THE QUALITY ASSESSMENT OF SOME HERBAL PREPARATIONS PRODUCED IN GHANA

By

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CERTIFICATION

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

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DEDICATION

This thesis is dedicated to my Guardians, Mr. and Mrs. Nsiah-Poku, the CEO of the KINAPHARMA Limited, whose assistance, if had been denied, my dream for the pursuance of this programme, would have been completely shattered.

I say, may the Creator, nourish you and your family and cause every disease of yours to be cured.

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ABSTRACT

The quality of some herbal products on the Ghanaian market was assessed using harmonized procedures for assessing the quality of herbal preparations issued by Ghana National Drug Programme (GNDP) and Ministry of Health, Ghana.

A total of thirty (30) samples consisting of six (6) herbal mixture (liquid), six (6) herbal powder, six (6) herbal creams, six (6) herbal ointments and six (6) soaps were obtained for the study. These samples were subjected to various analyses. The parameters measured, were grouped as follows: microbial type and levels; (Escherichia coli, aerobic bacteria, yeast/moulds and salmonella Typhi), Organoleptic properties; (clarity, appearance, colour, taste and odour), physicochemical properties; (pH, total ash, sulphated ash, acid-insoluble ash, total solids and specific gravity); phytochemical screening for major constituents; (glycosides, alkaloids, tannins, saponins, flavonoids, anthraquinones, anthracene) and heavy metals; (mercury, arsenic, lead and cadmium).

Results obtained in the study suggest that the parameters tested for all the samples from three batches of every herbal preparation showed reproducibility in the preparation of the products. When compared with quality standards where applicable, it was found out that the results were all below the WHO quality standards.

The quality of the herbal preparations assessed is acceptable based on comparison with the World Health Organization (WHO) quality standards.

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CHAPTER ONE

1 INTRODUCTION

1.1 DEFINITION OF HERBAL PREPARATION

According to the World Health Organisation (WHO), ‘’Herbal Preparations’’ contain plant parts or plant material in the crude or processed state as active ingredients and may contain excipients (foreign substances) (WHO, 1996a; Busse, 1999). Combinations with chemically defined active substances or isolated constituents are not considered herbal preparations (Busse, 2000; GNDP, 2004). Similarly, the European Medicine Evaluation Agency (EMEA) defines herbal preparations as medicinal products containing exclusively herbal drugs or herbal drug preparations as active substances (WHO, 1996b; Busse, 2000). Several constituents with different pharmacological targets are involved in the therapeutic action of herbal preparations. This characteristic may be an advantage compared to single isolated compounds, especially when the underlying disease has a multifactorial etiology which is the case in many chronic illnesses.

Herbal preparations are comminuted or powdered plant material, extracts, tinctures, fatty or essential oils, expressed juices, processed resins or gums and so forth prepared from different plant parts such as roots, bark, stems, leaves, and fruits whose production involves a fractional, purification, or concentration process (Evans, 1989; Evans, 1996). Based on the European Medicine Evaluation Agency (EMEA), Ghana Food and Drugs Board and WHO quality guidelines, the herbal drug or preparation in its entirety must be considered as the active ingredient. It is evident from this and from the fact that herbal medicines are complex mixtures of substances that great effort must be made to ensure quality.

1.2 Difference between Herbal Preparation and the Orthodox Drugs

The classical pharmaceutical development concept is targeted at isolating a single component from a medicinal plant which can later be manufactured synthetically or extracted on a large scale (e.g. taxol from taxus baccata for anticancer therapy). Such a product does not fall under herbal preparation. It is a fact that complex herbal extracts have been shown to be therapeutically active and safe (Linde et al., 1996; Oken et al., 1998).

1.3 Marker Compounds and uses of Herbal Preparations

Some compounds referred to as marker compounds in herbal preparations have been identified and are known to have some relationship to the reported health benefit of herbal preparations. Such compounds have since become useful parameters for assessing the quality of herbal preparations (WHO, 2005a).

Herbal preparations have been used in the treatment of many diseases including malaria, jaundice, menstrual pain, waist pain, piles, delay in ejaculation, hypertension, rheumatism, and many others in Ghana.

Herbal preparations are not only used in Ghana but also in developed countries such as UK, China, United States, India, for the treatment of mood disorders, particularly depression; for relief of anxiety and stress, insomnia; treatment of urinary tract infection, decrease kidney stone; to lower cholesterol levels and blood pleasure, as immune stimulants that help increase resistant to cold, relief from migraine headache and arthritis, healing of wounds; burns; skin ulcers; heart failure; hypertension (CSIR (TTC), 1992; Gulla et al., 2001).

Examples of these preparations include; ginseng, St. John wort, Dan shen, Kava, Comfrey, Dong quai, Siberian, Ginkgo biloba, Valerian, Saw palmetto, Feverfew, Garlic, Ginger, Cranberry, Aloe, Chan Su, Cat's claw, Asian ginseng, Soy milk, Ephedra, Calamus, Chaparral, Licorice, Shankhapushi, Borage oil, Senna, Pokeweed, Hawthorn (Wahed and Dasgupta, 2001; Dasgupta et al., 2002). Herbaquin, Angel Natural capsules, Angel Cream, Class Malacure, Engel Herbal mixture, Mighty power, Living bitters, Angel Fatwikɛkɛ, Top tonic, Stomach Care Ulcerplex, Karafi bitters, Ahuodzen ancient herbal brew, Yafo man capsules.

1.4 STATEMENT OF THE PROBLEM

Over the recent years, the worldwide interest in herbal products has grown significantly, particularly, in the United States and some developing countries such as Nigeria, Togo, Ghana (CSIR (TTC), 1992; GNDP, 2004).

The advanced knowledge about their composition has been acquired through the introduction of new analytical techniques. At present, however, there are still large international differences in the regulatory status of herbal preparations (Busse, 2000).

In the United States for example, herbal products may be introduced as dietary supplements with limited quality data on the package. In contrast to this, pre-market approval, which means the submission of quality and other therapeutic data, is mandatory for the authorization of herbal preparations in most European countries (Ko, 1998; Busse, 1999).

Germany, France, Sweden, Denmark and Switzerland have established specific national regulations concerning the evaluation of the quality parameters of herbal products. Other countries such as the Netherlands, the United Kingdom and Portugal

evaluate them in the same way as pharmaceuticals. In dietary supplement market in the United States, herbal products are easily available to the general public (Busse, 2000; Ang-Lee et al., 2001). A major drawback of a missing premarket control is that products which contain potentially unsafe or undeclared levels of toxic contaminants or do not contain the labeled amount of constituents may be introduced.

In countries such as India, China and UK work has been done in the field of quality assessment of herbal preparations (Busse, 2000).

In Ghana, however, even though, there is proliferation of herbal products on the market, not much has been done in this field. The producers of the herbal preparations in Ghana do not have the required laboratories or expertise to perform quality control on the preparations they produce. This brings the problem of inconsistency on the quality of the herbal preparations in the country. So far no information on the quality of the herbal preparations that have been produced in the country is available (GNDP, 2004). This work therefore, seeks to evaluate the quality of some herbal preparations on the Ghanaian market using harmonized procedures.

1.5 OBJECTIVES

- To determine microbial levels such as the microbial types including aerobic bacteria, yeasts/moulds, Escherichia coli and salmonella Typhi from some herbal preparations produced in Ghana.
- To evaluate organoleptic properties such as colour, odour, touch, taste and clarity of some herbal preparations produced in Ghana.

- To determine the Physico-chemical properties such as pH, Ash value, Total solids, in some herbal preparations produced in Ghana
- To determine some phytochemical constituents such as alkaloids, phenolic compounds, coumarins, saponins, glycosides in some herbal preparations produced in Ghana.
- To determine heavy metals Hg, As, Cd, Pb in the herbal preparations.
- To compare the results with the available quality standards of the World Health Organization (WHO) to see if they meet the quality requirements.

1.6 JUSTIFICATION

In the recent past, WHO has adopted a deliberate policy of encouraging the development and utilisation of traditional medicine in the Primary Health Care delivery system, particularly, in the third world countries.

This is based on the sound recognition of the role that traditional medicine is already playing in the health care programme in most developing countries such as Ghana.

In Ghana, it is estimated conservatively that between 60 – 90% of the general population rely on medicinal plants either totally or partially for their health care needs (CSIR (TTC), 1992).

It is therefore imperative to ensure quality of the preparations derived from the traditional plants by using modern techniques and applying suitable standards.

The Food and Drugs Law in Ghana mandates the Ghana Food and Drugs Board (FDB) to implement regulatory measures that aim at achieving high standards of quality of food and drugs, including herbal preparations (GNDP, 2004).

As the quality of the herbal preparations is the key parameter for safety and efficacy of the drugs (WHO, 1998), it is important to assess them all the time. This became evident when it was reported that more than 150 manufacturers, distributors and retailers received plantation potentially contaminated with digitalis (Slifman et al., 1998). This highlights the importance of the correct identification and quality assessment of herbal preparations.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 QUALITY REQUIREMENTS

Although there are differences between herbal preparations and chemically synthesized products, the basic quality requirements are the same, which is identity, purity, and content determination, the standards for good manufacturing practices (WHO, 1996b) and the labeling requirements for the containers. In 1968, the American Pharmaceutical Association and the Academy of Pharmaceutical Sciences published a book with definition of a quality of a drug product which is still valid and is also applicable for herbal medicinal products. The definition is quoted as follows:

1. The active ingredients labeled on the package should be contained in the respective tolerance range,
2. The same amount of active ingredient should be in each dose-unit and each batch.
3. The drug should be devoid of impurities,
4. The drug shall retain its active ingredients and efficacy until its usage,
5. The drug shall release the active ingredients on usage in such a way that they are bioavailable (Busse, 2000).

2.2 Quality of the Starting Material

Quality assessment starts at the level of raw material. The plant material is the most important factor in manufacturing herbal medicinal products. Plants are inevitably ‘irregular’ because their composition may be influenced by multiple factors, such as origin, growth, harvesting, drying, and storage conditions. Through the use of cultivated

plants, some causes of variability may be eliminated. To ensure a consistent quality of the product, however, it is necessary to utilize starting material from different geographical sources. This ensures compensation of annual variations which may occur despite a constant geographical source. Adulterants must be taken into consideration when the material is purchased from commercial sources. Cases of intoxication due to misuse of plantation and digitalis lanata raw material have been reported from the United States (Busse, 2000).

More than 150 manufacturers, distributors and retailers received raw materials potentially contaminated with digitalis (Slifman et al., 1998). This highlights the importance of the correct identification and quality assessment of herbal drug.

Consistent quality for products of herbal origin can only be assured if the starting materials are defined in a rigorous and detailed plant material use. The exclusion or the limitation of impurities such as other plants or foreign matter, microorganism, and their metabolites (aflatoxins) is important.

The European Pharmacopoeia has published limits of pesticides which must be taken into account. Limit for heavy metal content have not yet been harmonized in Europe, so that national regulations apply, for example, 5ppm for lead, 0.2ppm for cadmium and 0.1ppm for mercury in Germany (Baver, 1998). The United States Pharmacopoeia proposes a limit of 20ppm for total content of heavy metals in herbal extracts (USP, 1999). Control of heavy metals is of relevance as they have been involved continuously in reports on toxic reactions after administration of traditional Chinese and India herbal preparations (Shaw et al., 1997; Chu et al., 1998). The California State Department of Health published the results of an analysis of traditional Chinese

medicines marketed in United States as dietary supplements showing high amounts of the following heavy metals: lead (10 – 319ppm), arsenic (20 – 114000ppm), mercury (22 – 5070ppm) (Ko, 1998).

2.3 Quality of the active Ingredients

Usually, the crude plant material is processed further to yield an extract. Approximately 80% of herbal medicinal products contain extract mainly in the form of dry extracts. Due to the complex composition of herbal drugs, processing is a crucial step in maintaining the constancy of quality (Busse, 1999). Key parameters during the extraction process are: The ratio between crude herbal drug and the solvent, the type and the concentration of the solvent, the method of extraction, further purification steps.

2.4 Constituents of Herbal Preparations

The constituents of a herbal preparation can be categorized in several groups which may be relevant for analytical and therapeutic purposes such as:

1. Constituents with known clinical activity (active principle)
2. Constituents with known pharmacological activity or which otherwise contribute to the efficacy (active markers)
3. Constituents relevant for quality control (analytical markers)
4. Accompanying constituents like low amounts of inorganic salts, sugars, or amino acids (inert substances)
5. Constituents potential negative impact (allergens, toxins)
6. Matrix-substances, usually not soluble (cellulose, lignins)

Negative markers are unwanted constituents, for example, allergens or toxins. The third group consists of substances which interfere with the absorption of the active constituents. Examples of substances with toxicological relevance are valepotriates from valerian (Bos et al., 1998), ginkgolic acids from ginkgo (Busse, 1994), and pyrrolizidine alkaloids from a variety of herbs (Cheeke, 1988).

For further characterization of extracts, semi-quantitative fingerprints obtained by high-pressure liquid chromatography, gas chromatography, thin-layer chromatography or other techniques that are appropriate to document quality, and also to provide information on the batch-to-batch consistency, would be used.

2.5 Implications of Quality Aspects for Safety and Efficacy

The term herbal medicine covers a wide range of products, from traditional preparations such as teas, simple tinctures and capsules which contain comminuted plant parts to solid dosage forms containing more concentrated dry extracts, some obtained by multistage processing. Advocates with orthodox view accept that however complex the composition of a herbal medicinal product may be, its bioactive constituents remain chemical entities which must obey the same pharmacological rules as those which hold for synthetic molecules. Consequently, the safety and efficacy of the herbal products is determined by the pattern and concentration of the chemical components that they contain. Advocates of a more holistic approach favour the use of traditional forms of herbal products, as the plant itself is looked upon as a medicine and the ‘’natural’’ pattern and concentration of constituents should not be changed by processing (Busse, 2000).

2.6 PHYTOCHEMISTRY OF PLANTS USED IN HERBAL PREPARATION

Complex mixtures such as fats, fixed oils, volatile oils, tars and resins had been prepared and used, although, virtually nothing was known about their composition. Not all the chemical compounds elaborated by plants are of equal interest to the pharmacognosist. The active principles are frequently alkaloids or glycosides and these, therefore deserve special attention. Other groups such as carbohydrates, fats and proteins are of dietetic importance, and many such as starches and gums are used in pharmacy although lacking any marked pharmacological action. Other substances, such as calcium oxalate, silica, lignin and colouring matters, may be of assistance in the identification and the detection of adulteration.

The phytochemical investigation of a plant may involve the following:

- Extraction of the plant material;
- Separation and isolation of the constituents of interest;
- Characterization of the isolated compounds;
- Investigation of the biosynthetic pathways to particular compounds;
- Quantitative evaluations (Evans, 1989; Solomons, 1992).

2.6.1 Chemistry of Phytochemicals

Though plant kingdom represents an extraordinary reservoir of novel molecules which is estimated at 250000 – 500000 plant species around the globe, only a small percentage has been investigated phytochemically and the fraction subjected to biological or pharmacological screening is even lower (Nunoo, 2004)

2.6.2 Alkaloids

Alkaloids have been defined in various ways, but one definition comes fairly close to actuality. An alkaloid is a plant-derived compound that is toxic or physiologically active, contains nitrogen in a heterocyclic ring, is basic, has a complex structure, and is of limited distribution in the plant kingdom. It can also be described as group of mildly alkaline compounds containing nitrogen, mostly of plant origin and of moderate molecular complexity, which produce various physiological effects on the human body. Nearly 3,000 alkaloids have been recorded; the first to be prepared synthetically in 1886 was one of the simplest, called coniine, or 2-propyl piperidine, $C_5H_{10}NC_3H_7$. It is highly poisonous; less than 0.2 g (0.007 oz) is fatal. Coniine, obtained from seeds of the hemlock, was the poison used in the execution of Socrates. Some 30 of the known alkaloids are used in medicine. For example, atropine, obtained from deadly nightshade, causes dilation of the pupils; morphine is a painkiller; quinine is a specific remedy for malaria; nicotine is a potent insecticide; and reserpine is a valuable tranquilizer (Robbers, 1988; Wikipedia, 2006).

2.6.3 Alkaloid-Containing Drugs

Most plant alkaloids are derivatives of tertiary amines, while others contain primary, secondary or quaternary nitrogen. The basicity of the individual alkaloids varies greatly, depending on which of the four types is represented (GNDP, 2004) and also the medium in which it is found. For example, in the gas phase the basicity or the alkalinity of the following amines increase in the order indicated by the (‘’greater than’’ sign ‘’ >’’): $(CH_3)_3N > (CH_3)_2NH > CH_3NH_2 > NH_3$, whereas in the aqueous phase for the

same compounds the order is: $(CH_3)_2NH$ > CH_3NH_2 > $(CH_3)_3N$ > NH_3, (Solomons, 1996).

2.6.4 Flavonoid-containing Drugs

The main constituent of flavonoid drugs are 2-phenyl-γ-benzopyrones or structurally related, mostly phenolic, compounds. Most flavonoids are present in herbal preparations as mono-or diglycosides.

2.7 GENERAL CONCEPTS

Quality assessments of herbal preparations generally cover several areas such as descriptive tests of the preparation, general identity tests, purity tests and chemical assays. The quality tests and acceptable criteria such as organoleptic evaluation, chemical identification by spectroscopic or chromatographic fingerprints and maker components, chemical assay (or assay) for active constituents or characteristic markers if available, physico-chemical characteristic e.g. refractive index, pH, ash values, extractive values, determination of microbial levels, qualitative and quantitative determination of heavy metals are considered generally applicable to all herbal products. A chemical assay and/or assay for biological activity should be performed if the herbal product is considered potent (i.e. highly active) or toxic. In addition all herbal products intended for internal use should be free from any form of undesirable foreign organic matter (GNDP, 2004)

2.8 Heavy Metal

Heavy metals which of course, cannot be metabolized persist in the body and exert their toxic effects by combining with one or more reactive groups (ligands) essential for normal physiological functions. Heavy metals, particularly those in the transition series, may react with O-, S- and N- containing ligands which in the body take the form of: -OH, $-COO^{(-)}$, $-OPO_3H^{(-)}$, >C=O, -SH, -S-S-, $-NH_2$, and >NH. The resulting metal complex or (coordination compound) is formed by a coordinate bond – one in which both electrons are contributed by the ligand (Gilman, 1991).

2.8.1 Effects of Lead on Man

After absorption, inorganic lead is distributed initially in the soft tissues particularly in the tubular epithelium of the kidney and in the liver. In time, it is redistributed and deposited in bone, teeth and hair. About 95% of the body burden of the metal is eventually found in bone. Only small quantities of inorganic lead accumulate in the brain, with most of that in grey matter and basal ganglia (Task Group on Metal Accumulation, 1973). In man lead is excreted into urine more than faeces, and the concentration in urine is directly proportional to that in plasma (Kehoe, 1987). Its half-life is 1 to 2 months, however, its concentration in bone appears to increase and its half-life in bone has been estimated to be 20 to 30 years (Gross et al., 1975).

2.8.2 Effects of Mercury on Man

Mercury readily forms covalent bonds with sulfur, and it is this property that accounts for most of the biological properties of the metal. Organic mercurials form mercaptides of the type RHg – SR'. Even in low concentrations it is capable of inactivating sulfhydryl enzymes and thus interfering with cellular metabolism and

functions. The metal is excreted in the urine and faeces with a half-life of about 60days, (Friberg and Vostal, 1972), but excretion in faeces is more pronounced (Klaassen, 1975). Over 90% of methyl mercury is absorbed from the human gastrointestinal track (Klaassen, 1975). The biological half-life of methyl mercury in man is about 65 days (Rahola et al., 1972; Bakir et al., 1973; Friberg et al., 1974).

2.8.3 Effects of Arsenic on Man

The toxicity of a given arsenical is related to the rate of its clearance from the body and therefore to its degree of accumulation in tissues. In general, the toxicity increases in the sequence of: Organic arsenicals < As^{5+} < As^{3+} < arsine (AsH_3). The organic arsenicals contain arsenic linked to a carbon atom by a covalent bond, where arsenic exists in trivalent or pentavalent state. Trivalent arsenicals inhibit many enzymes by reacting with –SH groups. The arsenite salts are more soluble in water and better absorbed than their oxides (Gilman, 1991). Experimental evidence has shown a high degree of gastrointestinal absorption of both trivalent and pentavalent forms of arsenic (Tam et al., 1979; Elinder et al., 1983)

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 General cleansing of glassware used

All glassware were washed thoroughly in soap solution and soaked in 10% (v/v) nitric acid overnight, rinsed with distilled water and dried before used.

3.2 Washing and sterilization of glassware for microbial analysis

Glassware such as pipettes and spatula were disinfected with Camel antiseptic solution. Petri dishes, culture media, and other equipment were autoclaved to kill all contaminating bacteria. They were then soaked and washed in soapy water also containing disinfectant, after which they were packed into suitable receptacles to dry. Disposable gloves were worn during these operations. The Petri dishes were placed inverted in the canisters to ensure a thorough dryness before sterilised. The sterilisation was done by placing them in an oven at 170ºC for 1 hour. The pipettes in canisters were also sterilised in an autoclave at 121ºC for 15 minutes, after which the canisters were stored in a clean sterile environment.

3.3 Preparation of media/Reagents for microbial analysis

1. For Plate count agar (PCA), 23.0g of PCA powder was dissolved in 1litre of distilled water and boiled with frequent stirring. It was then sterilised by autoclaving at 121^{o}C for 15 minutes and cooled to room temperature before use.

2. For Dichloran Bengalrot Chloramphenicol Agar (DRBC), 15.75g of DRBC powder was dissolved in 500ml of distilled water and heated for complete dissolution.

One vial of the chloramphenicol supplement (SR78) was added and then autoclaved at 121^{o}C for 15 minutes. It was cooled to 50^{o}C and stirred well before use.

3. For Escherichia coli broth (ECB), 37.0g of ECB powder was dissolved in 1litre of distilled water in a beaker. It was then sterilised by autoclaving at 121^{o}C for 15 minutes and allowed to cool to room temperature before use.

4. For Triple sugar iron (TSI), 63.0g of TSI powder was suspended in 1litre of distilled water and boiled while stirring for complete dissolution. After cooling, it was distributed into sterilised Petri dishes and sterilised in an autoclave at 121^{o}C for 15 minutes before use.

5. For Tetrathionate broth base, 77.0g of Tetrathionate broth powder was suspended in a litre of distilled water and boiled. It was cooled to room temperature and 20ml of iodine solution was added and mixed thoroughly before use.

6. For Selenite cystine broth base, 4g of sodium biselenite (L121) was mixed with 19.0g of Selenite cystine broth base (CM699) and dissolved in a litre of distilled water and warmed to dissolve completely. It was sterilised under stream of hot air for 15minutes before use.

7. Buffered peptone water was prepared by dissolving 20.0g of the powder in a litre of distilled water with stirring. It was then sterilised by autoclaving at 121^{o}C for 15 minutes before use.

8. The Xylose lysine desoxycholate (XLD) was prepared by suspending 53.0g in 1litre of distilled water, warmed with frequent agitation till it just started to boil. It was then immediately warmed in a water bath at 50^{o}C and poured into Petri dishes.

9. The Bismuth sulphite agar (modified) was prepared by dissolving 20.0g in 500ml of distilled water and gently heated with frequent stirring until it just began to boil. It was allowed to simmer for 30 minutes and cooled to 55^{o}C before use.

3.4 Preparation of Reagents for Phytochemical Analysis

1. Bomtrager reagent

Potassium hydroxide pellets (10.00g) were dissolved in 50ml of distilled water and made up to 100ml with distilled water.

2. Dragendorff's reagent

Hydrated bismuth nitrate (8.00g) was dissolved in 20ml of concentrated nitric acid. It was then added slowly with stirring, to a solution of 27.20g potassium iodide dissolved in 50ml of distilled water. The precipitates of crystalline potassium nitrate were filtered off, and the solution made up to 100ml with distilled water.

3. Dragendorff's spray

Basic bismuth nitrate (0.85g) was dissolved in 40ml of distilled water and added to 10ml of glacial acetic acid. The resulting solution was added to 8.00g of potassium iodide dissolved in 20ml of distilled water.

4. Gelatin-salt reagent

Gelatin (1.00g) was added to 10.00g of sodium chloride and dissolved in 100ml of distilled water.

5. Liebermann – Buchard reagent

Redistilled acetic anhydride (5ml) was carefully mixed with 5ml of ice cold concentrated sulphuric acid, and 50ml of absolute ethanol was then added slowly to the resulting mixture, while cooling in ice.

6. Mayer's reagent

Mercuric iodide (1.36g) was dissolved in 60ml of distilled water and added to 10ml solution containing 5.00g of potassium iodide. The resulting solution was made up to 100ml with distilled water

3.5 Preparation of solutions for Heavy Metal Determination

1. Reducing agent for Hg analysis

The 1.00% (w/v) sodium borohydride in 0.05% (w/v) sodium hydroxide solution was prepared by mixing 10.00g of sodium borohydride with 0.50g sodium hydroxide in one (1) litre of deionized water.

2. Oxidation solution for Hg analysis

The 8% (w/v) potassium bromide in 2.24% (w/v) potassium bromate was prepared by mixing 8.00g potassium bromide with 2.24g potassium bromate in 100ml of deionized water.

3. Metal standard solutions

The concentration of each standard solution of the four heavy metals analyzed was 1000mg/L (1000ppm) purchased from MERCK, New Jersey. A 1000µg/L (1000ppb) solution of each metal was prepared out of the stock solution, by pipetting 1ml from each stock solution into a 1litre volumetric flask and diluting it to the mark with 10%v/v nitric acid. Working standard solutions of 0.0, 0.1, 0.2, 2.0, 5.0, 10.0, 20.0 and 50.0µg/L were prepared for each by using the dilution relation, ($C_1V_1 = C_2V_2$, (where 'C_1', 'V_1' are the concentration and volume respectively, of the stock solutions and 'C_2', 'V_2,' the concentration and volume of the dilute solutions), using 10%v/v nitric acid.

3.6 Sample Collection

The samples were collected in batches from the factory of the Angel Herbal Product Industry Limited, Kumasi between November, 2006 and May, 2007 and analyzed at the Ghana Standards Board and Ghana Atomic Energy Laboratories in Accra. Three different batches of five (5) brands were collected. For each brand, two samples were collected making a total of thirty (30) samples. The five brands collected were Angel Soap and Angel Herbal Mixture (Tonic). The rest were Angel Natural Capsules (Powder), Angel Cream and Angel Fatwekeke Ointment.

3.7 INFORMATION ON THE SAMPLES USED FOR THE STUDY

3.7.1 Angel Cream

Angel Cream is a herbal product of Angel Herbal Product Industry Limited, Kumasi-Ghana and is a combination of local herbs. It is for the treatment of the following diseases; Candysis, White (Odeepua), Boil (Mpompo), Shingles (Ananse), Ringworm (Eyam), Eczema (Ekro), Foot rot (Apropro). The instruction for use indicates that one must apply thinly on the affected parts, and the ingredients for the formulation include; *Cassia alata, Funtumia elastica*, Petroleum jelly and perfume.

3.7.2 Angel Herbal Mixture

This is another herbal product of Angel Herbal Product Industry Limited and is a combination of local herbs, which treat the following diseases; Jaundice, Menstrual pain, Malaria, Fever, loss of appetite and body pain. This comes in a syrup form and composed of; Cola *gigantea*, *Solanum torvum*, *Spathodéa Campanulata*, *Bombax buonopozense*, *Veronica amygdalina*.

3.7.3 Angel Natural Capsules

Another product of Angel Herbal Product Limited is also a combination of local herbs which treats body and waist pain and sexual weakness. It is in capsules form and the compositions are; *Eudenia eminens, Pisonia aculeata, Paulina pinata, Aichomea cordifolia, cocos nucifera, Heliotropium indicum, sida acuta.*

3.7.4 Angel Soap

Angel Soap is a combination of local herbs which treats; Dermalophytosis (Ewefare), Shingles (Ananse), Anal sore (Kokobo), Body itching (ahokeka), and Ringworm. The rest are Eczema, Boils, Foot rot and Fresh wounds. It comes in a soap form, and composes of *Casia alata and Funtumia elastica*.

3.7.5 Angel Fatwikeke Ointment

This is another formulation of local herbs from Angel Herbal Product Limited which treats; Muscular pain, Rheumatism, Backache, Joint and Waist pain. It is an ointment with compositions of; *Paulina pinata, Oesistapholis patens, Cassia alata* and petroleum jelly.

3.8 Sample Preparation and Treatment

The samples were kept sealed until the time of analysis. The freshly opened samples were first prepared for microbiological analysis before the rest of the analyses.

3.9 MICROBIOLOGICAL ANALYSIS

3.9.1 Aerobic bacteria

Plate Count Agar (PCA) and Maximum Recovery Diluent (MRD) count media methods were used (British Standards (BS) 5763: Part 5 (1981)

1. SOAP

For each soap, 10g was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution, 1:10), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions, that is first and second, was aseptically pipetted unto sterile labeled PCA agar plates in duplicates and spread with a stick. Petri dishes were inverted and incubated at 35°C for 24 hours and observed.

2. CREAM

For each cream, 10g was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution, 1:10), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions, that is first and second, was aseptically pipetted unto sterile labeled PCA agar plates in duplicates and spread with a stick. Petri dishes were inverted and incubated at 35°C for 24 hours and observed.

3. OINTMENT

A 10g of each ointment was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution, 1:10), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions, that is first and second, was aseptically

pipetted into sterile labeled PCA agar plates in duplicates and spread with a stick. Petri dishes were inverted and incubated at 35°C for 24 hours and observed.

4. MIXTURE

For each sample, 10ml was aseptically pipetted into 90ml sterile MRD count media in a sample bottle (first dilution, 1:10), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions, that is first and second, was aseptically pipetted into sterile labeled PCA agar plates in duplicates and spread with a stick. Petri dishes were inverted and incubated at 35°C for 24 hours.

5. CAPSULES

A 10g of the opened capsules was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution, 1:10), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions, that is first and second, was aseptically pipetted into sterile labeled PCA agar plates in duplicates and spread with a stick. Petri dishes were inverted and incubated at 35°C for 24 hours.

3.9.2 Test for Yeasts/Moulds

Dichloran Bengalrot Chloramphenicol Agar (DRBC) and Maximum Recovery Diluent (MRD) media were used according ISO 7954 (1987) methods.

1. SOAP

A 10g sample was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution,

1:100), 0.1ml each of the dilutions that is first and second, was aseptically pipetted unto labeled sterile DRBC Agar plates in duplicates and spread with a stick. Petri dishes were incubated at 25°C for 5 days and observed.

2. CREAM

A 10g sample was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions that is first and second, was aseptically pipetted into labeled sterile DRBC Agar plates in duplicates and spread with a stick. Petri dishes were incubated at 25°C for 5 days and observed.

3. OINTMENT

A 10g sample was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions that is first and second, was aseptically pipetted into labeled sterile DRBC Agar plates in duplicates and spread with a stick. Petri dishes were incubated at 25°C for 5 days and observed.

4. MIXTURE

A 10ml sample was aseptically pipetted into 90ml sterile MRD count media in a sample bottle (first dilution), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions that is first and second, was aseptically pipetted into

labeled sterile DRBC Agar plates in duplicates and spread with a stick. Petri dishes were incubated at 25°C for 5 days and observed.

5. CAPSULES

A 10g of the opened capsules was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution), 1ml of inoculum from the first dilution was aseptically pipetted into 9ml of sterile MRD count media in McCartney bottle (second dilution, 1:100), 0.1ml each of the dilutions that is first and second, was aseptically pipetted into labeled sterile DRBC Agar plates in duplicates and spread with a stick. Petri dishes were incubated at 25°C for 5 days and observed.

3.9.3 Test for Escherichia Coli

The following reagents were used: Maximum Recovery Diluent (MRD), Tryptone Water, Kovac's reagent, Lauryl Tryptose Broth (LTB) and Escherichia Coli Broth (ECB) using a method by ISO 6579 (1993).

1. SOAP

A 10g sample was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution), Serial dilutions were made to obtain 10^{-2}, and 10^{-3}; 1ml of each of the dilutions was aseptically pipetted into three sets of labeled test tubes containing 9ml each of LTB. Each of the test tubes had Durham tubes which allowed the presence of gas produced by the organism to be seen and the test tubes were incubated at 35°C for 24 hours before observing any growth.

2. CREAM

A 10g sample was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution), Serial dilutions were made to obtain 10^{-2}, and 10^{-3}, 1ml of

each of the dilutions was aseptically pipetted into three sets of labeled test tubes containing 9ml each of LTB. Each of the test tubes had Durham tubes which allowed the presence of gas produced by the organism to be seen and the test tubes were incubated at 35°C for 24 hours before observing any growth.

3. OINTMENT

A 10g sample was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution), Serial dilutions were made to obtain 10^{-2}, and 10^{-3}, 1ml of each of the dilutions was aseptically pipetted into three sets of labeled test tubes containing 9ml each of LTB. Each of the test tubes had Durham tubes which allowed the presence of gas produced by the organism to be seen and the test tubes were incubated at 35°C for 24 hours before observing any growth.

4. MIXTURE

A 10ml sample was aseptically pipetted into 90ml sterile MRD count media in a sample bottle (first dilution); Serial dilutions were made to obtain 10^{-2}, and 10^{-3}, 1ml of each of the dilutions was aseptically pipetted into three sets of labeled test tubes containing 9ml each of LTB. Each of the test tubes had Durham tubes which allow the presence of gas produced by the organism to be seen and the test tubes were incubated at 35°C for 24 hours before observing any growth.

5. CAPSULES

A 10g of the opened capsules was aseptically weighed into 90ml sterile MRD count media in a sample bottle (first dilution), Serial dilutions were made to obtain 10^{-2}, and 10^{-3}; 1ml of each of the dilutions was aseptically pipetted into three sets of labeled test tubes containing 9ml each of LTB. Each of the test tubes had Durham tubes which

allow the presence of gas produced by the organism to be seen and the test tubes were incubated at 35°C for 24 hours before observing any growth.

3.9.4 Test for Salmonella Typhi

The reagents used were: Peptone Water, Xylose Lysine Desoxycholate (XLD), Selenite Cystine Broth, Bismuth Sulphite Agar (BSA), Triple Sugar Iron (TSI), and Tetrathionate Broth stated by the ISO 6579 (1993).

1. SOAP

A 10g sample was aseptically weighed into 90ml sterile Peptone Water (first dilution) in sterile sample bottle and incubated at 35°C for 24 hours. 1ml of the first dilution was aseptically pipetted into 10ml Tetrathionate Broth in sterile McCartney bottle and incubated at 35°C for 24 hours. Again, 1ml of the first dilution was aseptically pipetted into 20ml Selenite Cystine broth in sterile McCartney bottle and incubated at 44°C in a water bath for 24 hours. Each of the incubated test sample was streaked on sterile BSA and XLD plates and incubated at 35°C for 24 hours. 1ml each of Salmonella Typhi stock culture (positive control) were diluted in the same way as the samples and applied alongside.

2 CREAM

A 10g sample was aseptically weighed into 90ml sterile Peptone Water (first dilution) in sterile sample bottle and incubated at 35°C for 24 hours. 1ml of the first dilution was aseptically pipetted into 10ml Tetrathionate Broth in sterile McCartney bottle and incubated at 35°C for 24 hours. Again, 1ml of the first dilution was aseptically pipetted into 20ml Selenite Cystine broth in sterile McCartney bottle and incubated at 44°C in a water bath for 24 hours. Each of the incubated test sample was streaked on

sterile BSA and XLD plates and incubated at 35°C for 24 hours. 1ml each of Salmonella Typhi stock culture (positive control) were diluted in the same way as the samples and applied alongside.

3 OINTMENT

A 10g sample was aseptically weighed into 90ml sterile Peptone Water (first dilution) in sterile sample bottle and incubated at 35°C for 24 hours. 1ml of the first dilution was aseptically pipetted into 10ml Tetrathionate Broth in sterile McCartney bottle and incubated at 35°C for 24 hours. Again, 1ml of the first dilution was aseptically pipetted into 20ml Selenite Cystine broth in sterile McCartney bottle and incubated at 44°C in a water bath for 24 hours. Each of the incubated test sample was streaked on sterile BSA and XLD plates and incubated at 35°C for 24 hours. 1ml each of Salmonella Typhi stock culture (positive control) were diluted in the same way as the samples and applied alongside.

4 MIXTURE

A 10ml sample was aseptically pipetted into 90ml sterile Peptone Water (first dilution) in sterile sample bottle and incubated at 35°C for 24 hours. 1ml of the first dilution was aseptically pipetted into 10ml Tetrathionate Broth in sterile McCartney bottle and incubated at 35°C for 24 hours. Again, 1ml of the first dilution was aseptically pipetted into 20ml Selenite Cystine broth in sterile McCartney bottle and incubated at 44°C in a water bath for 24 hours. Each of the incubated test sample was streaked on sterile BSA and XLD plates and incubated at 35°C for 24 hours. 1ml each of Salmonella Typhi stock culture (positive control) were diluted in the same way as the samples and applied alongside

5 CAPSULES

A 10g of the opened capsules was aseptically weighed into 90ml sterile Peptone Water (first dilution) in sterile sample bottle and incubated at 35°C for 24 hours. 1ml of the first dilution was aseptically pipetted into 10ml Tetrathionate Broth in sterile McCartney bottle and incubated at 35°C for 24 hours. Again, 1ml of the first dilution was aseptically pipetted into 20ml Selenite Cystine broth in sterile McCartney bottle and incubated at 44°C in a water bath for 24 hours. Each of the incubated test sample was streaked on sterile BSA and XLD plates and incubated at 35°C for 24 hours. 1ml each of Salmonella Typhi stock culture (positive control) were diluted in the same way as the samples and applied alongside

3.10 ORGANOLEPTIC TEST

The organoleptic test was performed on the physical properties of all the formulations for the three batches of samples collected. The sensory perceptions or properties were determined by physical examination of colour, odour, touch, taste and clarity of all the samples in six months at three days interval for the first batch. The tasting perception was not applied to the soap, cream and the ointments. The judgment of these properties was also done by 20 staffs from four different scientific institutions alongside. The second batch was performed in three months at three days interval and the third batch in six weeks at three days interval.

3.11 DETERMINATION OF PHYSICOCHEMICAL PROPERTIES

3.11.1 pH

1. Mixture:

For each mixture, the pH measurements were carried out directly on the preparations, placing the electrode in 20ml of the liquid at room temperature using JENWAY 3310 digital pH meter.

2. Capsules

A 1% (w/v) aqueous extract was prepared in warm water by weighing one gram (1g) of the herbal powder and extracted in 100 ml of warmed distilled water. It was vigorously shaken to ensure maximum extraction, and allowed to cool to room temperature before filtering. The pH was then taken.

3. Creams, Soaps and Ointments

One gram (1g) of each of these preparations was dispersed in 100ml of distilled water in three separate 150ml beaker, and warmed to melt the samples, followed by vigorous shaking. The mixture was then allowed to cool to room temperature to enable the insoluble parts to separate from the aqueous phase. They were then decanted and the pH determined

3.11.2 Total Ash

1. Capsules

The air-dried herbal powder was uniformly mixed and about 2g weighed in a tared platinum crucible, previously ignited and weighed. The above was repeated to obtain triplicate samples for all the three batches analyzed.

The samples were incinerated in a furnace by gradually increasing the temperature to 450ºC until it was free from carbon; the ash was then cooled in a desiccator and reweighed. The percentage ash was calculated in gram per 100g of the air-dried sample and reported as Mean ± SD% (w/w).

3.11.3 Sulphated Ash

1. Capsules

The air-dried herbal powder was uniformly mixed and about 2g weighed in a tared platinum crucible, previously ignited and weighed. The sample was evenly spread in the crucible and 2ml of concentrated Sulphuric acid added. The above was repeated to obtain triplicate samples for all the batches of the herbal powder analyzed.

The samples were then heated first on a hot plate until the samples were carbonized and then incinerated to 800ºC, until they were carbon-free. They were then cooled and a few drops of concentrated sulphuric acid added to moisten the residue. They were again heated on a hot plate and ignited as before. 1ml of ammonium Carbonate, R, was added to each and ignited to constant weight. (3ml of ammonium carbonate, R, was added to each residue to neutralize any acid that might remain). The percentage sulphated ash for each was calculated in gram per 100g of the air-dried sample and reported as Mean ± SD% (w/w).

3.11.4 Acid-insoluble Ash

1. Capsules

25ml of 2MHCl was added to each of the total ash obtained in crucibles, and covered with watch-glass and then boiled gently for 5 minutes. The watch-glass in each

case was washed with 5ml of hot water into the crucible. The insoluble matter was collected on an ash less filter paper for each and washed with hot water until the filter papers were neutral. The filter papers were then transferred to the original crucibles, dried and ignited to a constant weight. The percentage acid-insoluble ash was calculated for each in gram per 100g of the air-dried sample and reported as Mean ± SD% (w/w).

3.11.5 Loss on drying by Gravimetric method

1. Capsules

The air-dried powder was thoroughly mixed and about 1g weighed in a tared flat weighing vessel previously dried and kept in a desiccator. This was prepared in triplicate and dried in an oven at 105ºC for 5 hours before the first weighing was done. This continued until two consecutive weighing did not differ by more than 5mg, and the percentage loss of water calculated in gram per 100g of air-dried herbal powder as Mean ± SD % (w/w).

3.11.6 Total Solids

1. Mixture

The mixture was thoroughly mixed by shaking it gently to provide a uniform mixture.10ml of the mixture was pipetted into a tared nickel dish. This was repeated in triplicate for all the batches. They were heated on a water-bath until the residues were apparently dried. They were then transferred to an oven and dried to a constant weight at 105ºC, and then cooled in a desiccator. The percentage of the total solid was calculated in gram per 100ml of the liquid preparations and reported as Mean ± SD% (w/w).

3.11.7 Relative density

1. Mixture

An empty, cleaned and dried density bottle was weighed accurately (W_0). It was then filled with the mixture, and weighed accurately (W_1). It was thereafter emptied, cleaned and dried. The above was repeated with distilled water (W_2). This determination was carried out in triplicate for all the herbal mixture, and the relative density was reported as Mean ± SD.

3.12 PHYTOCHEMICAL SCREENING

3.12.1 Glycosides

Test 1**: (Soap, Cream, Ointment, Mixture and Capsules)**

A 0.2g of each sample was warmed in a test tube with 5ml of 10% (v/v) sulphuric acid on a water bath at 100ºC for 2 minutes. They were then filtered and neutralized with 5% (w/v) solution of NaOH. The volume of the aqueous NaOH added to each test tube was noted and 0.1ml of Fehling's solutions A and B were added to each. They were then heated on a water bath at 100ºC for 2 minutes. The intensity of the red precipitate formed in each case was observed and compared with the following in test 2.

Test 2: (**Soap, Cream, Ointment, Mixture and Capsules**)

A 0.2g of each sample was warm extracted using 5ml of distilled water instead of sulphuric acid. After cooling to room temperature, the volume of water equivalent to the volume of NaOH used in Test 1 was added, followed by the addition of 0.1ml of Fehling's solutions A and B. They were then heated on the water bath for 2 minutes. The intensity of the red precipitate formed was noted and compared with that of Test 1 above.

Test 2 represents the amount of free reducing sugars already present in the crude drug, whereas Test 1 represents free reducing sugars plus those released on acid hydrolysis of any glycosides in the crude drug. The precipitate in Test 1 for all the samples except the ointment was greater than that in Test 2, meaning glycoside was present in all the samples except the ointment).

3.12.2 Alkaloids

1. (**Soap, Cream, Ointment, Mixture and Capsules**)

A 0.5g of each sample was weighed and each mixed with 5ml of 1% (v/v) aqueous HCl. They were then warmed on the water bath and filtered. Two portions of 1ml each of the filtrates were transferred into two test tubes. To one test tube, two drops of Mayer's reagent were added and to the other, two drops of Dragendorff's reagent were added. The formation of brown precipitates with each reagent indicated the presence of alkaloids.

3.12.3 Alkaloids

2. Confirmatory Test for (**Soap, Cream, Ointment, Mixture and Capsules**)

A 0.5g of each sample was partitioned between 10ml of 2% H_2SO_4/$CHCl_3$ (1:1, v/v) in a separating funnel. The chloroform phase was washed with (2x3ml) of the acid, and all the acid washings combined. The acid phase was adjusted to pH 9 with 10% (v/v) ammonia solution. Each phase was then extracted with chloroform (3x10ml). The chloroform phase was combined and evaporated using stream of air. The residues were spotted on filter paper, dried and sprayed with freshly prepared Dragendorff's spray. The appearance of dark coloured spots against a pale yellow background indicates the

presence of alkaloids. The residues were acidified with 2% (v/v) H_2SO_4, and 3 drops of Mayer's reagent added to each. The formation of precipitates indicates the presence of quaternary alkaloid.

3.12.4 Tannins

1. Preliminary test (**Soap, Cream, Ointment, Mixture and Capsules**)

A 0.5g of each sample was dissolved in 10ml of warm distilled water and 3 drops of 1% (w/v) aqueous $FeCl_3$ was added to each filtrate after cooling. A purple colour that appeared with the herbal mixture indicated the presence of tannins.

2. Confirmatory Test: **Mixture**

A 0.5g of each extract was divided into three portions. Three drops of 1% gelatin solution was added to one portion. To the second portion, gelatin-salt reagent was added and to the third, 10% aqueous NaCl added. The appearance of white precipitate with gelatin-salt reagent indicated the presence of tannins in the mixture.

3.12.5 Saponins

1. Preliminary Test (**Soap, Cream, Ointment, Mixture and Capsules**)

A 0.5g of each sample was shaken with distilled water in a test tube. The development of froth, which persisted for over 15 minutes, indicated the presence of saponins.

2. Confirmatory Test: **Mixture**

A 0.1g of each mixture was extracted with 5ml of 70% (v/v) methanol. The extract was applied on silica gel $60F_{254}$ pre-coated TLC plates. The plates were allowed to dry and then sprayed with Liebermann-Buchard reagent and placed in an oven at

110^{o}C for 15 minutes. The appearance of a blue-green colour indicated the presence of steroidal saponins.

3.12.6 Flavonoids

1. Preliminary Test (**Soap, Cream, Ointment, Mixture and Capsules**)

For the mixture, a strip of filter paper was directly dipped into it. For the solids, 0.5g of each was extracted with 10ml distilled water. A strip of filter paper was then dipped into each extract. Each paper was dried and exposed to ammonia solution and then fumes of HCl.

2. Confirmatory Test: **Mixture**

This was performed by adding 0.2g of each to 1ml of 95% ethanol. Three drops of concentrated HCl and 6 pieces of magnesium turnings were added to each mixture. The appearance of a pinkish colour confirmed the presence of flavonoid.

3.12.7 Anthraquinones and anthracene-derivatives

(**Soap, Cream, Ointment, Mixture and Capsules**)

A 0.2g of each sample was boiled with a mixture of 2ml 0.5M sulphuric acid and 2ml 5% (w/v) aqueous ferric chloride (5% (w/v) $FeCl_{3(aq)}$) in a test tube for 5 minutes and filtered while hot. It was cooled and extracted with 3ml of dichloromethane. The lower organic layer was separated by pipetting and shaking with half its volume of 10% ammonia.

3.13 DETERMINATION OF HEAVY METALS

3.13.1 Heavy metals digestion procedures

3.13.2 Soap

A 1.20g sample was weighed into a 50ml digestion vessel and 8ml of concentrated 65% (w/w) HNO_3 (VWR Suppliers, Britain) was added followed by the addition of 4ml of 96% of H_2SO_4 (VWR Suppliers, Britain). The mixture was then placed in a programmable Ethos 900 Milestone Microwave Labstation for 25 minutes following a five step temperature programme; Step 1: Time 2 minutes, Power 250 watt; Step 2: Time 2 minutes, Power 0 watt; Step 3: Time 6 minutes, Power 250 watt; Step 4: Time 5 minutes, Power 400 watt; Step 5: Time 5 minutes, Power 600 watt; Vent Time 5 minutes. Each solution was made up to the 50ml mark with distilled water after the digestion.

3.13.3 Cream and Ointment

A 1.00g sample was weighed into 50ml digestion vessel and 10ml of concentrated 65% (w/w) HNO_3 was added, followed by 2ml of 30% H_2O_2. The mixture was placed in a programmable Ethos 900 Milestone Microwave Labstation for 22 minutes following a five step temperature programme; Step 1: Time 1 minute, Power 250 watt; Step 2: Time 1 minute, Power 0 watt; Step 3: Time 5 minutes, Power 250 watt; Step 4: Time 5 minutes, Power 400 watt; Step 5: Time 5 minutes, Power 650 watt; Vent Time 5 minutes. Each solution was made up to the 50ml mark with distilled water after the digestion.

3.13.4 Capsules

A 1.00g sample was weighed into a 50ml digestion vessel and 12ml of concentrated 65% (w/w) HNO_3 was added, after which 2ml of 30% H_2O_2 was also added. It was then placed in a programmable Ethos 900 Milestone Microwave Labstation for 25 minutes. The microwave was programmed as follows; Step 1: Time 2 minutes, Power 250 watt; Step 2: Time 2 minutes, Power 0 watt; Step 3: Time 6 minutes, Power 250 watt; Step 4: Time 5 minutes, Power 400 watt; Step 5: Time 5 minutes, Power 600 watt; Vent 5 minutes. Each solution was then made up to the 50ml mark with distilled water.

3.13.5 Herbal Mixture

Twenty milliliters of the herbal mixture was evaporated to dryness using a stream of nitrogen in a 50ml digestion vessel. 12ml of concentrated 65% (w/w) HNO_3 was added to the residue followed by 2ml of 30% H_2O_2. It was then placed in a programmable Ethos 900 Milestone Microwave Labstation for 25 minutes.

The microwave was programmed as follows; Step 1: Time 2 minutes, Power 250 watt; Step 2: Time 2 minutes, Power 0 watt; Step 3: Time 6 minutes, Power 250 watt; Step 4: Time 5 minutes, Power 400 watt; Step 5: Time 5 minutes, Power 650 watt; Vent 5 minutes. Each solution was then made up to the 50ml mark with distilled water.

3.13.6 Determination of Heavy Metals

Lead, Cadmium and Arsenic were determined by Graphite Furnace Atomic Absorption Spectrometry using the Thermo Elemental M5 Atomic Absorption Spectrophotometer (AAS) fitted with Graphite furnace and an autosampler. The blank

and working standards were first run followed by the samples. The concentrations were obtained on the instrument readout and recorded in an analysis book.

Mercury was determined by Cold Vapour Atomic Absorption Spectrometry (CV – AAS) using the Thermo Elemental M5 Atomic Absorption Spectrophotometer (AAS) fitted with a VP90 continuous flow hydride system. The samples were prepared by taking 10.0ml each of the already digested solutions and adding 0.4ml of the oxidation reagent (8% potassium bromide with 2.24% potassium bromate). The solutions were then allowed to stand at room temperature for 12 hours to ensure the quantitative oxidation of all mercury to the inorganic form. The solution was then aspirated into the reaction cell where the reductant (1% $NaBH_4$ in 1N NaOH) was added and the mercury vapour generated. The vapour was then swept by means of argon gas into the T-cell where absorption was measured.

3.14 QUALITY ASSURANCE

The accuracy and precision of the determination of the heavy metals were estimated by the analysis of blanks calibration standards and spiked samples. Some known concentrations of the heavy metals were spiked in some of the herbal preparations and digested. The solutions were then quantified.

3.14.1 Limit of Detection

For mercury, standard concentrations of 1.0µg/L, 0.8µg/L and 0.5µg/L were prepared and analyzed. The limit of detection of the instrument was then found to be 1.0µg/L. The same procedure was followed for As, Pb and Cd and their limits of detection were found to be 1.0µg/L, 1.8µg/L and 1.5µg/L respectively.

3.14.2 Percentage Recovery

The recovery test was performed on five selected herbal preparations after some known amounts of the heavy metals have been added and digested. The results are presented in table 23.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

A total of thirty (30) samples consisting of six (6) herbal mixture (liquid), six (6) herbal powder, six (6) herbal creams, six (6) herbal ointments and six (6) soaps were obtained for the study. These samples were subjected to various analyses. The parameters measured, were grouped as follows: microbial type and levels; (Escherichia coli, aerobic bacteria, yeast and moulds and salmonella Typhi), Organoleptic properties; (clarity, appearance, colour, taste and odour), physicochemical properties; (pH, total ash, sulphated ash, acid-insoluble ash, total solids and specific gravity); phytochemical screening for major constituents; (glycosides, alkaloids, tannins, saponins, flavonoids, anthraquinones, anthracene) and heavy metals; (mercury, arsenic, lead and cadmium).

The World Health Organization (WHO) has adopted a deliberate policy of encouraging the development and utilization of traditional medicine in the Primary Health Care delivery, particularly in third world countries. This policy is based on the sound recognition of the role that the traditional medicine is already playing in the health care programme as in the case of Ghana.

Some scientific papers and pamphlets have been published locally on traditional medicines in Ghana (CSIR (TTC), 1992). However, except for a manual (GNDP, 2004), which has recently published harmonized procedures for assessing the quality of herbal preparations; none addressed the quality which is very important in the production of herbal preparations. The quality of some of the herbal preparations on the Ghanaian market was assessed, based on the following quality parameters:

4.1 MICROBIAL LEVELS

Quite often, herbal preparations are contaminated by a large number of bacteria and moulds from the soil and the environment. The practices of harvesting, handling and production often cause additional microbial growth. Among these bacterial loads are aerobic spore-forming bacteria and the fungi, the pathogenic bacteria and the indicator bacteria such as the Escherichia coli and the salmonella species. Microbial contamination of preparations could result in possible risk or serious infections, if administered orally or through other means by which the organisms could have access to the body (GNDP, 2004; ISO, 2005; WHO, 2005b).

Herbal ingredients particularly those with high starch contents may be prone to increased microbial growth. It is not uncommon for herbal preparations to have aerobic bacteria present at 10^2 to 10^8 colony formed unit per gram (cfu/g) (The Scientific Community of England, 1990; Barnes et al., 2002). Many of these bacteria or microorganisms are needed in the body to keep it function properly. Some of these help to digest food, fight off sicknesses and keep the skin clean. But when they are above certain safety levels cause infections (Ryan and Ray, 2004).

These infections can in turn cause diseases such as urinary tract infections (UTI), meningitis, peritonitis, mastitis, septicemia, pneumonia, skin diseases, strep throat, tuberculosis (TB), vaginal infections and athlete's foot with symptoms such as severe bloody diarrhea, abdominal cramps, belly pains, vomiting, headache, nausea and feverish (Gianella, 1996; Centers for Diseases Control and Prevention CDC, 2005). It has been documented in the 1999 estimate that, 73000 cases of microbial infections and 61 deaths resulting from microbial infections occur in the United States each year (Centers for

Diseases Control and Prevention CDC, 2006). This is serious enough to call for the attention for the assessment of these organisms in all herbal preparations before use.

The soaps are to fight against shingles, ringworm, eczema, boils and foot rot. The capsules are for relief of body pains, waist pains, and to prevent sexual weakness. The cream is to prevent itches, shingles, boils, anal sores, candidiasis, after shaving and for quick hair growth. The indications of the mixture are jaundice, menstrual pain, malaria, loss of appetite, and body pains while the ointment fights against muscular pains, rheumatism, backache, joints and waist pain.

The Aerobic Bacteria, Yeast/Moulds, Escherichia Coli and Salmonella Typhi were the microbial loads assessed for all the preparations. The WHO standard for aerobic bacteria in preparations is $1x10^7$. The ranges of the values obtained for the aerobic bacteria in the samples analyzed were; Soap $3x10^2$ – $7x10^2$; Cream $1x10^2$ – $6x10^2$; Ointment $2x10^2$ – $3x10^2$; Mixture $1x10^3$ – $9x10^3$ and Capsules $3x10^4$, and are presented in tables 1, 2, 3, 4 and 5.

The WHO standard for yeast/moulds in preparations is $1x10^4$, and the ranges of the figures obtained in the samples are; Soap $<1x10^1$; Cream $<1x10^1$ - $3x10^2$; Ointment $<1x10^1$ - $2x10^2$; Mixture $<1x10^1$ - $1x10^3$ and Capsules $1x10^1$ - $1x10^3$.

For Escherichia coli, the WHO standard is $1x10^2$ but none was detected in any of the samples analyzed. By the WHO standard (GNDP, 2004) Salmonella Typhi should be negative in all preparations. None of the preparations analyzed contained these microbes. The microbial loads analyzed from all the preparations were below the acceptable limits, and so infections leading to these diseases, would not occur when administered.

Table 1 Results for the microbiological analysis of Soap

Item No.	Herbal Sample	Microbiological analysis			
		Aerobic bacteria (cfu/g)	**Yeast/ Moulds (cfu/g)**	**Escherichia Coli (cfu/g)**	**Salmonella Typhi (cfu/g)**
1	Angel Soap, Batch No. AHPS 004 (A)	$4.0x10^2$	$<1.0x10^1$	None detected	Negative
2	Angel Soap, Batch No. AHPS 004 (B)	$3.0x10^2$	$<1.0x10^1$	None detected	Negative
3	Angel Soap, Batch No. AHPS10 (A)	$6.0x10^2$	$<1.0x10^1$	None detected	Negative
4	Angel Soap, Batch No. AHPS 10 (B)	$6.0x10^2$	$<1.0x10^1$	None detected	Negative
5	Angel Soap, Batch No. AHPS 004B (A)	$7.0x10^2$	$<1.0x10^1$	None detected	Negative
6	Angel Soap, Batch No. AHPS 004B (B)	$6.0x10^2$	$<1.0x10^1$	None detected	Negative

Table 2 Results for the microbiological analysis for Angel Cream

Item No.	Herbal Sample	Microbiological analysis			
		Aerobic bacteria (cfu/g)	Yeast / Moulds (cfu/g)	Escherichia Coli (cfu/g)	Salmonella Typhi (cfu/g)
1	Angel Cream Batch No. AHPC 1006(A)	$3.0x10^2$	$<1.0x10^1$	None detected	Negative
2	Angel Cream Batch No. AHPC 1006(B)	$1.0x10^2$	$3.0x10^2$	None detected	Negative
3	Angel Cream Batch No. AHPC 11 (A)	$6.0x10^2$	$<1.0x10^1$	None detected	Negative
4	Angel Cream Batch No. AHPC 11 (B)	$1.0x10^2$	$3.0x10^1$	None detected	Negative
5	Angel Cream Batch No. AHPC 1206 (A)	$5.0x10^2$	$<1.0x10^1$	None detected	Negative
6	Angel Cream Batch No. AHPC 1206 (B)	$2.0x10^2$	$3.0x10^1$	None detected	Negative

Table 3 Results for the microbiological analysis for Angel Fatwikeke Ointment

Item No.	Herbal Sample	Microbiological analysis			
		Aerobic bacteria (cfu/g)	Yeast/Moulds(cfu/g)	Escherichia Coli (cfu/g)	Salmonella Typhi (cfu/g)
1	Fatwikeke Ointment. Batch No. AHPFO 0706 (A)	$3.0x10^2$	$<1.0x10^1$	None detected	Negative
2	Fatwikeke Ointment. Batch No .AHPFO 0706 (B)	$3.0x10^2$	$2.0x10^2$	None detected	Negative
3	Fatwikeke Ointment. Batch No .AHPFO 0706B (A)	$2.0x10^2$	$<1.0x10^1$	None detected	Negative
4	Fatwikeke Ointment. Batch No .AHPFO 0706B (B)	$3.0x10^2$	$3.0x10^1$	None detected	Negative
5	Fatwikeke Ointment. Batch No .AHPFO 0107 (A)	$2.0x10^2$	$<1.0x10^1$	None detected	Negative
6	Fatwikeke Ointment. Batch No .AHPFO 0107 (B)	$3.0x10^2$	$3.0x10^1$	None detected	Negative

Table 4 Results for the microbiological analysis for Angel Herbal Mixture

Item No.	Herbal Sample	Microbiological analysis			
		Aerobic bacteria (cfu/ml)	Yeast/Moulds (cfu/ml)	Escherichia Coli (cfu/ml)	Salmonella Typhi (cfu/ml)
1	Angel Herbal Mixture Batch No. AHPHM 0906 (A)	$1.0x10^3$	$<1.0x10^1$	None detected	Negative
2	Angel Herbal Mixture Batch No. AHPHM 0906 (B)	$7.0x10^3$	$4.0x10^2$	None detected	Negative
3	Angel Herbal Mixture Batch No. AHPHM 1106 (A)	$1.0x10^3$	$<1.0x10^1$	None detected	Negative
4	Angel Herbal Mixture Batch No. AHPHM 1106 (B)	$7.0x10^3$	$4.0x10^2$	None detected	Negative
5	Angel Herbal Mixture Batch No. AHPHM 0107 (A)	$1.0x10^3$	$4.0x10^2$	None detected	Negative
6	Angel Herbal Mixture Batch No. AHPHM 0107 (B)	$9.0x10^3$	$1.0x10^3$	None detected	Negative

Table 5 Results for the microbiological analysis for Angel Natural Capsules

Item No.	Herbal Sample	Microbiological analysis			
		Aerobic bacteria (cfu/g)	Yeast/Moulds(cfu/g)	Escherichia Coli(cfu/g)	Salmonella (cfu/g)
1	Angel Natural Capsules Batch No.003 (A)	$3.0x10^4$	$2.0x10^3$	None detected	Negative
2	Angel Natural Capsules Batch No.003 (B)	$3.0x10^4$	$2.0x10^1$	None detected	Negative
3	Angel Natural Capsules Batch No.1106 (A)	$3.0x10^4$	$2.0x10^3$	None detected	Negative
4	Angel Natural Capsules Batch No.1106 (B)	$3.0x10^4$	$<1.0x10^1$	None detected	Negative
5	Angel Natural Capsules Batch No.1206 (A)	$3.0x10^4$	$2.0x10^3$	None detected	Negative
6	Angel Natural Capsules Batch No.1206 (B)	$3.0x10^4$	$2.0x10^1$	None detected	Negative

4.2 ORGANOLEPTIC PROPERTIES

The organoleptic properties of herbal preparations are sensory perceptions which involve tasting, inhaling and visual inspection of the preparations (GNDP, 2004).

They are quick indicators for determining consistency in the production of herbal preparations (Homoeopathic Pharmacopoeia, 1984; GNDP, 2004). The following were assessed; colour, odour, touch, taste and clarity on all the preparations and the results obtained are presented in tables 6, 7 and 8.

The organoleptic properties are unique for every preparation. Inconsistencies in the properties for a particular preparation would mean that the preparation has been adulterated (GNDP, 2004). The distinctive properties of each category of sample analyzed were the same for all the batches which means that there was consistency in producing the preparations analyzed.

Table 6 Results of the organoleptic properties for the herbal preparation

Item No.	Herbal Sample	Colour	Odour	Touch	Taste	Clarity
1	• Angel Herbal Soap • Batch NO. AHPS 004 (A)	Dull Green	Aromatic	Smooth	N/A	N/A
2	• Angel Herbal Soap • Batch NO. AHPS 004 (B)	Dull Green	Aromatic	Smooth	N/A	N/A
3	• Angel Herbal Soap • Batch NO. AHPS 10 (A)	Dull Green	Aromatic	Smooth	N/A	N/A
4	• Angel Herbal Soap • Batch NO. AHPS 10 (B)	Dull Green	Aromatic	Smooth	N/A	N/A
5	• Angel Herbal Soap • Batch NO. AHPS 004B (A)	Dull Green	Aromatic	Smooth	N/A	N/A
6	• Angel Herbal Soap • Batch NO. AHPS 004B (B)	Dull Green	Aromatic	Smooth	N/A	N/A
7	• Angel Cream • Batch No. AHPC 1006 (A)	Green	Aromatic	Smooth	N/A	N/A
8	• Angel Cream • Batch No. AHPC 1006 (B)	Green	Aromatic	Smooth	N/A	N/A
9	• Angel Cream • Batch No. AHPC 11 (A)	Green	Aromatic	Smooth	N/A	N/A
10	• Angel Cream • Batch No. AHPC 11 (B)	Green	Aromatic	Smooth	N/A	N/A
11	• Angel Cream • Batch No. AHPC 1206 (A)	Green	Aromatic	Smooth	N/A	N/A
12	• Angel Cream • Batch No. AHPC 1206 (B)	Green	Aromatic	Smooth	N/A	N/A

NB: N/A = Not Applicable

Table 7 Results of the organoleptic properties for the herbal preparation

Item No.	Herbal Sample	Colour	Odour	Touch	Taste	Clarity
13	• Angel Fatwikeke Ointment • Batch No. AHPFO 0706 (A)	Orange	Pungent Aromatic	Smooth	N/A	N/A
14	• Angel Fatwikeke Ointment • Batch No. AHPFO 0706 (B)	Orange	Pungent Aromatic	Smooth	N/A	N/A
15	• Angel Fatwikeke Ointment • Batch No. AHPFO 0706B (A)	Orange	Pungent Aromatic	Smooth	N/A	N/A
16	• Angel Fatwikeke Ointment • Batch No. AHPFO 0706B (B)	Orange	Pungent Aromatic	Smooth	N/A	N/A
17	• Angel Fatwikeke Ointment • Batch No. AHPFO 0107 (A)	Orange	Pungent Aromatic	Smooth	N/A	N/A
18	• Angel Fatwikeke Ointment • Batch No. AHPFO 0107 (B)	Orange	Pungent Aromatic	Smooth	N/A	N/A
19	• Angel Natural Capsules • Batch No. 003 (A)	Brown	Characteristic	Smooth to Slightly Coarse	Slightly Bitter	N/A
20	• Angel Natural Capsules • Batch No. 003 (B)	Brown	Characteristic	Smooth to Slightly Coarse	Slightly Bitter	N/A
21	• Angel Natural Capsules • Batch No. 1106 (A)	Brown	Characteristic	Smooth to Slightly Coarse	Slightly Bitter	N/A
22	• Angel Natural Capsules • Batch No. 1106 (B)	Brown	Characteristic	Smooth to Slightly Coarse	Slightly Bitter	N/A

NB: N/A = Not Applicable

Table 8 Results of the organoleptic properties for the Herbal Preparations

Item No.	Herbal Sample	Colour	Odour	Touch	Taste	Clarity
23	• Angel Natural Capsules • Batch No. 1206 (A)	Brown	Characteristic	Smooth to Slightly Coarse	Slightly Bitter	N/A
24	• Angel Natural Capsules • Batch No. 1206 (B)	Brown	Characteristic	Smooth to Slightly Coarse	Slightly Bitter	N/A
25	• Angel Herbal Mixture • Batch No. AHPHM 0906 (A)	Chocolate Brown	Characteristic	N/A	Bitter	Opaque, Non-Viscous
26	• Angel Herbal Mixture • Batch No. AHPHM 0906 (B)	Chocolate Brown	Characteristic	N/A	Bitter	Opaque, Non-Viscous
27	• Angel Herbal Mixture • Batch No. AHPHM 1106 (A)	Chocolate Brown	Characteristic	N/A	Bitter	Opaque, Non-Viscous
28	• Angel Herbal Mixture • Batch No. AHPHM 1106 (B)	Chocolate Brown	Characteristic	N/A	Bitter	Opaque, Non-Viscous
29	• Angel Herbal Mixture • Batch No. AHPHM 0107 (A)	Chocolate Brown	Characteristic	N/A	Bitter	Opaque, Non-Viscous
30	• Angel Herbal Mixture • Batch No. AHPHM 0107 (B)	Chocolate Brown	Characteristic	N/A	Bitter	Opaque, Non-Viscous

4.3 PHYSICOCHEMICAL PROPERTIES

The physicochemical properties assessed were pH, Total Ash, Sulphated Ash, Acid Insoluble Ash, Loss on drying, Total Solids and Relative Density. The results are presented in tables 9 to 16.

4.3.1 pH

pH indicates the hydrogen ion concentration of a solution and it is the measure of the acidity of the solution. Uncontrolled amounts of it in preparations can pose health problems (GNDP, 2004). Some of these health problems that can result from uncontrolled pH are acidosis which is a condition of decreased alkalinity of the blood tissues. The symptoms of acidosis may include sickly sweet breath, headache, nausea, vomiting, disturbances of tissues and central nervous system (CNS) functions (IUPAC, 2000). If the pH is above or below the acceptable limits or range for a particular preparation, it can also damage the kidneys and the liver, can irritate the skin or tissues, and can cause inflammation of skin and tissues. It can also penetrate the skin to cause deep sores (MSDS, 2004)

The pH values are presented in tables 9 and 10.

Table 9 Results of pH for the Herbal Preparations

Item No.	Herbal Sample	pH
1	• Angel Natural Capsules • Batch No. 1106 (A)	5.64
2	• Angel Natural Capsules • Batch No. 1106 (B)	5.59
3	• Angel Natural Capsules • Batch No. 1206 (A)	5.71
4	• Angel Natural Capsules • Batch No. 1206 (B)	5.70
5	• Angel Herbal Mixture • Batch No. AHPHM 0906 (A)	4.09
6	• Angel Herbal Mixture • Batch No. AHPHM 0906 (B)	4.05
7	• Angel Herbal Mixture • Batch No. AHPHM 1106 (A)	4.07
8	• Angel Herbal Mixture • Batch No. AHPHM 1106 (B)	4.03
9	• Angel Herbal Mixture • Batch No. AHPHM 0107 (A)	4.07
10	• Angel Herbal Mixture • Batch No. AHPHM 0107 (B)	4.07

4.3.2 The three categories of pH environments

The standards of the pH for these preparations are in three different categories:

- The herbal soaps are prepared for external use and for brief skin contact. The pH ranged from 9 – 11, which is within the WHO standard range of 5.0 – 11.0. This category is more of alkaline than acidic since soap washes best in this pH region. The long skin contact according to MSDS, (2004), can cause skin irritation to inflammation. It can also cause skin to blacken, a condition where a fair coloured skin blackens due to reaction caused by the alkaline, (Pamplona-Roger, 1998).

Table 10 Results of pH for the Herbal Preparations

Item No.	Herbal Sample	pH
11	• Angel Herbal Soap • Batch NO. AHPS 004 (A)	10.10
12	• Angel Herbal Soap • Batch NO. AHPS 004 (B)	10.10
13	• Angel Herbal Soap • Batch NO. AHPS 10 (A)	9.85
14	• Angel Herbal Soap • Batch NO. AHPS 10 (B)	9.95
15	• Angel Herbal Soap • Batch NO. AHPS 004B (A)	9.79
16	• Angel Herbal Soap • Batch NO. AHPS 004B (B)	9.76
17	• Angel Cream • Batch No. AHPC 1006 (A)	6.00
18	• Angel Cream • Batch No. AHPC 1006 (B)	5.41
19	• Angel Cream • Batch No. AHPC 11 (A)	6.52
20	• Angel Cream • Batch No. AHPC 11 (B)	6.83
21	• Angel Cream • Batch No. AHPC 1206 (A)	6.25
22	• Angel Cream • Batch No. AHPC 1206 (B)	6.32
23	• Angel Fatwikeke Ointment • Batch No. AHPFO 0706 (A)	7.93
24	• Angel Fatwikeke Ointment • Batch No. AHPFO 0706 (B)	7.89
25	• Angel Fatwikeke Ointment • Batch No. AHPFO 0706B (A)	6.98
26	• Angel Fatwikeke Ointment • Batch No. AHPFO 0706B (B)	6.72
27	• Angel Fatwikeke Ointment • Batch No. AHPFO 0107 (A)	6.95
28	• Angel Fatwikeke Ointment • Batch No. AHPFO 0107 (B)	7.63
29	• Angel Natural Capsules • Batch No. 003 (A)	5.61
30	• Angel Natural Capsules • Batch No. 003 (B)	5.62

- Cream and Ointments are also prepared for external use, but for long continuous skin contact. The pH ranged from 6 – 8, which is within the standard range 5.0 – 8.0, that is safe for use.

- The Natural capsules and the herbal mixtures are prepared for internal use. These gave values with a range of 4 – 6. The pH of all the preparations were within the WHO acceptable limits of 4.0 – 7.0, and so when administered may not cause any pH health related problems.

4.3.3 Ash Value

Incineration of herbal ingredients produces ash which constitutes inorganic matter. Treatment of the ash with hydrochloric acid results in acid-insoluble ash which consists mainly of silica and may be used as a measure of soil present in a herbal preparation (Barnes et al., 2002; GNDP, 2004). Treatment of the ash with sulphuric acid results in sulphated ash which determines the amount of inorganic substances such as metals contained as impurities (Barnes et al., 2002). The ash values of preparations at certain levels can cause health hazards (The Scientific Committee of England, 1990; Barnes et al., 2002). The results of the ash values are presented in tables 11, 12 and 13. The range for the data obtained for the total ash was 6.5 – 7%, which fell within the acceptable limit of 3 – 10% (WHO, 2002). The range for the results obtained for the sulphated ash is 1.1 – 1.6% while the Ghana Food and Drugs Board (FDB) allows up to 1%. The range for the figures obtained for the acid insoluble ash was 1.1 – 1.4%. This also gave values a little above the Ghana FDB's acceptable limit of 1%.

4.3.4 Loss on drying

The presence of excessive water in solid preparations will promote the growth of microbes, fungi or insects and the hydrolysis of constituents leading to deterioration of the powder. It is important to set limit of moisture for herbal powder (GNDP, 2004). The results for Loss on drying presented in table 14 ranged from 7 – 10%. The acceptable range is 8 – 14% (GNDP, 2004). Since the moisture content of the powder analyzed did not exceed the acceptable limit of 14%, the powder would not deteriorate. An extensive work on loss on drying has been done in India on different herbal powders and presented in the form of monogram, by the Scientific Community of India. The data are captured in a herbal pharmacopoeia, Homoeopathic Pharmacopoeia (1984). The data were in the range of 7 – 13%.

Table 11 Results of the Total Ash for Herbal Powder (Capsules)

Item No.	Herbal Sample	Total Ash (%)						Mean (X')	Standard Deviation (σ)
		1	2	3	4	5	6		
1	Angel Natural Capsules Batch No.003	6.596	6.613	6.599	6.731	6.834	6.601	6.662	7.18×10^{-2}
2	Angel Natural Capsules Batch No.1106	6.696	6.738	6.713	6.794	6.758	6.808	6.752	4.5×10^{-2}
3	Angel Natural Capsules Batch No.1206	6.743	6.734	6.694	6.703	6.741	6.693	6.718	2.6×10^{-2}

Table 12 Results of the Sulphated Ash for Herbal Powder (Capsules)

Item No.	Herbal Sample	Sulphated Ash (%)						Mean (X')	Standard Deviation (σ)
		1	2	3	4	5	6		
1	Angel Natural Capsules Batch No.003	1.1426	1.4650	1.5090	1.5391	1.5073	1.6267	1.532	5.04×10^{-2}
2	Angel Natural Capsules Batch No.1106	1.5028	1.4894	1.4604	1.5236	1.3795	1.3310	1.448	1.59×10^{-2}
3	Angel Natural Capsules Batch No.1206	1.5944	1.5356	1.5339	1.4948	1.4735	1.5359	1.528	3.31×10^{-2}

Table 13 Results of the Acid-Insoluble Ash for Herbal Powder (Capsules)

Item No.	Herbal Sample	Acid-Insoluble Ash (%)						Mean (X')	Standard Deviation (σ)
		1	2	3	4	5	6		
1	Angel Natural Capsules Batch No.003	1.2042	1.3027	1.1480	1.1326	1.2589	1.3133	1.227	8.55×10^{-2}
2	Angel Natural Capsules Batch No.1106	1.1526	1.0424	1.822	1.1149	1.1007	1.0749	1.095	3.81×10^{-2}
3	Angel Natural Capsules Batch No.1206	1.1729	1.0883	1.0783	1.1330	1.1726	1.327	1.1132	3.75×10^{-2}

Table 14 Results of the Loss on drying for Herbal Powder (Capsules)

Item No.	Herbal Sample	Loss on Drying (%)						Mean (X')	Standard Deviation (σ)
		1	2	3	4	5	6		
1	Angel Natural Capsules Batch No.003	9.849	9.868	9.809	9.206	9.208	9.214	9.436	1.72×10^{-2}
2	Angel Natural Capsules Batch No.1106	7.928	7.939	8.561	7.311	8.684	8.665	8.182	5.75×10^{-2}
3	Angel Natural Capsules Batch No.1206	8.162	8.175	8.155	8.669	8.683	8.649	8.416	1.36×10^{-2}

4.3.5 Total Solids

The term total solid is applied to the residue obtained when the prescribed amount of liquid preparations are evaporated and dried to constant weight under specific conditions of temperature (GNDP, 2004; Homoeopathic Pharmacopoeia, 1984). Total solids is manufacturer specific due to formulation but must be consistent (Homoeopathic Pharmacopoeia, 1984). The values obtained for total solids ranged from 1 – 3%. All the three batches of the herbal mixture analyzed gave values which were close to each other, and that means there was consistency in production. The results of the total solids are presented in table 15.

4.3.6 Relative Density

Relative density is applicable to liquid preparations and it is defined as the ratio of the mass of a given volume of a liquid substance to the mass of an equal volume of water, both weighed at room temperature. The relative density is manufacturer specific and if it is found to be the same all the time, it shows consistency in production (GNDP, 2004). The results of the relative density obtained for the herbal mixture were all approximately one (1), indicating that there was consistency in production. The results are presented in table 16.

Table 15 Results of the Total Solids (TS) for the Angel Herbal Mixture

Item No.	Herbal Sample	Total Solids (%)						Mean (X')	Standard Deviation (σ)
		1	2	3	4	5	6		
1	Angel Herbal Mixture Batch No. AHPHM 0906	2.198	2.199	2.188	2.082	2.179	2.197	2.174	6.14×10^{-3}
2	Angel Herbal Mixture Batch No. AHPHM 1106	1.977	1.986	1.969	2.056	1.998	2.011	2.000	5.76×10^{-3}
3	Angel Herbal Mixture Batch No. AHPHM 0107	2.059	2.058	2.054	2.085	2.080	2.091	2.069	4.08×10^{-3}

Table 16 Results of the Relative Density (RD) for the Angel Herbal Mixture

Item No.	Herbal Sample	Relative Density (RD)						Mean (X')	Standard Deviation (σ)
		1	2	3	4	5	6		
1	Angel Herbal Mixture Batch No. AHPHM 0906	1.0097	1.0101	1.0097	1.0099	1.0099	1.0098	1.0099	4.07×10^{-5}
2	Angel Herbal Mixture Batch No. AHPHM 1106	1.0095	1.0096	1.0095	1.0098	1.0099	1.0098	1.0097	7.07×10^{-5}
3	Angel Herbal Mixture Batch No. AHPHM 0107	1.0097	1.0097	1.0096	1.0096	1.0095	1.0096	1.0097	7.07×10^{-5}

4.4 Phytoconstituents

In plants, several chemicals are produced after metabolism, some of which are called secondary metabolites. Most of the time it is these secondary metabolites which are physiologically active and so they become therapeutically important in medicine. Examples are glycosides, saponins, flavonoids, alkaloids anthraquinones, tannins, coumarins, anthracene etc. Most of these metabolites are biosynthesized by enzymes such as acetyl co- enzyme A. A lot of these can be found in one particular plant species with varying polarity and functional groups. Chemical tests are available for screening plants extracts for the presence of a range of secondary metabolite. These tests are useful in the identification and evaluation of herbal products and are based on colour reaction or precipitation in response to a particular reagent (GDNP, 2004).

Some medical applications of saponins are analgesic, cicatrizing and hemorrhoids and that of glycosides are hemorrhoids, heart insufficiency, cardio tonic, scabies, cicatrice, emollient and healing of wounds. Tannins and flavonoids are for untreatable aches, antidepressant, antiviral and antibacterial, diarrhea and fever. Anthracene and anthraquinones are remedies for constipation and also used as laxatives. Alkaloids are for anti inflammatory, stimulant, vulnerary, healing wounds, allergy, chronic aches, analgesic, and cancer (Pamplona-Roger, 1998; Barnes et al., 2002). The phytochemical screening was performed on all the samples to determine which metabolites are present or absent. The results are tabulated in tables 17, 18, 19, 20 and 21.

Table 17 Results for the phytochemical screening for the Angel Herbal Mixture

Item No.	Herbal Sample	Test						
		Glycosides	**Alkaloids**	**Tannins**	**saponins**	**Flavonoid**	**Anthraquinones**	**Anthracene**
1	Angel Herbal Mixture Batch No. AHPHM 0906A	Present	Absent	Present	Present	Present	Present	Absent
2	Angel Herbal Mixture Batch No. AHPHM 0906B	Present	Absent	Present	Present	Present	Present	Absent
3	Angel Herbal Mixture Batch No. AHPHM 1106A	Present	Absent	Present	Present	Present	Present	Absent
4	Angel Herbal Mixture Batch No. AHPHM 1106B	Present	Absent	Present	Present	Present	Present	Absent
5	Angel Herbal Mixture Batch No. AHPHM 0107A	Present	Absent	Present	Present	Present	Present	Absent
6	Angel Herbal Mixture Batch No. AHPHM 0107B	Present	Absent	Present	Present	Present	Present	Absent

Table 18 Results for the phytochemical screening for the Angel Natural Capsules

Item No.	Herbal Sample	Test						
		Glycosides	**Alkaloids**	**Tannins**	**saponins**	**Flavonoid**	**Anthraquinones**	**Anthracene**
1	Angel Natural Capsules Batch No.003 (A)	Present	Present	Absent	Absent	Absent	Present	Absent
2	Angel Natural Capsules Batch No.003 (B)	Present	Present	Absent	Absent	Absent	Present	Absent
3	Angel Natural Capsules Batch No.1106 (A)	Present	Present	Absent	Absent	Absent	Present	Absent
4	Angel Natural Capsules Batch No.1106 (B)	Present	Present	Absent	Absent	Absent	Present	Absent
5	Angel Natural Capsules Batch No.1206 (A)	Present	Present	Absent	Absent	Absent	Present	Absent
6	Angel Natural Capsules Batch No.1206 (B)	Present	Present	Absent	Absent	Absent	Present	Absent

Table 19 Results for the phytochemical screening for the Angel Soap

Item No.	Herbal Sample	Test						
		Glycosides	**Alkaloids**	**Tannins**	**saponins**	**Flavonoid**	**Anthraquinones**	**Anthracene**
1	Angel Soap, Batch No. AHPS 004 (A)	Present	Present	Absent	Absent	Absent	Absent	Absent
2	Angel Soap, Batch No. AHPS 004 (B)	Present	Present	Absent	Absent	Absent	Absent	Absent
3	Angel Soap, Batch No. AHPS10 (A)	Present	Present	Absent	Absent	Absent	Absent	Absent
4	Angel Soap, Batch No. AHPS 10 (B)	Present	Present	Absent	Absent	Absent	Absent	Absent
5	Angel Soap, Batch No. AHPS 004B (A)	Present	Present	Absent	Absent	Absent	Absent	Absent
6	Angel Soap, Batch No. AHPS 004B (B)	Present	Present	Absent	Absent	Absent	Absent	Absent

Table 20 Results for the phytochemical screening for the Angel cream

Item No.	Herbal Sample	Test						
		Glycosides	**Alkaloids**	**Tannins**	**saponins**	**Flavonoid**	**Anthraquinones**	**Anthracene**
1	Angel Cream Batch No. AHPC 1006(A)	Present	Present	Absent	Absent	Absent	Absent	Absent
2	Angel Cream Batch No. AHPC 1006(B)	Present	Present	Absent	Absent	Absent	Absent	Absent
3	Angel Cream Batch No. AHPC 11 (A)	Present	Present	Absent	Absent	Absent	Absent	Absent
4	Angel Cream Batch No. AHPC 11 (B)	Present	Present	Absent	Absent	Absent	Absent	Absent
5	Angel Cream Batch No. AHPC 1206 (A)	Present	Present	Absent	Absent	Absent	Absent	Absent
6	Angel Cream Batch No. AHPC 1206 (B)	Present	Present	Absent	Absent	Absent	Absent	Absent

Table 21 Results for the phytochemical screening for the Angel Fatwikeke Ointment

Item No.	Herbal Sample	Test						
		Glycosides	**Alkaloids**	**Tannins**	**saponins**	**Flavonoid**	**Anthraquinones**	**Anthracene**
1	Fatwikeke Ointment. Batch No. AHPFO 0706 (A)	Absent	Present	Present	Absent	Present	Absent	Absent
2	Fatwikeke Ointment. Batch No. AHPFO 0706 (B)	Absent	Present	Present	Absent	Present	Absent	Absent
3	Fatwikeke Ointment. Batch No .AHPFO 0706B (A)	Absent	Present	Present	Absent	Present	Absent	Absent
4	Fatwikeke Ointment. Batch No. AHPFO 0706B (B)	Absent	Present	Present	Absent	Present	Absent	Absent
5	Fatwikeke Ointment. Batch No. AHPFO 0107 (A)	Absent	Present	Present	Absent	Present	Absent	Absent
6	Fatwikeke Ointment. Batch No. AHPFO 0107 (B)	Absent	Present	Present	Absent	Present	Absent	Absent

The indications on the herbal mixtures were jaundice, menstrual pains, malaria, loss of appetite and body pain. It was found to contain the following phytoconstituents, glycosides, tannins, saponins, flavonoids and anthraquinones. The soap was meant for curing shingles, ringworm, eczema, boils and foot rot and was found to contain glycosides and alkaloids. The capsules were for body and waist pains and sexual weakness. It was also found to contain glycosides, alkaloids and anthraquinones. The cream and the ointments were meant for shingles, itches, boils, anal sore, candidiasis, after-shave, quick hair growth, muscular pain and rheumatism. They were found to contain glycosides, alkaloids tannins and flavonoid. The presence of these phytoconstituents makes the preparations therapeutically important in curing diseases (Pamplona-Roger, 1998; Schulz et al., 1999; Barnes et al., 2002). The preparations could therefore be effective for their therapeutic claims since they contained the necessary phytoconstituents.

4.5 Heavy Metals

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low or very high concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb). They are natural components of the Earth's crust. They cannot be degraded or destroyed and can find their way in plants. When heavy metals are ingested they pose health problems (Gilman, 1991; Barnes et al., 2002). Diseases caused by the poisoning of heavy metals when they enter the body through the skin or by oral means are well documented (Bakir et al., 1973; IUPAC, 2000; Barnes et al., 2002). Heavy metals poisoning can cause irritation of the lungs, eyes and the skin. It can damage

the nervous system, and it can cause cancer of the skin, lung, liver and nose. The poisoning can cause dermatitis, can harm the brain and nerve function; can cause anaemia and can damage the kidney.

The work done by Bakir et al., (1973) to investigate the cause of an outbreak in Iraq, revealed the following concentrations of methyl mercury in the blood of some people and their consequences. It was found out that for methyl mercury concentration levels of 0.1 – 0.5ppm, 5 people suffered from paresthesia and from, 0.5 – 1.0 ppm, 42 people suffered from paresthesia, 11 people ataxia, 21 people visual defects, 5 people dysarthria and 5 people, hearing defects. From 1.0 – 2.0ppm, 60 people suffered from paresthesia, 47 people, ataxia 53 people, visual defects, 24 people, dysarthria and 5 people hearing defects. Concentrations between 3.0 – 4.0 ppm; 17 people died and between 4.0 – 5.0ppm 28 people died (Bakir et al., 1973). This work shows clearly that the higher the concentration of the mercury the lethality.

A United States survey reported widespread inconsistencies and adulterations in imported Asian herbal preparations (Ko, 1998). Of 260 imported preparations tested, at least 83 (32%) contained undeclared amounts of heavy metals such as lead, arsenic and mercury. Another survey found evidence of a continuing problem with 10% of 5000 herbal preparations containing high amounts of lead, mercury or arsenic (Au, 2000). Also, a survey conducted in Singapore between 1990 and 1997 on herbal preparations reported that 42 different preparations were found to contain excessive amounts of heavy metals such as mercury, arsenic and lead (Koh and Woo, 2000). In 2001, the United Kingdom Medicines Control Agency (MCA) reported the presence of mercury in some preparations on the United Kingdom market. A patient from Taiwan developed a unique

syndrome of multiple renal tubular dysfunctions after taking a Chinese herbal preparation contaminated with cadmium (Wu, 1996). In the United Kingdom cases have been reported of two patients with heavy metal intoxication following ingestion of an Indian preparation containing inorganic arsenic and mercury (Kew, 1993), and of a patient with lead poisoning after taking Indian preparation containing high amounts of lead, arsenic and mercury (Sheerin, 1994). In a case reported from Macau, death of a 13-year old girl from arsenic poisoning has been linked with a Chinese herbal preparation (Cuncha, 1998).

The four heavy metals whose health hazards through herbal preparations have been known worldwide and are a major problem, were determined. These are Hg, As, Cd and Pd. The standards for internal and external use respectively for arsenic are 10ppb and 50ppb. The range for the values obtained for arsenic is 1.6 – 2.8ppb. The arsenic levels were lower than the standards. Mercury and cadmium were not detected in any of the samples. The detection limits for Hg and Cd respectively were 1.0 and 1.5ppb. Lead which was detected in only few of the samples gave values with a range of 2.0 – 2.3ppb and a detection limit of 1.8ppb. They also fell below the standards of 10 and 100ppb for internal and external use respectively. By WHO standards, the herbal preparations

analyzed were not contaminated by these heavy metals and so may not cause any of the associated health hazards above. The results are presented in table 22.

Table 22 Concentration of heavy metals in the 30 herbal preparations analyzed

No	Sample and Batch No.	Mercury (ppb)	Arsenic (ppb)	Lead (ppb)	Cadmium (ppb)
1	Capsules 003 (A)	None detected	2.6	2.3	None detected
2	Capsules 003 (B)	None detected	2.7	2.3	None detected
3	Capsules 1106 (A)	None detected	2.4	2.1	None detected
4	Capsules 1106 (B)	None detected	2.4	2.0	None detected
5	Capsules 1206 (A)	None detected	2.6	2.1	None detected
6	Capsules 1206 (B)	None detected	2.6	2.1	None detected
7	Soap AHPS 004 (A)	None detected	2.6	None detected	None detected
8	Soap AHPS 004 (B)	None detected	2.8	None detected	None detected
9	Soap AHPS 10 (A)	None detected	2.7	None detected	None detected
10	Soap AHPS 10 (B)	None detected	1.6	None detected	None detected
11	Soap AHPS 004B (A)	None detected	1.8	None detected	None detected
12	Soap AHPS 004B (B)	None detected	2.0	None detected	None detected
13	Cream AHPC 1006 (A)	None detected	2.0	None detected	None detected
14	Cream AHPC 1006 (B)	None detected	1.9	None detected	None detected
15	Cream AHPC 11 (A)	None detected	2.1	None detected	None detected
16	Cream AHPC 11 (B)	None detected	2.0	None detected	None detected
17	Cream AHPC 1206(A)	None detected	1.8	None detected	None detected
18	Cream AHPC 1206 (B)	None detected	1.8	None detected	None detected
19	Ointment AHPFO 0706 (A)	None detected	2.4	None detected	None detected
20	Ointment AHPFO 0706 (B)	None detected	2.2	None detected	None detected
21	Ointment AHPFO 0706B(A)	None detected	2.4	None detected	None detected
22	Ointment AHPFO 0706B (B)	None detected	2.4	None detected	None detected
23	Ointment AHPFO 0107 (A)	None detected	2.2	None detected	None detected
24	Ointment AHPFO 0107 (B)	None detected	2.4	None detected	None detected
25	Mixture AHPHM 0906 (A)	None detected	1.6	None detected	None detected
26	Mixture AHPHM 0906 (B)	None detected	1.6	None detected	None detected
27	Mixture AHPHM 1106 (A)	None detected	1.8	None detected	None detected
28	Mixture AHPHM 1106 (B)	None detected	1.6	None detected	None detected
29	Mixture AHPHM 0107 (A)	None detected	1.8	None detected	None detected
30	Mixture AHPHM 0107 (B)	None detected	1.8	None detected	None detected

For accuracy and precision of the determination, some known concentrations of the heavy metals were added to some selected herbal preparations and digested. The digested solutions were then analyzed for the heavy metals. The recoveries were about 81 to 99% indicating how efficient the methods were. The results obtained for the recoveries are presented in table 23.

Table 23 Recovery of the four (4) heavy metals from five (5) selected samples

Sample	Cd added (ppb)	Cd recov. (ppb)	%Cd recov.	Pb added (ppb)	Pb recov. (ppb)	%Pb recov.	Hg added (ppb)	Hg recov. (ppb)	%Hg recov	As added (ppb)	As recov. (ppb)	%As recov.
Angel Soap	0.50	0.4501	90.02	1.00	0.8601	86.02	2.00	1.6932	84.66	2.00	1.8314	91.57
Angel Cream	0.50	0.4877	97.54	1.00	0.9012	90.12	2.00	1.8637	93.19	2.00	1.8943	94.97
Angel Fatwikeke Ointment	0.50	0.4918	98.36	1.00	0.8993	89.93	2.00	1.9274	96.37	2.00	1.9875	99.38
Angel Natural Capsules	0.50	0.4912	98.24	1.00	0.9039	90.39	2.00	1.6152	80.76	2.00	1.8847	94.24
Angel Herbal Mixture	0.50	0.4814	96.28	1.00	0.8795	87.95	2.00	1.8973	94.87	2.00	1.8936	94.68

NB: recov. = Recovery

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The parameters tested for all the three batches for every herbal preparation assessed were reproducible. This means that there was consistency in production. When compared with their respective standards where applicable, it was found out that there was not much difference among the samples and the standards. When compared with quality standards where applicable, it was found out that the results were all below the World Health Organization (WHO) quality standards. The quality of the herbal preparations assessed is therefore acceptable based on the WHO quality standards.

5.2 Recommendation

The work could not extend to the analysis by thin layer chromatography (TLC) and the high performance liquid chromatography (HPLC) to determine the fingerprints and profiles of the individual phytoconstituents present in the preparations analyzed. It is therefore recommended that later work be extended to cover these two areas.

Manufacturers should be encouraged to send their products regularly to laboratories for quality assessment to ensure consistency and quality before marketed.

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GLOSSARY

GLOSSARY

Capsules
It is a solid preparation with hard or soft shells, of various shapes and capacities. The contents of capsules may be of solid, liquid or paste-like consistency. They are intended for oral administration.

Concoction
It is any extract made by combining different ingredients (sample plant materials).

Cream
It is a homogeneous, viscous or semi-solid preparation usually consisting of solutions or dispersions of one or more active ingredients in suitable bases. It is intended to be applied to the skin or certain mucous membranes for protective, therapeutic (Tending to cure or restore to health) or prophylactic (Preventing or contributing to the prevention of disease, conception or impregnation) purposes.

Decoction
It is a water extract in which the sample plant material is boiled for at least 15minutes.

Infusion
It is a water extract in which hot or cold water is added to sample plant material and allowed to soak at room temperature for a period of time.

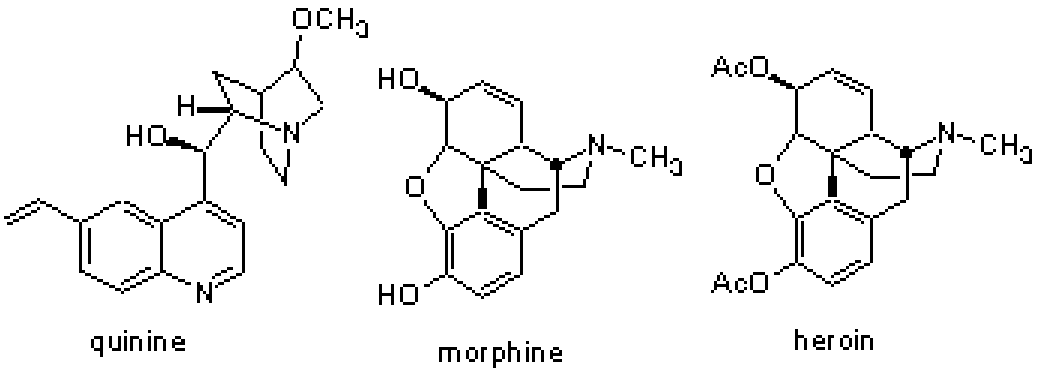
Maceration
It is a process of extracting a sample plant material with a solvent, with several daily agitations at room temperature.

Ointment
It is a homogeneous semi-solid preparation, usually consisting of solution or dispersions of one or more active ingredients in suitable bases. It is intended to be applied to the skin or certain mucous membranes for emollient, protective, therapeutic or prophylactic purposes.

Paste
It is a homogeneous semi-solid preparation usually containing a high proportion of solids finely dispersed in suitable bases.

APPENDICES

Appendix 1


OCH3
H
HO
N
N
quinine
HO
O
N
CH3
HO
morphine
AcO
O
N
CH3
AcO
heroin

Appendix 2

The Standards for herbal preparations as quoted by the respective reference

No.	Parameter	For External use: **(GNDP, 2004, GS238, 2001)**	For Internal use: **(GS238, 2001, WHO, 2004)**	For external and internal use in **Germany (Busse W, 2000)**
1	**pH** 1.Brief skin Contact 2.Long skin contact	 5.0 – 11.0 5.0 – 8.0	4.0 – 7.0	---------
2	Mercury	20ppb (0.02ppm)	1ppb (0.001ppm)	100ppb (0.1ppm)
3	Cadmium	0.2ppm	3ppb (0.003ppm)	200ppb (0.2ppm
4	Lead	0.1ppm	10ppb (0.01ppm)	5000ppb (5ppm)
5	Arsenic	50ppb (0.05ppm)	10ppb (0.01ppm)	----------
No.	Parameter	For Boiled Preparations/(cfu/g) **(GNDP, 2004)**	For Un boiled Preparations/(cfu/g) **(GNDP, 2004)**	
1	Salmonella	Negative	Negative	
2	Escherichia Coli	10^2	10^1	
3	Aerobic bacterial	10^7	10^5	
4	Yeast and Mould	10^4	10^3	

Standards for Phytochemical Screening

Loss on Drying, 8 – 14 %, Total Ash, 6%, Acid Insoluble Ash, 1%,

Specific Gravity – Solvent Dependent (example, H_2O = 1), Phytochemical Contents – Manufacturer Specific, Total Solids – Manufacturer Specific, Extractive Values- (Alcohol) – Not Less than 25%, Sulphated Ash – Not more than 1%, (Ministry of Health (GNDP) Ghana, 2004), SCE, 1983, SCE, 1990, WHO, 2002)