

**Determination of some toxic metals in some herbal preparation sold by hawker in Ilorin
and Environs**

Table of Contents

Abstract	5
Background	7
1.2 Statement of the problem	9
1.3 Aims and Objectives of the study	9
1.4 Research Questions	9
1.5 Significance of the Study	10
1.6 Scope of the study	10
1.7 Operational Definition of Terms.....	11
CHAPTER TWO	13
Introduction	13
Tradition and safety of herbal medicines	15
Carcinogenic effects of traditional herbal medicines.....	16
Kinetic interactions of herbal medicines with conventional medicines.....	17
Factors Responsible for Increased Patronage and Self Medication with Herbal Medicine.....	18
Influence of Regulatory Policies on Safety of Herbal Medicines	19
Toxicity and Adverse Health Effects of Some Common Herbal Medicines	20
Challenges Associated with Monitoring Safety of Herbal Medicines.....	21
Challenges Related to the Regulatory Status of Herbal Medicines.....	22
Challenges Related to the Assessment of Safety and Efficacy.....	23
Challenges Related to Quality Control of Herbal Medicines	23
Challenges Related to Safety Monitoring of Herbal Medicines	24
CHAPTER THREE	26
Materials and Methods	26
3.1 Equipment and reagents.....	26
3.2 METHODS.....	26
3.2.1 Collection of samples.....	26
3.2.2 Preparation of calibration curve.....	26
3.2.3 Sample pre-treatment	27
3.3 Sample analysis	28
3.4 Statistical analysis.....	28
CHAPTER FOUR	29

4.1 RESULTS	29
CHAPTER FIVE.....	31
5.1 DISCUSSION.....	31
CONCLUSION.....	34
REFERENCES.....	35

List of tables

Table of concentration of Iron (Fe), Lead (Pb), Cadmium (Cd), Copper (Cu), and Chromium (Cr)

Abstract

The use of herbal medicines has increased in recent years and has gained much attention in the health sectors, scientific community and the public alike. The safety and quality of these products become questionable even when the efficacy and potency may be guaranteed. In this study the presence of heavy metals in herbal preparations was determined. Ten different herbal preparations were purchased from different parts of Ilorin. Samples were prepared from these preparations and analyzed for the presence of Cadmium (Cd), Iron (Fe) and Lead (Pb) using Atomic Absorption Spectrophotometer. Their concentrations were compared with WHO permissible limits. The concentration of heavy metals (Pb, Fe, Cu, Cr and Cd) in the samples were compared with permissible limit of 10 mg/L, 0.3 mg/L and 0.1 mg/L, 2mg/L and 40mg/L for Pb, Cd, Fe, Cr and Cu respectively. The concentration of lead in herbal samples ranged from 0.010 ± 0.027 and 0.320 ± 0.035 mg/L, The highest and the lowest concentrations were found in H3 and H4 samples which were used by traditional healers to treat hypertension and sexual impotency. The concentration of Cadmium in herbal samples ranged from 0.000 ± 0.000 and 0.250 ± 0.065 mg/L. It is important to note that seven (7) samples from the herbal preparations analyzed contained iron in concentrations significantly ($P=0.01$) higher than the 0.1 mg/L permissible limit recommended by WHO. In this study, the concentration of chromium was found in the range of 0.180 ± 0.062 and 0.860 ± 0.027 mg/L. The highest amount was reported in the H10 sample while the lowest amount was reported in the H8 sample. The concentration range of copper in tested samples was 0.370 ± 0.006 and 0.010 ± 0.013 mg/L as shown in Table 1. The maximum concentration was reported in the H3 sample while the minimum amount was reported in the H4 sample preparation. This result revealed that the concentration of copper in all traditional herbal products was found below the WHO permissible limit (40 mg/L). The results

obtained from this study showed that Iron was present in all the samples with some concentrations significantly higher than the WHO permissible limit. Lead, copper and cadmium were present in some of the samples with concentrations below the WHO permissible limit.

CHAPTER ONE

Background

The use of homeopathic and herbal medicines has increased in recent years (Chikezie et al, 2015). This has probably arisen as a result of a number of factors including; disillusionment with conventional drugs, growing confidence in complementary medicine, and a belief that the products are safe, often on the grounds that 'natural' equates to safe (Enuh *et al.*, 2012). Herbal medicine and traditional treatment are the main source of health care and sometimes the only source of care for millions of people in developing countries (WHO, 2013). A survey carried out in Lagos, Nigeria also reported that majority of the respondents use herbal medicines (WHO 2013; Oreagba *et al.*, 2011). However, the challenges associated with the use of these medicinal plants and herbal products obtained from them include safety and quality even when efficacy and potency are ascertained. The presence of heavy metals in the body can be detrimental to health particularly at concentrations above tolerance level. Most are considered toxic to living organism due to their accumulative tendency in tissues resulting in various disorders (Bakare-Odunola and Mustapha, 2014). It has been reported that the presence of heavy metals in either orthodox or herbal drugs often compound the original ailments for which they were administered with resultant increased morbidity for the patient (Topliss *et al.*, 2002). There are various sources of contamination that renders the safety and quality of medicine questionable and notable amongst them is that from heavy metals. Contamination of traditional medicines by heavy metals is of major concern because of the toxicity, persistence and bio-accumulative nature of these metals

(Osegbe and Amaku, 1985). Examples of heavy metals include Arsenic (As), Lead (Pb), Mercury (Hg), Copper (Cu), Iron (Fe), Cadmium (Cd) and Zinc (Zn).

Three main sources have been proposed to be responsible for heavy metal contamination of medicinal herbal products (Saeed *et al.*, 2010), viz; contamination during the process of cultivating the medicinal plant itself (Sharma and Dubey, 2005 Tong *et al.*, 2000), unintentional cross contamination during processing, (Chan, 2003) and the deliberate addition of heavy metals as therapeutic agents (Mohammad *et al.*, 2012; Ernst, 2002). The WHO has formulated guidelines for quality assurance and control of herbal medicine but most traditional practitioners lack this information (WHO, 2007). This sometimes results in medicinal plants and products derived from them being contaminated with various types and concentration of heavy metals. Some reported works established the fact that there are both heavy metal pollution of the environment and contamination of herbal products in Nigeria and the world at large (Nweke and Sanders, 2009). In a study conducted in Nigeria to determine the cadmium (Cd) concentrations of herbal drugs used as antimalarial and that of chloroquine syrup, it was revealed that Cadmium was detected in 100 % of the herbal products and 75 % of the chloroquine syrups (Mohammad *et al.*, 2012). The concentration of Cd in all the herbal products exceeded the WHO limit, while the concentration in all the chloroquine syrups were within the British Pharmacopeia (BP) 2002 limit. Another study reported the concentration of Fe ranged from 65.68-1652.89 µg/g in different products of herbal medicine purchased from various places in Karachi city of Pakistan (Tong *et al.*, 2000). A lot of cases of poisoning from heavy metal such as cadmium, lead, iron, mercury and manganese remain underreported while some are undocumented due to poor record keeping in the developing nations (Sharma and Dubey, 2005). Therefore, the need for more studies investigating the safety and quality of the herbal products becomes imperative.

This research focused on various herbal bitters acclaimed to be used for a variety of health conditions within Ilorin Metropolis of Nigeria. The safety and quality of different manufacturing batches of herbal preparations, in terms of their level of contamination by heavy metals were ascertained. The heavy metals selected for this study include four biologically non-essential elements: Lead (Pb), Cadmium (Cd), Copper (Cu), Chromium (Cr) and one biologically essential element: Iron (Fe) which are some of the heavy metals implicated to be toxic in human.

1.2 Statement of the problem

The use of herbal medicines has increased in recent years and has gained much attention in the health sectors, scientific community and the public alike. The safety and quality of these products become questionable even when the efficacy and potency may be guaranteed.

1.3 Aims and Objectives of the study

The aim of the study is to determine the presence of heavy metals in herbal preparation obtained from hawkers within Ilorin metropolis.

The specific objectives are

1. To determine the presence of lead, iron, copper, cadmium, and chromium in herbal preparation consume within Ilorin metropolis.
2. To compare the concentration of the heavy metals present in the preparation consume within Ilorin metropolis with WHO permissible concentration.

1.4 Research Questions

1. Does heavy metals present in herbal preparation?

2. What are the regulatory standard requirements of the heavy metals concentration?
3. Is herbal preparation contaminated?

1.5 Significance of the Study

The presence of heavy metals in the body can be detrimental to health particularly at concentrations above tolerance level. Most are considered toxic to living organism due to their accumulative tendency in tissues resulting in various disorders (Bakare-Odunola and Mustapha, 2014). It has been reported that the presence of heavy metals in either orthodox or herbal drugs often compound the original ailments for which they were administered with resultant increased morbidity for the patient (Topliss *et al.*, 2002). There are various sources of contamination that renders the safety and quality of medicine questionable and notable amongst them is that from heavy metals.

1.6 Scope of the study

This research focused on various herbal preparations acclaimed to be used for a variety of health conditions within Ilorin Metropolis of Nigeria. The safety and quality of different herbal preparations, in terms of their level of contamination by heavy metals were ascertained. The heavy metals selected for this study include four biologically non-essential elements: Lead (Pb), Cadmium (Cd), Copper (Cu), Chromium (Cr) and one biologically essential element: Iron (Fe) which are some of the heavy metals implicated to be toxic in human.

1.7 Operational Definition of Terms

Herbal medicine: this is the use of plants to treat disease and enhance general health and wellbeing. Herbs can interact with other pharmaceutical medications and should be taken with care.

Herbal preparations: Preparations obtained by subjecting herbal substances to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices and processed exudates.

Heavy metals: refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb).

Quality control is a term used to describe all measures designed to ensure the output of uniform batches of drugs that conform to established specifications of identity, strength, purity, and other characteristics.

Quality assurance: is a wide range of concept that individually or collectively influences the quality of a product. It also includes sum of the organized arrangement with the objective that medicinal products are of required quality for their intended use. It is a totality of arrangement deliberately designed and intended to ensure that products will be consistent with the quality appropriated for their use.

Good Manufacturing Practice: commonly referred to as GMP, is the part of quality assurance, which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use as required by the marketing authorization.

Tablet: A solid unit dosage form of medication with suitable excipients. It comprises a mixture of active substances and excipients, usually in powder form, that are pressed or compacted into a solid dose.

Active pharmaceutical ingredients (APIs): are the active components in a pharmaceutical drug that produce the required effect on the body to treat a condition. APIs are produced by processing chemical compounds. In a biologic drug, the active ingredient is known as a bulk process intermediate (BPI).

CHAPTER TWO

Introduction

Traditional or folk medicine comprises practices, approaches, knowledge and beliefs not based on scientific evidence that are applied to treat, diagnose and prevent illness within a society. It is defined by a culture's knowledge and values and thus is context-specific, as are social constructions and negotiations of risk. When modern societies adopt such long-standing health practices outside of their traditional context, these practices become “complementary, non-conventional or alternative medicine” (Crellin, 2001). The extent to which the traditional use of an herb ensures that a corresponding herbal drug is safe, however, is a debatable matter. A new draft regulation published by the Brazilian National Health Surveillance Agency (Anvisa) brought this controversial topic to the center stage. The proposed regulation, which has recently undergone a public consultation for reviews and comments, defines two categories of herbal drugs for registration: “phytotherapeutic medicines” and “traditional phytotherapeutic products” (TPTP) (Anvisa, 2013). A product fits into the latter category if a long-standing (traditional) use is identified that has not been proven unsafe and is recognized in the literature or demonstrated by ethnopharmacological and/or ethno-botanical studies. Once the traditional use is recognized, safety and efficacy data from pre-clinical and/or clinical studies are no longer essential requirements to obtain approval for commercialization.

The proposed new rules for herbal medicine registration in Brazil are, to some extent, similar to the regulation released by the European Parliament in 2004. According to EC Directive 2004/24, herbal medicines with traditional use that are acceptably safe, albeit not having a recognized level of efficacy, can be classified as “traditional herbal medicines products” (THMP), as

detailed by Calapai (2008), Quintus and Schweim (2012) and Silano et al. (2004). In order for a medicinal product, or its corresponding products (*i.e.*, products having the same active ingredients, the same or similar intended purpose, equivalent strength and posology and the same or similar route of administration), to be classified as THMP, it must have been in medicinal use for a period of at least 30 years, of which more than 15 years must relate to the European Union. Herbal drugs in the THMP category undergo a simplified registration procedure. Aspiring herbal products are required to demonstrate the traditional use of the herb, but this requirement is lifted if the product complies with the European positive list established by the Committee on Herbal Medicinal Products (HPMC) (Knöss and Chinou, 2012). Herbal monographs prepared by HPMC are not binding, and member state agencies may not agree with every single aspect of the monograph. For instance, applicants that refer to a HPMC monograph may be required to provide additional points on safety (*e.g.*, on genotoxicity). Drug safety and efficacy In reference to medications, safety is the likelihood of not causing harm under the proposed conditions of use, while efficacy is the capacity to induce a clinical benefit. Both safety and efficacy depend on the drug's therapeutic indication; in principle, a substance has no clinical usefulness if it is "safe" but lacks efficacy or if it is active on a relevant therapeutic target but its use is unsafe (Bian *et al.*, 2006). Although these are recognized as equally essential attributes of any medicine, safety has taken precedence over proof of efficacy in drug regulation history.

In the US, for instance, the Federal Food Drug and Cosmetic Act of 1938 required that safety of new drugs had to be proven by pre-marketing testing, whereas similar requirements to demonstrate drug efficacy were introduced only 25 years later by the Kefauver-Harris Amendments of 1962. The idea that safety should come first in therapeutic interventions is conveyed by the famous Latin expression "*primum non nocere*" ("first, not do harm"), the origin

of which is uncertain (Smith, 2005). Similarly, the classical version of the Hippocratic Oath that physicians take upon entering medical practice contains a promise that expresses a similar idea (doctors are required to “keep [patients] from harm”).

Tradition and safety of herbal medicines

Many supporters of herbal medicines argue that products with a long history of popular use are generally safe when used properly at common therapeutic doses (Fong, 2002). A crucial question underlying this statement is the extent to which the absence of evidence of toxicity could be taken as evidence of the absence of toxicity or safety of herbal medicines. Whether the absence of records of adverse effects is an indication of lack of toxicity depends on the type of toxic effect and the likelihood of observing such an adverse outcome under the conditions prevailing in the traditional usage (Hao *et al.*, 2013). Acute symptoms and short term toxic effects, such as gastro-intestinal disturbances and dermatological effects, are likely to be recognized and associated to herbal medicine. Therefore, the absence of such observations provides some evidence of safety in these particular endpoints. Long-term adverse outcomes, such as cancer, liver and kidney damage, reproductive dysfunctions, birth defects and several morbidities that are more difficult to detect, however, are unlikely to be associated with the popular use of a medicine, unless an adequately designed epidemiology study (preferably, a prospective cohort study) is undertaken (Halicioglu *et al.*, 2011). Thus, the absence of evidence of these adverse effects within the context of traditional usage of herbal medicines is not evidence of the absence of potential to cause them. As far as drugs are concerned, safety is assumed only when the null hypothesis (absence of toxicity) has not been disproved after being challenged by properly designed and comprehensive set of pre-clinical and clinical studies, that had enough statistical power to reject it if it were false (Gurley *et al.*, 2005).

Carcinogenic effects of traditional herbal medicines

During R&D of conventional drugs, carcinogenic potential is assessed through a battery of *in vitro* and *in vivo* short-term genotoxicity tests, and long-term rodent carcinogenicity assays. Carcinogenicity tests in rats and mice are the longest and most costly non-clinical safety studies commonly required for a new drug marketing approval. A waiver of long-term carcinogenicity studies for marketing approval can be obtained if results of short-term genotoxicity tests are negative and the drug is intended to be used continuously for less than three months, or intermittently for less than six months. Long-term carcinogenicity studies have seldom been performed with herbal products, and the data of their genotoxic potential are scant as well (Teschke, 2010). Owing to their limited duration, clinical trials do not shed light on the carcinogenic potential of drugs. In principle, evidence on the carcinogenicity of traditional herbal medicines could be obtained from observational epidemiological studies. Nonetheless, epidemiological studies have rarely been undertaken to address this question.

Liver toxicity associated with herbal medicines Severity of drug-induced liver toxicity (DILI) ranges from mild dysfunction leading to raised serum levels of alanine aminotransferase (ALT) unaccompanied by increases in bilirubin levels and clinical symptoms reversible upon treatment discontinuation, to jaundice and overt hepatic failure that could culminate in liver transplantation or death. DILI can be broadly divided into two types: liver toxicity that depends on the dose and can be predicted by pre-clinical and clinical studies; and an idiosyncratic DILI that is a rare, but severe form of liver damage that occurs only in susceptible individuals (presumably involving immuno-allergic mechanisms) and cannot be predicted by pre-marketing safety studies. Similar to conventional drugs, herbal medicines are capable of causing both predictable and idiosyncratic DILI (Teschke *et al.*, 2012).

It should be noted that the degree of evidence for establishing a causal link between intake of a medicinal herb and liver impairment is variable, as it is for conventional drugs. In addition to the difficulties and limitations of pharmacovigilance, the vigilance related to herbal products faces additional problems, such as: self-medication, products that contain a diverse number of different plants, use in combination with classical pharmaceutical agents, the widespread belief that natural products are intrinsically safe so that patients often forget to disclose this information to their physicians, and poor quality issues, including misidentification of the plants, selection of the wrong part of the plant, adulteration, mislabeling, inadequate storage conditions, and contamination by fungi, pesticides, metals and other potential toxins (Larrey and Faure, 2011; Shaw *et al.*, 2012). The fact that several hepatotoxic plants and their constituents have a long-standing use in folk medicine is also consistent with the notion that traditional use on its own does not guarantee the safety of a phytopharmaceutical.

Kinetic interactions of herbal medicines with conventional medicines

Herbal medicines are often used concomitantly with conventional drugs, making potential pharmacokinetic interactions a cause for concern. Herbal drug co-administration with medicines of narrow therapeutic indices (*e.g.*, digoxin, warfarin) raises even deeper safety concerns (Silano *et al.*, 2004). Common herbal medicines known to interact with conventional drugs include St. John's wort (*Hypericum perforatum* L.), ginkgo (*Ginkgo biloba* L.), ginger (*Zingiber officinale* Rosc.), ginseng (*Panax ginseng* C.A. Meyer) and garlic (*Allium sativum* L.). St. John's wort, an herbal antidepressant, is possibly the most notorious example. It is a potent inhibitor of CYP3A4, and when co-administered with drugs metabolized by this enzyme, it decreases their clearance and increases their plasma concentrations (AUC). Because St. John's wort also induces the expression of CYP3A4 and the transmembrane transporter protein PgP (P-glycoprotein) in the

liver and intestines, previous and repeated administrations have opposite effects; enhancement of clearance and decrease in AUC. The kinetic and clinical effects of a number of drugs that are substrates for CYP3A4 are altered by St. John's wort, and these drugs include cyclosporine, midazolam, oxycodone, methadone, imatinib, finasteride, bupropion, tacrolimus, digoxin, atorvastatin, and verapamil, among others (Leach and Moore, 2012).

Factors Responsible for Increased Patronage and Self Medication with Herbal Medicine

Essentially, herbal remedies consist of portions of plants or unpurified plant extracts containing several constituents which are often generally believed to work together synergistically. The recent resurgence of public interest in herbal remedies has been attributed to several factors some of which include (i) various claims on the efficacy or effectiveness of plant medicines, (ii) preference of consumers for natural therapies and a greater interest in alternative medicines, (iii) erroneous belief that herbal products are superior to manufactured products, (iv) dissatisfaction with the results from orthodox pharmaceuticals and the belief that herbal medicines might be effective in the treatment of certain diseases where conventional therapies and medicines have proven to be ineffective or inadequate, (v) high cost and side effects of most modern drugs, (vi) improvements in the quality, efficacy, and safety of herbal medicines with the development of science and technology, (vii) patients' belief that their physicians have not properly identified the problem; hence the feeling that herbal remedies are another option, and (viii) a movement toward self-medication (Bandaranayake, 2006).

The increasing utilization of herbs for self-medication by patients or individuals is also attributed to a number of other reasons such as (i) patients being uncomfortable about discussing their medical problems and fear lack of confidentiality in handling their health information, (ii) fear of possible misdiagnosis and wrong treatment by patients with non-specific symptoms or general

malaise, and (iii) lack of time to see a physician; this is usually a reason where prior visit did not yield any positive experience (Studdert *et al.*, 1998). Furthermore, patients' freedom of choice of a practitioner is also encouraging their utilization of alternative treatments and herbal remedies, although many select herbal medicines from a deductive approach based on anecdotal information, that is, "it worked for my friend or relative" (Parle and Bansal, 2006). So also, because of the influence of religion and greater level of spiritual consciousness, many individuals tend to be increasingly disposed to accepting therapeutic value of a treatment based on faith or intuition rather than scientific reasoning (Astin, 1998; Zeil, 1999). Herbal medicines, therefore, become particularly alluring when the body's natural capacity for self-repair, given appropriate conditions, is emphasized (Parle and Bansal, 2006).

Influence of Regulatory Policies on Safety of Herbal Medicines

It has been observed that most of the problems associated with the use of traditional and herbal medicines arise mainly from the classification of many of these products as foods or dietary supplements in some countries. As such, evidence of quality, efficacy, and safety of these herbal medicines is not required before marketing. In the same vein, quality tests and production standards tend to be less rigorous or controlled and in some cases, traditional health practitioners may not be certified or licensed. The safety of traditional and herbal medicines has therefore become a major concern to both national health authorities and the general public (Kasilo and Trapsida, 2011).

Until 2011, there were three possible regulatory routes by which an herbal product could reach a consumer in the UK. The unlicensed herbal remedy is the commonest route which does not have to meet specific standards of safety and quality neither is it required to be accompanied by safety information for the consumer (Raynor *et al.*, 2011). Recently, the European Union (EU)

implemented a directive after a 7-year transition period to harmonize the regulation of traditional herbal medicine products across the EU and establish a simplified licensing system in order to help the public make informed choices about the use of herbal products. This requires that all manufactured herbal products either gain a product license of the type needed to manufacture “conventional” products or become registered as a “traditional herbal medicinal product” (Routledge, 2008; Raynor *et al.*, 2011).

Toxicity and Adverse Health Effects of Some Common Herbal Medicines

In most countries, herbal medicines and related products are introduced into the market without any mandatory safety or toxicological evaluation. Many of these countries also lack effective machinery to regulate manufacturing practices and quality standards. These herbal products are continuously made available to consumers without prescription in most cases and the potential hazards in an inferior product are hardly recognized (Bandaranayake, 2006). The general perception that herbal remedies or drugs are very safe and devoid of adverse effects is not only untrue, but also misleading. Herbs have been shown to be capable of producing a wide range of undesirable or adverse reactions some of which are capable of causing serious injuries, life-threatening conditions, and even death. Numerous and irrefutable cases of poisoning have been reported in the literature (Vanherweghem and Degaute, 1998; Cosyns *et al.*, 1999; Ernst, 2002). The toxicity evaluation of the polyherbal formula, Yoyo “Cleanser” Bitters®, conducted recently in our laboratory (Ekor *et al.*, 2010), was prompted by an unpublished case report of a young male adult who had been on self-medication with this herbal product and was subsequently admitted to the hospital on account of liver failure. Yoyo “Cleanser” Bitters® is one of the herbal remedies that is widely advertised in the various Nigerian media and as such has gained so much public acceptance over time and continues to enjoy increased patronage among consumers,

especially in the southwestern part of the country. Our study revealed that this herbal formula was capable of elevating plasma levels of liver enzymes and inducing hypokalemia following 30 days administration in rats. From our observation, the potassium loss (which is capable of predisposing to dangerous arrhythmias) was a greater risk associated with this herb during this sub-acute exposure or toxicity study. Prior to this study, we had evaluated the safety of “super B blood purifier” and “super B seven keys to power” mixtures in experimental model over a decade ago (John *et al.*, 1997). These herbal mixtures were marketed by a registered Nigerian company which cultivated medicinal plants and manufactured medicinal herbal preparations. The herbal blood tonics were well patronized by common folks who claim their efficacy according to the manufacturer’s stipulation that “they are safe, give strength and cleanse the blood and body of infection.” We obtained the herbal constituents (*Entandrophragma utile* and *Anacardium occidentale*) and investigated the individual plant extract as well as the herbal tonics made from them. Although, all the extracts and tonics proved safe during acute toxicity study, chronic toxicity testing revealed splenic enlargement in 10% of mice that received *E. utile* or either of the two tonics and one case of lung tumor (John *et al.*, 1997).

Challenges Associated with Monitoring Safety of Herbal Medicines

With the enormous global consumption of herbal products and medicines, it is high time they were included in pharmacovigilance systems. In terms of population exposure alone, it is essential to identify the risks associated with the use of herbal medicines, and in this regard, the safety of these products has become an issue of great public health importance (WHO, 2004, 2005b). There is no doubt that the increasing cases of poisoning associated with the use herbal medicines in many parts of the world in recent times, is necessitating the need to ensure thorough

toxicity assessment alongside active pharmacovigilance on these products in order to promote their safe use and protect public health (Zhou *et al.*, 2013).

The development as well as implementation of the regulation of traditional or herbal medicines in different parts of the world is often confronted with several challenges. Challenges often encountered and common to many countries are those related to regulatory status, assessment of safety and efficacy, quality control, safety monitoring and inadequate or poor knowledge about traditional, complementary/alternative, and herbal medicines within national drug regulatory authorities (WHO, 2005b).

Challenges Related to the Regulatory Status of Herbal Medicines

The definition and categorization of herbal medicines vary from one country to another. Depending on the regulations applying to foods and medicines, a single medicinal plant may be categorized as a food, a functional food, a dietary supplement, or a herbal medicine in different countries. This introduces serious difficulty in the definition of the concept of herbal medicines for the purposes of national drug regulation while at the same time also confusing patients and consumers (WHO, 2005b). In the United States, for example, natural products are regulated under the Dietary Supplement Health and Education Act (DSHEA) of 1994 (U.S. Food and Drug Administration, 2012). By definition, a dietary supplement is a product that is ingested and is intended to supplement the diet and contains a “dietary ingredient.” The dietary ingredients in these products may include vitamins, minerals, herbs, or other botanicals (U.S. Food and Drug Administration, 2011). Under the DSHEA, additional toxicity studies are generally not required if the herb has been on the market prior to 1994 (National Institute of Health (NIH) Office of Dietary Supplements, 2011). In this regard, the FDA bears the burden to prove that a herbal medicinal product or “dietary ingredient” is toxic or not safe for use. Additional major challenge

in many countries is the fact that regulatory information on herbal medicines is often not shared between regulatory authorities and safety monitoring or pharmacovigilance centers (WHO, 2004).

Challenges Related to the Assessment of Safety and Efficacy

There is no gainsaying the fact that the requirements as well as the research protocols, standards and methods needed for the evaluation of the safety and efficacy of herbal medicines are much more complex than those required for conventional or orthodox pharmaceuticals (WHO, 2005b; Zhou *et al.*, 2013). A single herbal medicine or medicinal plant may contain hundreds of natural constituents, and a mixed herbal medicinal product may contain several times that number. Suppose every active ingredient were to be isolated from individual herb from which the herbal medicine is formulated or produced, the time and resources required would be tremendous. Such an analysis may practically be impossible especially where an herbal product is a mixture of two or more herbs (WHO, 2005b).

Challenges Related to Quality Control of Herbal Medicines

The quality of the source materials used in the production of herbal medicines determines to a large extent the safety and efficacy of these herbal remedies. Generally, the quality of source materials is dependent not only on intrinsic (genetic) factors, but also on extrinsic factors like environmental conditions, good agricultural, and good collection practices (GACP) for medicinal plants, including plant selection and cultivation. It is the combination of these factors that makes it difficult to perform quality controls on the raw materials of herbal medicines (WHO, 2004, 2005b). According to good manufacturing practice (GMP), correct identification of species of

medicinal plants, special storage, and special sanitation and cleaning methods for various materials are important requirements for quality control of starting materials.

One of the major challenges often encountered in the quality control of finished herbal medicinal products, especially mixture herbal products, is the difficulty in ascertaining the inclusion of all the plants or starting materials (WHO, 2005b). Thus, the general requirements and methods for quality control of finished herbal products remain far more complex than for other pharmaceuticals (WHO, 2003, 2004, 2005b). To ensure safety and efficacy of herbal medicines, therefore, WHO continues to recommend the institution of quality assurance and control measures such as national quality specification and standards for herbal materials, GMP for herbal medicines, labeling, and licensing schemes for manufacturing, import and marketing, in countries where herbal medicines are regulated (WHO, 2004).

Challenges Related to Safety Monitoring of Herbal Medicines

In recent years, issues relating to increasing use of herbal products in developed countries, dependence of many people living in developing countries on plants as a major source of medicines coupled with absence or weak regulation of herbal medicines in most countries and the occurrence of high-profile safety concerns, have increased awareness of the need to monitor safety and deepen understanding of possible harmful as well as potential benefits associated with the use of herbal medicines (Rodrigues and Barnes, 2013). Adverse events arising from consumption of herbal medicines are attributable to several factors among which include the use of the wrong species of plant by mistake, adulteration of herbal products with other, undeclared medicines, contamination with toxic or hazardous substances, overdose, misuse of herbal medicines by either healthcare providers or consumers and use of herbal medicines concomitantly with other medicines. Although, the assessment of the safety of herbal medicines

has become an important issue for consumers, regulatory authorities, and healthcare professionals, analysis of adverse events related to the use of these products is much more complex than in the case of conventional pharmaceuticals (WHO, 2005b; Zhou *et al.*, 2013).

It is also recognized that evaluation of safety is complicated by factors such as the geographical origin of plant material, different processing techniques, route of administration, and compatibility with other medicines (Zhang *et al.*, 2012). Furthermore, there is lack of the knowledge and/or poor emphasis on the importance of taxonomic botany and documentation by most manufacturers of herbal medicines and this poses peculiar challenges during identification and collection of medicinal plants used for herbal remedies (Farah *et al.*, 2000). In order to eliminate the confusion created by the common names, it is necessary to adopt the most commonly used binomial names (including their binomial synonyms) for medicinal plants. For example, *Artemisia absinthium* L., which contains an active narcotic derivative and capable causing CNS disorders and generalized mental deterioration, has at least 11 different common names. Seven of the common names bear no resemblance to its botanical name. Because common names are mainly used, *Heliotropium europaeum* (heliotrope), which contains potent hepatotoxic pyrrolidine alkaloids, is often confused with *Valerian officinalis* (garden heliotrope), known to contain valepotriates with sedative and muscle relaxant properties. This explains why it is important to provide the exact scientific name of the plant, the plant part used, and the name of the manufacturer when reporting adverse drug reactions of herbal medicines. Therefore, effective monitoring of safety of herbal medicine will require effective collaboration between botanists, phytochemists, pharmacologists, and other major stake-holders.

CHAPTER THREE

Materials and Methods

3.1 Equipment and reagents

Buck Accusys model 211 Atomic Absorption Spectrophotometer (AAS) equipped with corresponding hollow cathode lamp (Lead, Cadmium and Iron) at the time of analysis, De-ionized water, H_2SO_4 , HNO_3 , NO_2 . All experiments were performed using analytical grade of the reagents.

3.2 METHODS

3.2.1 Collection of samples

Five locations were selected using convenience sampling method from the three local Government Areas that make up Ilorin metropolis, viz: Ilorin South, West and East local Government Areas. Herbal preparations were purchased from hawkers. Five different herbal preparation having two batches were selected for this study and were coded H1, H2, H3, H4, H5, H6, H7, H8, H9, and H10. The herbal preparations were collected in a clean container and kept for further evaluation.

3.2.2 Preparation of calibration curve

De-ionized water was used in preparing solutions. Stock solutions of each metal Pb, Fe (undifferentiated), and Cd were prepared by dissolving each solid metal sample (1.0 g) in 10 mL 1:1 nitric acid solution. The solution was transferred into a 1000 mL volumetric flask and made

up to mark with de-ionized water. Standard solutions were prepared from each metal stock solution of 1000 mg/L.⁵ A 100 mL quantity of the standard solution of each metal was adjusted to pH of 2.5 by adding 1M nitric acid. Each standard solution and blank was transferred into an individual 250 mL separating funnel. 1 mL ammonium pyrrolidine dithiocarbamate was added followed by the addition of 10 mL methyl isobutyl ketone and the solution was shaken vigorously for 2 minutes and allowed to settle. The aqueous layer was discarded; while the organic layer was then aspirated directly into the flame and the absorbance was recorded using an Atomic Absorption Spectrophotometer equipped each with hollow cathode lamp, at 283.2 nm, 248.3 nm and 228.8 nm wavelengths for Pb, Fe and Cd respectively. The nebulizer, atomizer and burner were flushed each time with de-ionized water after each sample was aspirated before the next. The stability of the equipment was checked at intervals by introducing the highest working standard solution and the blank.⁵

3.2.3 Sample pre-treatment

A 10 mL quantity of each herbal bitter sample was measured into a digestion flask and digested with 10 mL of tri-acid mixture (HNO_3 : HClO_4 : H_2SO_4) in the ratio 25:4:2 i.e. 8.1 mL HNO_3 , 1.3 mL HClO_4 , 0.6 mL H_2SO_4 on a hot plate under a fume cupboard at 100°C until dense white fumes appeared. The flask was allowed to cool, a quantity of de-ionized water was added and digested until a clear solution was obtained. The solution was cooled and filtered into a 100 mL volumetric flask and made up to mark with de-ionized water. ¹⁹ This pre-treatment was done in triplicate for each sample and a total of thirty (30) digests were obtained.

3.3 Sample analysis

The digested samples were analyzed using BUCK ACCUSYS 211 Atomic Absorption Spectrophotometer equipped with hollow cathode lamp, at 283.2 nm, 248.3 nm and 228.8 nm wavelengths for Pb, Fe and Cd respectively. The absorbance for each metal (Pb, Fe, and Cd) in each digested sample was recorded and concentration of metal determined from the calibration curve.

3.4 Statistical analysis

The results were expressed as the mean $\pm$ S.E.M. Data was analyzed using GraphPad Prism (Version 7). Statistical analysis was carried out using Student's t-test to compare heavy metal content in the herbal bitters with the WHO permissible limit. The statistical significance was taken at $P < 0.01$

CHAPTER FOUR

4.1 RESULTS

The concentration of heavy metals (Pb, Fe and Cd) in the samples were compared with permissible limit of 10 mg/L, 0.3 mg/L and 0.1 mg/L, 2mg/L and 40mg/L for Pb, Cd, Fe, Cr and Cu respectively. The concentration of lead in herbal samples ranged from 0.010 ± 0.027 and 0.320 ± 0.035 mg/L, The highest and the lowest concentrations were found in H3 and H4 samples which were used by traditional healers to treat hypertension and sexual impotency. The concentration of Cadmium in herbal samples ranged from 0.000 ± 0.000 and 0.250 ± 0.065 mg/L. It is important to note that seven (7) samples from the herbal preparations analyzed contained iron in concentrations significantly higher ($P=0.01$) than the 0.1 mg/L permissible limit recommended by WHO, 2006. In this study, the concentration of chromium was found in the range of 0.180 ± 0.062 and 0.860 ± 0.027 mg/L. The highest amount was reported in the H10 sample while the lowest amount was reported in the H8 sample. The concentration range of copper in tested samples was 0.370 ± 0.006 and 0.010 ± 0.013 mg/L as shown in Table 1. The maximum concentration was reported in the H3 sample while the minimum amount was reported in the H4 sample preparation. This result revealed that the concentration of copper in all traditional herbal products was found below the WHO permissible limit (40 mg/L).

Table of concentration of Iron (Fe), Lead (Pb), Cadmium (Cd), Copper (Cu), and Chromium (Cr)

Sample code	Concentration in mg/mL				
	Pb	Fe	Cd	Cu	Cr
H1	0.030±0.000	0.007±0.021	0.250±0.065	0.140±0.021	0.450±0.056
H2	0.160±0.053	0.130±0.067	0.220±0.041	0.150±0.004	0.720±0.046
H3	0.320±0.035	0.230±0.028	0.000±0.000	0.370±0.006	0.540±0.026
H4	0.010±0.027	0.056±0.051	0.000±0.000	0.010±0.013	0.640±0.085
H5	0.210±0.030	0.120±0.072	0.160±0.058	0.050±0.017	0.260±0.031
H6	0.130±0.002	0.230±0.084	0.000±0.000	0.060±0.012	0.730±0.063
H7	0.100±0.001	0.270±0.063	0.000±0.000	0.070±0.009	0.760±0.084
H8	0.160±0.068	0.040±0.086	0.000±0.000	0.250±0.021	0.860±0.027
H9	0.140±0.021	0.310±0.047	0.170±0.063	0.330±0.005	0.270±0.063
H10	0.240±0.052	0.2400±0.071	0.000±0.000	0.130±0.012	0.180±0.062

CHAPTER FIVE

5.1 DISCUSSION

The concentration of lead in herbal samples ranged from 0.010 ± 0.027 and 0.320 ± 0.035 mg/L (Table 1). The highest and the lowest concentrations were found in H3 and H4 samples which were used by traditional healers to treat hypertension and sexual impotency. The WHO maximum permissible limit for lead in traditional herbal preparations is 10 mg/L (WHO, 2007). Hence, all samples analyzed were within this permissible limit. Maghrabi reported similar results from different samples of herbal drugs marketed in Saudi Arabia, with the levels of Pb below the WHO permissible limit (Maghrabi, 2014). However, contrary to this finding, the concentration of lead above the WHO maximum permissible limit was reported in previous studies (Anim *et al.*, 2012; Mousavi *et al.*, 2014; Mulaudzi *et al.*, 2017; Zamir *et al.*, 2019). The presence of this amount of lead in the herbal bitters may not pose health threat, although human users should be advised not to use these products over a long period of time. This result is similar to the findings obtained from a study carried out in Ghana on hazardous concentration of lead in traditionally used herbal drugs. Lead is one of the most toxic heavy metals and progressive exposure may cause poor muscle coordination, gastrointestinal symptoms, brain and kidney damage, hearing and vision impairments, and reproductive defects. Lead bioaccumulates in biological tissues; patients who use medicinal herbs with even low concentrations of Pb over a long period of time might be at risk of chronic Pb toxicity and should be monitored for any signs of lead poisoning (Nourmoradi *et al.*, 2013). The WHO maximum permissible limit of cadmium in traditional herbal products is 0.3 mg/L (WHO, 2007).

The concentration of Cadmium in herbal samples ranged from 0.000 ± 0.000 and 0.250 ± 0.065 mg/L. A study conducted in Zaria, Northern Nigeria showed that cadmium (Cd) concentration exceeded

the WHO limit by 100 % in all the herbal drugs used as antimalarial. Similar findings have been reported in Jordan and Bangladesh (Zamir *et al.*, 2015). However, high levels of cadmium above the WHO permissible limit have been reported in South Africa (Mulaudzi *et al.*, 2017), Iran (Mousavi *et al.*, 2014), and Nigeria (Afieroho *et al.*, 2018). An intake of high levels of cadmium is known to be very toxic and carcinogenic. Its ingestion at lower levels can accumulate in the kidneys, resulting in renal tract impairment, lung damage, and fragile bones (Mulaudzi *et al.*, 2017).

It is important to note that seven (7) samples from the herbal preparations analyzed contained iron in concentrations significantly higher than the 0.1 mg/L permissible limit recommended by WHO, 2006. Another study reported the concentration of iron to be between 0.0001 and 1.12 ppm in different herbal medicine purchased from various places in Karachi city of Pakistan and KwaZulu Natal Province, South Africa (Bakare-Odunola and Mustapha, 2014). Previous studies have shown that when consumers take above the permissible safe limit of iron, either from nutritional sources, or from orthodox or herbal drugs, untoward reactions may occur (WHO, 2007). Also, cases of iron ingestion leading to toxicity or poisoning have been reported in some studies (Oreagba *et al.*, 2011). Iron toxicity may cause gastrointestinal effects such as vomiting, diarrhea, melena, and abdominal pain which may occur and progress to life threatening hemorrhagic gastritis, perforation, and peritonitis.²⁶ Such cases of acute gastroenteritis may contribute to early cardiovascular toxicity through fluid and blood loss which manifests as decreased cardiac output, cardiac arrest and shock (Mousavi *et al.*, 2014 Mulaudzi *et al.*, 2017; Zamir *et al.*, 2019). Also, metabolic acidosis and CNS manifestations such as depressed sensorium, ranging from mild obtundation to profound coma are commonly seen with severe iron overdose. Iron preparations are beneficial in the management of iron deficiency anaemia and

a deliberate addition by the manufacturer of the herbal bitters cannot be overruled. The high concentrations of Fe found in the preparations may be due to factors such as contamination during the process of cultivating and/or harvesting the medicinal plants, and unintentional cross contamination during production. Critical evaluation is recommended to be carried out to detect the source(s) of iron contamination in the samples of herbal preparation analyzed. Public awareness and or enlightenment about the adverse effects of iron overload in herbal preparation may be extremely needful (Mulaudzi *et al.*, 2017; Zamir *et al.*, 2019).

In this study, the concentration of chromium was found in the range of 0.180 ± 0.062 and 0.860 ± 0.027 mg/L (Table 1). The highest amount was reported in the H10 sample while the lowest amount was reported in the H8 sample. The level of chromium in herbal products was above the WHO recommended permissible limits (2 mg/kg) (WHO, 2007). This might be due to contamination of the raw materials used in manufacturing and contamination during the harvesting and manufacturing process of herbal plants. Similar results of high levels of chromium have been reported in a study conducted in South Africa. Exposure to high levels of chromium can result in respiratory tract problems, lung cancer, dermatitis, and permanent nose damage (Mulaudzi *et al.*, 2017). The health risk due to metal contamination, in general, depends on the average daily dietary intake. Chromium is lethal and toxic to humans even at lower concentrations.

The concentration range of copper in tested samples was 0.370 ± 0.006 and 0.010 ± 0.013 mg/L as shown in Table 1. The maximum concentration was reported in the H3 sample while the minimum amount was reported in the H4 sample preparation. This result revealed that the concentration of copper in all traditional herbal products was found below the WHO permissible limit (40 mg/L) (WHO, 2007). Similar results were reported in Brazil, Nigeria, and Ghana (Anim

et al., 2012). Copper is an essential element for the human metabolic system. It regulates various biological processes like redox reactions, energy production, connective tissue formation, iron metabolism, and synthesis of neurotransmitters. However, chronic exposure to a high concentration of copper causes irritation of nasal mucosa, vomiting, nausea, diarrhea, kidney, and liver damage (Zamir *et al.*, 2019).

CONCLUSION

The results obtained from this study showed that iron was present in all the samples with some concentrations significantly higher than the WHO permissible limit. Lead and cadmium were present in some of the samples with concentration below the WHO permissible limit. This study recommends that regulatory bodies should ensure herbal products are free from heavy metal contaminants and safe for human consumption.

REFERENCES

- Afiero, O. Achara, F. Adewoyin, B. and Abo, K. (2018) "Determination of cadmium, chromium and lead in four brands of herbal bitters preparation sold in Benin-city, Southern Nigeria," *African Journal of Environmental Science and Technology*, vol. 12, no. 5, pp. 186–190.
- Anim, A. Laar, C. Osei, J. Odonkor, S. and Enti-Brown, S. "Trace metals quality of some herbal medicines sold in Accra, Ghana," *Proceedings of the International Academy of Ecology and Environmental Sciences*, vol. 2, no. 2, p. 111, 2012.
- Anvisa, 2013. Consulta Pública No 34 de 6 de Agosto de 2013. Agência Nacional de Vigilância Sanitária. Diário Oficial da União, Brasília, em 7 de Agosto de 2013.
- Astin, J. (1998). Why patients use alternative medicines: results of national study. *J. Am. Med. Assoc.* 279, 1548–1553. doi: 10.1001/jama.279.19.1548
- Bakare-Odunola MT, Mustapha KB (2014). Identification of heavy metals in local drinks in Northern Zone of Nigeria. *Journal of Toxicology and Environmental Health Sciences*, 6(7):126-131.
- Bandaranayake, W. M. (2006). "Quality control, screening, toxicity, and regulation of herbal drugs," in *Modern Phytomedicine. Turning Medicinal Plants into Drugs*, eds I. Ahmad, F. Aqil, and M. Owais (Weinheim:Wiley-VCH GmbH & Co. KGaA), 25–57. doi: 10.1002/9783527609987.ch2
- Bian, Z.X., Li, Y.P., Dagenais, S., Liu, L., Wu, T.X., Miao, J.X., Kwan, A.K., Song, L., 2006. Improving the quality of randomized controlled trials in Chinese herbal medicine, part I: clinical trial design and methodology. *J. Chin. Integr. Med.* 4, 120-129.
- Bunchorntavakul, C., Reddy, K.R., 2013. Review article: herbal and dietary supplement hepatotoxicity. *Aliment. Pharmacol. Ther.* 37, 3-17.
- Calapai, G., 2008. European legislation on herbal medicines: a look into the future. *Drug Saf.* 31, 428-431.
- Carmona, F., Pereira, A.M.S., 2013. Herbal medicines: old and new concepts, truths and misunderstandings. *Rev. Bras. Farmacogn.* 23, 379-385.
- Chikezie PC, Ojiako OA (2015) Herbal Medicine: Yesterday, Today and Tomorrow, *Alternative & Integrative Medicine*, 4:3.

- Cosyns, J. P., Jadoul, M., Squifflet, J. P., De Plaen, J. F., Ferluga, D., and van Ypersele de Strihou, C. (1994). Chinese herbs nephropathy: a clue to Balkan endemic nephropathy? *Kidney Int.* 45, 1680–1688. doi: 10.1038/ki.1994.220
- Cosyns, J. P., Jadoul, M., Squifflet, J. P., Wese, F. X., and van Ypersele de Strihou, C. (1999). Urothelial lesions in Chinese-herb nephropathy. *Am. J. Kidney Dis.* 33, 1011–1017. doi: 10.1016/S0272-6386(99)70136-8
- Crellin, J.K., 2001. Social validation: an historian's look at complementary/ alternative medicine. *Pharm. Hist. (Lond).* 31, 43-51. Fong, H.H., 2002. Integration of herbal medicine into modern medical practices: issues and prospects. *Integr. Cancer Ther.* 1, 287-293.
- Ekor, M., Osonuga, O. A., Odewabi, A. O., Bakre, A. G., and Oritogun, K. S. (2010). Toxicity evaluation of Yoyo 'cleanser' bitters and fields Swedish bitters herbal preparations following sub-chronic administration in rats. *Am. J. Pharmacol. Toxicol.* 5, 159–166. doi: 10.3844/ajptsp.2010.159.166
- Enuh H, Oragwu C, Okeke C, Elu C, Orisakwe O (2012). Semen Abnormality and Nigeria Herbal Remedies: A Preliminary Investigation. *The Internet Journal of Toxicology*, 8(2).
- Ernst E (2002). Toxic heavy metals and undeclared drugs in Asian herbal medicines. *Trends in Pharmacological Sciences*, 23(3):136- 139.
- Ernst, E. (2002). Toxic heavy metals and undeclared drugs in Asian herbal medicines. *Trends Pharmacol. Sci.* 23, 136–139. doi: 10.1016/S0165-6147(00)01972-6
- European Union Herbal Medicines Directive. (2004). Directive 2004/24/EC of the European Parliament and of the Council of 31 March 2004 amending, as regards traditional herbal medicinal products, Directive 2001/83/EC on the Community code relating to medicinal products for human use. *Off. J. Eur. Union* 47, 85–90.
- Gurley, B.J., Gardner, S.F., Hubbard, M.A., Williams, D.K., Gentry, W.B., Cui, Y., Ang, C.Y., 2005. Clinical assessment of effects of botanical supplementation on cytochrome P450 phenotypes in the elderly: St John's wort, garlic oil, *Panax ginseng* and *Ginkgo biloba*. *Drugs Aging* 22, 525-539.
- Halicioglu, O., Astarcioglu, G., Yaprak, I., Aydinlioglu, H., 2011. Toxicity of *Salvia officinalis* in a newborn and a child: an alarming report. *Pediatr. Neurol.* 45, 259-260.

- Hao, C.Z., Wu, F., Lu, L., Wang, J., Guo, Y., Liu, A.J., Liao, W.J., Zheng, G.Q., 2013. Chinese herbal medicine for diabetic peripheral neuropathy: an updated meta-analysis of 10 highquality randomized controlled studies. *PLoS One*. 8, e76113.
- John, T. A., Okoli, C. C., and Ekor, M. (1997). Some phytochemical, safety, antimicrobial and haematological studies of super b and seven keys to power blood purifier herbal tonics. *Nig. J. Med. Res.* 1, 37–43.
- Kasilo, O. M. J., and Trapsida, J. M. (2011). Decade of African traditional medicine, 2001–2010. *Afr. Health Mon. (Special Issue)* 14, 25–31.
- Larrey, D., Faure, S., 2011. Herbal medicine hepatotoxicity: a new step with development of specific biomarkers. *J. Hepatol.* 54, 599-601.
- Leach, M.J., Moore, V., 2012. Black cohosh (*Cimicifuga* spp.) for menopausal symptoms. *Cochrane Database Syst. Rev.* 12, CD007244. doi: 10.1002/14651858.
- Maghrabi, I. A. (2014) “Determination of some mineral and heavy metals in Saudi Arabia popular herbal drugs using modern techniques,” *African Journal of Pharmacy and Pharmacology*, vol. 8, no. 36, pp. 893–898.
- Mohammad R, Reza F, Mojib SB (2012). Effects of heavy metals on the medicinal plant. *International Journal of Agronomy and Plant Production*, 3(4):154-158.
- Mousavi, Z. Ziarati, P. Esmaeli Dehaghi, M. and Qomi, M. (2014). “Heavy metals (lead and cadmium) in some medicinal herbal products in Iranian market,” *Iranian Journal of Toxicology*, vol. 8, no. 24, pp. 1004–1010.
- Mulaudzi, R. B. Tshikalange, T. E. Olowoyo, J. O. Amoo, S. O. and Du Plooy, C. P. (2017). “Antimicrobial activity, cytotoxicity evaluation and heavy metal content of five commonly used South African herbal mixtures,” *South African Journal of Botany*, vol. 112, pp. 314–318.
- National Institute of Health (NIH) Office of Dietary Supplements. (2011). *Kava*. Available at: http://ods.od.nih.gov/Health_Information/kava.aspx.
- Nourmoradi, H. Foroghi, M. Farhadkhani, M. and Vahid Dastjerdi, M. (2013) “Assessment of lead and cadmium levels in frequently used cosmetic products in Iran,” *Journal of Environmental and Public Health*, vol. 2013, Article ID 962727, 5 pages.
- Nweke OC, Sanders WH (2009). Modern Environmental Health Hazards: A Public Health Issue of Increasing Significance in Africa. *Environmental Health Perspectives*.

- Oreagba IA, Oshikoya KA, Amachree M (2011). Herbal medicine use among urban residents in Lagos, Nigeria. *BMC Complementary and Alternative Medicine*, **11**:117.
- Osegbe DN, Amaku EO (1985). The causes of male infertility in 504 consecutive Nigeria patients. *International urology and nephrology*, 17(4):349- 358.
- Saeed M, Muhammad N, Khan H, Khan SA (2010). Analysis of toxic heavy metals in branded Pakistani herbal products. *Journal of the Chemical Society of Pakistan*, 32(4):471.
- Quintus, C., Schweim, H.G., 2012. European regulation of herbal medicinal products on the border area to the food sector. *Phytomedicine* 19, 378-381
- Raynor, D. K., Dickinson, R., Knapp, P., Long, A. F., and Nicolson, D. J. (2011). Buyer beware? Does the information provided with herbal products available over the counter enable safe use? *BMC Med.* 9:94. doi: 10.1186/1741- 7015-9-94
- Rodrigues, E., and Barnes, J. (2013). Pharmacovigilance of herbal medicines: the potential contributions of ethnobotanical and ethnopharmacological studies. *Drug Saf.* 36, 1–12. doi: 10.1007/s40264-012-0005-7
- Sharma P, Dubey RS (2005). Lead toxicity in plants. *Brazilian Journal of Plant Physiology*, 17(1):35-52.
- Shaw, D., Graeme, L., Pierre, D., Elizabeth, W., Kelvin, C., 2012. Pharmacovigilance of herbal medicine. *J. Ethnopharmacol.* 140, 513-518.
- Silano, M., De Vincenzi, M., De Vincenzi, A., Silano, V., 2004. The new European legislation on traditional herbal medicines: main features and perspectives. *Fitoterapia* 75, 107-116.
- Smith, C.M., 2005. Origin and uses of *primum non nocere*--above all, do no harm! *J. Clin. Pharmacol.* 45, 371-377
- Teschke, R., 2010. Kava hepatotoxicity-a clinical review. *Ann. Hepatol.* 9, 251-265.
- Teschke, R., Wolff, A., Frenzel, C., Schulze, J., Eickhoff, A., 2012. Herbal hepatotoxicity: a tabular compilation of reported cases. *Liver Int.* 32, 1543-1556.
- Tong S, Von Schirnding YE, Prapamontol T (2000). Environmental lead exposure: a public health problem of global dimension. *Bulletin of the*
- Topliss JG, Clark AM, Ernst E, Hufford CD, Johnson GAR, Rimoldi JM, Weimann BJ (2002). Natural and synthetic substances related to human health (IUPAC Technical Report). *Pure and Applied Chemistry*, 74(10): 1957-85.

- U.S. Food and Drug Administration. (2011). *Regulatory Framework of DSHEA of 1994*. Available at: <http://www.fda.gov/NewsEvents/Testimony/ucm115163.htm>.
- U.S. Food and Drug Administration. (2012). *Dietary Supplement Health and Education Act of 1994*. Available at: <http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148003.htm>.
- Vanherweghem, J. L., and Degaute, J. P. (1998). The policy of admission to the education in medicine and dentistry in the French-speaking community of Belgium. *Acta Clin. Belg.* 53, 2–3.
- WHO. (2002a). *WHO Monographs on Selected Medicinal Plants*, Vol. 2. Geneva, Switzerland: World Health Organization.
- WHO. (2002b). *Traditional Medicine Strategy (2002–2005)*. WHO/EDM/TRM/2002.1. Geneva, Switzerland: World Health Organization.
- WHO. (2003). *WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants*. Geneva, Switzerland: World Health Organization.
- WHO. (2004). *WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems*. Geneva, Switzerland: World Health Organization.
- WHO. (2005a). “WHO global atlas of traditional, complementary and alternative medicine,” in *Map Volume*, eds C. K. Ong, G. Bodeker, C. Grundy, G. Burford, and K. Shein (Geneva, Switzerland: World Health Organization).
- WHO. (2005b). *National Policy on Traditional Medicine and Regulation of Herbal Medicines*. Report of a World Health Organization Global Survey. Geneva, Switzerland: WHO.
- World Health Organization (2007). *Guideline for Good Manufacturing Practices for Herbal Medicine*, Geneva
- World Health Organization (2013). *Traditional Medicine Strategy: 2014-2023*. Retrieved September, 2014 http://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/.
- World Health Organization, 78(9):1068-1077 Chan K (2003). Some aspects of toxic contaminants in herbal medicines. *Chemosphere*, 52(9):1361- 1371.

- World Health Organization, WHO (2007). *Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues*, World Health Organization, Geneva, Switzerland.
- Zamir, R. Hosen, A. Ullah, M. O. and Nahar, N. (2015) “Microbial and heavy metal contaminant of antidiabetic herbal preparations formulated in Bangladesh,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 243593, 9 pages.
- Zamir, R. Islam, N. and Faruque, A. (2019) “Comparison of toxic metal concentrations in antidiabetic herbal preparations (ADHPs) available in Bangladesh using AAS and XRF analytical tools,” *2e Scientific World Journal*, vol. 2019.
- Zeil, H. (1999). Complementary alternative medicine boon or boondoggle. *Skeptic* 7, 86–90.
- Zhang, L., Yan, J., Liu, X., Ye, Z., Yang, X., Meyboom, R., et al. (2012). Pharmacovigilance practice and risk control of traditional Chinese medicine drugs in China: current status and future perspective. *J. Ethnopharmacol.* 140, 519–525. doi: 10.1016/j.jep.2012.01.058
- Zhang, Y., Huang, F., Wu, D., and Zhang, M. (2008). Hepatotoxicity of *Flos Farfarae* and the contained alkaloid to mice. *Lishizhen Med. Mater. Med. Res.* 19, 1810– 1811.
- Zhang, Z. J., Ye, F., Wiseman, N., Mitchell, C., and Lun, S. H. (1999). *On Cold Damage Paradigm Publications*. Brookline, MA: Paradigm.
- Zhou, J., Ouedraogo, M., Qu, F., and Duez, P. (2013). Potential genotoxicity of traditional Chinese medicinal plants and phytochemicals: an overview. *Phytother. Res.* doi: 10.1002/ptr.4942 [Epub ahead of print].
- Zhou, S., Koh, H. L., Gao, Y., Gong, Z. Y., and Lee, E. J. (2004). Herbal bioactivation: the good, the bad and the ugly. *Life Sci.* 74, 935–968. doi: 10.1016/j.lfs.2003.09.035

