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Forensic Investigations of Antibiotic Adulteration in Herbal Medicinal Products sold in Kumasi Metropolis

By

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DECLARATION

I, Linda Akosua Boahemaah, hereby declare that I have wholly undertaken the project documented herein under the supervision of Dr. F. C. Mills-Robertson and Dr. S.O. Bekoe that except portions where references have been duly cited this dissertation is the outcome of my research work.

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ABSTRACT

Adulterated herbal medicines, are threat to public health, consumer safety, and undermine national health policies with serious economic consequences. In Ghana and most other African countries, herbal medications are commonly used as remedies for a wide variety of conditions and are highly patronized nationwide. However, these medications are also prone to adulterations with a variety of chemicals and substances. In light of this, the present study investigated the adulteration of selected antimicrobial herbal medicines sold in the Kumasi metropolis. In all, 21 samples (5 liquid samples, 6 capsules, 3 powdered samples and 7 creams) were analysed for the presence of four antibiotics using HPLC at the Aflatoxin Laboratory, Kwame Nkrumah University of Science and Technology. Results showed that 71.43% of the herbal creams were contaminated with at least one of selected antibiotics (trimethoprim, chloramphenicol, amoxicillin and ciprofloxacillin). Also, 75% of the liquid samples had various concentrations of the four antibiotics. Furthermore, 42.86% of the powdered samples had various concentrations of the selected antibiotics while only 16.67% of the herbal capsules showed adulteration. Thus, some herbal medications on the Ghanaian market contain antibiotics and therefore proper surveillance is needed to avoid antibiotic abuse which may subsequently lead to antibiotic resistance.

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CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND INFORMATION

Herbal drugs are medicines made from parts or whole plants with active ingredients in their leaves, bark, stem, flowers or roots. The discovery of plant medicine has become essential in view of the advent of increased resistance to several orthodox therapeutic agents in use. Plant medicines with proven efficacy and safety have been used in the management of some ailments in some countries where orthodox medicines have failed (Ekor, 2014; P. Saxena, 2001). If not used correctly, just like the conventional medications, herbal medicines can have harmful effects on the consumer (Ernst, 2008).

Adulterated herbal medicines are a threat to public health, consumer safety, and undermines national health policies and systems (Blackstone *et al.*, 2014). Previous studies have shown account of falsification of medical products throughout history, particularly among nations with weak regulatory and control systems. These countries are usually nations in transition, particularly many African, Asian and Latin American countries (Kovacs *et al.*, 2014). In West Africa alone, estimations for illegal antimalaria drugs exceed US$400 million (Gostin *et al.*, 2013) while the international marketplace for fake drugs may range between $75 and $200 billion (Mackey and Liang, 2013). However, given the pervasiveness of the internet and e-commerce, the proportion of counterfeit drug markets in developing countries which accounted for 10% is predicted to exceed 50% taking together internet sales (Gautam *et al.*, 2009). The evidence elaborates more on the significance of the problem and, therefore, call for garnered efforts to halt the widespread of counterfeit drugs on the market. Although this is uncommon in many advanced republics, due to real supervisory schemes and market control, the incidence of adulterant herbal materials is estimated to be of the minimal accounting for less than 1% of the market value (Medina *et al.*, 2016). Counterfeit drugs or the incidence of adulterant herbal

materials are rising nowadays given the increasing global cost of drugs (Jackson, 2009). Generally, medications that are on top of demand lists become an easy target for counterfeiters (Jackson *et al.*, 2012). Although many definitions of the term counterfeit exist in many countries and jurisdictions, World Health Organization defines drugs as counterfeit when they have deliberately been mislabelled with regards to their content, identity/sources (www.who.int/medicines/services/counterfeit/overview/en/). Most adulterated medical products are very often manufactured under poor and unhygienic conditions by unqualified personnel (Ayukekbong *et al.*, 2017).

The health and economic cost of counterfeit medication are high and unbearable most of the times (Blackstone *et al.*, 2014). Consumption of counterfeit drugs could lead to treatment failure, incidence of serious side effects, possible cases of added contamination and even death (Blackstone *et al.*, 2014; Pullirsch *et al.*, 2014). For instance, the microbial content of counterfeit or unapproved drugs in Canadian and Austrian markets were found to be above pharmacopoeia limits (Pullirsch *et al*., 2014). Moreover, the prevalence of adulterated herbal medicines contributes to mistrust of pharmaceutical manufactures and various health facilities across the country. More importantly, counterfeit drugs could possibly undermine major global health initiatives such as the fight against malaria, HIV, tuberculosis, neglected tropical diseases and other non-communicable diseases which are now gaining grounds in Ghana and other developing countries (Deye *et al.*, 2016).

Antibiotics are used in treating a host of bacterial infections. Thus, because of their antibacterial efficacies, they easily become a target as adulterants for herbal preparations with claims of antimicrobial action.

1.2. PROBLEM STATEMENT

The health and economic consequences of infectious and non-communicable diseases in developing countries have inspired the development of cost effective, readily available and prevailing medications for population use. However, the high cost of some orthodox medicines, the country's relatively weak drug monitoring systems and negligence of the population have promoted the proliferation of counterfeit and substandard drugs as well as adulterated herbal medicines on the Ghanaian market (Buckley & Gostin, 2013). Although previous studies have investigated the prevalence of counterfeit medical products such as antimalarial and some generic medications, the exact prevalence of adulterated herbal medicines on the Ghanaian market is lacking. Moreover, despite local and international efforts to fight the explosion of adulterated herbal medical products, tracking of adulterated herbal medicines on the Ghanaian market has been met with numerous challenges such as lack of cost-effective tools and personnel. Moreover, a database or list of adulterated herbal medical products and their perpetrators is lacking. Furthermore, there is limited information about the general population's knowledge, perceptions and practices with regards to herbal medicine adulteration on the Ghanaian market. Finally, most of the current technologies being used to identify adulterated herbal medicines lack adequate forensic evidence, thus the focus of this study.

1.3. JUSTIFICATION

Adulteration of medical products is still a challenge in Ghana and even globally. There is the need for researches into various adulterations to provide a list or collection of commonly adulterated medications either locally manufactured or of foreign origin that can be used as basis for drug surveillance. It is also expected that a list of drug types and manufacturers generally involved in this illegal business could be generated. The prevalence of adulterated herbal medications provided by the study, could affect policies as part of the national

pharmacovigilance activities to clamp down the proliferation of these drugs. This would in turn promote the safety of herbal medicines for general use and to also promote trust in herbal medicine manufacturers. Also expected from this study will be its contribution to the reduction of high rates of antibiotic resistance in Ghana.

1.4. MAIN OBJECTIVE

- To evaluate the prevalence of antibiotic adulteration of herbal antimicrobial medicinal products sold in the Kumasi Metropolis using forensic analytical tool methods.

1.5. SPECIFIC OBJECTIVES

- To determine the presence or absence of selected synthetic antibiotics in selected antimicrobial herbal medications in the Kumasi Metropolis.
- To determine the various concentrations of the selected synthetic antibiotics in antimicrobial herbal medicines in the Kumasi Metropolis.
- To determine the prevalence of antibiotic adulteration in herbal antimicrobial medicines in the Kumasi Metropolis.

CHAPTER TWO

LITERATURE REVIEW

2.1. HERBAL MEDICINES

Considered the most ancient form of treatment regimen, herbal medications have been used mostly in indigenous traditional cultures with irrefutable influence on many systems of medicine. Herbs are plants or plant parts often times the leaves or bark harvested for their therapeutic properties, flavour or scent (Masevhe *et al.*, 2015). There are many individuals who use herbal medicines to maintain or improve their health. Sold as fresh or dried plants, they also form some kind of dietary supplements in forms of tablets, capsules, powders, teas, etc (Plus, 2017).

There is a general believe that products labelled "natural" are relatively safer and good for consumption. This assertion is not being necessarily true. Most herbal preparations do not go through the extensive testing that most orthodox drugs go through. Whilst some herbs' active ingredients interact with over-the-counter drugs, others such as ephedra and comfrey can result in serious harm (Wachtel-Galor, 2011). Different cultures around the globe have developed various types of herbal medicines today. All the different types of herbal medicine employ medicinal plant-based products only varying in the type of plants or plant parts they use, how they are prepared and applied taking into account their treatment philosophies and approach referred to as "alternative medicine" in Western countries, herbal medications remain the only form of medication patronised by majority of our world's population (Wachtel-Galor, 2011).

2.1.1. How Does Herbal Medications Work?

Herbs naturally contain active ingredients that have some form of biochemical tendencies to influence biological activities in organisms. For example, quinine is extracted from cinchona tree bark whilst morphine is obtained from the opium poppy as a pain killer. These exemplify

that synthetic medications have their roots in herbs and thus thy work in similar fashion (Gilani, 2010).

Some herbalists profess that herbs must be used in their natural state to ensure balance of ingredients in the usage of the various parts in treating chronic conditions. They argue that herbs are most effective when used this way. They also believe herbal fixings, though they are not quick-fixes are very effective in treating many conditions without the accompanying side effects that come with orthodox pharmaceutical treatments. Notably is the caution against the potency of these herbs should they be used wrongly (Poornima, 2010). In addition, some herbs like St. John's wort can interrupt with birth control pills as gingko biloba is noted to increase risks of bleeding with blood thinning medicines. Thus, their effects can either increase or decrease the potency of normal over-the-counter drugs that are often used. (https://www.mydr.com.au/complementary-medicine/herbal-medicine).

2.1.2. Antimicrobial Herbal Medicines

Across the globe, infectious diseases account for the premature deaths of almost 50,000 people (World Health Organization, 2011). Morbidity and mortality due to diarrhoea, as an example, continually plaque developing countries as a major problem, especially amongst children. Infections owing to variability of microbial etiologic agents such as pathogenic *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* are most common (Pawlowski *et al.*, 2009). With the continuous prescriptions of antibiotics, bacterial agents have become resistant to certain drugs and this resistance have been reported from around the world in the scientific community. Allergic reactions, hypersensitivity and other adverse reactions have added to the problem associated with antibiotics usage in the treatment of infectious diseases (Fair & Tor, 2014a). This highlights the need to find substitute antimicrobial drugs to tackle infectious diseases; one method being to screen medicinal plants for potential antibiotic properties. Plants remain

important resource to fight critical diseases on earth. According to (WHO, 2011) about 80% of global population depends on traditional medications which involves the utilization of plant extracts. Surprisingly, scientific studies of herbs for their antimicrobial properties seems new. Traditional methods of using herbal plants as cure for ailments still play a vital role in covering some health needs in less developed countries. Searching for potent antibacterial agents have been shifted to plants lately (Namita & Mukesh, 2012). Even though only 1% of plant-based medicines is estimated to have gained meaningful recognition by modern scientists, there are suggestions about the fact that 10% of all flowering plants must been used at a point in time for healing purposes. As most herbs are used in treating diseases in body, some have overarching efficacy claims as well (Ojo *et al.*, 2006).

From just 95 plant species, there are about 120 plant-based prescriptions the world over. In the over 250,000 species of flowering plants, only 5000 have over the years been assessed for their pharmaceutical potentials. Continually, the treatment of infectious microbes with mainstream antibiotics presents increased bacterial resistance to these antibiotics in modern medicine with may studies showing a steady rise globally (Alavijeh *et al.*, 2012). It has become desirable to look to plants for chemical compounds that can fight these microbes due to the resistance against established antibiotics.

Traditional herbs have been reportedly effective against Gram-positive and Gram-negative microbes and by these plants still remain the bedrock for modern medicine to treat infectious diseases (Mills-Robertson *et al.*, 2014; 2015 Adjapong *et al.*, 2017).

2.2. ADULTERATION OF DRUGS

Adulterating drugs qualifies as the means by which original drugs are substituted wholly or partially with similar replica but with no use or worse negative effects in its healing potentials. Adulterating drugs may involve diverse implications such as worsening, admixture, swap,

inferiority and degeneration (Fig. 2.1) (Sreelekshmi *et al.*, 2017). Degeneration is the impairment in drug quality while the addition of one substance to the original content due to accident or carelessness is referred to as admixture. Substitution, on the other hand, ensues once something completely unalike is added in place of the original drug while inferiority denotes the substandard version of the target active ingredient. Spoilage deterioration may be due to the attack of microorganisms (Kumar, 2017).

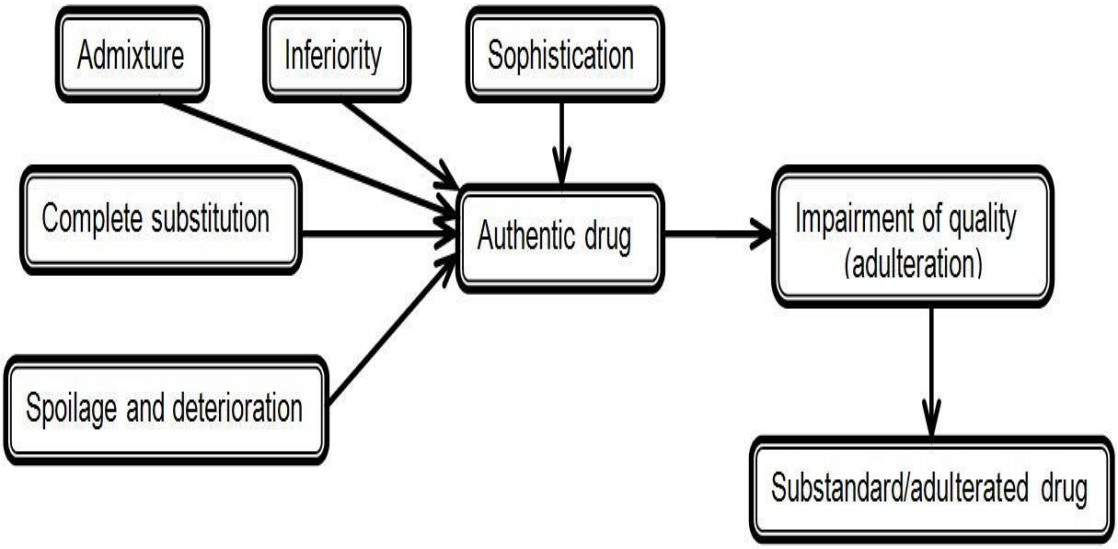


Figure 2.1. Factors creating adulteration in authentic crude drug

Pictorial representation of how "Authentic Drugs" are manipulated to result in impairment of their quality which then results in adulterations.

Source: (Ahmed & Hasan, 2015)

2.2.1. Types of Adulterants

Largely, most adulterated drugs are just ones that have active ingredients replaced with inferior commercial alternatives. There are many common types of adulteration (Fig. 2.1).

2.2.1.1. Substitution with Sub-Standard Commercial Varieties

Resembling the original crude drugs in morphology, chemical and therapeutic traits, the adulterants are inferior in nature and thus cheaper in costs (Prakash *et al.*, 2013). This happens to be the most common adulterating practice. For example, *Arabian sena* is sometimes substituted with African ginger (Kumar, 2017).

2.2.1.2. Substitution with Superficially Similar inferior Drugs

The counterfeit drugs may not have any therapeutic value as opposed to the genuine drug. Owing to their similar morphology, they are then referred to as adulterants. Japan wax is used in place of bee wax, *Ailanthus* leaves used as though it is *Bellandonna* amongst others (Ahmed & Hasan, 2015).

2.2.1.3. Substitution with Artificially Manufactured Substances

For the kind of drugs that are expensive, it is observed that artificially prepared substances are made to look like the original drug. Well known examples are basswood shaved to look like nutmeg, paraffin wax that are coloured to replace bees' wax and Chicory compressed to be used as coffee (Kumar, 2017).

2.2.1.4. Substitution with Exhausted Drug

Often times, natural characteristics of exhausted drugs as their colour and taste are changed by the addition of irrelevant additives. This is done to drugs containing volatile oils as in the case of coriander, clove, caraway and others. Such same drugs are added but is devoid of their medicinal properties as they are extracted out already (Preethi *et al*., 2014). Exemplified in this case is the use of artificial colouring of saffron and exhausted gentian made bitter by adding aloes are common practices. Other times too, the use of synthetic chemicals are placed into the

mixture to enhance their supposed efficacy as in the case of benzyl benzoate to Peru balsam and citral to citrus oils, among others (Coomber & Avery, 1997).

2.2.1.5. Presence of Vegetative Matter from the Same Plant

Other times miniature plants that grow alongside medicinal plants are added to the authentic drugs due to their semblance of odour or colour and constituents. Lower shrubs like moss and liverworts growing on tree barks are mixed with cascara and stems portion mixed with stramonium and lobelia leaves (Kumar, 2017).

2.2.1.6. Harmful Adulterants

Particularly noticed for liquids and unorganized drugs is the admixture of wastes from markets from which perpetrators add white oil to coconut oil, stearin is mixed with cocoa butter and led shot are put in opium. Harmful amongst them is the addition of rodent faeces to cardamom seed (Kumar *et al.*, 2008).

2.2.1.7. Adulteration of Powders

The powdered forms of drugs are the frequently adulterated that have exhausted ginger in colocynth, red-sanders wood in capsicum and gentian added to powdered olive stones (Evans, 2009).

2.2.2. Adulteration of Antimicrobial Herbal Drugs

The major challenge posing a threat to the growing herbal drug industry and its complementing research on commercialization of natural products is the adulteration schemes adopted by peddlers. Whether intentional or accidental, the term adulteration of a given medication covers a list of conditions. Substituting the original natural active ingredient with commercial varieties

that are mostly inferior, it leads to the supposed drug not having any therapeutic potential as would the natural crude drug (Poornima, 2010).

2.2.3. Reasons for Adulteration

Aside the greed that comes with people adulterating drugs for unwarranted profits, some reasons why drugs get adulterated include unscientific collection, similar morphology, unavailability of authentic plants, confusions in vernacular name, inadequate knowledge about authentic sources and high prices on the drug market (Kumar, 2014).

2.3. HEALTH AND SOCIO-ECONOMIC EFFECTS OF ADULTERATED HERBAL MEDICINES

Herbal medicines, also termed as phytomedicines or botanical medicines, are naturally occurring, plant-derived products through negligible or no manufacturing processing and could be used for curative purposes (Firenzuoli & Gori, 2007). Herbal medicines are popular in every country and were previously passed on from one generation to the other; however, the advent of high-through-put technologies and modern drug screening and discovery techniques, have significantly impacted the exploration of herbal products of better quality (Chavan *et al.*, 2006; Rossi *et al*., 2017). The widespread use and culturally embedded nature of herbal medicinal products suggest that these medicines are safe for use in treatment and prevention of diseases. However, the efficacy of the treatment and potency is something that remains debated among traditional practitioners and contemporary medicine users (Liu *et al*., 2017). Conversely, the use of herbal medicine is challenged by the lack of definite and complete information about the composition of the extracts (Li, 2018). There is, therefore, the need to measure the pharmacological properties and safety of these medicinal products to ensure that they are able to perform the intended purposes in consumers with minimal side effects.

Collective evidences suggest that some herbal medicines are contaminated with other drugs, toxins or heavy metals and some even lack the listed ingredients on the label. These additional ingredients may interact with the drugs which may lead to severe adverse reactions when consumed. Moreover, some manufacturers add artificial medications in the recipe of their products and are later branded, advertised or sold as herbal medicine. This is done to improve the efficacy and effectiveness of their products. Taken together, these practices are what we define as adulteration and such herbal products are termed as adulterated herbal medicine. According to the US Food and Drug Administration, any drug which falls below conforming levels of ideals of quality, strength, or concentration, after testing with compendia methods, is considered as adulterated except the difference in the standards are openly stated on the labels on the drug (FDA, 2017). Standard tests may include various tests to investigate drug properties such as effectiveness, sterility, suspension, variation in weight and uniformity in content.

Over the years, there have been reports of approved drugs and unapproved analogues in herbal medicine such as tadalafil, sildenafil, and glibenclamide (Bogusz *et al.*, 2006). Surprisingly, these synthetic products have been in several food supplements on the market (Venhuis and de Kaste, 2012). These compounds function as phosphodiesterase type 5 (PDE-5) enzyme inhibitors and have been used for erectile dysfunctional treatments (Patel *et al.*, 2014). The prevalence of such drugs to enhance sexual performance has increased in recent times while these perpetuators are increasingly finding new ways to create novel synthetic analogues to evade national drug regulatory authorities. PDE-5 enzyme inhibitors may lead to serious drug-drug interactions. For instance, when taken with nitrates or alpha-blockers, this could lead to severe hypotension and syncope thereby people with cardio problems may take these drugs unknowingly leading to severe health problems (Gur *et al*., 2013).

The surge in falsified and adulterated herbal medicine products are noteworthy public health complications around the globe predominantly in most unindustrialized states such as Ghana.

This is largely due to weak pharmacovigilance and drug regulatory systems in these countries thereby promoting the proliferation of such drugs in developing countries. The general public's limited knowledge on the identity and the health implications of these drugs are also factors that facilitate the growth of the counterfeit drug industry in low-and middle-income countries (LMiCs) (Mhando *et al*., 2016). Adulterated herbal medicine has serious health implications on the consumer which may range from mild to fatal in certain instances (Blackstone et al., 2014). The effect of counterfeit medicinal products on the body's kidney and liver has been widely documented in recent times.

The motivation of counterfeit drug manufacturers and dealers are enormous including huge profit incentives accrued from the sale of such drugs in countries with loose drug regulatory and public health systems. Conversely, it is important to distinguish adulterated from counterfeit medicinal products. Any medicine deliberately and fraudulently not labelled properly with respect to identity and source is considered adulterated or counterfeit. Counterfeit drugs extend to those that may even have correct ingredients but wrong labels and packaging in as much as those with wrong ingredients may have good labels. Second-rate remedies are sometimes defined as authentic medicine that did not pass quality tests and standards (Almuzaini *et al*., 2013). Such standard tests have been documented in the WHO pharmacopoeias.

Adulteration may cause unwanted side effects in patients. Adulteration may also lead to the alteration of nature of drugs. It may also cause denaturation and degradation of product and may completely destroy the active constituents.

2.3.1. Health and Socio-Economic Effects of Selected Antibiotics

2.3.1.1. Trimethoprim

Trimethoprim is a white to cream concealed scentless compound accessed as 100 mg tablets for oral intake as an antibiotic. It contains accompanying fixings like sodium starch glycolate, silicon dioxide, anhydrous lactose and magnesium stearate. It is used for the treatment of early signs of urinary tract infections in the presence of weak strains of microbes like *Escherichia coli.* Treatment might be started before acquiring the after-effects of these tests (Fair & Tor, 2014b).

The standard oral adult estimation is 100mg of trimethoprim at regular intervals or 200 mg10 days each. It is not good for persons with creatinine clearance of under 15 mL/min (Ho & Juurlink, 2011). The antagonistic impacts experienced much of the time with trimethoprim are rashes and skin expulsions. At the prescribed measurement batches of 100 mg twice daily or on the other hand 10 days for 200 mg. In medical examinations which used high dosages of trimethoprim pill, a raised frequency of rash was noted. Uncommon reports of exfoliative dermatitis, Stevens-Johnson disorder, erythema multiforme, harmful epidermal necrolysis, and hyperactivity have additionally been chronicled.

Gastrointestinally, epigastric pain, queasiness, retching, and glossitis have been accounted for. Cholestatic jaundice has been once in a while revealed. Hematologically, there can be thrombocytopenia, leukopenia, neutropenia, megaloblastic weakness, and methemoglobinemia. Trimethoprim may limit the hepatic biological processing of phenytoin. Trimethoprim, given at a typical clinical dose, broadened the phenytoin half-life by 51% and reduced the phenytoin metabolic freedom rate by 30%. When controlling these meds all the while, one should be alert for possible pointless phenytoin impact (Antoniou *et al.*, 2011).

Careful frequency records are not obtainable since no precise information of treated patients is open. The most often announced antagonistic responses have been consuming, stinging, visual

bothering, and conjunctival hyperaemia. Blood dyscrasias, ominously vulnerable or fiery responses on account of individual excessive touchiness, angioneurotic oedema, urticaria, vesicular and maculopapular dermatitis have likewise been accounted for (Antoniou *et al.*, 2011).

2.3.1.2. Chloramphenicol

Chloramphenicol should be used particularly in those certifiable diseases for which less conceivably hazardous medications are unable or contraindicated. Chloramphenicol is shown for the treatment of surface visual diseases including the conjunctiva and additionally cornea brought about by chloramphenicol-defenceless life forms (RxList, 2018).

The prolonged use of antibiotics may once in a while bring about abundance of non-susceptible organisms, including fungi. In the event that new infections show up during medicine or clinical improvement is not seen within a week, the medication ought to be ceased and suitable measures ought to be taken. No long-haul revisions have been directed in creatures or in people to assess the cancer-causing impacts on ripeness with chloramphenicol. In any case, there is some clinical proof that aplastic frailty because of chloramphenicol might be related with ensuing improvement of leukaemia (RxList, 2018).

2.3.1.3. Amoxicillin

Amoxicillin with imperial blue hazy top and pink obscure body, comprises 250 mg and 500 mg amoxicillin as the trihydrate. The top and body of the 250 and 500 mg containers are engraved with the name amoxicillin respectively. Every amoxicillin contains 500 mg or 875 mg amoxicillin as the trihydrate. Each film-covered, container formed, pink tablet is debossed with amoxillin focused more than 500 or 875, individually (RxList, 2018).

Amoxicillin is designated in the treatment of contagions because of vulnerable (only β-lactamase–negative) isolate species of *Streptococcus*, *Staphylococcus* spp. *Streptococcus pneumoniae*, or *Haemophilus influenzae*. Amoxicillin is used to treat infections due to the nature of susceptibility of isolates of *Proteus mirabilis*, *Escherichia coli*, or *Enterococcus faecalis*. It is also observed in treating infections due to the susceptible isolates of *Strepto* (α- and β-haemolytic isolates only), *S. pneumoniae*, *H. influenza*, or *Staphylococcus* spp.

Amoxicillin, when combined with clarithromycin in addition to lansoprazole given as treatment therapy, is recorded in its usage in patients with *H. pylori* infection and colonic abscess. The risk of recurrence of duodenal ulcer has been shown to be reduced as well. Amoxicillin, has been recorded to be active in patients with *H. pylori* when combined with lansoprazole capsules as dual therapy when such patients are intolerant to clarithromycin or are suspected to have some form of resistance (RxList, 2018).

Continuing treatment even when the patient has been observed to be asymptomatic is slated for between 48 to 72 hours beyond proof of bacterial eradication. Recommendable is for treatment to be at least 10 days when the infection is confirmed to be caused by *Streptococcus pyogenes* to avoid the risk of acute rheumatic fever. Therapy may be required for several weeks in some infections when it becomes necessary to continue clinical monitoring after therapy has been discontinued.

2.3.1.4. Ciprofloxacillin

Ciprofloxacillin comes as a mixture of the sesquihydrate and a monohydrate. $C_{17}H_{18}FN_3O_3$ $HCl.H_2O$ is the empirical formula of the monohydrate with a molecular weight of 385.8 whilst the sesquihydrate also has a formula of $C_{17}H_{18}FN_3O_3$ $HCl.1.5$ H_2O a with a molecular weight of 394.8 respectively (RxList, 2018). Being yellowish crystalline substance, the placebos in there include titanium dioxide, magnesium stearate amongst others. Ciprofloxacillin is known

for the treatment of unproblematic urinary tract diseases (UTIs) brought about by *Escherichia coli*.

Since fluoroquinolones have been incidental with genuine unfavourable responses and for certain patients, uncomplicated UTI (intense cystitis) is self-constraining, this is saved for treatment of uncomplicated UTIs (intense cystitis) in patients who have no elective treatment alternatives. Ciprofloxacillin is demonstrated for the treatment of confounded urinary tract contaminations (cUTI) brought about by *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and acute uncomplicated pyelonephritis (AUP) caused by *Escherichia coli* (RxList, 2018).

The successfulness and practicality of treating contaminations other than urinary tract diseases has not been illustrated.

To decrease the development of drug-resistant bacteria and maintain their efficacy, other antibacterial drugs are advised for proven strains or those known to be strongly susceptible. Should culture and susceptibility data be available, these must be well-thought-out in adjusting the therapy. In their absence, resident epidemiology can contribute to empiric choice of therapy.

Accurate susceptibility tests must be performed in order to isolate and identify the causative agents. However, treatment can be started before outcomes are known and continued when they are verified.

Likewise, with different medications, some isolates of *Pseudomonas aeruginosa* can acquire resistance rapidly in the presence of treatment. Symptoms may include possible irreversible grave antagonistic reactions, tendinitis and rupture of tendons, hypersensitivity, hepatotoxicity, development of drug resistant bacteria amongst others.

The human cytochrome P450 1A2 (CYP1A2) mediated metabolism is inhibited by Ciprofloxacillin and therefore given in combination with medications basically metabolized by

CYP1A2 brings about plasma concentration increment of such drugs and could prompt to clinically critical adverse effects of the co-administered medication. Ciprofloxacillin has a contraindication record in persons with hypersensitivity to quinolone class of antibacterial medications (RxList, 2018).

2.4. TOOLS FOR SCREENING COUNTERFEIT DRUGS

The proliferation of adulterated drugs particularly in resources limited countries with poor pharmacovigilance strategies have necessitated the need for innovative strategies and technologies for routine monitoring of the drug market. The popularity of the internet and e-commerce have even compounded current efforts of fighting the high incidence of adulterated herbal medicines on the Ghanaians market. A recent systematic review compared a collection of technologies and methods that can be used in Low-and Middle-income Countries (LMiC) and assigned a suitability score which ranges from 0-8 to each of the methods (Kovacs, 2014). The scores were based on some parameters such as the need for electricity, need for sample preparation, need for reagents, portability, level of training required, speed of analysis. In addition, the scores were augmented with prices of these tools where the lowest cost was <$10,000, medium cost $10,000-100,000 and highest cost was >$100,000.

Tools ranging from expert checklist to mass spectrometry were compared. The study highlighted the fact that no single tool could serve all requirements under resource-limited conditions. At the moment there are 42 distinct technologies that could be used for detecting substandard or counterfeit drugs. These technologies range from commercial portable tools requiring basic laboratory skills to ones which require high expertise or domain knowledge (Roth *et al.,* 2018). It must be emphasized that not all tools are capable of being deployed on the field for routine investigation or testing of adulterated drugs, largely due to their size,

sample preparation needs, expertise and need for power or electricity (Jackevicius & Glassman, 2014).

Conversely, recent spike in the counterfeit or adulterated drugs worldwide has inspired the institution of the Counterfeit Drug Forensic identification Network (CODFiN), which is a group of laboratories around the world that promotes the testing of suspected substandard or adulterated drugs (Almuzaini *et al.,* 2013). Therefore, a standard workflow has been developed by CODFiN for detecting substandard or adulterated medicines. The first step of the workflow is visual inspection of the packaging using standard protocols or a checklist. Afterwards, quantitative High-Performance Liquid Chromatography (HPLC), Raman and Near-infrared spectroscopy and colorimetric tests are performed for the detection of the correct active pharmaceutical ingredient (API). Henceforth, in order to ensure that the correct amount of API is present, dissolution testing is carried out. However, given that some of the drugs do not pass visual inspection, ambient mass spectrometry (MS) analysis is performed on such drugs to approve the occurrence of a forged drug. In the same way for drugs samples that have been established to be a counterfeit drug, isotope ratio MS, X-ray Diffraction (XRD), and nuclear magnetic resonance (NMR) are carried out to help categorise the geographic source of production of the falsified medicine for forensic purposes (Quist & Östling, 2002).

2.4.1. Technologies for Visual inspection of Adulterated Drugs

The WHO medicine testing guidelines recommends both quantitative and qualitative approaches in order to support the authenticity of the medicine (Kosack *et al.,* 2017). Some of these qualitative tests include visual inspection, colourimetric test or checking for tablet or capsule disintegrations. However, the initial steps in determining whether a drug is counterfeit or adulterated, is by visual inspection of the packaging or some security features deposited on the packaging or drugs. This is also the first step in the CODFiN workflow for the detections

of counterfeit or adulterated medicine in the market or on the field. Although this technique is subjective and could vary from person to person, it has served as one of the first line detection techniques that would decide whether subsequent or confirmatory tests should be carried out on the drug. So far, the widely used method has been the WHO checklist for visual inspection of medicine to identify suspicious products for further examination (Buckley & Gostin, 2013). The tool was designed to support health professionals in spotting signs of imitation such as inappropriate packaging, cataloguing, and portrayal of dosages and physical characteristics of tablets or capsules. In addition, misspelling or absence of expiry date or batch number are key indicators for checking whether the drug is genuine or compromised. It also offers guidelines on how to report such incidence to appropriate food and drugs regulatory authorities. However, one key challenge with visual inspection tests is that prior knowledge of the authentic manufacturer's packaging is required in order to compare market or field observations with. This limitation challenges proper and routine identification of adulterated medicines by most patients, particularly, in developing countries. Therefore, combining visual inspection with other quantitative and laboratory approaches remains one of the surest ways of properly detecting adulterated or counterfeit medicines in the Ghanaian market (Ofori-Kwakye, 2014).

2.4.1.1. Atomic force microscopy (AFM)

The atomic force microscopy (AFM) instrument is another tool used for visual inspection of counterfeit drugs (Lal & Arnsdorf, 2010). This minimally destructive nanotechnology functions by reading molecular watermarks embossed in the course of authentic manufacture of drugs (Kovacs et al., 2014). Conversely, its application extends to forensic examinations of bloodstains, investigation of textile fibres, fingerprints examinations, document forgery detection and gunshot or explosive residues analysis largely due to its greater resolution and minimal sample preparation and destruction requirements (Rapp-Wright et al., 2017) AFM,

however, requires a climate-controlled facility and experienced chemists for its operations in addition to its average medium cost (~USD100,000) therefore making its use challenging (Senapati & Lindsay, 2016).

2.4.1.2. GPHF Minilab kits

The introduction of the Minilab by the Global Health Pharma Fund (GHPF) has significantly affected the way verification of the authenticity of drugs are carried in the market. The tool combines both physical and chemical screening tests to ensure that the findings from these tests comply with the information on the label, at least for drug identity and content. Any inconsistencies render the decision making indecisive; therefore, the entire batch of such medications are transported to reference laboratories or centre of excellences across the countries for further testing (Galea, 2016). The Minilab protocol comprises three steps for detecting substandard or falsified medications. The first step includes series of physical examinations of the dosage forms and packaging of medicine for prompt rejection of counterfeits or substandard drugs. The second step, which applies to tablets or capsules, involves a simple disintegration test of tablet or capsules to confirm claims on enteric-coating and other modified-release systems. The final step of the Minilab protocols employs a chemical test to supplement visual inspections and involves a simple thin-layer chromatographic test to verify the contents of the drug in line with the label information about the potency of the drug (Jähnke, 2004).

The flexibility, low-cost, minimal sample preparation requirements, relatively small size of test kits, minimal expertise requirements are reasons making it an ideal tool in low-middle-income countries such as Ghana. The GPHF Minilab is reproducible and can be adapted to any setting of choice. A recent study reported on the surveillance of falsified and sub-standard dugs in several African and Asian countries by resident establishments using the GPHF Minilab kits

(Petersen *et al*., 2017). In all, 10 organizations across Africa and Asia were selected to collect approximately 100 medicine sample each for preliminary analysis using Minilab at the respective institutions followed by confirmatory studies at a WHO references laboratory in Kenya. Unsurprisingly, out of the about 869 drug samples analysed, it was revealed that 21 were inferior or counterfeit therapeutic goods with some 12 not containing the API and six (6) without sufficient volumes of the API. However, another 3 samples were reported to show unsatisfactory dissolution of the API. In addition, Lalani and colleagues (2015), used the GPHF Minilab kits for detection of substandard antimalarials (including tablets, ampoules, and syrups) available on the Afghanistan market, another resource limited country. Out of the total of 7,740 medicine samples checked with the GPHF Minilab, it was showed that 134 failed the preliminary authenticity check (Jähnke, 2004). GPHF Minilab has also been used in Ghanaian settings (Accra) where 24 drugs failed the test out of the 94 medications sampled (Jahnke, 2004).

2.4.1.3. Counterfeit Detection Device #3 (CD3)

Counterfeit Detection Device #3 (CD3) is an addition to the range of tools based on visual inspection for the detection of falsified or adulterated drugs (Kovacs, 2014). This technology works on the principle of being able to allow examination of the labels and packaging together with the core capability of detecting the active pharmaceutical ingredient. CD3 is one of the handheld devices that can be deployed on the field at country borders, warehouses and pharmaceutical outlets for routine and inexpensive detection of adulterated medications. The device is made of series of single wavelength light-emitting diode (LED) light sources which includes the spectral regions from ultraviolet (UV) to infrared (iR). In addition, the device has been fixed with two charge couple device (CCD) cameras for real time imaging of samples under investigation allowing for videos as well. The cameras have been designed in such a way

that one operates in the UV-visible spectral region while the other one functions in the iR spectral region. However, it can be connected to high powered microscope to examine suspected drugs under greater magnifications.

The principle behind the tool is that the light from the LED interacts with the ink and tablet colour on the package and dosage form surfaces therefore permitting the user to observe differences between samples and genuine drugs for verification. Images could then be taken of any observation with the camera. The device has memory storage chip to store images following analysis for subsequent views and could also be connected to a personal computer for data management and version control. The device is battery powered and could last between 3 and 8 hours depending on the frequency and intensity of use in a day (Ben Amar *et al.*, 2015). Conversely, although visual inspection remains one of the cost-effective and portable ways of detecting counterfeit or adulterated medications, perpetuators have also devised sophisticated ways to evade drugs monitoring and testing authorities. Therefore, pinning down the authenticity of such drugs may involve a combination tools such as including visual inspection and chemical or laboratory analysis.

2.4.2. Technologies for Detection of Correct Active Pharmaceutical Ingredient (API)

Aside the examination of packaging and dosage to infer the genuineness of a medicine, there are now several tools that could be employed to identify the correct active pharmaceutical ingredient in any medication (Kiivet, 2007). This is to help establish the presence or absence and even quantify or estimate the amounts of API in the medication. Others can even detect the functional groups or establish novel chemical groups of the API. Taken together, this step-in drug testing might help individuals know whether the drug is potent or harmful for its intended consumers. Therefore, the rest of the section focuses on techniques for detecting the correct API in counterfeit or adulterated herbal medicines. Technologies for correct API

detection may differ in size, cost requirement for sample preparations, requirement of highly skilled personnel to operate. While some of these tools are portable and can be adopted for field or drug surveillance at pharmacies, ports, borders, and other gateways to the market, some require a laboratory to operate, therefore, cannot be used in the field (Hamilton *et al.*, 2016).

2.4.2.1. Portable Techniques for the Detection of the Correct API

Portable devices include Raman and near-infrared (NIR) light spectroscopy, the counter detection device #3 (CD3) nuclear quadrupole resonance (NQR) spectroscopy, thin layer chromatography techniques such as fast chemical identification systems (FCIS), the GPHF-MiniLab and speedy apparatus and paper chromatography test cards. Portable and low-cost detection devices are well suited for use in low resources settings or countries for rapid screening of adulterated medicine. Among the above-mentioned techniques, CD3 remains the widely used tool deployed for field testing and both laboratory testing of adulterated drugs. This is because of its light weight, minimal cost, and no sample preparation requirements and relatively little expertise requirement to operate compared to NIR and Raman portable devices. Despite, the several accolades of this technique, its sensitivity and specificity remain to be widely studied and compared to other portable detection techniques such as Raman and NIR devices. However, when NIR and Raman were compared, both technologies were reported to be superior, in terms performance, to CD3 but NIR performs relatively better than Raman (Kovacs *et al.*, 2014).

In the previous section, the GPHF-MiniLab and counter detection device #3 (CDD3) were discussed and found that FTIR, Raman and NIR are all devices which can be employed in the field by personnel with limited coaching or expertise while their cost may not exceed $50,000. However, all these portable devices, including CD3, are limited by the fact that they rely on utilizing reference libraries of pharmaceutical catalogues to fish out substandard medical

products which therefore needs to be curated regularly whenever new generics or compounds emerge on the market. There are thus serious practical limitations which warrants co-operation by many players in the anti-counterfeit or anti-adulterated drug industries, particularly to share data on the sources, activities and current trends in counterfeit or adulterated drug market. However, some laboratory-based techniques such as x-ray diffraction and NQR are being developed as portable devices for routine or field examination of adulterated medicine (Kiivet, 2007).

2.4.3. Laboratory Based Devices for the Detection of Counterfeit Medicines

Laboratory-based devices include Fluorescence Spectroscopy, powder X-ray diffraction, high performance liquid chromatography (HPLC), Anion Exchange Chromatography, Capillary Electrophoresis, nuclear magnetic resonance (NMR) and gas chromatography (Fernandez *et al.*, 2011). Among these techniques, HPLC serves as the bench mark for biochemical separation, quantification and identification in most laboratories (Deconinck *et al*., 2013). HPLC requires high expertise and sample preparation. Its energy costs (electricity) are quite high and requires specialized reagents for running experiments. HPLC initial cost, approximately $50,000 restricts its use to specialized laboratories in most developing countries (Deconinck *et al*., 2013). HPLC testing forms an integral part of the CODFIN workflow and remains the first test to be run when suspected medical products arrive at the laboratory.

Another addition to the technologies for confirmatory studies of counterfeit or adulterated medications is the mass spectrometry (MS). Mass spectrometers are limited by their high operating cost and requirements for highly trained technicians to work on samples. In addition, the technique requires sample preparation and processing which relies on experts to achieve the best results. It can, therefore, be used for identification and quantification of APIs, adulterants. In a previous study, high-pressure liquid chromatography-electrospray tandem

mass spectrometry (LC-ESI-MS-MS) was used to detect synthetic adulterants in herbal medicinal products (Bogusz *et al.*, 2006). The products that were tested included analgesics, antimicrobial agents, aphrodisiacs, hormonal and anabolic drugs, weight altering drugs, and psychotropic drugs. Moreover, core-shell column that is coupled to tandem mass spectrometer was used in a recent study to determine common adulterants in food and herbal medicinal samples. From the study it was revealed that 7 out of the 33 herbal medicinal products sampled contained adulterants of different types. The major adulterants were tadalafil, sildenafil, and vardenafil as well as sibutramine (Al Lawati *et al.*, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

YMC Jsphere ODS-H80, 150 × 4.6mm (YMC, USA). Antibiotic Standards (Sigma-Aldrich). Binary Pump HPLC with a Wave Quest (CE 4300 UV/ViS Detector, Cecil-Adept, UK). Acetronitrile (HPLC Grade). Acetic Acid (Sigma-Aldrich, USA), C18 300mm × 3.9mm, 5um (Waters incorporated, USA). C18 SPE Catridge (Waters incorporated, USA).

3.1.1 Study Site

In Ghana, Kumasi Metropolis being one of the major capital cities was chosen as the site to conduct the study. Ghana happens to be one of the popular countries in the West African sub-region. Gold Coast used to be its colonial name. One of its big cities is Kumasi with English as the official language (Ernest, 2019). The population is approximately 28 million (Ghana Statistical, 2019). Ghana has a very steady growing economic prosperity and improving political inheritance has stated its place in the regional power forming alliances with Commonwealth Nations, the African Union and Economic Community of West African States.

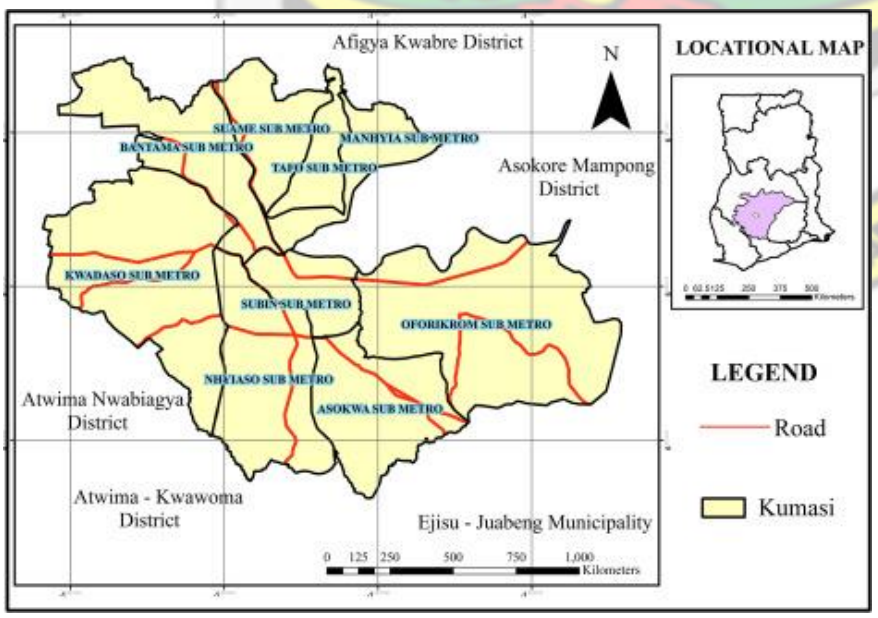


Figure 3. 1 Map of Kumasi Metropolis

Ashanti region of Ghana has Kumasi as its capital. It is the second biggest city in Ghana after Accra. Kumasi Metropolis constitute a total population of 2 million people (Ghana Statistical Services, 2019) spanning a vast area of 500 kilometers. Kumasi Metropolis is situated 200 kilometers north from the Gulf of Guinea. In Kumasi there are several endowed pharmaceutical and licensed local drugs and chemical drug sellers. Most of these pharmaceutical and standalone drug sellers have in stock local herbal medicine. Drug sellers are normally untrained with an inefficient drug regulation enforcement agency (Deye *et al.*, 2014). Some virtually cannot read and write at all and only rely on info passed on by predecessors. Producers of these drugs are commonly termed as traditional herbalist.

3.1.2 Study Design

For the purpose of this study, a random sampling design was deployed in Kumasi Metropolis. Samples of various herbal antimicrobial medicines were acquired from the market and analysed for the detection of chloramphenicol, amoxicillin ciprofloxacillin and trimethoprim.

3.1.3 Sampling

A total number of 21 herbal medications in the form of liquids, powders, capsules and creams were sampled. The following products were acquired for the research; Five (5) liquid samples code named LS-1 to LS-5 were obtained as part of the sampling. One of them had an FDA number whilst the others did not have any notable FDA numbers. All samples had manufactured dates with their corresponding expiry dates. The powdered samples code named PS-1 to PS-3 were three in number. Though they were acclaimed to be herbal and not expired by some consumers and the salesmen, none of these powdered samples had FDA numbers. All samples also did not have manufacturing and expiry dates. Herbal medicines in the form of capsules were identified as CS-1 to CS-6. Five of them had no FDA numbers with only one

showing a readable FDA number. With the exception of CS-3 which did not have manufacturing date and an expiry date, the other samples had these dates. Cream samples were seven in all. They were code named CrS-1 to CrS-7 with four of them lacking FDA numbers. There were five of them with inscribed manufacturing and expiry dates and two that did not have.

3.1.3.1. Inclusion criteria

For the purpose of this study, all samples or products used constituted both locally and foreign manufactured herbal medicines that can be found on Kumasi Metropolis, Ghana. At the time of visit there was a strict observation of selecting only new and sealed products.

3.1.3.2. Exclusion criteria

Old and expired products, including broken sealed herbal products formed part of the exclusion criteria. All orthodox medication of any brand was not considered for the purpose of this study.

3.2. METHODS

Samples considered for the study had to go through a two-step selection criterion, namely; visual inspection and laboratory analysis.

3.2.1. Visual Inspection

There was a purposive sampling of the products used. The area chosen for the study is the hub of herbal medicine sales and can be adequately accepted to be where most buyers will visit. To make sure the right samples are selected the visual inspection protocol was used. The World Health Organization (WHO) 'tool for visual inspection of medicine' was used as first point reference for detecting spurious, substandard, falsified, falsely labelled and counterfeit (SSFF)

medical products. The packaging of the products was inspected to ensure they complied with the inclusion criteria stated for the purposes of this work.

3.2.2. Laboratory Analysis

For the purpose of this study, the high-performance liquid chromatography (HPLC) was deployed as the laboratory test for the chemical detection of selected adulterating components.

3.2.2.1. Sample Preparations

The liquid samples were loaded on hydrophilic-lipophilic balance (HLB) solid-phase extraction (SPE) cartridges (200 mg sorbent, 30 µm, 6 cm^3) procured from Waters Oasis (Massachusetts, USA). The SPE cartridges were conditioned with 2 mL MeOH twice and lastly with 2 mL Milli-Q water. A 100 mL portion of the samples were loaded onto SPE cartridges at a flow rate of 1.5 mL/min and allowed to dry for 10 mins. The washing of the dried SPE cartridges was done with 3 mL of 5% MeOH in water and then allowed to dry under vacuum for about 10 minutes. The sorbents were eluted with 3 mL MeOH at a flow rate of 1 mL/min and collected into a vial prior to HPLC analysis.

For the solid samples 100 mL of HPLC grade methanol was added to 10 g of samples and sonicated for 15 min. The solution was allowed to cool and SPE extracted using the same protocol for the liquid samples.

3.2.2.1. HPLC Conditions for the Chloramphenicol

Model: A Cecil-Adept Binary Pump HPLC with a Wave Quest CE 4300 UV/Vis Detector

Mobile Phase: Acetonitrile: Acetic Acid (4% v/v), 40:60 (v/v)

Column: YMC Jsphere ODS-H80, 150 x 4.6mm, 4um

Column Temperature: 30 ^{0}C

Flow rate: 0.5 mL/min

Wavelength: 275nm

3.2.2.2 HPLC Conditions for the Trimethoprim

Model: A Cecil-Adept Binary Pump HPLC with a Wave Quest CE 4300 UV/Vis Detector

Mobile Phase: Acetonitrile: Acetic Acid (10% v/v), 80:20 (v/v)

Column: Waters 300mm x 3.9mm, 5um

Column Temperature: 30 ˚C

Flow rate: 1.0 mL/min

Detector: UV/Vis

Wavelength: 275nm

Similar conditions as used for Trimethoprim were employed for the remaining two antibiotics (ciprofloxacillin and amoxicillin).

3.3. STATISTICAL ANALYSIS

From the study datasets, the figures were computed to means and standard deviations. These were compared across the various sample forms. All p values less than 0.05 were considered significant.

CHAPTER FOUR

RESULTS

4.1. VISUAL CHARACTERISTICS OF SAMPLES

Based on the inclusion and exclusion criteria, the tables below show the visual results of the various samples. The product types are liquid, powdered, capsule and cream samples with manufacturing and expiry dates for those that had and none applicable (N/A) for those that did not have. The food and drugs authority (FDA) and Ghana standards authority (GSA) numbers were also observed and stated where they could be recognized and N/A used in place of the ones that could not be seen. There were some ingredients such as *Vernonia amygdalina, Allium sativum, Vernonia conforta* and *Rauvolfia vomitoria* amongst others which were listed as part of the constituents of the various samples. Notably, synthetic antibiotics were not seen as part of the list of ingredients that were recorded on the samples.

4.1.1. Visual Characteristics of the Liquid Samples

As shown in Table 4.1, all five (5) liquid products had both manufacturing and expiry dates. None of the products had expired before the commencement of the study. Of the 5 liquid products, only one (20%) was certified by the Food and Drugs Authority (FDB/HD 17-03131). All the products had labels imprinted with product name as well as ingredients used for the manufacturing of the product.

Table 4.1. Visual Characteristics of the Liquid Herbal Samples

SAMPLE ID/CODE	Mfg Date / Expiry Date	FDA/GSA Certification/ ingredients
LS-1	Mfg Date: 10/04/2018 Expiry Date: 10/12/2020	N/A *Vernonia amygdalina, Mussaenda erthrophylla, Anthocleista nobilis*
LS-2	Mfg Date: 25/07/2017 Expiry Date: 25/12/2019	FDB/HD 17-03131 *Vernonia conforta, Ancistrophyllum secundiflorum, Securidaca longepedunculata*
LS-3	Mfg Date: 20/03/2018 Expiry Date: 20/03/2020	N/A *Daucus carota, Carica papaya, Butryrospermum parkli, Allium sativum*
LS-4	Mfg Date: 15/03/2017 Expiry Date: 15/03/2019	N/A *Momordica charantia, Rauwolfia vomitria*
LS-5	Mfg Date: 5/05/2017 Expiry Date: 5/05/2020	N/A N/A

4.1.2. Visual Characteristics of the Powdered Samples

None of the powdered samples had manufacturing dates and also no expiry dates. They also did not have FDA/GSA certification in as much as they did not have any ingredients listed on them.

Table 4.2. Visual Characteristics of the Powdered Herbal Samples

SAMPLE ID/CODE	Mfg Date / Expiry Date	DA/GSA Certification / ingredients
PS-1	Mfg Date: N/A Expiry Date: N/A	N/A
PS-2	Mfg Date: N/A Expiry Date: N/A	N/A
PS-3	Mfg Date: N/A Expiry Date: N/A	N/A

4.1.3. Visual Characteristics of the Capsule Samples

As shown in Table 4.3, five (5) capsule products had both manufacturing and expiry dates. None of the products had expired before the commencement of the study. Of the 6 capsule products, only three (50%) was certified by the Food and Drugs Authority with supposed numbers (FDB/HD 12-11184), (FDB/HD 16-9260) and (FDB/HD 17-04146) respectively. All

the products had labels imprinted with product name as well as ingredients used for the manufacturing of the product.

Table 4.3. Visual Characteristics of the Capsule Herbal Samples

SAMPLE ID/CODE	PRODUCT NAME/ Mfg Date / Expiry Date	FDA/GSA Certification / ingredients
CS-1	Mfg Date: 08/2017 Expiry Date: 08/2021	N/A *Crytolepsis sanguinolenta*
CS-2	Mfg Date: 09/01/2017 Expiry Date: 08/01/2019	FDB/HD 16-9260 N/A
CS-3	Mfg Date: N/A Expiry Date: N/A	FDB/HD 12-11184 N/A
CS-4	Mfg Date: 02/02/2015 Expiry Date: 02/02/2019	*N/A* *Momordica charantia, Ocimum viride, Micro demis zenker*
CS-5	Mfg Date: 01/2017 Expiry Date: 12/2021	FDB/HD 17-04146 *Momordica charantia, Ocimum viride, Microdemis zenker*
CS-6	Mfg Date: 07/11/2017 Expiry Date: 07/11/2020	N/A N/A

4.1.4. Visual Characteristics of the Cream Samples

Two of the seven cream samples had no manufacturing date and expiry dates. Apart from those without the dates, none of the products had expired per the dates stated on them. Of the 7 capsule products, only four (57%) were certified by the Food and Drugs Authority with numbers (FDB/HD 11-6048), (FDB/HD – 08-9151), (FDB/HD 13-916) and (FDB/HD 07-4051), respectively.

Table 4.4. Visual Traits of the Cream Herbal Samples

SAMPLE ID/CODE	PRODUCT NAME/ Mfg Date / Expiry Date	FDA/GSA Certification / ingredients
CrS-1	Mfg Date: 07/04/2017 Expiry Date: 07/04/2019	FDB/HD 11-6048 *Azadirachta indica, Hyslundia opposita, Cassia occindentalis, Ocimum greatissimum, Alchamea cardifolia, Citrus aurantifolia*
CrS-2	Mfg Date: 03/04/2018 Expiry Date: 03/04/2020	N/A *Cymbopogam citratus*
CrS-3	Mfg Date: 01/02/2017 Expiry Date: 01/02/2019	FDB/HD – 08-9151 *Daniellia ogea, Khaya ivorensis,Mineral oil, Paraffin wax*
CrS-4	Mfg Date: N/A Expiry Date: N/A	N/A Wonder powder and base
CrS-5	Mfg Date: 01/02/2016 Expiry Date: 01/02/2019	FDB/HD 13-916 Cassia alata, Aloe forex
CrS-6	Mfg Date: 12/2017 Expiry Date: 11/2019	FDB/HD 07-4051 *Cassia alata, Gossypium arboreum, Daucus careta, Carica papaya,* Petroleum jelly
CrS-7	Mfg Date: N/A Expiry Date: N/A	N/A N/A

4.2 LABORATORY ANALYSIS OF THE SAMPLES

4.2.1. Concentration of Antibiotics in the Liquid Herbal Samples

In all, 5 liquid samples (LS) were analysed using the HPLC for the occurrence of four antibiotics, namely trimethoprim (ug/g), chloramphenicol (ug/g), amoxicillin (ug/g) and ciprofloxacillin (ug/g). Two of the antibiotics were detected in liquid sample-1 (LS-1) which were chloramphenicol (140.66 ±1.67 ug/g) and to a lesser extent trimethoprim (0.36 ±0.01 ug/g). However, amoxicillin (ug/g) and ciprofloxacillin (ug/g) were not detected. Liquid sample-2 (LS-2) contained chloramphenicol (110.10 ± 0.18 ug/g) and to a lesser extent trimethoprim (0.16 ± 0.07 ug/g) but not amoxicillin (ug/g) and ciprofloxacillin (ug/g). LS-3 contained only chloramphenicol (2.59 ± 0.86 ug/g). LS-4 did not contain amoxicillin (ug/g) and ciprofloxacillin (ug/g) but had some levels of chloramphenicol (0.34 ± 0.03 ug/g) and trimethoprim (127.17 ± 0.57 ug/g). LS-5 did not contain three of the antibiotics

(chloramphenicol, amoxicillin and ciprofloxacillin) except trimethoprim. Generally, LS-1 recorded the highest concentration of chloramphenicol (140.66 ± 1.67ug/g) among the samples. Amoxicillin and ciprofloxacillin were absent from all liquid samples analysed (Table 4.5).

Table 4.5. Levels of Antibiotics in Liquid Herbal Samples

	Sample ID	TTP (ug/mL)	Chlo (ug/mL)	Amx (ug/mL)	Cipro (ug/mL)
Liquid	Sample 1	0.36±0.01	140.66±1.67	not detected	not detected
	Sample 2	0.16±0.07	110.10±0.18	not detected	not detected
	Sample 3	not detected	2.59±0.86	not detected	not detected
	Sample 4	0.34±0.03	127.17±0.57	not detected	not detected
	Sample 5	0.29±0.91	not detected	not detected	not detected

Values are mean ± SD of 7 determinations

TTP = Trimethoprim, **Chlo** = Chloramphenicol, **Amx** = Amoxicillin, **Cipro** = Ciprofloxacillin

4.2.2. Concentration of Antibiotics in the Powdered Herbal Samples

In the powered sample 1 (PS-1), Trimethoprim (356.00 ± 1.93 µg/g) and Chloramphenicol (2.07 ± 0.73 µg/g) were detected while the only antibiotic detected in powdered sample 2 (PS-2) was Trimethoprim (247.72 ± 1.04 µg/g). Sample 3 (PS-3) also contained only Trimethoprim (4.65 ± 0.94 µg/g) (Table 4.6). All powdered herbal samples examined did not contain amoxicillin and ciprofloxacillin.

Table 4.6. Levels of Antibiotics in the Powdered Herbal Samples

±	Sample ID	TTP (ug/g)	Chlo (ug/g)	Amx (ug/g)	Cipro (ug/g)
	Sample 1	356.00 ± 1.93	2.07 ± 0.73	not detected	not detected
	Sample 2	247.72 ± 1.04	not detected	not detected	not detected
	Sample 3	4.65 ± .94	not detected	not detected	not detected

Values are mean ± SD

TTP = Trimethoprim, **Chlo** = Chloramphenicol, **Amx** = Amoxicillin, **Cipro** = Ciprofloxacillin

4.2.3. Concentration of Antibiotics in the Capsule Herbal Samples

In total, six capsules were analysed to determine the occurrence of antibiotics and their respective concentrations. No antibiotic was detected in capsule samples 1, 2, 3, 4, and 5. The only antibiotics detected was Chloramphenicol (0.95 ± 0.04 ug/g) in sample-6 (CS-6) (Table 4.7).

Table 4. 7 Levels of Antibiotics in Capsule Herbal Samples

	Sample ID	TTP (ug/g)	Chlo (ug/g)	Amx (ug/g)	Cipro (ug/g)
Capsules	Sample 1	not detected	not detected	not detected	not detected
	Sample 2	not detected	not detected	not detected	not detected
	Sample 3	not detected	not detected	not detected	not detected
	Sample 4	not detected	not detected	not detected	not detected
	Sample 5	not detected	not detected	not detected	not detected
	Sample 6	not detected	0.95 ± 0.04	not detected	not detected

Values are mean ± SD

TTP = Trimethoprim, **Chlo** = Chloramphenicol, **Amx** = Amoxicillin, **Cipro** = Ciprofloxacillin

4.2.4. Concentration of Antibiotics in the Creamy Herbal Samples

As shown Table 4.8, samples 1 (CrS-1) and 6 (CrS-6) did not contain any antibiotic. CrS-3 and CrS-5 contained only Chloramphenicol antibiotics with concentrations of 0.41 ± 0.04 ug/g and 0.29 ± 0.03 ug/g, respectively. Similarly, samples 2 (CrS-2) and 4 (CrS-4) contained only amoxicillin with concentrations of 0.13 ± 0.07 ug/g and 0.12 ± 0.05 ug/g, respectively. Sample 7 (CrS-7) was observed to contain amoxicillin (0.16 ± 0.01 ug/g).

Table 4.8. Levels of Antibiotics in the Creamy Herbal Samples

	Sample ID	TTP (ug/g)	Chlo (ug/g)	Amx (ug/g)	Cipro (ug/g)
Creams	Sample 1	not detected	not detected	not detected	not detected
	Sample 2	not detected	not detected	0.13 ± 0.07	not detected
	Sample 3	not detected	0.41 ± 0.04	not detected	not detected
	Sample 4	not detected	not detected	0.12 ± 0.05	not detected
	Sample 5	not detected	0.29 ± 0.03	not detected	not detected
	Sample 6	not detected	not detected	not detected	not detected
	Sample 7	not detected	not detected	0.16 ± 0.01	not detected

Values are mean ± SD

TTP = Trimethoprim, **Chlo** = Chloramphenicol, **Amx** = Amoxicillin, **Cipro** = Ciprofloxacillin

CHAPTER FIVE

DISCUSSION

Some people believe that when they see products labelled “natural”, it signifies that they are safe and good. The debasement and substitution of herbal medications as “natural” is a serious issue threatening the herbal medication industry. The peculiar challenge is the way some proclaim the efficacy of their herbal products only for science to investigate and discover the use of synthetic and semi-synthetic substituents in place of the original natural ingredients (Little, 2009).

Natural drugs make up a significant segment of the trend toward alternative prescriptions. Herbal medicine is becoming even famous in this day and age as individuals search out normal cures to their infirmity. To rival the developing pharmaceutical market, there is a direness to use and scientifically approve all the more restoratively helpful natural items. The ascendency of the herbal industry is considered among the quickest developing ventures in Ghana and Africa in general. With the journey to give alternative health services and handle chronic conditions that are inert to standard prescriptions, home grown offices like centres and clinics are built up. The majority of these offices produce their very own prescriptions and furthermore may make a few items for the market. Some natural focuses or organizations anyway deal just in various types of herbal products.

In the present study, the occurrence and concentration of the above described antibiotics were investigated in the selected herbal samples on the Ghanaian market, specifically in the Kumasi Metropolis. Adulteration of herbal medicine is a major concern in most African and developing countries all over the world and may have negative consequences on the lives of individuals while straining public health budgets. While there may be several types of adulterants in herbal medicines, the present study focused on selected antibiotics within various forms of local medicinal products such as capsules, liquid, powdered and creams on the Ghanaian market,

specifically Kumasi. Some works done by Ofori-Kwakye and others with topical medications also revealed adulterations. The herbal ingredients in four 7/16 (25 %) of their samples were missing from the item labels. Eight of the topical herbal medications (50 %) were enlisted with the statutory medicines' regulatory authority in Ghana while the staying eight examples were unregistered (Ofori-Kwakye *et al.*, 2014).

Herbal medicines in liquid form are quite common on the Ghanaian market and used for the treatment of several ailments. They may be used as remedies for conditions such as malaria, typhoid fever, chronic diseases such as diabetes, heart diseases and many others among the population.

Adulterations have serious health implications to the body. Medication, for example, Sibutramine and fenfluramine, utilized in treating obesity have been found in some common 'natural' products for weight reduction. Sibutramine expands the danger of cardiovascular conditions like heart rate and blood pressure. Fenfluramine is also related with extreme lung and heart valve illness. Regular healthy skin items have additionally been found to contain corticosteroids; steroidal physician endorsed drugs used to treat provocative conditions, similar to joint pain, sensitivities, and skin issues like psoriasis and dermatitis. Corticosteroids have genuine reactions and these incorporate sporadic heartbeats, expanded circulatory strain, stomach ulcer, blood issue, skin, muscle and bone harm, and sensory system issue. Antidiabetic drugs like glibenclamide, and metformin have likewise been found in some natural items utilized for the administration of diabetes in different nations.

To determine the occurrence of selected antibiotics and their respective concentrations in liquid herbal medicine on the Ghanaian market, the HPLC was used to identify and quantify these antibiotics from selected samples. LS-1 had no FDA number on its container that could be observed. It had a visible expiry date of 10/12/2020 from a manufacturing date of 10/04/2018 which meant that according to the producers this product was good for consumption at the time

this research was conducted. The challenge here, however, is that the antibiotics found in the sample were not listed as active ingredients in the medicine. The listed active ingredients have a general effect on some bacterial infections. For example, *Vernonia amygdalina* as a perennial herb belongs to the plant family Asteraceae. Extracted active ingredients from this plant has scientific records of use in traditional medicine practice as cures against protozoal, bacterial and helminthic infections. *Vernonia amygdalina* has phytochemicals as numerous as flavonoids, lignans, edotides, steroids, sesquiterpenes, xanthones, phenolic acids, and saponins extracted and isolated from it (Farombi & Owoeye, 2011). *Mussaenda erythrophylla*, generally branded as Ashanti blood, red flag bush and tropical dogwood, is an evergreen West African shrub. The juice from the plant is used to treat eye infections. A decoction of the leaves is used to rid the body of intestinal worms. The root is used as a treatment for leprosy. The juice of the roots, combined with about 10% by volume of cow's urine, is used in the treatment of jaundice. The juice is also used to treat blemishes on the tongue while the juice of the bark is used in the treatment of body pain, diarrhoea and dysentery. The flowers are diuretic. They are used in the treatment of cough (Sheat & Schoefield, 1995). According to (Ngwoke *et al.*, 2018), the root bark of *A. nobilis* possess anti-diabetic activity, antiviral and anti-plasmodial activities, anti-Leishmanial activity, antibacterial, antioxidant activity, and wound healing properties. Aside these effects of the listed active ingredients, one may be tempted to question the source of the synthetic antibiotics that were found in LS-1. Thus, the presence of trimethoprim (0.36±0.01ug/ml) and chloramphenicol (140.66±1.67 ug/ml) is questionable as to how they found their way into the supposed herbal product without it being listed as a component even as amoxicillin and ciprofloxacillin were absent.

In LS-2 the active ingredients that were visibly listed on the packaging are *Vernonia conforta* (rightly spelt as *V. conferta*), *Ancistrophyllum secundiflorum* and *Securidaca longepedunculata.* The *Vernonia conferta* also known as Owudifukete in Akuapem is a Shrub

believed to heal diabetes when the root and bark are boiled as a decoction. This product had the 25/07/2017 as its manufacturing date and 25/12/209 as its expiry date with FDB/HD 17-03131 as the registered ID from the FDA. LS-2 contained Chloramphenicol (110.10±0.18 ug/mL) and to a lesser extent Trimethoprim (0.16±0.07 ug/mL) but not amoxicillin and Ciprofloxacillin. The antibiotics present may account for why it serves some of the purposes for which this drug is sold under the guise of herbal medicine.

LS-3 has *Daucus carota* (Carrots) listed as its active ingredient. *Daucus carrota* holds numerous phytochemical constituents like dietary fibres and carbohydrates. Also present are fats and proteins coupled with vitamins A, C, B6, K, and minerals like calcium, sodium, iron, zinc etc. Carrots are goldmines of antioxidants with the carotenoids, vitamins and polyphenols neutralizing the effects of free radicals which can neutralize the effect of free radicals which might be a cofactor for its claimed effect on bacterial that causes STD (Satish, 2017).

Carica papaya (Pawpaw) has widely been used in traditional practices to fight many ailments: warts, cancers, tumours, corns, and thickened skins are treated with the juice from pawpaw; in treating uterine cancers, syphilis haemorrhoids and removing mineral concentrations in urine its roots are known to be effective and even the unripe fruit used as a diuretic or mild laxative (Wolters, 2018). The bark of the shea tree (*Butyrospermum parkii*) is cooked and drank as a brew. The boiled shea tree bark is said to be potent against diabetes and STIs in some societies in Ghana. It is not surprising to come across it as listed on the sample (Muotono & Maanikuu, 2017). *Allium sativum* (Liliaceae), known as garlic, is a powerful aromatic bulb crop thought to originate from Kazakhstan, Uzbekistan, and Western China. Antioxidant capacity of garlic extract has been clearly demonstrated by several investigators. Against a wide range of conditions and throughout history, garlic has been used. Earlier studies have assessed its effect on a broad variety of organisms, including viral, bacterial, fungal, and parasitic infections. Common urinary pathogens are fought with garlic which contains allicin, a sulphur-containing

compound thought of as the most active ingredient in the bulb of a garlic. Even so, there are almost 100 compounds that may act in synergy to give its noted effects (Amagase, 2013). However, from the results table, it can be seen that LS-3 has Chloramphenicol present in the tested sample with the concentration of 2.59±0.86 µg/mL. The other antibiotics were not detected. This means there is a potential tainting of the already active ingredients as listed with this know antibiotic to complement its effectiveness.

LS-4 has Trimethoprim and Chloramphenicol detected in the sample. They had concentrations of 0.34±0.03 ug/mL and 127.17±0.57 ug/mL, respectively. LS-5 on the other hand, has Trimethoprim detected at a concentration of 0.29±0.91 µg/mL. Rauwolfia *vomitoria*, has its roots, stem and leaves being used in West African medicines. People use *Rauwolfia vomitoria* to induce vomiting and promote sleep in others while others use it to treat fever, arthritis pain and weakness. Countries including Canada and others have disqualified supplements containing *Rauwolfia vomitoria* extracts from the market. This is because some *Rauwolfia vomitoria* extracts comprise high levels of chemicals that are prescription drugs (Bisong, Brown, & Osim, 2011). In the presence of the listed active ingredients, the presence of these two antibiotics forensically makes this sample fall into the category of adulterated herbal medicine.

Momordica charantia grows in tropical areas of Asia, Amazon, East Africa, and the Caribbean. It is grown as a vegetable and for its medicinal potency. Some of its shared uses in most countries are for diabetes, as a carminative and in treatment of colic. Extracts from leaves of bitter melon have shown broad-spectrum clinical and experimental antimicrobial potencies (Grover & Yadav, 2004).

Three samples had no amoxicillin and ciprofloxacillin antibiotics in them. This is quite interesting given the most commonly prescribed class of antimicrobials in Ghana are the beta-lactam class of antibiotics in the order of amoxicillin, ampicillin and cefuroxime (Tagoe &

Attah, 2009). Therefore, the absence of amoxicillin in all five liquid herbal medications samples implied that this specific antibiotic may have limited contributions towards antibiotics resistance burden in Ghana. On the other hand, the two other antibiotics (Trimethoprim and Chloramphenicol) were found in three liquid samples. The concentration of Chloramphenicol was found to be higher than 95.13 ± 62.95 µg/mL than that of Trimethoprim 0.2867 ± 0.1102 µg/mL.

Following analysis with the HPLC, it was observed that capsule samples 1-5 contained none of the selected antibiotics understudy. This suggests that patients who consumed these medications were not at a higher risk of antibiotics resistance from trimethoprim, chloramphenicol, amoxicillin and ciprofloxacillin. Sample 6, however, contained chloramphenicol antibiotics which could have negative consequences on individuals who take them. This may be due to the fact that the stated infections that the sample claims to cure are indicative of the use of the antibiotic chloramphenicol that was found in the sample. Generally, none of these medications had expired per the stated expiry dates on them. They were expected to expire by 2020.

Trimethoprim (µg/g) was found in powdered samples 1, 2 and 3. Chloramphenicol was absent in two powdered samples but was present in one powdered sample.

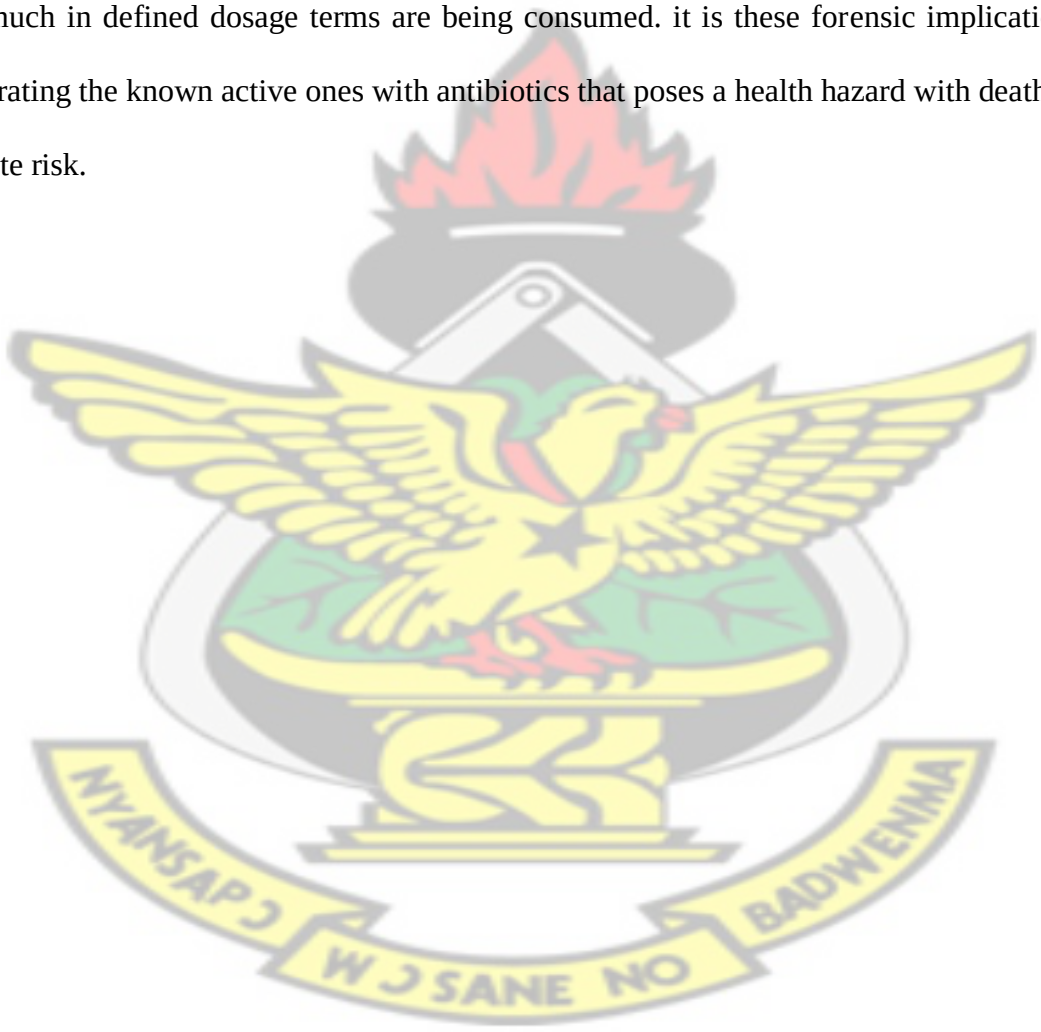
Moreover, despite the fact that the cream herbal medications samples were not primarily targeted towards antimicrobial treatments, 5 samples (samples 2,3,4,5, and 7) contained various amount of the antibiotics being studied including amoxicillin, the most frequently prescribed antimicrobial in Ghana. This suggests a lot of the cream samples sold on the market may be contaminated and could pose serious health risk to human life in the community. This is also the risk of antibiotic resistance which could threaten various infectious disease eradication programmes in the country such as tuberculosis. However, samples 1 and 6 recorded no antibiotics contamination.

Looking at the results, it confirms what a former health minister alluded to in 2014 that, "Adulteration of herbal preparations with orthodox medicines is inimical to the process and systems we are trying to build". There has been some banning of selected herbal samples in the past even as the FDA tried to regulate the adulterations of herbal medicines. The herbal medicines affected by the ban included *Rockman Capsules* produced by RockCare Clinic Limited; Mars for men imported by Joe D. Adventures and Tinatett Be4 Herbal Capsules produced by Tinattet Herbal Manufacturing and Marketing Co. Ltd. The rest were, Angel Natural Capsules, made by Angel Herbal Products Ltd.; Adom Gentleman Power Capsules fabricated by Dependable Pharmacy Ltd.; and Laud P Capsules made by Stephen Gyan Herbal Centre. Observedly, these listed banned products were not found in the area this research covered. This may be considered an effective compliance with the ban.

The FDA said the drugs were listed as herbal therapeutic items noted for male potency. However post-market surveillance exercises, led by the FDA, uncovered that the drugs have been tainted with Vardenafil, a manufactured drug utilized in the treatment of erectile dysfunctions. The unfavourable impacts of Vardenafil may have genuine consequences which incorporate cerebrovascular drain which can prompt strokes; heart attacks; palpitations and genuine heart problems including abrupt cardiovascular passing, as indicated by the FDA.

In India, Alam (2007), reports that the herbal medications are adulterated on the grounds that more often than not the efficacy of the herbal medications are not known and they act relatively slowly. So, to create snappy help from a side effect, some are contaminated with allopathic drugs. The normal allopathic medications utilized for adulterations are paracetamol, glibenclamide, ibuprofen, indomethacin, caffeine, and oral corticosteroids. Also, herbal medications are not regulated carefully by the regulatory authorities and subsequently it turns out to be simple for the general population to adulterate these drugs.

The ones that fell into the category of adulterated drugs under this research, however, raises questions about how frequent the checks are done. The fact that all these products were manufactured locally could be indicative of how traditional herbal medicines are widely used within the Kumasi Metropolis and the belief in the efficacy of these herbs as long as they are kept in such forms as were bought from the market. The other challenge is not knowing the exact dose of the adulterant in these herbal products. In the first place as the adulterants are not even stated on the medication packages, it is impossible for any "innocent" consumer to know how much in defined dosage terms are being consumed. it is these forensic implications of adulterating the known active ones with antibiotics that poses a health hazard with death as an ultimate risk.



CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

It was observed that 71.4% of the cream herbal medicines were contaminated with at least one of the selected antibiotics. Also, 75.0% of the liquid samples had various concentrations of the four antibiotics. All (100%) three powdered samples had various concentrations of selected antibiotics while the capsule herbal medicines showed the least (16.7%) contamination by the test antibiotics used. in all, 66.7% of the 21 different samples (in liquid, powdered, capsule, and cream forms) were adulterated with at least one of the selected antibiotics (chloramphenicol, amoxicillin, ciprofloxacillin and trimethoprim). The study recorded that most herbal medications on the Ghanaian market contained orthodox antibiotics and may predispose those who consume these medications without proper medical prescription to antibiotic resistance.

6.2. RECOMMENDATIONS

From the study it was observed that almost all forms of traditional antimicrobial products studied contained synthetic antibiotics which could put the entire population at risk of antibiotics resistance and its consequent effects. Herbal medications are commonly consumed in most Ghanaian communities even without prescription. Further studies must be conducted in other regions to determine if this is a national phenomenon. Although herbal medicine forms a major component of African traditional medicine, there is the need to regulate their proliferation and ensure they are prepared safely and under strict hygienic conditions. Following analysis, the study recorded various concentrations of selected antibiotics and, therefore, recommends that people who patronize herbal medicine should first of all be educated to look at the labels of these medications to determine whether they are buying the

correct medication from the manufacturer with respect to what is recommended by the FDA. Batch numbers printed on medications are also quick visual inspection methods that could be employed even before purchased. There is the need to educate people, especially in rural communities to visit only approved chemical stores and not to take these medications until necessary. Further recommendation is also being suggested to the Ministry of Health as well as the Food and Drugs Authority to ensure strict regulation in the licensing of some of the companies. Random checks at manufacturing houses and point of sales services could also mitigate the proliferation of these drugs and their negative effects on the entire population.

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