca schidigera*. Saponins have many health benefits that includes their beneficial effects on bone health, blood cholesterol levels, cancer, and stimulation of the immune system (Han et al., 2007). The effect of saponins from specific plant sources have been scientifically investigated but the results cannot be applied to other saponins (Talcott et al., 1999). Studies have shown that saponins have anti-mutagenic and antitumor activities and can lower the risk of human cancers, by preventing cancer cells from growing (Liu et al., 2011) (Jun et al., 2002). Saponins seem to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Bhattacharya et al., 2010). Some studies have shown that saponins can cause apoptosis of leukemia cells by inducing mitotic arrest ( Sindambiwe et al., 1998). The direct antioxidant activity of the non-sugar part of saponins may results in other benefits such as reduced risk of heart diseases and cancer (Joseph. et al., 2003). Saponins from *Yucca* and *Quillaja* are used in some beverages,

such as beer, to produce stable foam. The detergent properties of saponins have led to their use in shampoos, facial cleansers and cosmetic creams (He et al., 2009).



## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Study site**

The study site comprises two health facilities; Dodowa District and Lekma Hospital. Dodowa District hospital is located in the heart of the Shai Osudoku district and was established in 1985. Attendance at the hospital increased from 13,080 to over 42,000 between 2009 and 2012. From this estimation, it was extrapolated that hospital attendance will increase further by 50% in 2015 (Blay, 2016). As one of the largest hospitals in Greater Accra, it is designed to be capable of expansion from 120 beds to 200 beds.

Lekma hospital is a Municipal hospital for Ledzokuku-Krowor Municipality. It is a Government of Ghana facility built by the Chinese Government in 2010. It is a hundred bed capacity hospital that has all the units of a general hospital including Herbal Medicine Unit (Sagoe, 2017).

#### **3.2 Study population**

Dodowa District, Lekma hospital and Backyard garden in Afiencya, Accra were the source population of the study. The study population included cancer cell lines, patients from Out Patient Department (OPD) and health workers aged greater than 18 years.

#### **3.3 Plant collection**

Fresh leaves, roots and lime of good quality were obtained from a backyard garden at Afiencya, Accra where VA was accessible.

### **3.4 Sample size calculation**

Sample size was calculated based on the 70% prevalence of TM users in Ghana (Abdullah, 2011). The value of Z (1.96) derived from the standard normal distribution corresponding to desired confidence level of 95%, margin of error ( $e= 5\%$ ). Using the formula for estimation of single population proportions;  $n = (Z^2 \times P(1 - P))/e^2$  and adding a non-response rate of 10%, the final sample size became 355 patients from OPD and Health workers.

### **3.5 Sampling technique**

A systematic random sampling technique was used to select patients from OPD and health workers. The first participant was selected from a list of initial five individuals by random selection. Then every third person was selected and interviewed using a semi-structured questionnaire (refer to Appendix).

#### **3.5.1 Data Collection Procedure**

Data were collected using semi- structured interviewer administered questionnaire adapted from national studies such as published articles in peer-reviewed journals. The Principal Investigator conducted the face-to-face interview.

#### **3.5.2 Study Variables**

The outcome variables of the fieldwork were knowledge and use of *Vernonia amygdalina*. The outcome variables of the laboratory aspect of the study were the free radical scavenging and the anti-proliferative activity of *Vernonia amygdalina*.

### **3.5.3 Data Management and Statistical Analysis**

Data were checked for completeness and consistency using Microsoft Excel. The data were entered into Stata version 13.0, cleaned, and analyzed. The results were presented using simple frequencies with proportions in appropriate tables to display the descriptive part. The data from biological assays were subjected to regression analysis to calculate the dose-response relation between the extracts. Linear regression analysis was performed to find out the correlation between mean percent antioxidant activity and the various concentration of *Vernonia amygdalina*. The procedures were presented as mean  $\pm$  standard deviation (n=3) and  $p < 0.05$  were considered significant using the Student's t-test.

### **3.5.4 Ethical Consideration**

A certificate of approval was obtained from the Ethical Review Board of Noguchi Memorial Institute for Medical Research (NMIMR) and Ghana Health Service (GHS) with the approval numbers; 070/16-17 and GHS/RDD/ERC/admin/App/17/335 respectively. Each participant of the study agreed to participate voluntarily after reading the consent form. Participants were allowed to discontinue the interview when they needed. All participants of the study declared their willingness to participate and approved by appending their signatures.

## **3.6 Materials**

### **3.6.1 Cell lines, Chemicals and Reagents**

The cell lines used were obtained from NMIMR, Department of Clinical Pathology. Dimethyl Sulfoxide (DMSO), 2,2, diphenyl-1-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye, trypan blue solution, absolute ethanol, HCl,

methanol, isopropanol, phosphate buffer saline, 96 well plates, antibiotics (penicillin, streptomycin and L-glutamine), Foetal Bovine Serum (FBS), culture media Dulbecco's modified eagle medium and RPMI "were obtained from Sigma-Aldrich Company (St. Louis, MO, USA)".

### **3.6.2 Plant materials**

The roots and the leaves of VA were collected from backyard garden at Afienya, Accra. A Botanist at the Botany Department of the University of Ghana, Legon, authenticated the selected plant samples.

### **3.6.3 Extraction of plant materials**

The roots and the leaves were separated into two and weighed into two batches of 50g each. Each of the plant material in the first batch was blended and extracted with 500ml of distilled water by cold maceration. The root and the leaves in the second batch were similarly extracted with 495ml of distilled water and 5ml of lime juice for 5 minutes. Extracts were filtered, frozen and freeze-dried. Solubility test was performed by dissolving 20mg of the powdered extract in 0.5ml absolute DMSO, ethanol and methanol for 30 min. The DMSO produced the best yield.

### **3.6.4 Cell Culture and Treatment**

Liver cancer cells (HepG2), Leukemia cells (Jukart), breast cancer cells (MCF-7) and normal human liver cell (Chang liver cell) lines were obtained from the Department of Clinical

Pathology, Noguchi Memorial Institute for Medical Research. The MCF and HepG2 cells were maintained in Dulbecco's modified eagle medium (DMEM) whilst Chang and Jurkat were maintained in RPMI. Both media were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/ L-glutamine. The cells were maintained in a humidified incubator, with 5% CO<sub>2</sub> concentration at 37°C and sub-cultured on reaching about 90% confluence.

### **3.6.5 *In vitro* cytotoxicity assay**

The DMSO extracts from the selected plant samples were evaluated for their cytotoxicity against leukemia, breast cancer, liver cancer and normal liver human cell lines. The cytotoxicity assay was performed according to the modified MTT (3,4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. RPMI and DMEM culture media were supplemented with 10% foetal bovine serum (FBS) containing penicillin, streptomycin and L-glutamine and maintained in culture at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. The tetrazolium-based colorimetric assay (MTT) was used to determine the cytotoxicity of VA extracts on the cancer and normal cell lines (Ayisi et al., 2011). Cells were seeded into the 96-well plates at the concentration of  $1 \times 10^5$  cells/well, treated with varying concentrations of the plant extracts (0-1000 µg/mL) and incubated for 72 h. A color control plate was also setup for each extract including the positive control, curcumin. The MTT solution (0.5 mg/mL in PBS) was added to each well on the plate, and incubation continued for further 4 h. The reaction was stopped with acidified isopropanol solution, and the plate incubated in the darkness overnight at room temperature (26°C) before reading the absorbance at 570 nm using a microplate reader (Tecan Infinite M200 Pro, Austria). The percentage cell viability was determined as follows.

% Cell Viability = Mean absorbance [(treated cells-blank/ (untreated cells -blank)] × 100

The inhibition concentration at 50% (IC<sub>50</sub>) values, that is, concentration of test substance that caused 50% inhibition of various cell lines were determined from the plot of percent cell viability on the y-axis against extract concentrations on the x-axis.

### **3.6.6 *In vitro* free radical scavenging activity-DPPH assay**

The antioxidant activities of VA leaf and root extracts was determined using the free radical scavenging activity by DPPH method ((Appiah-Opong et al., 2016)). Methanolic solution of DPPH (0.5 mM) was added to equal volumes of various concentrations of each extract (concentration range 0-1 mg/mL). After 20 mins incubation at room temperature, the absorbance was read at a wavelength of 517 nm (Tecan Infinite M200 Pro plate reader, Austria). The IC<sub>50</sub> value of each extract was calculated from the following formula.

% Antioxidant activity = [(A<sub>0</sub>-A<sub>1</sub>)/A<sub>0</sub>] × 100

Where A<sub>0</sub> is the absorbance of negative control (methanol/DMSO) and A<sub>1</sub> is the absorbance of test sample with DPPH. Butylated hydroxytoluene (BHT) was used as standard control. Triplicate experiments were performed. The EC<sub>50</sub> value, which is the concentration of the extracts that can cause 50% free radical scavenging activity, was determined.

## CHAPTER FOUR

### 4.0 RESULTS

#### Demographic characteristics (Table 1)

Most of the respondents were females 73.4% (259/353) with 44.6% (158/354) mean age of  $39 \pm 1.33$  years. These respondents were Akans 43.2% (148/343), Ewes 24.5% (84/343), Ga Adangme 23.9% (82/343), Guans 2.3% (8/343), Frafra 5.8% (20/343) and Pete 0.3% (1/343) a tribe in La Cote d'Ivoire. A total of 356 respondents were Christians, 95.5% (340/356), Islam 3.9% (14/356) and Traditional religion 0.6% (2/356). The educational level of 343 participants comprises tertiary education 36.4%, Junior High School (JHS) 32.9%, Senior High School (SHS) 16.6%, primary 10.5% and vocational/technical 3.5%. Out of 327 respondents, 21.7% are students, 72.8% are workers consisting of 30.0% artisans, 21.7% traders, 16.5% office workers and 4.6% health workers.

**Table 1: Distribution of Demographic Characteristics**

	Number (N)	Percentage(%)
<b>Sex</b>		
Male	94	26.6
Female	259	73.4
<b>Ethnicity</b>		
Akan	147	43.2
Ewe	84	24.7
Ga Adangme	80	23.5
Guan	8	2.4
Frafra	20	5.9
Pete	1	0.3
<b>Religion</b>		
Christianity	337	95.5
Islamic	14	3.96
Traditionalist	2	0.6

**Table 2: Distribution of Demographic Characteristics**

	Number (N)	Percentage(%)
<b>Educ. Level</b>		
Primary	36	10.6
JHS	112	32.9
SHS	56	16.5
Voc/Technical	12	3.5
Tertiary	124	36.5
<b>Occupation</b>		
Health worker	14	4.3
Trader	71	21.9
Artisan	97	29.9
Office worker	53	16.4
Student	71	21.9
Unemployed	18	5.6

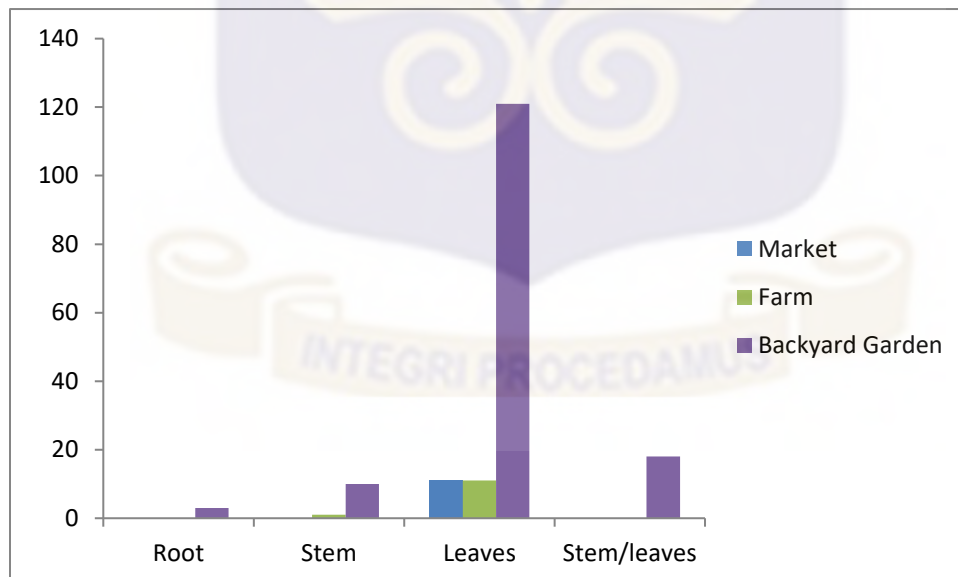
**Knowledge and use of *Vernonia amygdalina* (Table 2)**

In the study sites, 307 (98.7%) of the respondents, have heard about *Vernonia amygdalina*. Fifty four percent (168/311) of these respondents have heard from relatives, 34.0% (106/311) have heard from friends and 8.7% (27/311) from media whilst others who have heard about the plant from Doctors and herbalist constitute 2.89%. Out of 184 (51.3%) respondents who uses *Vernonia amygdalina*, 78.8% (145/184) uses the leaves, 10.9% (20/184) uses both leaves and stem and others (10.3) uses either the root or the stem of the plant. *Vernonia amygdalina* is obtained from the shop 1.13% (2/177) or the market 6.2% (11/177) but usually from the farm 6.8% (12/177) and backyard gardens 85.9% (152/177). Three percent (5/175) of the people use it raw by washing the leaves with saline, 72% (126/175) crashes or blends with water and filter before drinking. Fourteen percent (25/175) boil the leaves and 6.9% (12/175) also chew the stems after washing. Majority (92.0%) of the people use *Vernonia amygdalina*

leaves as vegetable due to its nutritional benefit, others use it as vegetable because of its ability to prevent and treat diseases.

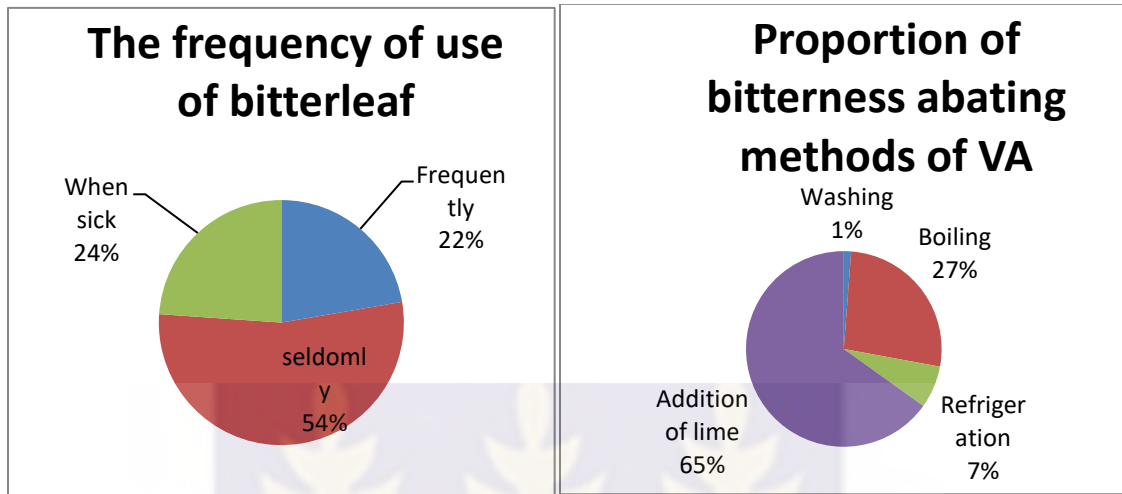
**Table 3: Names of bitterleaf by ethnic groups in Ghana**

<b>Ethnicity</b>	<b>Names of bitterleaf</b>	<b>N(%)</b>
Akan	Awonyono Aborwin Anyanule Asla ba	148(43.2)
Ewe	Egborti	84(25)
Ga Adangme	Ayigbetatso Agbatso Kintsoba	82(24)
Guan	Otsande	8(2.3)
Frafra	Shuwaaka	20(5.8)



**Figure 1: The distribution of parts and source of bitterleaf use**

The study participants commonly used leaves and stem of bitterleaf and they are usually obtained from the farm and the backyard garden.



**Figure 2: The frequency of use and the bitterness abating methods of VA**

Fifty four percent of the respondents use the plant seldom, 22% use it frequently as the rest use it when they are sick.

Few of the users (24.8%) abate the bitterness of the leaves by washing in several changes of water, boiling, refrigeration and addition of lime juice that is commonly used by 64.8% (57/88) of the people.

**Table 4: Demographic variables with the use of *Vernonia Amygdalina***

	Ever use VA N(%)	Never use VA N(%)	P Value
<b>Sex</b>			0.841
Male	48(50.1)	47 (49.5)	
Female	135(51.7)	126 (48.)	
<b>Age</b>			0.454
18-30	79(22.3)	79(22.3)	
31-40	43(12.1)	41(11.6)	
41-50	21(5.9)	26(7.3)	
51-60	20(5.6)	12(3.4)	
60+	20(5.6)	13(3.7)	
<b>Ethnicity</b>			0.008
Akan	73(21.3)	75(21.9)	
Ewe	30(8.7)	54(15.7)	
Ga Adangme	53(15.5)	29(8.4)	
Guan	4(1.1)	4(1.10)	
Frafra	12(3.5)	8(2.3)	
<b>Nationality</b>			0.093
Ghanaian	173(49.3)	170(48)	
Togolese	2(0.6)	0	
Nigerian	3(0.8)	0	
<b>Religion</b>			0.241
Christian	173(48.6)	167(46.9)	
Islam	9(2.5)	5(1.4)	
Traditional	2(0.56)	0	
<b>Educ level</b>			0.035

Primary	14(4.1)	22(6.4)
JHS	72(21)	41(12)
SHS	27(7.9)	30(8.7)
Voc/Tech	6(1.8)	6(1.8)
Tertiary	59(17.2)	66(19.2)
<b>Occupation</b>		0.692
Health worker	7(2.1)	8(2.4)
Trader	41(12.5)	30(9.2)
Office Worker	53(16.2)	45(13.8)
Student	29(8.9)	25(7.6)
Unemployed	34(10.4)	37(11.3)

#### **Attitude characteristics (Table 5)**

For 354 respondents, 48.6% have poor and 43.8% have good knowledge about herbal medicine. Others described their knowledge as neither good nor poor. In comparison of knowledge of prescribed medicines to the knowledge of herbal medicine, 30.6% (108/353) and 21% (74/353) of the participants described their knowledge of herbal medicines as good and better, respectively. Eighty-five percent (301/355) of the respondents worry that people take herbal medicines without telling their Medical Health Providers. However, 7.9% of them have not considered it. Out of 352 participants, 94.3% agree that patients should be asked if they are already taking herbal medicine when planning or reviewing patients' drug therapy. According to their response, this practice could prevent about 67.5% (183/271) complications, assist in determining 18.8% efficacy of herbal medicine, ensure 13.8% monitoring of treatment and enhance education. Respondents were willing to seek reliable information about

herbal medicine from books, internet searches and herbalist meanwhile about 73.6% (259/352) will contact Health workers for their information.

Herbal medicine is useful in some 15.9% (56/353) and most circumstances 76.2% (269/353).

Sixty seven percent (235/352) of the respondents described the generally public attitude towards herbal medicine as generally positive, whilst 22.2% neither have positive nor negative attitude towards the medicine. According to 42.1% (147/349) of the respondent, some health workers have misplaced faith in herbal medicine because they are not well informed.

**Table 5: Summary of attitude characteristics that influence the use of VA**

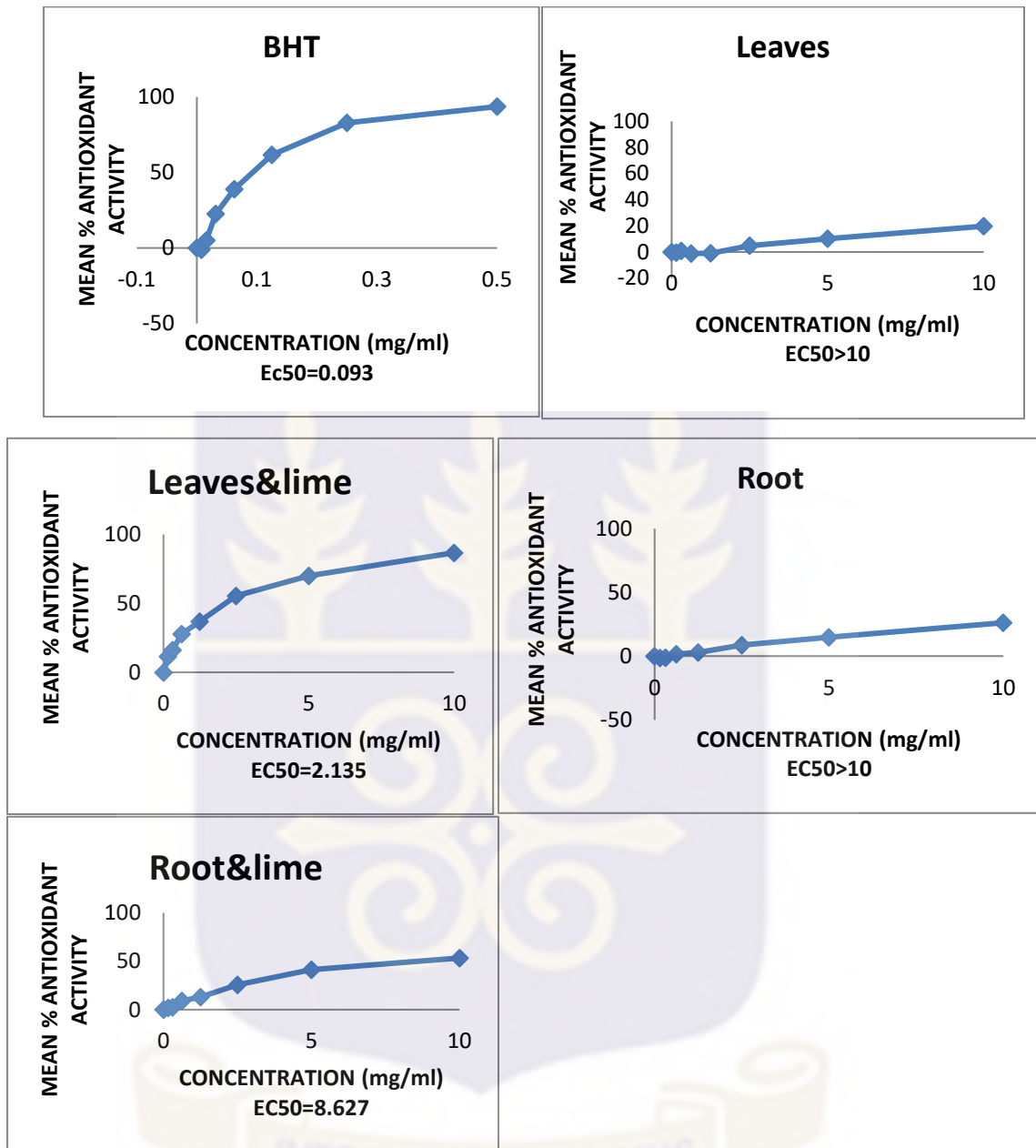
Attitude of General public	Usefulness of herbal medicine		
	Rarely/Never N (%)	In some circ N (%)	Most circ N (%)
Generally Positive	9(3.9)	32(13.7)	188(80.7)
Neither positive nor negative	6(7.8)	21(27.3)	48(62.3)
Generally negative	2(11.8)		15(88.2)
Don't know	3(13.6)	2(9.1)	16(72.7)
Misplaced faith of Health workers in herbal medicine			
Have misplaced faith	9(6.1)	34(23.1)	103(70.1)
Do not have misplaced faith	9(7.5)	13(10.8)	97(80.8)
Don't know	2(2.5)	9(11.4)	63(52.5)
How informed Health workers are about herbal medicine			
Poorly informed	3(6.5)	7(15.2)	35(76.1)
Moderately well informed	4(4.8)	7(8.3)	73(86.9)
Well informed	1(4.5)	4(18.1)	16(72.7)
Don't know	1(4.5)	1(4.5)	18(81.8)
Respondents current knowledge about herbal medicine			
Poor	15(8.8)	29(17.0)	122(71.3)
Good	4(2.6)	14(9.2)	133(86.9)
Neither good nor poor	2(7.4)	12(44.4)	13(48.1)

**Table 6: Summary of attitude characteristics that influence the use of VA**

Attitude of General public	Usefulness of herbal medicine		
	Rarely/Never N (%)	In some circ N (%)	Most circ N (%)
Comparison of the knowledge of prescribed medicine to herbal medicine			
Poor	16(9.6)	30(18.1)	116(69.9)
Good	4(3.7)	15(13.9)	87(80.6)
Better		8(11.3)	63(88.7)
Don't know	1(20)		3(60.0)
Sources of information about herbal medicine			
Books	1(7.1)	6(42.9)	7(50.0)
General internet searches	4(14.3)	8(28.6)	16(57.1)
Herbalist	10(3.9)	26(10.1)	217(84.1)
Health workers	3(9.1)	10(30.3)	20(60.6)
Don't know	2(12.5)	5(31.3)	7(43.8)

#### 4.1 Antioxidant activity of VA extracts

The DPPH free radical scavenging activity of the VA leaves, root and mixture of root, leaves and lime extracts was used to assess antioxidant activity. The leaf of VA and lime extract exhibited the strongest antioxidant activity with an EC<sub>50</sub> value of 2.135 ±0.056 mg/ml, producing 23% the effect of 0.1M BHT (p<0.05). “The antioxidant activity” of the standard (BHT), “stem”, leaf, “and” the mixture of root, leaves and lime are shown in figure 3.



**Figure 3: Antioxidant activity of BHT, VA leaves, root, leaves & lime and root & lime extracts. Each point represents a mean of three determinations.**

### Anti-proliferative activity of VA extracts and curcumin

The various extracts exhibited cytotoxicity towards, Jurkat, HepG2, MCF and Chang cell lines in a dose dependent manner. The combination of leaves and lime juice extract was active against all the tested cell lines with its strongest inhibition against Jurkat ( $IC_{50}$  value =  $96.341\mu\text{g/mL}$ ) and Chang ( $IC_{50}$  value =  $90.97\mu\text{g/mL}$ ).

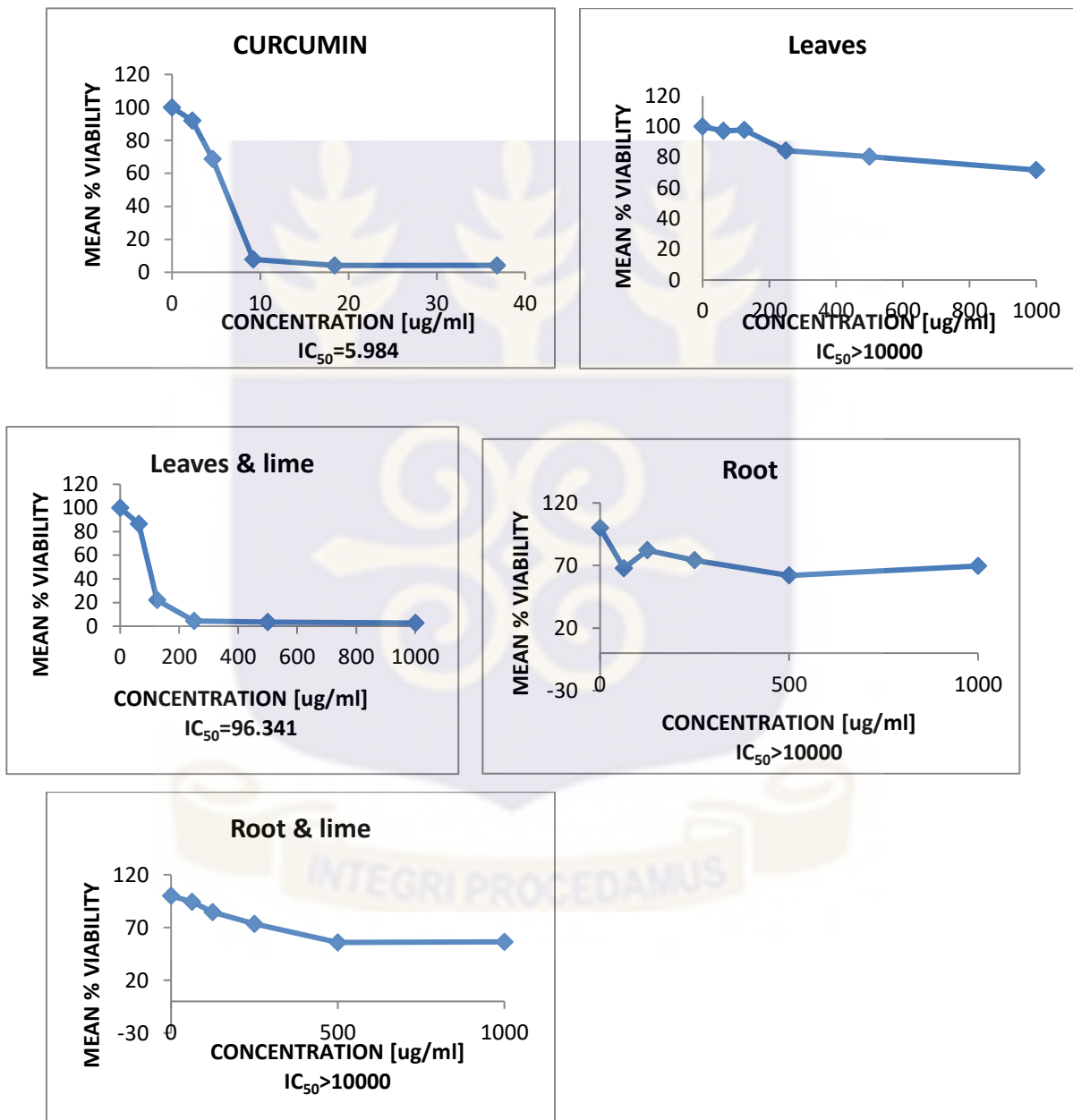


Figure 4: Anti-proliferative activity of VA extracts against Jurkat cell line

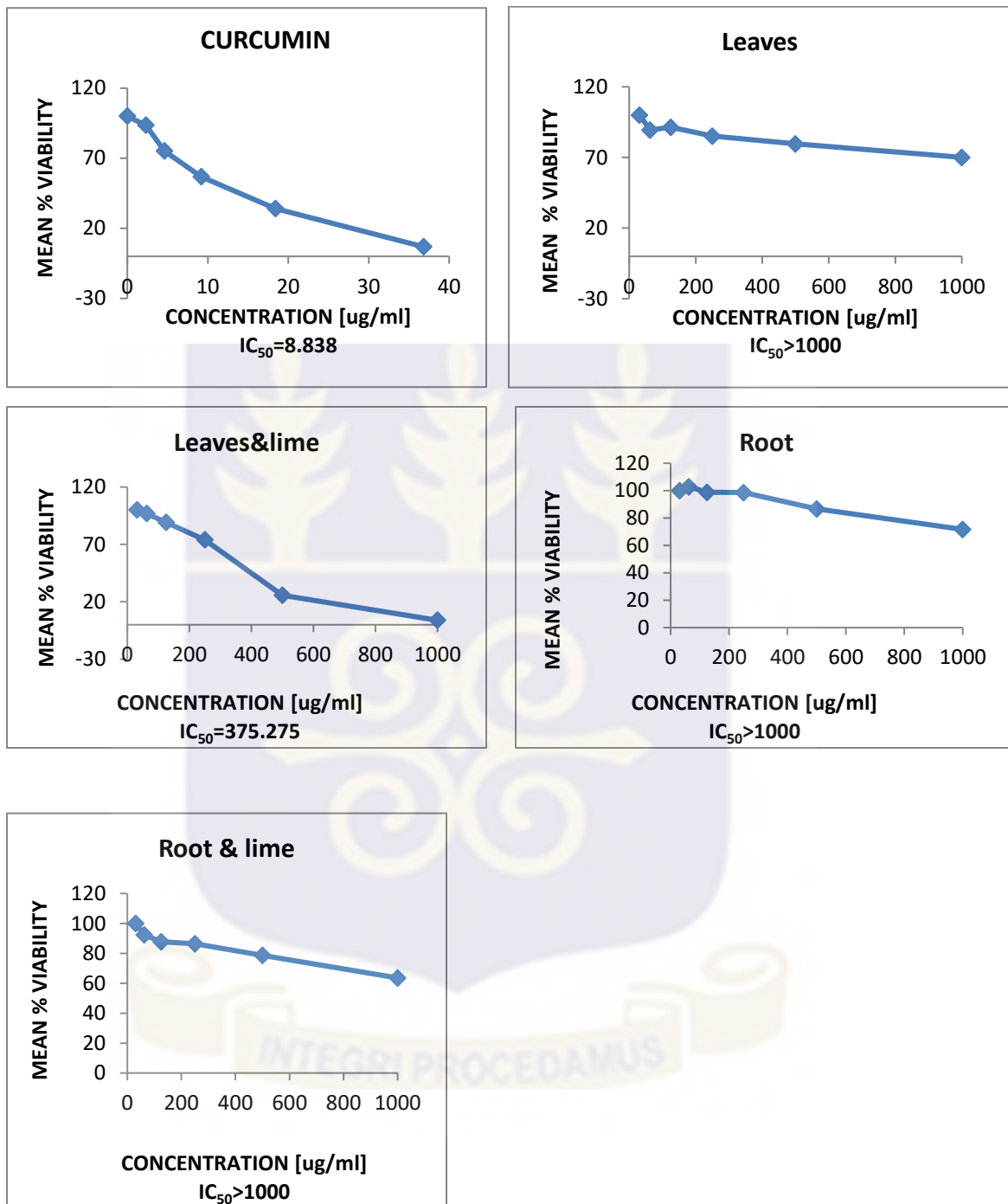
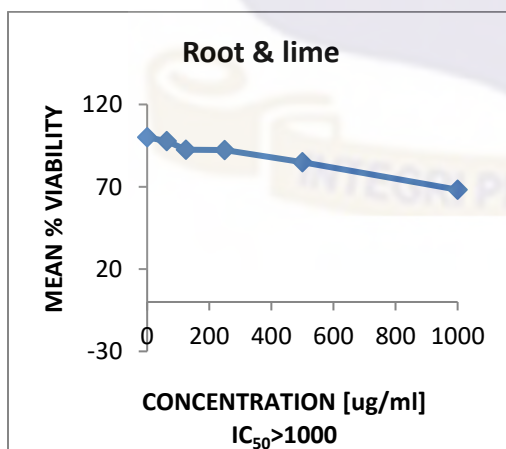
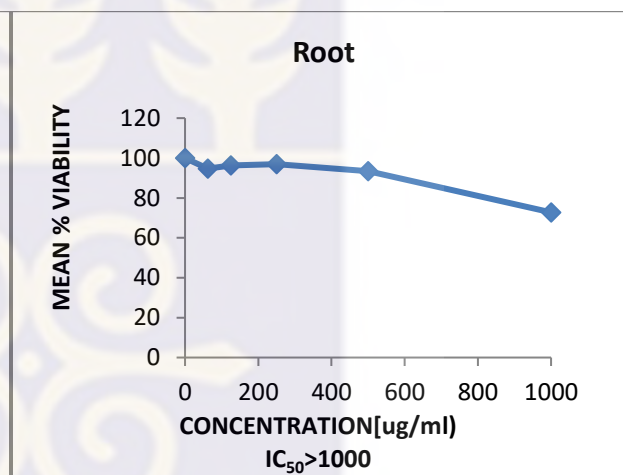
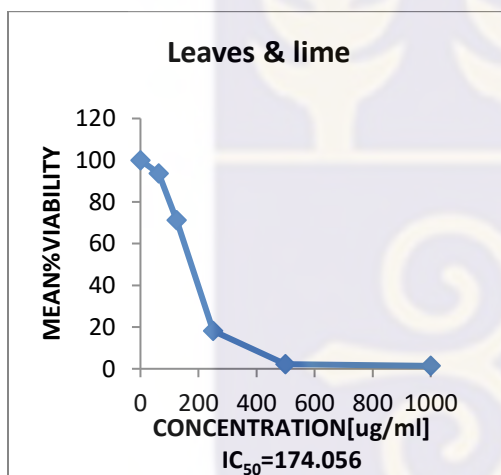
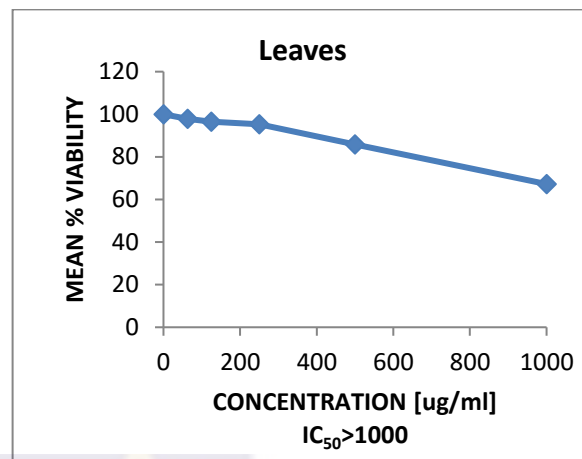
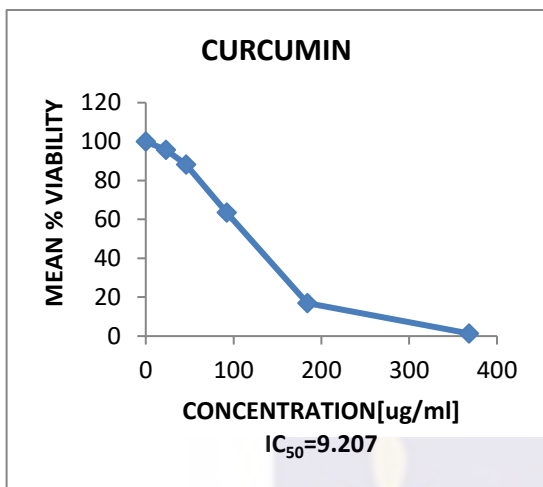
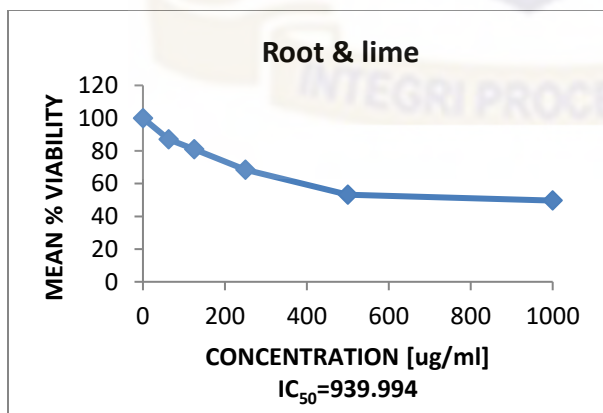
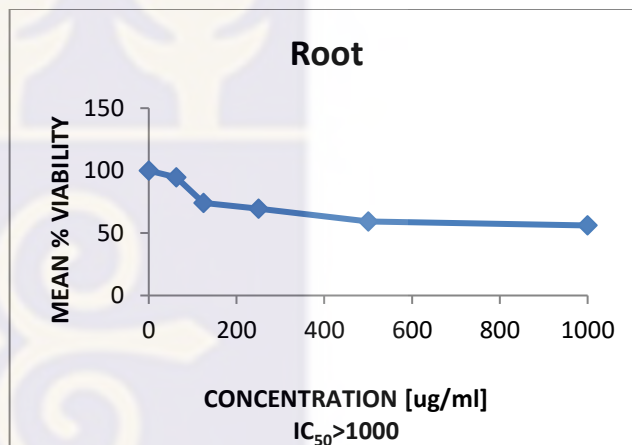
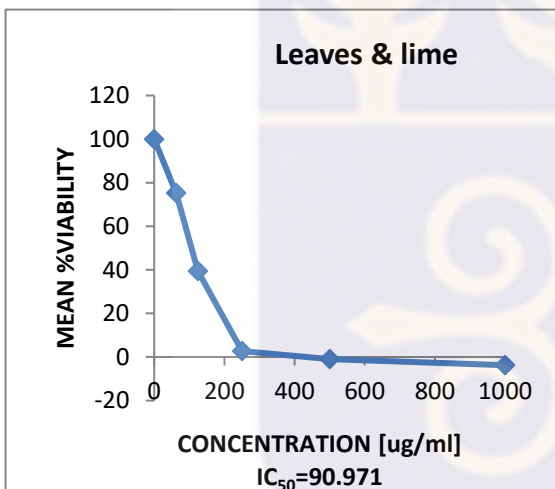
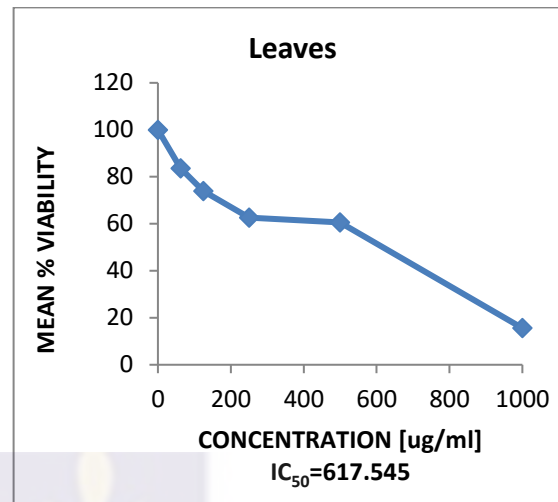
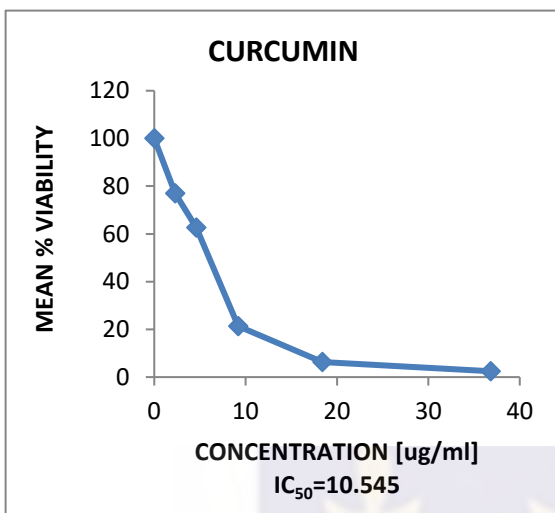


Figure 5: Anti-proliferative activity of VA extracts against MCF cell line



**Figure 6: Anti-proliferative activity of VA extracts against HepG2 cell line**



**Figure 7: Anti-proliferative activity of VA extracts against Chang cell line**

All the extracts analyzed were cytotoxic towards the normal human (Chang) liver cell, exhibiting a rather poor selectivity.

**Table 7: Selectivity index (S.I.) of VA extracts against cell lines**

CELL LINES							
SAMPLES	CHANG	HEPG2	S.I.	MCF-7	S.I.	JURKAT	S.I.
	IC50	IC50		IC50		IC50	
<b>CURCUMIN</b>	10.545	9.207	1.145	8.838	1.193	5.984	1.762
<b>V1</b>	617.545	***	***	***	***	***	***
<b>V2</b>	90.971	174.056	0.523	374.275	0.243	96.341	0.944
<b>V3</b>	>1000						
<b>V4</b>	939.994	***	***	***	***	***	***

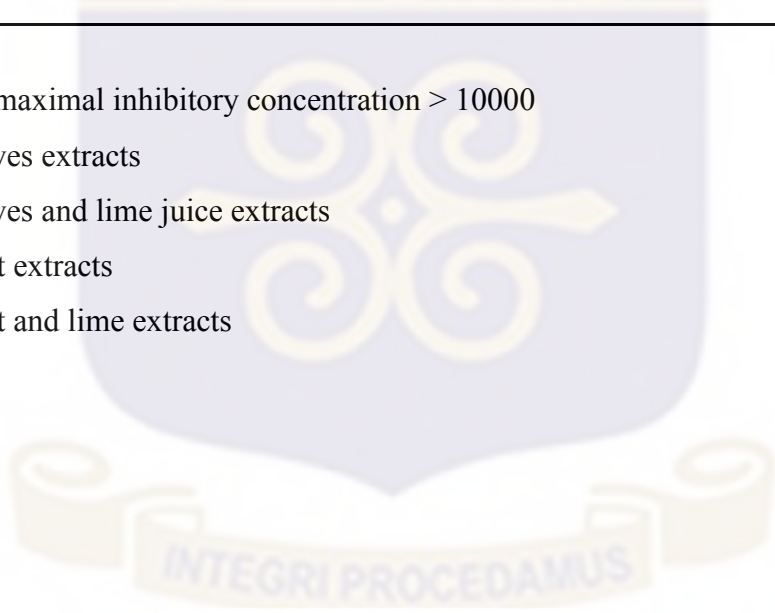
\*\*\* Half maximal inhibitory concentration > 10000

V1 Leaves extracts

V2 Leaves and lime juice extracts

V3 Root extracts

V4 Root and lime extracts



## CHAPTER FIVE

### 5.0 DISCUSSION

The prevalence of the use of VA in this study was 51.3% which is although lower when compared to the prevalence of traditional medicine use in Ghana from another study (Yarney et al., 2013) . This is probably due to the sample size difference where the latter takes larger sample size. A prevalence of 51.3% is still high and this can be attributed to the fact that majority of the participants (76%) accepts the usefulness of traditional medicine. Also this study has revealed that 43.7% of the population has good knowledge about herbal medicine which is similar to 44.7% knowledge of Respondents observed in earlier studies conducted in Lagos, Nigeria (Agbaje et al., 2005). The respondents' sources of knowledge of VA were mainly from relatives, friends, media, herbalist and health workers.

The use of VA is widely accepted due to its availability in the farms, shops, markets and especially in the backyard gardens of family houses. Various ethnic groups in Ghana have different names for the plant and apart from the stem and the root; the leaves are commonly used for either their nutritional purposes or their ability to treat and to prevent diseases. Most people use the above-mentioned parts raw by washing the leaves with saline, crashing or blending with water and filter before drinking. Others also boil the leaves and chew the stems after washing. Different studies have also reported similar practices (Ezuruike et al., 2014). Due to the bitterness of the plant, users abate the bitterness of the leaves by squeeze washing in several changes of water, boiling, refrigeration and addition of lime juice that is commonly used by most people to treat diseases including cancers. Few people also use the leaves for soups, salads and stew (Audu et al., 2012).

VA as described as a multipurpose and rapid regenerating shrub in previous work (Adebayo et al., 2014), is not only used for the treatment of cancer but for colds, coughs, headache, stomachache, ulcers, injuries, skin diseases, typhoid, diabetes, hypertension, heart diseases and predominantly malaria and Fever. In this study we found out that the use of VA was significantly associated with ethnicity and educational level with p values of 0.008, and 0.004, respectively.

According to the respondents, drinking the VA decoction prepared from its fresh leaves or roots in addition to lime juice for the treatment of ailments like cancer is more effective. VA and lime juice extracts contain phytochemicals such as phenols and polyphenolic compounds like flavonoids that are generally present in medicinal plants. These compounds have been shown to possess good antioxidant and anticancer activities (Audu et al., 2012). An earlier study revealed that the temperature at which the plant was harvested, processed and preserved influences the bioavailability of these compounds (Kamiloglu et al., 2013). Results from this research showed that VA indeed has great antioxidant activity, predominantly found in its leaf which agrees with other findings (Erasto et al., 2007). There was significant difference between the observed extracts of various parts and the portion with lime ( $p < 0.05$ ) suggesting a synergistic effects of the phytochemicals present in both VA and lime juice.

Meanwhile the MTT cell viability assay also indicated that only the extracts of the VA leaves and lime juice had anti-proliferative activity against all the three cancer cells (Jurkat, MCF-7 and HepG2) and the normal liver cell (Chang). The various extracts exhibited cytotoxicity

towards MCF, HepG2, Jurkat and Chang cell lines in a dose dependent manner. The mixture of leaves and lime extract was active against all the tested cell lines with its strongest inhibition against Jurkat ( $IC_{50}$  value = 96.341  $\mu\text{g}/\text{mL}$ ) and Chang ( $IC_{50}$  value = 90.97  $\mu\text{g}/\text{mL}$ ). An earlier publication has shown an anti-proliferative activity of *Vernonia amygdalina* against MCF-7 in dose-dependent manner. The highest dose was 200  $\mu\text{g}/\text{ml}$  (Chanda et al., 2013). However, in this study, *Vernonia amygdalina* leaves extracts and the mixture of the leaves and lime showed cytotoxic effect against MCF-7 at a higher dose, thus >1000 & 374.28  $\mu\text{g}/\text{ml}$  respectively. Again, the mixture poses to be more desirable chemotherapeutic alternative, which could be attributed to the fact that the constituents of the lime juice complemented the phytochemicals of the leaves extracts. On the other hand, it is possible the solvents and the methods used for the plant extraction in both studies were different. Meanwhile, the mixture exhibited anti-proliferative activity against all cell lines in a dose-dependent manner (90.97, 96.34, 174.06 & 374.28  $\mu\text{g}/\text{ml}$ ) which was significantly different from  $IC_{50}$  of the leaves extract ( $p < 0.005$ ). The potency of a compound is determined by its Half-maximal inhibitory concentration ( $IC_{50}$ ) the lower the  $IC_{50}$  value, the more potent the compound is. According to previous authors (Shital et al., 2017), the fact that normal cell line, was susceptible to the extract suggests that it has no selectivity between cancer and non-cancer cells, thus exhibiting poor selectivity. This denotes some level of toxicity whenever the plant is use for the purpose of its nutritional benefit, treatment or prevention of a disease condition.

The plant is used for treating ailments such as colds, coughs, headache, stomachache, ulcers, injuries, skin diseases, typhoid, diabetes, hypertension, heart diseases, and predominantly malaria (60%) and Fever (30%)

## CHAPTER SIX

### 6.0 CONCLUSION AND RECOMMENDATIONS

“The” population studied has good knowledge about VA, the prevalence of the use of VA is high, and this is related to the acceptability of the usefulness of traditional medicine and easy accessibility. Ethnicity and educational level was found to be highly associated with the use of *Vernonia amygdalina*.

The results obtained from the DPPH and MTT assay revealed that the combination of *Vernonia amygdalina* leaf extract and lime juice has a good antioxidant properties and it is effective in inhibiting Jurkat cancer cell lines. However, it does not appear as a suitable chemotherapeutic alternative for cancer due to its cytotoxic effects towards the normal liver cell. More work should be done to ascertain the antioxidant and anticancer properties of the mixture.

### Recommendations

The findings of the DPPH and MTT assay confirmed that the various samples of *Vernonia amygdalina* has antioxidant and anticancer property. Further studies should be directed at:

1. Antioxidant and anticancer activity of *Vernonia amygdalina* extracts *in vivo*.
2. The hepatic effect of *Vernonia amygdalina* extracts in addition to lime juice *in vivo*.

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## APPENDIX

### QUESTIONNAIRE

Title: Determination of anticancer properties of bitter leaf (awonyono).

Good morning (afternoon/evening). Thank you for agreeing to take part in this study. Our questions will cover your perception about herbal medicine, your demographics, knowledge and use of bitter leaf. We understand that some things will be difficult to remember. We would like to have your best possible answer, so please take the time you need to think things over. Everything you tell me in the interview will be kept private and confidential, as is required by law. But, if for any reason you would rather not answer a question, we can skip it and go on to the next. Do you have any questions before we begin?

Date: ..... ID:..... Contact  
No.:.....

#### 1. DEMOGRAPHICS

1. Name: .....

2. Sex: Male: [ ] Female: [ ]

3. Age: 18-30 years [ ] 31-40 years [ ] 41- 50 years [ ] 51-[ ] 60 + years [ ]

4. Residence: ..... Ethnicity: ..... Nationality: .....

5. Religion: .....

6. What is your highest level of education?

Primary [ ] JHS [ ] SHS [ ] Vocational/Technical [ ] Tertiary [ ]

7. Occupation .....

#### KNOWLEDGE AND USE

8. Have you heard of bitter leaf? Yes [  ] No [  ]
9. If yes, where did you hear about it?
  - a) Doctor [  ]
  - b) Herbalist [  ]
  - c) Relative [  ]
  - d) The media [  ]
  - e) Friend [  ]
  - f) Other [  ]
10. What other names do you have for bitter leaf?.....
11. How do you call bitter leaf in your language.....
1. Does bitter leaf prevent diseases? Yes [  ] No[  ] Don't know [  ]
2. If yes, mention a disease preventable by bitter leaf.....
3. Does bitter leaf cure diseases? Yes [  ] No[  ] Don't know [  ]
4. If yes, mention a disease curable by bitter leaf.....
5. Have you ever use bitter leaf? Yes [  ] No[  ]
6. If yes, how often do you use bitter leaf?.....
7. How do you process the bitter leaf before use.....
8. Do you use bitter leaf as a vegetable? Yes [  ] No[  ]
9. If yes, why do you use it?.....
10. Do you use bitter leaf for treating ailment Yes [  ] No[  ]
11. What kind of ailment do you treat with bitter leaf?.....
12. Do you abate the bitterness before use? Yes [  ] No[  ]
13. How do you abate the bitterness? Boiling [  ] Refrigeration[  ] Addition of lime juice[  ]
14. Which part of the bitter leaf do you use? Root[  ] Stem[  ] Leaves[  ]

15. From where do you obtain the bitter leaf for use? Market[  ] Shop[  ] Farm[  ]  
Backyard gardens[  ]

ATTITUDE

16. Do you think herbal medicines are useful? Rarely or never [  ] In some circumstances [  ]  
In most circumstances [  ] Don't know [  ]

17. How will you describe the General Public attitudes to herbal medicine? Generally  
positive[  ] Neither positive nor negative[  ] Generally negative[  ] Don't know[  ]

18. Do you think some Health workers have misplaced faith in herbal medicine?  
Yes [  ] No [  ] don't know [  ]

19. If no how well informed do you think Health workers are about herbal medicine  
Poorly informed [  ] moderately well informed [  ] well informed [  ] Don't know  
[  ]

20. How will you describe your current knowledge about herbal medicine?  
Poor[  ] Good[  ] Neither good nor poor[  ]

21. Compared to your knowledge of prescribed medicine, describe your knowledge of Herbal  
medicine. Poor [  ] Good [  ] Better [  ] Don't know[  ]

22. Do you worry that people take herbal medicines without telling their medical health  
providers? Yes [  ] No[  ] Have not considered it [  ] Don't know [  ]

When planning or reviewing patient's drug therapy, is it advisable to ask them if they are  
already taking herbal medicine? Yes [  ] No [  ] don't know [  ]

23. If yes, why.....

24. Where will you seek reliable information on herbal medicine? Books[  ] General internet  
searches[  ] Herbalist[  ] Health worker[  ]Don't know[  ]

25. Does the clinical prescribing system provide any information on herbal medicine?

Yes [  ] No [  ] don't know [  ]



