DETERMINATION OF SELECTED CHEMICAL PARAMETERS AND ANTI-NUTRITIONAL FACTORS OF BRANDED HERBAL MEDICINES SOLD IN MINNA

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ABSTRACT

Many herbal medicines are available in Minna for human consumption with little or no adequate information provided on their qualities and safety. In this study, analysis was carried out on thirteen liquid and seven powdered branded samples of the herbal medicine sold in Metropolis, in order to determine their selected heavy metal concentrations, phytochemical, physicochemical and anti-nutrition factors using standard methods. The concentrations ($\mu g/dm^3$) of the selected heavy metals; Cd, Fe, Cu, Mn and Zn in branded liquid samples ranged from 0.12±0.10 to 0.02±0.10, 1280.02±1.99 to 8730.04±1.98, 250.02±2.10 to 600.01±2.23, 160.14±2.01 to 2186.01±3.13, 1346.02±1.99 to 9810.11±1.86 respectively while lead was not detected in any of the samples analyzed. The concentrations (mg/kg) of these metals in the powdered samples ranged from: 0.08±0.02 to 0.14±0.05, 21.70±0.13 to 193.80±3.18, 8.00±0.02 to 38.10±0.01, 12.10±0.32 to 127.70±1.20, and 96.10±0.30 to 338.10±3.10 respectively. The concentrations of cadmium obtained for both liquid and powdered samples were below the permissible limit of 0.30 mg/kg for powdered and 0.30 mg/dm^3 forliquid respectively as recommended by WHO while the concentrations of iron in the samples were above the respective permissible limits; $100\mu g/dm^3$ and 20 mg/kg. The concentrations of copper in the liquid samples were found to be below the permissible limit of 1000 $\mu g/dm^3$ while for manganese, the values for the samples: HM_2, HM_3, HM_7 and HM_8 are below the permissible limit of 260 $\mu g/dm^3$, other liquid samples had values above the permissible limit. For the concentrations of zinc in both liquid and powdered samples, values obtained were above the permissible limit 1500 $\mu g/dm^3$(liquid) and 50 mg/kg (powdered). The respective phytochemical contents; flavonoids, alkaloids, and saponnins of the liquid samples ranged from 0.35±0.06 to 4.30±0.06, 3.69±0.04 to 10.10±0.03 and 0.28±0.32 to 2.27±0.11 % while those of the powdered samples ranged from 14.40±0.36 to 0.35±0.01, 16.67±0.02 to 10.36±0.04 and 14.24±0.23 to 3.43±0.07 % respectively. The physicochemical values of the liquid samples; pH, viscosity, and electrical conductivity which ranged from; 7.41±0.25to 4.21±0.15, 23.45±0.46 to 0.55±0.01mm^2/s, 23.43±0.13 to 2.18±0.15ml/cm and the pH, bulk density, and electrical conductivity of powdered samples which ranged from 7.44±0.01 to 5.03±0.02, 0.67±0.00 to 0.43±0.01mm^2/s, 39.08±0.32 to 9.31±0.33 ml/cm respectively. The pH values obtained for both liquid and powdered samples are within the permissible limit of 4.0 - 7.50. The mean percentages of the selected anti-nutritional contents; tannins, oxalate and phytate with the nitrate and nitrite of the liquid samples ranged from 1.94±0.10 to 5.40±0.06, 0.32±0.06 to 1.48±0.32 and 3.16±0.01 to 10.81±0.01 with 4.24±0.01 to 8.37±0.02 and 4.24±0.01 to 0.35±0.01% respectively while the values for the powdered samples ranged from 11.37±0.21 to 6.15±0.10, 1 7.46±0.14 to 6.15±0.10 and 24.63±0.03 to 1.87±0.03 with 4.25±0.01 to 40.73±0.37 and 1.25± 0.01 to 79±0.04 respectively. Since the phytate, tannins and saponin contents of the samples analyzed in this study are not above the minimum recommended values, could lead to the interferences of these phytochemicals with essential micronutrients of man. The physicochemical parameters show a level of the safety and quality of these samples.

TABLE OF CONTENTS

Content		Page
Cover Page		
Title Page		i
Declaration		ii
Certification		iii
Dedication		iv
Acknowledgements		v
Abstract		vi
Table of Contents		vii
List of Tables		xi
List of Figures		xii
Abbreviation		xiii
CHAPTER ONE		
1.0	**INTRODUCTION**	**1**
1.1	Background to the Study	1
1.2	Statement of the Research Problem	3
1.3	Justification for the Study	4
1.4.	Aim and Objectives of the Study	4
CHAPTER TWO		
2.0.	**LITERATURE REVIEW**	**6**
2.1	Herbal Medicine	6
2.1.1	Paraherbalism	6
2.1.2	Herbal preparations	7
2.1.3	Brief history of herbal medicines	7
2.1.4	Preference of herbal drugs in modern societies	8
2.1.5	Categorization of herbal medicines	8

2.1.5.1 Traditional herbal medicines 8
2.1.5.2 New herbal medicines 8
2.1.6 Difference of herbal and conventional drugs; 9
2.1.7 Herb-drug interactions 9
2.1.7.1 Modes of action of herbs compared to those of drugs 10
2.1.7.2 Whole Plants 11
2.1.7.3 Herbal combination 11
2.1.8 Why people use herbal medicines 12
2.1.9 Safety issues of herbal medicines 13
2.2 Present Status of Herbal Medicine 13
2.3 Bioavailability of Herbal Drugs 14
2.4 The Need for Clinical Trials 15
2.5 Regulatory Control of Herbal Medicine 16
2.6 Metals in Herbal Medicinal Products 16
2.7 Metals of Interest 17
2.7.1 Lead 17
2.7.2 Cadmium 17
2.7.3 Iron 18
2.7.4 Copper 18
2.7.5 Manganese 19
2.8 Phytochemicals 19
2.8.1 Flavonoids 20
2.8.1.1 Functions of Flavonoids 20
2.8.2 Saponins 21
2.8.2.1 Functions of Saponins 22
2.8.3 Alkaloid 22
2.9 Anti-nutritional Factors 22
2.9.1 Tannins 22
2.9.1.1 Applications of tannins 23

2.9.2 Oxalates 24

2.9.3 Phytate 25

2.9.4 Nitrate and nitrite 25

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials 27

3.2 Samples Collection 31

3.2.1 Sample preparation 31

3.2.2 Preparation of standard metal solutions of the metals 31

3.2.2.1 Iron (Fe) 31

3.2.2.2 Cadmium (Cd) 31

3.2.2.3 Copper (Cu) 32

3.2.2.4 Manganese (Mn) 32

3.2.2.4 Zinc (Zn) 32

3.2.2.5 Lead (Pb) 32

3.2.3 Samples digestion 32

3.2.4 Heavy metals determination in the herbal samples 33

3.3. Determination of Phytochemicals 33

3.3.1 Determination of flavonoids 33

3.3.2 Determination of saponins 34

3.3.3 Determination of alkaloids 34

3.4 Evaluation of Anti-Nutritional Factors and Nitrogen Compounds 35

3.4.1 Evaluationof oxalates 35

3.4.2 Evaluation of tannin 36

3.4.3 Evaluation of phytate 37

3.4.4 Evaluation of nitrate 37

3.4.5 Evaluation of nitrite 38

3.5 Assessment of Physicochemical parameters 38

3.5.1. Assessment of pH 38

3.5.2 Assessment of viscosity 39

3.5.3. Assessment of bulk density 39

3.5.4 Assessment of electrical conductivity 39

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION 41

4.1 Results 41

4.1.1 Concentrations of selected heavy metals in herbal samples **41**

4.1.2 Selected phytochemical contents of herbal samples in g/100g 48

4.1.3 Selected anti-nutritional factors and nitrogen compounds of the herbal samples 50

4.1.4 The selected physicochemical parameters of the herbal samples 52

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS 54

5.1 Conclusion 54

5.2 Recommendations 55

REFERENCES 56

LISTS OF TABLES

Table		Page
3.1	List of the Reagents used in the Study	27
3.2	List of Analytical Equipment used in the Study	27
3.3	List of the Selected Herbal Samples	28
4.1	Concentrations of Selected Heavy Metals in Herbal Samples	42
4.2	Selected Phytochemical contents of Herbal samples.	47
4.3	Selected Anti-nutritional factors and Nitrogen compounds of Herbal samples	50
4.4	Selected physicochemical parameters of the Herbal Samples	51

LIST OF FIGURES

Figure		Page
2.1	Structure of flavonoids	20
2.2	Structure of Steroids saponins	21
2.3	Structure of Tannins	23
2.4	Structure of Oxalate ion	24
2.5	Structure of Phytic acid	25

ABBREVIATION

WHO:	World Health Organization
DNA:	Deoxyribonucleic Acid
ATDSR:	Agency for Toxic Substances and Disease Registry
GACP:	Good Agricultural and Collection Practice
THMPD:	Traditional Herbal Medicinal Products Directives
TCM:	Traditional Chinese Medicine
HMP:	Herbal Medicinal Products

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Herbal medicines are plant derived materials and preparations with therapeutic or other human health benefits which contain either raw or processed ingredients from one or more plants, inorganic materials or animal origin. Herbal medicine preparations are developed and created drugs by the modern pharmaceutical industries. Nowadays, they are manufactured and sold most widely on the pharmaceutical market for curing diseases and promoting public health (Ezekwesili and Okaka, 2019).

Various plants are used in the formation of herbal medicines, these include, but not limited to, leaves, roots, bark, fruits and seeds (Nkasa *et al*., 2016). Due to their natural origin, herbal remedies are perceived by people who patronize them to be safer than western pharmaceutical products. The World Health Organization (WHO) reckons that over 80% of the population in Africa and developing countries depend on herbal remedies for their healthcare needs (WHO, 2018). For most people in Africa, the costs of orthodox pharmaceuticals put modern health care services out of their reach and therefore heavily rely on herbal medicines to meet their healthcare needs**.** In addition, western pharmaceuticals are often times inaccessible to most people in Africa and so herbal medicines have become one of the major options for treating various diseases. In parallel with the increasing interest in the therapeutic benefits of herbal products, there has been an increasing concern over the safety and toxicity of natural herbs and formulations available in the markets. There is a widespread misconception that natural herbs and plants are inherently safe, nevertheless, there has been a large volume of

reports on incidences of toxicity and adverse effects linked to the use of herbal plants and their formulations in different parts of the world (Nema *et al*., 2016).

Heavy metal is a term used to define metal that has density above 5.00 g/cm^{3}and is toxic or poisonous even at low concentrations. Contamination of herbal preparations with heavy metals is known to be associated with serious health hazards. Some of these metals, including lead, have been reported to be carcinogenic as a result of their inherent ability to form bonds with sulphydryl groups of proteins and depletion of glutathione, while others generate reactive radicals which may alter DNA bases as well as calcium and sulphydryl homeostasis. This implies that consumption of herbal preparations contaminated with these metals at concentrations beyond safety limits may be detrimental to human health (Iffah *et al.,* 2019). Besides, these metals are non biodegradable and long term exposure may result in bioaccumulation and still elicit effects which are detrimental to human health (Eniayewu *et al*., 2020).

Metals are notable for their wide environmental dispersion, their tendency to accumulate in selected tissues of human body and their overall potential to be toxic at high level of exposure. Some metals, such as copper and iron, are essential to life and play irreplaceable roles in the functions of critical enzyme systems. Other metals are xenobiotic (they have no useful roles in human physiology and most other living organisms) and, even worse, as in the case of lead and mercury, may be toxic even at trace levels of exposure. Metals that are essential have the potential to be toxic at very high level of exposure (Umar *et al*., 2016). One reflection of the importance of metals relative to other potential hazards is their ranking by the United States Agency for Toxic Substances and Disease Registry (ATDSR), which lists all hazards present in toxic waste sites according to their prevalence and the severity of their toxicity. The first,

second, third and sixth hazards on the list are heavy metals: lead, mercury, arsenic and cadmium respectively.

The toxicity of trace metals on human health and the environment has attracted considerable attention in recent years. Heavy metals have low excretion rates through the kidney which could result in damaging effects on humans even at very low concentrations. Metals such as zinc, copper, iron, manganese, and chromium are essential nutrients; they are important for the physiological and biological functions of the human body. However, an increase in their intake above certain permissible limits can be toxic (Odoh and Ajiboye, 2019).

.Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Azmatullah *et al*., 2018).

1.2 Statement of the Research Problem

Studies have shown that herbal medicines though, are of natural source, are not completely free of contaminants such as heavy metals present in the soil of the environment where the herbs exist or are grown, due to the various anthropogenic activities that are being carried out in the areas. Contaminants get into the body through the intake of herbal medicines and related products.

1.3 Justification for the Study

Most herbal product in the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness and some could contain certain heavy metals like mercury lead or arsenic and poisonous organic substances in harmful amount.

While a number of these products have safety and quality endorsement, some remain untested which could be of great health risk to their consumers. This is because improper or non-labeling of these products limit the understanding of the risks associated with their usage. Therefore, establishment of the quality and safety of herbal medicines by the determination of the presence and concentrations of heavy metals which pose significant health risks to their consumers alongside the physicochemical parameters, phytochemical contents and anti-nutritional factors is crucial.

1.4. Aim and Objectives of the Study

The aim of this research work is to determine selected chemical parameters and antinutritional factors of branded herbal medicines sold in Minna.

The above aim was achieved through the following objectives

i. Determination of selected heavy metals in the selected herbal samples

ii Determination of phytochemical contents of the selected herbal samples using standard methods.

iii. Evaluation of anti-nutritional factors of the selected herbal samples using standard methods.

iv. Determination of Nitrate and Nitrite of the selected herbal samples using standard methods.

v. Assessment of physicochemical parameters of the selected herbal samples using standard methods.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1 Herbal Medicine

Herbal medicine is the study of pharmacognosy and the use of medicinal plants. Plants have been the basis for traditional medical treatments through most of human history and such traditional medicine is still widely practiced today. This practice which most times has been referred to as herbalism is different from modern medicine that makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs. Although herbalism may apply modern standards of effectiveness testing to herbs and medicines derived from natural sources, few high-quality clinical trials and standards for purity or dosage exist. The scope of herbal medicine is sometimes extended to include fungal and bee products as well as minerals, shells and certain animal parts. Herbal medicine is also called phytomedicine or phytotherapy (Ezekwesili and Ajiboye, 2019).

2.1.1 Paraherbalism

This describes alternative and pseudoscientific practices of using unrefined plant or animal extracts as unproven medicines or health-promoting agents. It differs from plant derived medicines in standard pharmacology because it does not isolate or standardize biologically active compounds, but rather relies on the belief that preserving various substances from a given source with less processing is safer or more effective in the treatment of certain illnesses for which there are no evidences (Dorothy *et al.,* 2017).

2.1.2 Herbal preparations

Herbal medicines and their preparations have been widely used for thousands of years in developed and developing countries owing to their natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. Herbal medicine, also called botanical medicine, phytomedicine or plant medicine, is defined as the finished labeled medicinal product that contains as active ingredients aerial or underground parts of plants or other plant materials. Examples of such materials include fresh juice, gums, fixed oils, essential oils, resins and dry powders of herbs, leaves, bark, roots, rhizomes or other plant parts which may be entire, fragmented or powdered or combinations thereof whether in crude state or as plant preparations. Although herbal medicines have been used to treat many conditions such as asthma, eczema, premenstrual syndrome, rheumatoid, arthritis, migraine, menopausal symptoms, chronic fatigue, and irritable bowel syndrome, the qualities of these products are still points of concern by health authorities, pharmaceutical industries and the public because their sales and consumption are not properly regulated in developing countries especially Nigeria (Odoh and Ajiboye, 2019).

2.1.3 Brief history of herbal medicines

Since prehistoric times, humans have used natural products, such as plants as medicines to alleviate and treat diseases (Manishar *et al*., 2020). The use of natural products as medicines must, of course, have presented a tremendous challenge to early humans. It is highly probable that when seeking food, early humans often consumed poisonous plants, which led to vomiting, diarrhea, coma, or other toxic reactions (perhaps even death). However, in this way, early humans were able to develop knowledge about edible materials and natural medicines which led them to know how to develop new drugs

hence, enhancing the field of traditional medicine which has a millenary history, especially throughout the Orient (Haidan *et al.*, 2016).

2.1.4 Preference of herbal drugs in modern societies

Recent years have witnessed a renewed interest in plants as pharmaceuticals in the western world. In the global context, the use of herbal medicines flourishes as a therapeutic choice in many parts of the world. In recent years, the increasing demand for herbal medicines is being fueled by a growing consumer interest in natural products. Therefore, it is now finding new popularity as an alternative conventional medicine even in industrialized countries. Thus, the adoption of crude extracts of plants for self medication by the general public is in the increase (Tengku *et al*., 2019).

2.1.5 Categorization of herbal medicines

2.1.5.1 Traditional herbal medicines

These are defined as herbal substances (single or mixture of herbs) that have been widely used, supported by well-established safety and efficacy data, or have been used within the local community for a minimum period of 15 years. This category would also include traditional medicine formulations to which minor changes have been made (Josephine *et al*., 2019). Herbal medicines that are not indigenous to a particular country or region, for instance; ginseng, could also be included if they have been widely used within the region with sufficient knowledge about their safety and efficacy (Douglas *et al*., 2020).

2.1.5.2 New herbal medicines

Herbal medicines (single or mixture of herbs) can be considered “new” if never used within the community or region, used for only a short period of time, used to a very

small extent (few uses in a small number of patients), or used in a new combination of herbal substances never combined before Ezekwesili and Okaka (2019).

Types of traditional system of medicine vary greatly from region to region based on many historical, economical and cultural factors. Asia is the home to a number of codified systems that is, systems of practice where the preparation of medicines and their specific applications have been documented in written form. These texts written thousands of years before have been passed down the generations through many millennia (Siv. 2018).

2.1.6 Differences between herbal and conventional drugs

Herbal medicine are finished, labeled medicine containing pharmacologically active parts of a plant or plants either in crude form or physically modified during processing while conventional drug is a pharmacological single entities which have been derived by chemical synthesis (Glynn and Bhilkha, 2018).

Compared with modern allopathic medicine, herbal medicine is freely available and can easily be accessed by all. As a result, there is limited consultation with traditional healers because there is a fairly good knowledge of common curative herbs especially in the rural areas except in the case of treatment of chronic diseases. In Nigeria, and indeed the entire West Africa, herbal medicine has continued to gain momentum, some of the advantages being low cost, affordability, availability, acceptability, and apparently low toxicity (Okaka and Ozioma, 2019).

2.1.7 Herb – drug interactions

As there is a significant and increasing number of people taking herbal remedies simultaneously with conventional drugs, there is a distinct and real possibility of herbdrug interaction, analogous to the drug-drug interaction that is now well established

and documented (Rashid *et al.,* 2018).It is generally accepted that drugs can interact with food. The original indication that a herbal agent could interact significantly with the action of a conventional drug was the observation, more than 20 years ago, that the consumption of grapefruit juice could markedly reduce the metabolism of the immunosuppressant cyclosporine in patients following transplantation (John and Bhilka, 2018). It is now known that the metabolic enzymes of patient's liver are inhibited by drugs, so higher levels of active drugs are maintained for a longer period of time. As a result, a lower dose of drugs especially the very expensive ones could be employed without compromising clinical efficacy or outcome (Glynn and Bhilka, 2018).

2.1.7.1 Modes of action of herbs compared to those of drugs

Identifying the pharmaceutical mechanisms of herbal products activity poses numerous challenges not faced by those studying conventional drugs. For example, herbal products contain many active substances which can act in combination or synergistically, whereas conventional drugs are generally studied in isolation as single agents (Rashid *et al.,* 2018).

Another difference is that a single conventional drug may only have one major direct action on a particular receptor site. Indeed, the more specific for one type of receptor is the preferred outcome of research for new pharmaceutical agents. Conversely, herbal products probably have several different pharmacological actions and the one that predominates depends on the dosage employed, the part of plant selected and the presence of other active components. Moreover, the herbal product may contain a particular active agent, but it may not be present in sufficient quantity to elicit a pharmacological action (Glynn and Bhilka, 2018).

Another difference is that pharmacological synergy may occur when a multi-component herbal product is used in which case the net pharmacological response will be different when compared to the use of one active agent alone. In this case, the dominant mechanism may be potentiated by a separate mechanism (Glynn and Bhilka, 2018).

2.1.7.2 Whole plants

Herbalists generally use unpurified plant extracts with several constituents. It is claimed that these can work synergistically so that the effect of the whole herb is greater than the summed effects of its components. It is also claimed that toxicity is reduced when whole herbs are used instead of isolated active ingredients (buffering). Although two samples of a particular herbal drug may contain constituent compounds in different proportions, practitioners claim that this does not generally cause clinical problems. Although there are some experimental evidences for synergy and buffering in certain whole plant preparations, how far these are applicable to all herbal products is not yet known (Philips *et al.,* 2019).

2.1.7.3 Herbal combinations

Often several different herbs are used together with the assertion of the practitioners that the principles of synergy and buffering apply to combinations of plants thus claiming that combining herbs improves their efficacy and reduces adverse effects. This contrasts with convectional practice where polypharmacy is generally avoided whenever possible (Philips *et al.,* 2019).

For the last decades, it is well-accepted that combined drug therapies may provide better clinical outcomes in the treatment of some conditions such as hypertension, cancer, depression and HIV infection. Synthetic pharmaceutical drugs are usually single chemical entities acting on a single biological target. Combined drug therapies are

formulated in a fashion to augment the total effects in treating the target condition, reduce the side effect via dose-sparing of the active components, or address different metabolic interdependence, mediators or risk factors of diseases through a variation of independent bimolecular targets (Kelvin *et al*., 2016).

2.1.8 Why people use herbal medicines

The earliest evidence of human's use of plant for healing dates back to the Neanderthal period (Alexandra *et al.,* 2020). Herbal medicines are now being used by an increasing number of patients who typically do not report to their clinicians of their concomitant use (Quazi and Molvi, 2016). There are multiple reasons for patients turning to herbal therapies. Often cited is a "sense of control, a mental comfort from taking action," which helps explain why many people taking herbs have diseases that are chronic or incurable such as diabetes, cancer, arthritis or AIDS. In such situations, they often believe that conventional medicine has failed them. When patients use home remedies for acute, often self-limiting conditions, such as cold, sore throat, or bee sting, it is often because professional care is not immediately available, too inconvenient, costly or time consuming (Haidan *et al.,* 2016).

In rural areas, there are additional cultural factors that encourage the use of botanicals, such as the environment and culture, a "man earth relationship." People believe that where an area gives rise to a particular disease, it will also support plants that can be used to cure it (Fatemah *et al.,* 2018). Hundreds of primary health centers which are intended to serve rural areas, lack staff, diagnostic facilities and adequate supplies of drugs. The rural population is therefore heavily dependent on traditional medical systems (Jamshidi-kia and Lorigooini*,* 2017). Natural plant products are perceived to be healthier than manufactured medicines (Quazi and Molvi, 2016).

2.1.9 Safety issues of herbal medicines

Traditional herbal products are heterogeneous in nature. They impose a number of challenges to quality control, quality assurance and the regulatory process. Most herbal products on the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness. Some of them contain mercury, lead, arsenic (Irfrat *et al*., 2020) and poisonous organic substances in harmful amount. Hepatic failure and even death following ingestion of herbal medicine have been reported (Dilip 2018). A prospective study shows that 25% of the corneal ulcer in Tanzania and 26% of the childhood blindness in Nigeria and Malawi were associated with the use of traditional eye medicine (Mudasir *et al.,* 2020).

Sometimes patients use traditional and conventional medicine simultaneously. The interaction of these two types of drugs *in vivo* may be dangerous and have raised serious concern among the medical scientists about the safety of the patients (Mudasir *et al.,* 2020).There are case reports of serious adverse events after administration of herbal products. In most cases the herbs involved were selfprescribed and bought over the counter or obtained. As herbal medicines are used by increasingly number of people, pharmacist must be knowledgeable about their safety. This requires appreciation of the magnitude of use, as well as regulation under which the products are marketed (Mudasir *et al.,* 2020).

2.2 Present Status of Herbal Medicine

The wide spread use of herbal medicine is not restricted to developing countries, as it has been estimated that 70% of all medical doctors in France and German regularly prescribe herbal medicines (Mudasir *et al.,* 2020). The number of patients seeking herbal approaches for therapy is also growing exponentially (Mudasir *et al.,* 2020). The recent survey estimated that 39% of 520 new approved drugs in 1983-1994 were natural

products or derived from natural products and 60-80% of antibacterial and anticancer drugs were derived from natural products (Mudasir *et al.,* 2020).

The penicillin that replaced mercury in the treatment of syphilis and put an end to so many of the deadly epidemics comes from plant mold. Belladonna still provides the chemical used in ophthalmological preparations and in antiseptics used to treat gastrointestinal disorders. Rauvolfia serpentine (The Indian snake root) which has active ingredient, reserpine, was the basic constituent of a variety of tranquilizer first used in the 1950's to treat certain types of emotional and mental problems. Though reserpine is seldom used today for this purpose, its discovery was a breakthrough in the treatment of mental illness. It is also the principal ingredient in a number of modern pharmaceutical preparations for treating hypertension. But reserpine can have a serious side effect severe depression (Haidan *et al.,* 2016).

2.3 Bioavailability of herbal drugs

The bioavailability of the active constituents of the herb is another area of considerable importance. Before a compound can act systemically it must pass from the gastrointestinal tract into the blood stream. This is an area in which surprisingly little is known for herbal constituents (Manisha *et al.,* 2020).

Studies showing systemic effect in animals have all involved parental administration of these alkaloids. Yet goldenseal remains one of the best-selling herbs, is widely promoted, and is accepted by a misinformed public as a nonspecific immune stimulant (Bianca *et al.,* 2019).

Cinnabar has been for a long time in traditional medicine. The toxic effects of inorganic mercury are well recognized, but because of its insolubility have been assumed that this compound would not be significantly absorbed from the gastrointestinal tract. However,

investigation on the oral absorption of cinnabar in mice found a significant increase in mercury concentration in the liver and kidney. Concomitant use of drugs containing bromide,sulphates, sulphides, nitrates and iodine may enhance its toxicity by increasing the gastrointestinal absorption (Archana *et al.*, 2019).

2.4 The Need for Clinical Trials

To gain public trust and to bring herbal product into mainstream of today health care system, the researchers, manufacturers, and regulatory agencies must apply rigorous scientific methodologies and clinical trials to ensure the quality of the traditional herbal products (Abida *et al.,* 2015).Since the identities of the final products are not well defined and there are essentially no purification steps involved in the productions of herbal products, the quality of the products rely mostly on the quality control of source materials and their manufacturing into the final products. Using modern technologies the quality and consistency of the heterogeneous herbal products can be monitored (Olufunsho *et al.,* 2018). A well-designed clinical trial is the method of choice to prove the safety and effectiveness of therapeutic products. Manufacturers of the herbal products must adhere to the requirements of good manufacturing practices (GMPs) and pre-clinical testing before these products can be tested on human (Francis *et al.,* 2018). The basic principle and design of the clinical trials for herbal products are the same as those for single component chemical product. A number of randomized double-blinded controlled studies have been carried out using herbal formulations. These studies have proven the effectiveness of the herbal products tested and shown little side effects. Thousands of years of traditional use can provide us with valuable guidelines to the selection, preparation and application of herbal formulations. To be

accepted as viable alternatives to western medicines, the same rigorous methods of scientific and clinical validations must be applied (Sayeed *et al.,* 2015).

2.5 Regulatory Control of Herbal Medicine

The emergence of herbal medicines caused the needs for proper regulatory control and to avoid the lack of consistent terminology. Some of the herbal products are classified as food products and as dieter supplement (Saminathan *et al.,* 2018). Therefore, the need to identify the classification of herbal drugs and use of targeted treatment. World Health Organization (WHO) has conducted a global survey on the regulatory control of herbal medicines and has reported findings from 141countries. This survey confirmed that many countries established herbal drugs regulation during the past few years. The process of regulatory control, establishing herbal medicines national policy and regulatory status is most important to access the herbal medicines, assessment of safety and efficacy. Recently, World Health Organization (WHO) guidelines have developed in several important areas including Pharmacovigilance, consumer information and good agricultural and collection practices (GACP) (WHO, 2004). Several countries following herbal regulatory body such as Traditional Herbal Medicinal Products Directives (THMPD) Europe, which became effective in early 2011, Traditional Chinese Medicine (TCM), since 2004, Africa and Brazil are ongoing. These reviews concluded that regulation of herbal drugs could be able to monitor the preparation of herbal products and its clinical importance. Herbal medicinal products can offer an alternative to conventional medicines in non-life-threatening conditions, provided they are of adequate quality and safety and are used in appropriate manner (Vetriselvan *et al.,* 2018).

2.6 Metals in Herbal Medicinal Products

The reasons for the presence of metals in Herbal Medicinal Products (HMPs) are varied. Plants may accumulate heavy metals from the environment during growth on contaminated soil or by deliberate addition. Whatever the route by which heavy metals are introduced in HMPs, if the level of these metals is high, poisonings may occur. The toxicity of metals most commonly involves the brain and the kidney, but other manifestations occur, and some metals, such as arsenic, are clearly capable of causing cancer (Umar *et al.,* 2016).

2.7 Metals of Interest

2.7.1 Lead

Lead is involved in a large number of processes in the human body, and is toxic for many tissues: bone, heart, intestines, liver, kidney, reproductive and nervous systems. Interfering with the metabolism of the nervous system, it is therefore particularly hazardous to children. It can produce permanent behavior and learning disabilities. Symptoms may be mental confusion, irritability and headaches, abdominal pain, anemia, and in more dramatic cases, convulsions, coma and death. Lead is certainly among the most ubiquitous and widespread toxic metals. Its considerable spread is largely due to the fact that for many years the lead, as $Pb(C_2H_5)_4$, has been used in gasoline as anti knock. So, considering it strong toxicity, but at the same time, also the fact that herbal medicines are grown in the countries where un-leaded fuel is not used, there is an obvious danger of using herbal medicines heavily contaminated by lead. (Adewoyin *et al*., 2016).

2.7.2 Cadmium

Cadmium is an extremely toxic element. The exposure or inhalation of this metal quickly leads to serious respiratory problems such as; trachio-bronchitis, pneumonia,

and even pulmonary edema and serious damage to kidneys and liver. Cadmium is a metal of exclusively anthropogenic origin, resulting from different activities, for example cement plants, burning of fossil fuels, and waste incineration plant, uncontrolled discharge of sewage. In addition, agricultural chemicals containing cadmium, phosphate fertilizers in particular certainly contribute to contaminate cultivated plants. Several researchers from countries producing herbs used in medicinal preparations showed much concern and interest for the content of heavy metals in plant grown in their countries (Adewoyin *et al*., 2016).

2.7.3 Iron

Iron is the second most abundant metal on the earth's crust. It is a most crucial element for growth and survival of almost all living organisms (Viera *et al.,* 2016).It is one of the vital components of organisms like algae and catalase, as well as of oxygen transporting proteins, such as hemoglobin and myoglobin (Nkansa *et al.,* 2016). Iron is an attractive transition metal for various biological redox processes due to its interconversion between ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions (Nkansa *et al.,* 2016). The source of iron in surface water is anthropogenic and is related to mining activities. The following equations represent the simplified oxidation reaction for ferrous and ferric iron (Nkansa *et al.,* 2016).

$2FeS_2 + 7O_2 \rightarrow 2FeSO_4 + H_2SO_4$ (ferrous) 2.1

$4FeSO_4 + O_2 + 10H_2O \rightarrow 4Fe(OH)_3 + 4H_2SO_4$ (ferric). 2.2

Biologically it is the most important nutrient for most living creatures as it is the cofactor for many vital proteins and enzymes. Iron mediated reactions support most of the aerobic organisms in the respiration process. If it is not shielded properly, it can catalyze the reactions involving the formulation of radicals which can damage biomolecules, cells, tissues and the whole organism (Umoh *et al.*, 2020).

2.7.4 Copper

Copper is an essential element but when consumed in excess, cause toxicity. It's deficiency results in kinky and steely hair syndrome in humans and abnormal wool in sheep, while excessive Cu intake results to hepatolenticular degeneration with progressive impairment of Cu-laden tissues until death results. It also helps in interconversion of the major neurotransmitters, dopamine, nor-adrenaline, and in pigment production. Zinc-Cu interaction has shown hypothesis of ischemic heart disease, which proposes that decreased copper intake with excessive zinc may play an etiologic role in cardiac death in both animals and man (Viera *et al*., 2016).

2.7.5 Manganese

Manganese is one of essential metal which is normally found in traces in human as well as animals' bodies. It deficiency causes tissue damage and impairs central nervous system functions. However, it's excessive amount can results in breathing disorders like pneumonia and affects reproductive system, which may lead to infertility (Viera *et al.,* 2016).

2.8 Phytochemicals

Phytochemicals are a group of bioactive compounds naturally found in plant parts such as flowers, leaves, fruits, roots, barks, spices and medicinal plants (Saranja *et al*., 2016).

Phytochemicals such as alkaloids, phenols, aspirins are frequently used in chemotherapeutic treatment or may be used as chemo preventive agents with chemoprevention referring to the use of agents to constrain, reverse, or delay tumor genesis. For instance, saponins, flavonoids and phenolic compounds also play an important role in the growth and reproduction of most plants, these compounds also act as anti-feedants and anti-pathogens (Desmond *et al.,* 2018).

2.8.1 Flavonoids

Flavonoids are polyphenolic phytochemicals present in a variety of food products and beverages classified into six groups of different chemical structures and physiological properties (Sumanta and Syed, 2020). The potential health benefits arising from the antioxidant activities of these polyphenolic compounds have sparked recent interest in these substances. By scavenging free radicals and/or chelating metal ions, active hydroxyl groups in flavonoids mediate their antioxidant effects. Metal chelation may be crucial in preventing radical generation that damages target biomolecules. Because of their high antioxidant potential both in vivo and in vitro systems, flavonoids are thought to have health-promoting properties as a dietary element and the ability to activate human enzyme defense systems (Mondal and Syed, 2020). The structure of flavonoid is shown in figure one.

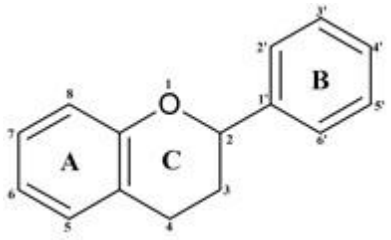

Figure 2.1: Structure of flavonoid (Source: *Avtar* and Bhawna, 2019 **)**

2.8.1.1. Functions of flavonoids

Plants produce a wide variety of organic compounds, the vast majority of which do not appear to be directly involved in growth and development. Traditionally referred to as secondary metabolites (flavonoids), these substances are often distributed differently

within the plant kingdom among small taxonomic groups. The flavonoids are known as alkaloids, terpenoids and phenolics in different classes. In the human body, flavonoids perform a variety of defensive roles. Most flavonoids have developed as bioactive compounds that interact with nucleic acid or proteins and have pharmacological and antimicrobial or insecticidal properties. Therefore, flavonoids are use in medicine as therapeutics (Mondal and Syed, 2020).

While there is considerable interest in the potential health benefits of flavonoid intake, there has not been extensive study of the potential adverse effects of consuming very large amounts of these phytochemicals (Rahaman and Sumanta, 2020).

2.8.2 Saponins

Saponins are naturally occurring bioorganic compounds having at least one glycosidic linkage (C-O-sugar bond) at C-3 between aglycone and a sugar chain. Hydrolysis of saponin molecule produces two portions, aglycone and a sugar moiety. Isolated amorphous solid saponins have a high molecular weight, and containing 27 to 30 carbon atoms in the non-saccharide portion (Maher *et al.,* 2019).

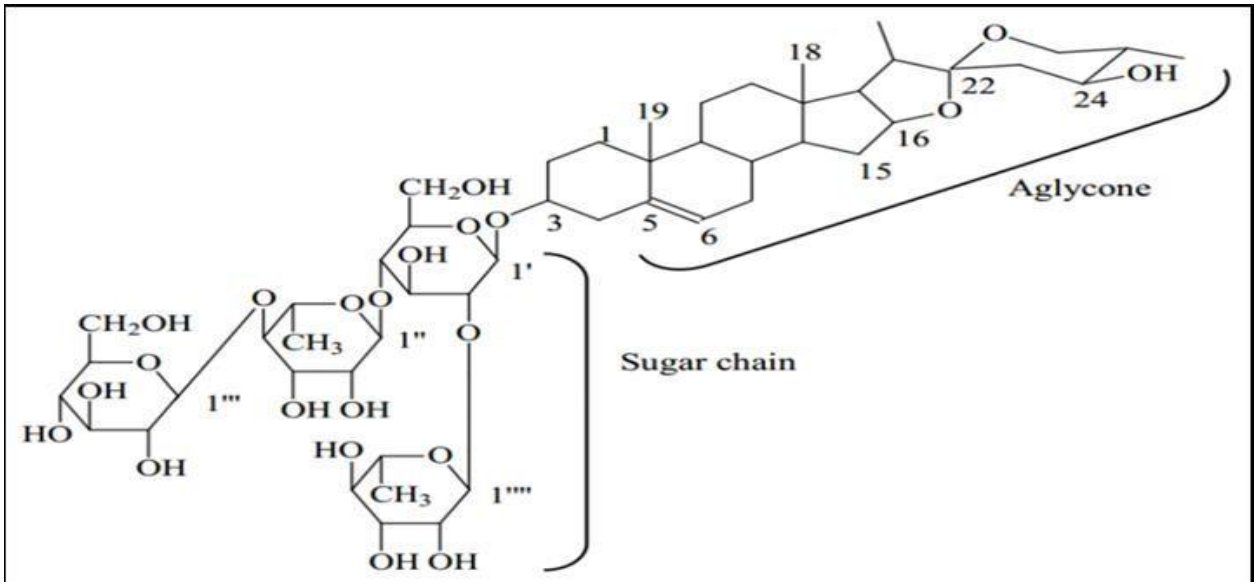

Figure 2.2: Structure of Steroid Saponin; (Source**:** Maher *et al.,* 2019)

These naturally occurring compounds form the backbone of modern medicine or drugs. Saponins are a class of bioorganic compounds found in particular abundance in the plant kingdom. Structurally saponins have one or more hydrophilic glycoside sugar moieties combined with a lipophilic triterpene molecule (Aziza *et al.,* 2019). The chemical structure of saponins is shown in Figure 2.

2.8.2.1 Functions of saponins

Saponins exhibit a biological role and medicinal properties such as hemolytic factor anti-inflammatory,antibacterial,antifungal,antiviral, insecticidal, anticancer, cytotoxic and molluscidal action. In addition, saponins are reported to exhibit cholesterol lowering action in animals and human (El Sadek *et al.,* 2019).

2.8.3 Alkaloid

Alkaloids are a group of naturally occurring chemical compounds that contains mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties that are produced by a large variety of organisms which includes bacteria, fungi, plants and animals. They rank among the most diverse, efficient and therapeutically significant plant substances. Generally, alkaloids are extremely toxic though they have a marked therapeutic effect in small quantities. Pure, isolated plant alkaloids and their synthetic derivates are used as basic medicinal agents all over the world for their analgesic, antispasmodic, and bactericidal effects. Some alkaloids are used as an antiseptics due to its antibiotic activity e.g. berberine in opthamics and sanguinarine in toothpastes (Arpital, 2017).

2.9 Anti-nutritional factors

2.9.1 Tannins

Tannins are water-soluble natural polyphenols mainly present in plant-based materials, including food. Tannins play a very significant role as a raw material for sustainable green industries. They are sort of secondary metabolites, available in various parts of plants such as bark, wood, leaves, seeds, roots, and even the plant galls are the major sources of tannin extractions used for various purposes (Akhlash and Sunil, 2019).

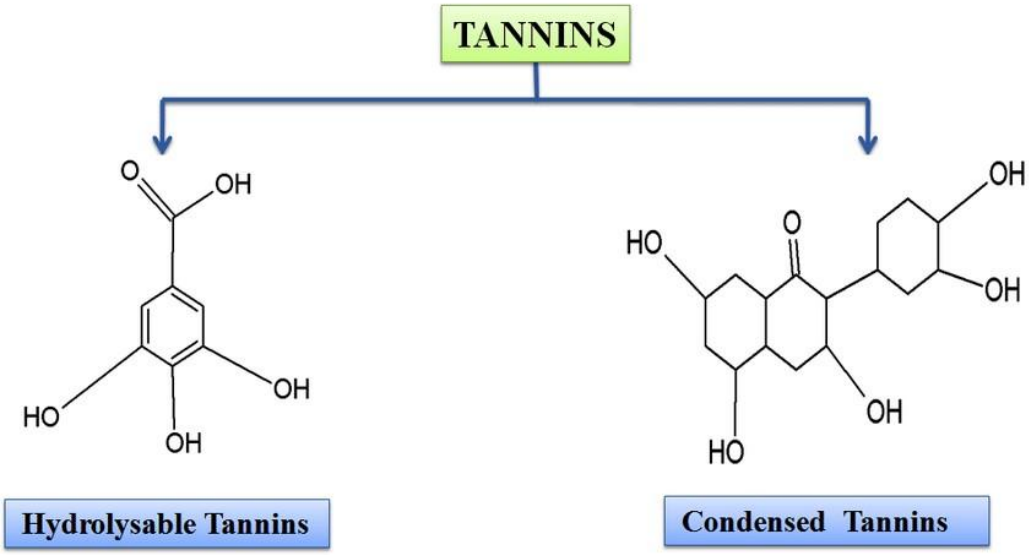

Figure 2.3: Structures of tannins, (Source: Harley *et al*., 2017)

Plant tannins are a large group of natural phenolic compounds which contain a range of molecular weight between 500 and 3000 Da. Currently, they have been divided into three main subgroups: (1) hydrolysable tannins, (2) condensed tannins, and (3) phlorotannins. Hydrolysable tannins are highly soluble in water; biochemically, they consist of a central core of a carbohydrate (D-glucose) with its hydroxyl groups or polyol esterified with phenolic compounds such as gallic acid (3,4,5-trihydroxybenzoic acid) or hexahydroxydiphenic acid, which also known as ellagic acid (Sunil and Akhlash, 2019). Structure of tannins is shown in Figure 2.3

2.9.1.1Applications of tannins

Chemically, it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers. It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin etc.), alkaloids, nucleic acids and minerals (Koche *et al.*, 2016).

Many studies have clearly shown that tannins are natural antioxidants linked with the prevention of degenerative diseases such as atherosclerosis, cardiovascular diseases, neurodegenerative diseases, and certain types of cancers by acting as antioxidants and antibacterial (Singh and Sunil, 2019).

2.9.2 Oxalates

Oxalate is a naturally occurring substance found in plants and in the human body. In chemical terms, oxalate belongs to a group of molecules called organic acids. Certain body tissues routinely convert other substances into oxalate, which is an end product of human metabolism. For example, vitamin C can be converted into oxalate. It is interesting to note that the leaves of a plant usually contain higher oxalate levels than its roots, stems, and stalks (James *et al.,* 2015).

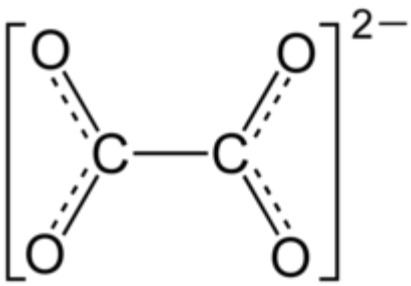

Figure 2.4: Structure of Oxalate ion (Source; Carla *et al.,* 2019).

It is found as salts of insoluble complexes with divalent cations, minerals and trace elements. Oxalates react with calcium to precipitate calcium oxalate and accumulation of oxalates in the body prevents the absorption and utilization of calcium; which in turn causes calcium imbalance, rickets and osteomalacia (Madhu *et al.,* 2018).

2.9.3 Phytate

Phytic acid (PA) is a unique natural substance found in plant seeds. It is a major form of phosphorus found in edible plants such as grains, nuts and legumes. It is found only in plant derived food and exist predominantly in its salt form: phosphate ester of inositol polyphosphate where itaccounts for about 60 to 70% total plant phosphorus.

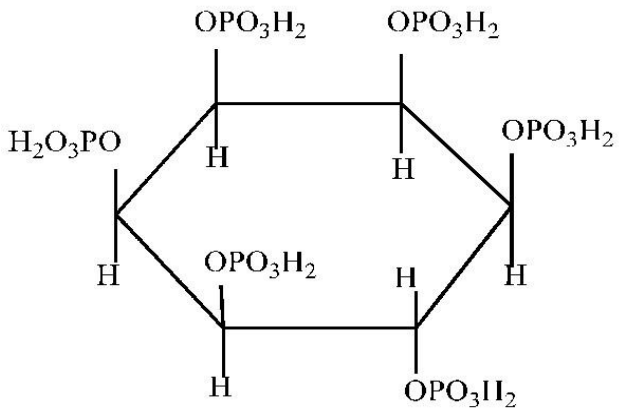

Figure 2.5: Structure of phytic acid (Source;Alexandra *et al.,* 2020)

At physiological pH the phosphate is partiallyionized. The resulting anions are colourless species that has significant nutritional role as the principal storage form of phosphorus in plant tissues. The composition of PA varies amongst plants and within plant species. The variability of PA composition depends on certain factors like: growing conditions, harvesting and processing techniques and age of the food grains

harvested (Abdulwaliyu *et al*, 2019). Chemical structure of phytic acid is shown in figure 5.

2.9.4 Nitrate and nitrite

Nitrate and nitrite exist naturally at low levels in the environment, yet human activities increase their levels, and can lead to both environmental and health problems. Elevated amounts of nitrate and nitrite can exist in plants which are direct source of herbal medicine, as a result of agricultural activities that involve the excessive use of fertilizers and animal waste, runoff, leaching and release of untreated sewage and industrial waste (Mohammed *et al*., 2017). Nitrate and nitrite are used as additives to improve food quality and protect against microbial contamination, and are source of N-nitroso compounds (NOCs) which are known carcinogens (Marie and Edmond, 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The materials, reagents and equipment used for the research are listed in Tables 3.1 and 3.2 respectively.

Table 3.1. List of Reagents Used in the Study

Materials and reagents	Brand
Herbal drugs	Powdered and liquid mixtures
Conc. hydrochloric acid, HCl	BDH chemical
Conc. Trioxonitrate(V) acid, HNO_3	BDH chemical
Phosphomolybdic acid	BDH chemical
Methanol	BDH chemical
Ethanol	BDH chemical

Table 3.2List of Analytical Equipment Used in the study

Equipment	Manufacturer/ Model
Atomic Absorption Spectrophotometer, AAS	Bulk Scientific, Model: Accusys 211 made in England
pH meter	Genway, model 3505. Manufacturers: Cole-parmer, UK

Analytcal weighing balance	Mettler pm 2000. Manufactured by Mettler Instrument Limited, England
UV Spectrophotometer	752 UV Spectrophotometer, Model; YM 1208 PTS1
Centrifuge	OHB Specimen, USA.

Table 3.3 List of Selected Herbal Samples

Sample code	Form	Brand name	Manufacturer	Uses	Dose
HM_1	Liquid	Sawaba herbal medicine	Sawaba herbal medicine; Sabon-Garimaain motor park, Kano	Typhoid, pile, malaria, gonorrhea, fever, pain, STD, man-power, ulcer and infections.	Adult; one bottle daily(80ml) Child; half bottle daily
HM_2	Liquid	Al-afwaUlcercline	Al-Afwa Botanical Company	Stomach problem, Back pain, Gastrointestinal and Chronic ulcer	Half bottle daily for days
HM_3	Liquid	Sahihinmaganinsanyinmara	Khalid Bin Walid Islamic Chemist.	Gonorrhea and Cold.	Adult: One bottle per day
HM_4	Liquid	Agajinabiyar	Sharda Pulse, Shagotara Bayan labi Kano	Stomach pain	10ml per day
HM_5	Liquid	Mai dabino	Mai Dobino	Anti rheumatism,Syphilis, virginal odour	Adult: 3 spoons twice dailyChildren: 1 spoon twice daily
HM_6	Liquid	Agajinatara	Islamic Medicine Centre.Add: Sharada phase 3, jainshagotaraBarkinladi Kano.	Typhoid,malaria	

HM_7	Liquid	Shajaratusshifa	Daru-shifa Herbal Medicine	Pile	Half a bottle
HM_8	Liquid	Al-himayah	Al-Himaya Herbal solution. Add: Km 13, Maguza Gwammeja Road Kano	STD, cold	Adult: One bottle per day
HM_9	Liquid	Sahihinmaganitari	Dutsinma Islamic Medicine, Katsina	Strong cough, TB, and chest pain.	Adult: 2-3 spoons daily.Children: 2 spoons daily.
HM_{10}	Liquid	Body defense	Hamdasa Fruits and Herbal Remedies. Add: NIPOST Quarters, Off Okada Road Minna.	Treatment of viral, bacteria, cancer and leukemia	3 table spoon daily
HM_{11}	Liquid	Stomach peace	Hamdasa Fruits and Herbal RemediesAdd: NIPOST Quarters, Off Okada Road Minna.	Stomach pain, cramps and menstrual challenge.	5ml three times daily
HM_{12}	Liquid	Malaria Ultimate Solution	Tauraro Herbal Medicine	Malaria, dysentery, typhoid and fever	Adult: 2 spoons twice daily Children: 1 spoon twice daily
HM_{13}	Liquid	Kudiratusshifa	Danladi Islamic Medicine Centre. Add: SabonGari, Kano	Ulcer	

HM_{14}	Powder	Seven-four-seven	Seven –for-seven Herbal Medicine	For cold	Seven-four-seven
HM_{15}	Powder	Jenyo Herbal Powder	Jenyo Herbal Medicine Add: Ifejenyo Comp.	Malaria and typhoid	Once daily
HM_{16}	Powder	Dan-buzu	Himmadiya Junction, Osogbo. Dan-buzuHerbal Centre	Typhoid and malaria	
HM_{17}	Powder	Pile Plus Dankanoma	Hamdasa Fruits and Herbal RemediesAdd: NIPOST Quarters, Off Okada Road Minna.	Pile	1 tea spoon trice daily
HM_{18}	Powder	Anti-stress plus	Hamdasa Fruits and Herbal RemediesAdd: NIPOST Quarters, Off Okada Road Minna.	Relief stress	Half teaspoon daily
HM_{19}	Powder	Anti-stooling	Hamdasa Fruits and Herbal Remedies Add: NIPOST Quarters, Off Okada Road Minna.	Prevents stooling	
HM_{20}	Powder	HDC Plus	Hamdasa Fruits and Herbal RemediesAdd: NIPOST Quarters, Off Okada Road Minna.	Headache and ringworm	1 tea spoon trice daily.

3.2. Samples Collection

Random sampling method was adopted for the collection of samples by which thirteen branded liquid samples and seven branded solid samples were purchased from different medicine stores and mobile drug dealers in Minna metropolis. The therapeutic effect(s) of each of the herbal samples were recorded and samples collected were carefully arranged and kept in a cool and dry place, away from the reach of rodents, so as to prevent contamination. The purchased samples of herbal mixtures were of two forms; liquid and solid respectively. Samples were coded as HM_1, HM_2, $HM_3$to HM_{20}.

3.2.1 Sample preparation

Samples were accurately weighed and measured using weighing balance and measuring cylinder and were neatly packed, coded and made ready for digestion.

3.2.2 Preparation of standard solutions of the metals

Standard solutions, 5ppm of each of the metals to be analyzed were prepared and the wavelengths at which the metals were determined in the samples were recorded.

3.2.2.1 Iron (Fe)

0.022 mol/dm^3 of Iron (III) chloride, Fe_2Cl_3 was prepared by weighing 4.840 g from the container of analytical grade iron (III) chloride, which was dissolved in 200 cm^3 of distilled de-ionized water and made up to 1000cm^3.

3.2.2.2 Cadmium (Cd)

0.008mol/dm^3 of Cadmium nitrate, $Cd(NO_3)_2$ was prepared by weighing 2.1032g from the container of analytical grade cadmium nitrate, dissolved in 250 cm^3 of distilled water and made up to 1000 cm^3.

3.2.2.3 Copper (Cu)

0.016 mol/dm^3 of hydrated copper (II) trioxonitrate (V) was prepared by weighing 3.7980g from the container of analytical grade hydrated copper (II) trioxonitrate (V), dissolved in 250 cm^3 with distilled de-ionized water and made up to 1000 cm^3.

3.2.2.4 Manganese (Mn)

0.015 mol/dm^3 of hydrated manganese chloride, $MnCl_2.6H_2O$ by weighing 3.6077g from the reagent bottle of the analyzed grade chemical, dissolved in 50cm^3 conc. Hydrochloric acid and made up to 1000cm^3.

3.2.2.5 Zinc (Zn)

0.015 mol/$dm^3$of Zinc oxide (ZnO) was prepared by weighing 1.2450g, dissolved in 50cm^3 of distilled de-ionized water followed by 25cm^3 of 5M hydrochloric acid, and made up to 1000cm^3 in a volumetric flask.

3.2.2.6 Lead (Pb)

0.004 mol/dm^3 of lead nitrate $Pb(NO_3)_2$by weighing 1.5980g from the analyzed grade container, dissolved in 100cm^3 distilled de-ionized water and made up to 1000cm^3.

3.2.3 Samples digestion

The digestion method, as recorded by Abim *et al.* (2016) (wet acid digestion in an open system) was used for the analytical treatment of the samples. In this method, 10.0cm^3 of each of the liquid coded samples and 0.50g of each of solid coded samples were accurately measured into 50.0cm^3 beakers and 250cm^3 conical flasks respectively. 10 cm^3 of aqua-ragia (3:1 mixture of trioxonitrate (V) and hydrochloric acid) was added to each samples and carefully set for digestion until clear solution of digested samples

were obtained. Each digested samples were filtered, made up to 50cm^{3}volumes, and neatly packaged for elemental analysis.

3.2.4 Heavy metals determination in the herbal samples

A Bulk scientific AAS (model; Accussys 211, USA), was employed for the determination of the heavy metals in the herbal samples. Bulk scientific hallow cathode lamps for Pb, Cd, Fe, Cu, Mn and Zn were used as recitation sources. From the prepared stock solutions of each of the metals, 5.0 ppm solutions were prepared by taking 5.0 cm^3 with the aid of a micro pipette and made up to 100 cm^3. Under optimum operating conditions, the metals were measured at 228.8, 248, 283.3, 279.4, and 213.9 nm respectively using air-acetylene flame (with exception of lead).

3.3. Determination of Phytochemicals

3.3.1 Determination of flavonoids

Flavonoid determination was by the method reported by Ejikeme and Chukwu (2016). Exactly 50 cm^3 of 80% aqueous methanol was added to 2.50 g of sample in a 250 cm^3 beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each herbal sample. Each herbal sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desicator and weighed until constant weight was obtained. The flavonoid was calculated as

$$\text{Flavonoid} = \frac{\text{Weight of flavonoid}}{\text{of sample}} \times 100 \qquad \text{3.1 Weight}$$

3.3.2 Determination of saponins

Saponin quantitative determination was carried out using the method reported by Ejikeme and Chukwu (2016). Exactly 100 cm^3 of 20% aqueous ethanol was added to 5.00 g of each herbal sample in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was re-extracted with another 100 cm^3 of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 cm^3 over water bath at 90°C. 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60 cm^3 of n-butanol was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium chloride layer the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as:

$$\text{Saponin} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} \times 100 \qquad 3.2$$

3.3.3 Determination of alkaloids

Quantitative determination of alkaloid was according to the methodology by Harborne *et al* (2016). Exactly 200 cm^3 of 10% acetic acid in ethanol was added to each herbal sample (2.50 g) in a 250 cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with

20 cm^3 of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). Using electronic weighing balance Model B-218, the residue was dried in an oven and the percentage of alkaloid is expressed mathematically as

$$\text{Alkaloid} = \frac{\text{Weight of alkaloid}}{\text{of sample}} \times 100 \qquad 3.3 \text{ Weight}$$

3.4 Evaluation of Anti-Nutritional Factors and Nitrogen Compounds

3.4.1 Evaluation of oxalates

Oxalate quantitative determination was carried out using the method reported by Ejikeme and Chukwu (2016). Exactly 20 cm^3 of 0.3 M HCl in each herbal sample (2.50 g) was extracted three (3) times by warming at a temperature of 50°C for 1 hour with constant stirring using a magnetic stirrer. For oxalate estimation, 1.0 cm^3 of 5 M ammonium hydroxide was added to 5.0 cm^3 of extract to ensure alkalinity. Addition of 2 drops of phenolphthalein indicator, 3 drops of glacial acetic acid, and 1.0 cm^3 of 5% calcium chloride to make the mixture acidic before standing for 3 hours was followed by centrifugation at 3000 rpm for 15 minutes. After discarding the supernatant, the precipitate was washed three times using hot water by mixing thoroughly each time centrifugation. Then, to each tube, 2.0 cm^3 of 3 M tetraoxosulphate (VI) acid was added 0.01 M potassium permanganate (KMnO4) was titrated against the content of each tube at room temperature until the first pink colour appears throughout the solution. The solution was allowed to stand until it returned colourless, after which it was warmed on an electric hot plate at 70°C for 3 minutes, and re-titrated again until a pink colour appears and persists for at least 30 seconds.

$$C_2O^{2-}_4 + 8H^+ + MnO^{2-}_4 \rightarrow 2CO_2 + 4H_2O + Mn^{2+}$$

Titration reaction of oxalate in sample was calculated as

Ratio of reacting ions = 1 : 1

From $M_1V_1 = M_2V_2$ 3.4

Where:

M_1 is molarity of KMnO4, M_2 is molarity of extract (oxalate), V_1 is volume of extract (oxalate), and V_2 is volume of KMnO4 (Titre Value).

Molecular weight of $KMnO_4$ = 100

Weight of Oxalate in titre = M_2x Molecular 3.5.

$$\text{Weight of Oxalate in titrand 2.0 cm}^3 = \frac{Xg}{1000} \times 2 = Y \quad 3.6$$

Oxalate extract 100 cm^3 = Y/2.5 x100= W 3.7

Oxalate composition mg/100g=W/2.5 x 100 3.8

3.4.2 Evaluation of tannin

Analytical method for quantitative determination of tannin was according to (Ejikeme and Chukwu, 2016). By dissolving 50 g of sodium tungstate (Na_2WO_4) in 37 cm^3 of distilled water, Folin-Denis reagent was made. To the reagent prepared above, 10 g of phosphomolybdic acid ($H_3PMO_{12}O_{40}$) and 25 cm^3 of orthophosphoric acid (H_3PO_4) were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500 cm^3 with distilled water. 1.00 g of powder sample in a conical flask was added to 100 cm^3 of distilled water. This was boiled gently for 1 hour on an electric hot plate and filtered using number 42 (125 mm) What man filter paper in a 100 cm^3 volumetric flask. Addition of 5.0 cm^3Folin-Denis reagent and 10 cm^3 of saturatedNa_2CO_3 solution into 50 cm^3 of distilled water and 10 cm^3 of diluted extract (aliquot volume) was carried out after being pipette into a 100 cm^3 conical flask for colour development. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25°C after

thorough agitation. With the aid of a Spectrum Lab 23A spectrophotometer absorbance was measured at 700 nm and compared on a standard tannic acid curve.

Dissolution of 0.20 g of tannic acid in distilled water and diluted to 200 cm^3 mark (1 mg/cm^3) was used to obtain tannic standard curve. 0.25 mg/cm^3 of the standard tannic acid solution was pipetted into five different test tubes to which Folin-Denis reagent (5.0 cm^3) and saturated Na_2CO_3 (10.0 cm^3) solution were added and made up to the 100 cm^3 mark with distilled water. The solution was left to stand for 30 minutes in a water bath at 25°C. Absorbance was ascertained at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer. The following formula was used in the calculation:

$$\text{Tannic acid}\left(\frac{mg}{100g}\right) = \frac{\mathbf{C} \times \mathbf{extract\ volume}}{\boldsymbol{Aliquot\ volume} \times \boldsymbol{weight\ of\ sample}} \times \mathbf{100} \qquad 3.9$$

Where C is concentration of tannic acid.

3.4.3 Evaluation of phytate

Phytate was evaluated using the method of Soetan *et al.* (2015) in which. 2.0 g of each powdered and 10 cm^3 of each liquid samples were taken. 100 ml of 2% conc. HCl was added to each samples in conical flasks for 3 hours and filtered. 50 ml of each filtrate was placed in 250 ml beakers and 100 ml of distilled water was added in each case to give proper acidity. 10 ml of 0.3% ammonium thiocyanate solution was added into each solutions, as indicators. This was titrated with standard iron (III) chloride solution which contained 0.00495 g iron per ml. The end point was slightly brownish-yellow which persisted for 5 minutes.

3.4.4 Evaluation of nitrate

A working standard of nitrate solution was prepared and 10 cm^3 was pipetted into 100 cm^3 volumetric flask and made up to mark with distilled water. The solution of

powdered samples was filtered and 2 cm^3 aliquots of each sample, standard and water blank were pipetted into 10 cm^3 volumetric flask and 1 drop of the sulfite urea reagent was added to each flask of the samples. The flasks were placed on a tray of cold water at a temperature of 15℃, 2 cm^3 of antimony reagent were added while swirling the flasks and were allowed to stand for 4 minutes, followed by the addition of 1 cm^3chromotropic acid. The flasks were swirled again and allowed to stand in the cooling bath for another 3 minutes. The solution was made up to mark with concentrated H_2SO_4 then the flasks were allowed to stand for 15 minutes before measuring the absorbance at 410 nm using 1 cm cell with water as the reference.

3.4.5 Evaluation of nitrite

In each case, 40 cm^3 of the samples was pipetted into a 50 cm^3 volumetric flask. 2 cm^3 of sulfanilic acid solution were added and allowed to stand for 20 minutes, 5 cm^3 of cleve's acid solution were added and made up to mark with distilled and allowed to stand for another 20 minutes. The absorbance was measured at 525 nm using 1 cm cell and water in reference cell. The amount of nitrite, (NO_2) in mg/cm^3 was read and the concentration of nitrite was calculated in the samples.

3.5 Assessment of Physicochemical Parameters

3.5.1 Assessment of pH

The pH meter electrode was rinsed with distilled water and wiped clean with tissue paper. The electrode was submerged into the pH 7 buffer solution until the meter reads 7. The electrode was rinsed with distilled water, cleaned and then inserted into the solutions of each of the herbal samples and the pH values were read and recorded (Odoh and Ajiboye, 2019). Jenway 3505 pH meter was used to determine the pH values of the herbal samples.

3.5.2 Assessment of viscosity

The method adopted for the determination of viscosity of liquid herbal samples was as recorded by Mali *et al*, (2019.) In this method, Ostwald viscometer was thoroughly clean with acetone. The viscometer was mounted vertically on a stand. The dry viscometer was filled with water to G –mark level. The time require for water to flow was determined. The viscometer was rinsed with a liquid sample whose viscosity was to be determined and filled up, the time require to flow was also determined. The formula used to determine the viscosity is given below:

$$\text{Viscosity, } \mu_2 = \frac{\mu_1 \text{ x } t_2}{t_1} \qquad 3.10$$

$\mu_1 \rightarrow$Viscosity of the reference fluid

$\mu_2 \rightarrow$Viscosity of sample fluid

$t_1 \rightarrow$ Time for reference fluid

$t_2 \rightarrow$ Time for sample fluid

3.5.3 Assessment of bulk density

The assessment was according to WHO (2011), 20.0 g of the powdered sample was passed through a sieve with aperture of 1.0 mm. The sample was introduced into 100 ml dry graduated cylinder without compacting and the unsettled apparent volume was read. The bulk density was calculated in g/ml, with the aid of the formula:

$$\text{Bulk density} = \frac{\text{weight of powder}}{\text{Volume occupied by the powder}} \qquad 3.11$$

3.5.4 Assessment of electrical conductivity

A standard working KCl solution (100µs/cm) was prepared to calibrate the conductivity meter. This working standard was placed in a small beaker and the conductivity cell was suspended in the solution and was held at 1.5cm above the bottom of the beaker and the conductivity was adjusted to 100 µs/cm. The cell was rinsed with distilled water and samples reading were performed in the same way, obtained values were read and recorded.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Concentrations of selected heavy metals in herbal samples

Table 4.1 represents the result of the concentrations of the determined selected heavy metals in herbal samples. For all samples, lead was not detected while the cadmium contents varied, ranging from 0.02±0.10 to 0.11±0.07µg/dm^3, for liquid samples (HM_1HM_{13}) and 0.08±0.02 to 0.14±0.05 mg/kg, for powdered samples (HM_{14}-HM_{20}). The iron contents ranged from 1280.02±1.99 to 8730.04±1.98 µg/dm^3 (liquid samples) and 21.70±0.70 to 193.80±3.18 mg/kg (powdered samples). The ranges of values for copper, manganese and zinc were respectively 290.01±2.01 to 600.01±2.23, 110.01±2.41to1850.01±0.01 and 4410.03±1.99 to 9350±2.00 µg/dm^3 (liquid samples) and 8.00±0.02 to 74.10±0.32, 12.10±0.32 to 127.70±1.20 and 96.10±0.30 to 338.10±3.10 mg/kg (powdered samples). These values differed significantly from samples to samples ($p \leq 0.05$).

The concentrations of cadmium in all the samples analyzed are below the permissible limit. The values obtained ranged from 0.02±0.10 to 0.11±0.07 µg/dm^3 for the liquid samples. Umar *et al*. (2016) recorded 0.0114±0.00 to 0.1601±0.04 mg/dm^3 for the liquid samples which were also below the permissible limit. For the powdered samples, the values ranged from 0.08±0.02 to 0.14±0.05 mg/kg which are also below the permissible limit of 0.3 mg/kg. Lower values of 0.0045±0.00 to 0.0068±0.00 ppm were however reported by Umar *et al*. (2016) for powdered herbal samples. The differences obtained

in this study and the literature could be as a result of differences in the environmental factors that is areas where the herbs were grown.

Table 4.1 Concentrations of the selected heavy metals in the herbal samples

Samples	Pb	Cd	Fe	Cu	Mn	Zn
HM_1	ND	0.10 ± 0.06^{e}	3560.01 ± 1.23^{h}	270.02 ± 5.61^{ab}	440.02 ± 2.01^{i}	6570.01 ± 2.23^{h}
HM_2	ND	0.11 ± 0.07^{e}	8730.04 ± 1.98^{m}	250.02 ± 2.93^{b}	220.01 ± 2.23^{c}	7910.03 ± 1.99^{j}
HM_3	ND	0.08 ± 0.10^{d}	1280.02 ± 1.99^{a}	290.01 ± 2.01^{abc}	110.01 ± 2.41^{a}	5660.02 ± 1.99^{e}
HM_4	ND	0.12 ± 0.10^{e}	5620.03 ± 1.99^{j}	440.02 ± 2.12^{d}	2816.01 ± 3.13^{m}	1346.02 ± 1.99^{m}
HM_5	ND	0.08 ± 0.10^{d}	4660.02 ± 1.99^{i}	320.02 ± 2.01^{c}	320.03 ± 2.01^{e}	1346.02 ± 1.99^{m}
HM_6	ND	0.08 ± 0.10^{d}	4660.02 ± 1.99^{i}	310.04 ± 2.01^{bc}	420.02 ± 1.99^{h}	5410.02 ± 1.99^{c}
HM_7	ND	0.06 ± 0.20^{bcd}	2440.01 ± 2.23^{d}	600.01 ± 2.23^{g}	160.04 ± 2.01^{b}	6360.01 ± 2.23^{g}
HM_8	ND	0.02 ± 0.10^{a}	1330.02 ± 2.00^{b}	330.01 ± 2.23^{c}	230.01 ± 2.23^{d}	4410.03 ± 1.99^{a}
HM_9	ND	0.03 ± 0.10^{a}	1870.04 ± 1.98^{c}	290.03 ± 2.02^{abc}	630.01 ± 2.98^{j}	5161.01 ± 2.43^{b}
HM10	ND	0.06 ± 0.20^{bcd}	5680.02 ± 1.99^{k}	453.37 ± 7.95^{d}	1850.01 ± 4.45^{l}	9810.11 ± 1.86^{l}
HM11	ND	0.05 ± 0.15^{bc}	5800.03 ± 2.01^{l}	533.35 ± 0.62^{f}	1150.02 ± 2.23^{k}	9350.02 ± 2.00^{k}
HM_{12}	ND	0.08 ± 0.10^{d}	2460.05 ± 1.97^{e}	460.01 ± 3.71^{de}	380.02 ± 2.32^{g}	6910.02 ± 1.99^{i}
HM13	ND	0.04 ± 0.11^{ab}	2500.06 ± 1.98^{f}	500.02 ± 2.23^{ef}	370.01 ± 2.23^{f}	5810.01 ± 2.32^{f}
HM14	ND	0.10 ± 0.05^{a}	21.70 ± 0.13^{a}	38.10 ± 0.01^{e}	69.20 ± 1.20^{e}	202.10 ± 2.10^{e}
HM15	ND	0.08 ± 0.03^{a}	193.80 ± 3.18^{g}	8.00 ± 0.02^{a}	12.10 ± 0.32^{a}	96.10 ± 0.30^{a}
HM16	ND	0.08 ± 0.02^{a}	177.50 ± 2.24^{f}	74.10 ± 0.32^{f}	127.70 ± 1.20^{g}	156.01 ± 1.01^{c}
HM_{17}	ND	0.14 ± 0.05^{b}	150.60 ± 1.62^{e}	32.03 ± 0.51^{d}	58.10 ± 0.24^{c}	291.01 ± 2.51^{f}
HM18	ND	0.13 ± 0.07^{b}	141.80 ± 2.02^{d}	8.00 ± 0.02^{b}	121.30 ± 1.20^{f}	182.01 ± 2.01^{d}
HM19	ND	0.08 ± 0.02^{a}	59.37 ± 1.01^{c}	22.30 ± 0.32^{c}	44.10 ± 0.72^{b}	139.10 ± 3.10^{b}
HM20	ND	0.14 ± 0.05^{b}	31.87 ± 2.01^{b}	11.70 ± 0.26^{g}	67.60 ± 0.42^{d}	338.10 ± 3.10^{g}
WHO* (2017)		0.30	100.00	1000.00	260.00	1500.00
WHO**(2012)		0-30	20.00	40.00	20.00	20.00

Values are expressed as mean ± standard deviation of triplicate determinations. Values in the same column bearing the same superscript are significantly the same ($p \leq 0.05$) while values in the same column that are having different superscript are significantly different ($p \geq 0.05$). ND = Not detected, WHO * (2017) = the permissible limit of heavy metal content for liquid herbal samples in $\mu g/dm^3$, WHO** ((2012) = the permissible limit of heavy metal content for powdered herbal samples in mg/dm^3

The concentration of iron in the liquid samples ranged from 1280.02±1.99 to 8730.04±1.98 μg/dm^3, which is above the permissible limit of 100 μg/dm^3. In the same vein the iron concentrations in the powdered samples ranged from 21.70±0.13 to 193.80±3.18 mg/kg, which are above the permissible limit of 20 mg/kg. Odoh *et al*. (2019) recorded a high concentration of iron, 230.49±0.38 mg/kg for their herbal samples. Though iron is a trace element, which is a component of the respiratory pigments (haemoglobin and myoglobin) and enzymes like cytochrome. It is also essential for oxygen and electron transport. However, ingestion of iron in large qualities may lead to severe necrotizing gastritis with vomiting, haemorrhage and diarrhea followed by circulatory shock with disease of aging.

The concentrations of Copper, ranged from 250.02±5.61 to 600.01±2.23 μg/dm^3 for the liquid samples and 11.70±0.26 to 74.10±0.32 mg/kg for the powdered samples. Concentrations of copper for the liquid samples are below the permissible limit likewise the powdered samples except for HM_{16} (74.10±0.32 mg/kg). Umar *et al*, (2016) recorded similar values of concentration of copper below the permissible limit. Copper has both beneficial and toxic effect depending on it level of consumption. High intake of copper may result in hair and skin discoloration, irritation of the upper tract and vomiting.

Manganese concentration range from 110.01±2.41 to 2816.01±3.13 μg/dm^3 for the liquid samples and 12.10±0.32 to 127.70±1.20 mg/kg for the powdered samples. All the powdered and liquid samples are above the permissible limit, as recommended by the WHO, except four of liquid samples that are below the permissible limit. There is significant difference in the concentration of manganese among the samples, both for liquid and powdered samples. Excess intake of manganese is toxic to the nervous

system. Chinaza *et al*, (2020) also recorded a high concentration of manganese above the permissible limit.

Concentrations of zinc, for liquid samples ranged from 1346.02±1.99 to 9810.11±1.86 μg/dm^3 and for powdered samples ranged from 96.10±0.30 to 338.10±3.10 mg/dm^3. These values are above the permissible limit, as recommended by WHO.

MH_1 is Sawaba Herbal medicine, the cadmium and copper contents of this sample are below the permissible limit, but other metals present are above the permissible limits which will alter the therapeutic effects of the active ingredients.

HM_2 is Al-afwaUlcercline. Cadmium, copper, and manganese contents of this sample are below the permissible limit while iron and zinc are above. These will pose challenge to the therapeutic effect of the sample.

HM_3 is Sahinhin-maganin-sanyinmara, Cadmium, copper and zinc have values below the permissible limit while iron and manganese are above the permissible limit, which will hinder the therapeutic effects of the active ingredients present in the sample.HM_4 is Agajinabiyar, the cadmium and copper contents are below the permissible limit while other metals present are above the permissible limit, excess of these metals will counter the the therapeutic actions of the active ingredients present in the herbal sample.

HM_5 is Maidabino, the cadmium and copper contents of this sample are below the permissible limit while other metals are above. The effects of these metals make the active ingredients inactive.HM_6 is Agaji natara, the cadmium and copper contents are below the permissible limit while iron, manganese and zinc are above the permissible limit. This condition will affect the therapeutic action of the drug sample.HM_7 is Shajaratusshifa. The cadmium, copper, and manganese contents are below the

permissible limit while iron and zinc contents are above the permissible limit. Excess of these metal ions is harmful because it will result to ailments in the body.HM_8 is Alhimaya, the cadmium, copper, and manganese contents are below the permissible limit while iron and zinc are above the permissible limit.

HM_9 is Sahihin-maganitari, the cadmium and copper are below the permissible limit while iron, manganese and zinc contents are above the permissible limit. High content of these metals will create more harm by lowering the effectiveness of the active ingredients. HM_{10} is Body defence, the cadmium and copper contents of the sample is below the permissible limit while the contents of iron, manganese and zinc are above the permissible limit. The contents of these metals will cause the active ingredients to be in active which will have harmful effects on the consumers. HM_{11} is Stomach peace, the content of cadmium, and copper are below the permissible limit, while iron, manganese, and zinc are above the permissible limit. Metals with concentration above the permissible limit could pose health risk when consumed in excess. HM_{12} is Malaria ultimate solution. The concentrations of cadmium and copper are below the permissible limit while concentrations of iron, manganese, and zinc are above the permissible limit. These high content metals will alter the effectiveness of the active ingredients. HM_{13} is Kudiratusshifa, cadmium and copper contents are below the permissible limit while iron, manganese, and zinc are above the permissible limit, a condition that negatively affect the active ingredients present.

HM_{14} is Seven-for-seven, the cadmium and copper concentrations are below the permissible limit while iron, manganese and zinc are above the permissible limit.HM_{15} is Jenyo, the concentrations of cadmium, copper and manganese are below the permissible limit while the concentrations of iron and zinc are above the permissible

limit. These metals with high concentration will alter the effectiveness of the active ingredients. HM_{16} is Dan-buzu. Among the metals detected in the sample, only cadmium has it concentration below the permissible limit while others are above the limit. This show a great risk because of the high concentration of the metals.

HM_{17} is Pile plus dankanoma, the cadmium and copper concentrations of this sample are below the permissible limit while the concentration of iron, manganese and zinc are above the permissible limit. HM_{18} is Anti-stress plus, the cadmium and copper concentrations of the sample are below the permissible limit while those of iron, manganese, and zinc are above the permissible limit. HM_{19} is Anti-stooling, the concentrations of cadmium and copper are below the permissible limit while those of iron, manganese and zinc are above the permissible limit, and HM_{20} is HDC plus, cadmium and copper concentrations of this sample are above the permissible limit while the concentrations of iron, manganese and zinc are above the permissible limit. Generally, high contents of heavy metals in herbal medicine usually have adverse effects on human body when consumed, such as depletion of glutathione.

4.1.2 Selected phytochemical contents of herbal samples in mg/100g

Table 4.2 represents the phytochemical contents of the herbal samples. The flavonoid, alkaloids and saponins contents ranged from 0.35±0.06 to 14.40±0.36, 3.69±0.04 to 16.67±0.02 and 0.28±0.32 to 14.24±0.23 mg/100g respectively for the liquid and solid samples respectively. These values significantly differed from samples to samples ($p \leq 0.05$).

Table 4.2: Selected phytochemical contents of the herbal samples in mg/100g

Samples	Flavonoid	Alkaloid	Saponin
HM_1	0.65 ± 0.02^{f}	5.92 ± 0.04^{a}	1.38 ± 0.01^{i}
HM_2	0.42 ± 0.06^{c}	5.42 ± 0.02^{d}	1.77 ± 0.06^{k}
HM_3	0.63 ± 0.07^{e}	9.34 ± 0.02^{j}	0.86 ± 0.03^{g}
HM_4	0.35 ± 0.06^{a}	7.21 ± 0.03^{g}	0.53 ± 0.03^{d}
HM_5	0.50 ± 0.04^{d}	10.10 ± 0.03^{k}	0.88 ± 0.03^{h}
HM_6	0.36 ± 0.03^{b}	8.90 ± 0.02^{i}	0.42 ± 0.07^{b}
HM_7	0.82 ± 0.03^{g}	4.07 ± 0.03^{b}	0.45 ± 0.21^{c}
HM_8	0.42 ± 0.06^{c}	3.69 ± 0.04^{a}	0.28 ± 0.32^{a}
HM_9	0.90 ± 0.04^{h}	5.42 ± 0.04^{d}	0.56 ± 0.33^{e}
HM_{10}	4.30 ± 0.06^{l}	5.54 ± 0.01^{e}	2.27 ± 0.11^{m}
HM_{11}	2.80 ± 0.31^{k}	4.52 ± 0.04^{c}	1.41 ± 0.21^{j}
HM_{12}	2.67±0.21j	7.28 ± 0.08^{h}	1.84 ± 0.06^{l}
HM_{13}	1.84 ± 0.11^{i}	5.52 ± 0.04^{e}	0.68 ± 0.04^{f}
HM_{14}	0.35 ± 0.01^{a}	13.32 ± 0.04^{c}	4.80 ± 0.03^{b}
HM_{15}	0.39 ± 0.03^{b}	16.67 ± 0.02^{f}	3.43 ± 0.07^{a}
HM_{16}	9.36 ± 0.06^{d}	13.79 ± 0.44^{d}	6.85 ± 0.09^{e}
HM_{17}	5.80 ± 0.07^{c}	12.80 ± 0.05^{b}	14.24 ± 0.23^{g}
HM_{18}	12.25 ± 0.16^{e}	10.36 ± 0.04^{a}	5.83 ± 0.06^{f}
HM_{19}	14.40 ± 0.36^{g}	14.92 ± 0.04^{e}	6.50 ± 0.09^{c}
HM_{20}	12.95 ± 0.28^{f}	12.69 ± 0.07^{b}	6.76 ± 0.16^{d}
WHO Standard	NE	NE	NE

NE: Not Established

The phytochemical contents of the herbal samples determined showed that the herbal medicines are rich in flavonoid, alkaloids and saponins. The contents of flavonoid for the liquid samples ranged from 0.63±0.07 to 4.30±0.06 and 0.35±0.01 to 14.40±0.36 for powdered samples. Evidence from various epidemiological studies support the notion that high flavonoid-rich diets may be associated with a reduced risk of certain chronic diseases, including cardiovascular, neurodegenerative and select cancers (Sumanta and

Syed, 2020). Flavonoid has anti-oxidative, anti-inflammatory, anti-mutagenic and anticarcinogenic properties (Panche *et al*., 2016). Metal chelation property of flavonoid is crucial in preventing radical generation that damages target biomolecules (Sumanta and Syed, 2020).

Alkaloids contents of liquid samples ranged from 3.69±0.04 to 10.10±0.03 and 10.36±0.04 to 16.67±0.02 mg/100 g for powdered samples. Alkaloids possess significant biological properties and are reported to have anti-cancerous as well as immune stimulant properties (Jaya, 2017). Alkaloids are used in medicines for reducing headache and fever; these are attributed for antibacterial and analgesic properties (Odoh and Ajiboye, 2019).

The saponin contents of the liquid samples ranged from 0.28±0.32 to 2.27±0.11 while those of the powdered samples ranged from 3.43±0.07 to 14.24±0.23 mg/100g.

Saponins exhibit a biological role and medicinal properties such as hemolytic factor, anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer. In addition, saponins are reported to exhibit cholesterol-lowering action in humans (Maher *et al.*, 2019).

The phytochemical contents of all the herbal samples including liquid and powdered samples are considerably high enough to chelae the high contents of the heavy metals present in the samples thus reducing their availability and enhance the therapeutic effects of the active ingredients present in them.

4.1.3. Selected anti-nutritional factors and nitrogen compounds of the herbal samples

Table 4.3 represents the result obtained for tannins, oxalate, phytate, nitrate and nitrite contents of the samples. These values ranged from 1.94±0.10 to 11.37±0.21, 0.32±0.06 to 7.44±0.17, 3.16±0.01 to 30.03±0.03, 4.24±0.01 to 66.23±0.20 and 0.35±0.01 to 22.79±0.04 mg/g respectively. These values significantly differed from samples to sample ($p \leq 0.05$).

The tannins contents of all the herbal samples are of high contents which will enable the active ingredients to be more effective, as tannins have therapeutic activities such as antiviral, anti-oncogenic, they are useful as an anti-inflammatory agent and in the treatment of burns and other wounds based on their anti-hemorrhagic and antiseptic potentials. Oxalates, phytate, nitrate and nitrite contents of the entire herbal samples are below the permissible limits and will not pose any health challenge to the ushers but are beneficial because phytates helps to bind with the excess metal ions and as a result reduce their availability in the herbal samples and thus enable the active ingredients to function maximally.

Table 4.3: Selected Anti-nutritional factors and nitrogen compounds of the herbal samples

Samples	Tannins (mg/100g)	Oxalate (mg/100g)	Phytate (mg/100g)	Nitrate (mg/100g)	Nitrite (mg/100g)
HM_1	4.07 ± 0.26^{def}	1.24 ± 0.12^{l}	10.81 ± 0.01^{l}	6.45 ± 0.11^{l}	1.36 ± 0.02^{g}
HM_2	4.77 ± 0.30^{gh}	1.48 ± 0.32^{m}	6.28 ± 0.03^{g}	4.71 ± 0.01^{g}	0.35 ± 0.01^{a}
HM_3	4.86 ± 0.31^{h}	0.65 ± 0.16^{e}	8.71 ± 0.01^{j}	6.17 ± 0.01^{d}	1.30 ± 0.02^{f}
HM_4	5.40 ± 0.06^{i}	0.44 ± 0.06^{c}	6.52 ± 0.03^{h}	8.24 ± 0.01^{k}	2.20 ± 0.02^{j}
HM_5	4.22 ± 0.21^{efg}	0.32 ± 0.06^{b}	5.22 ± 0.07^{e}	8.37 ± 0.02^{l}	1.35 ± 0.01^{g}
HM_6	3.16 ± 1.14^{b}	0.85 ± 0.06^{i}	3.41 ± 0.02^{b}	5.13 ± 0.02^{c}	0.75 ± 0.03^{c}
HM_7	2.01 ± 0.21^{a}	0.69 ± 0.03^{f}	3.85 ± 0.010^{c}	7.44 ± 0.01^{i}	1.94 ± 0.02^{i}
HM_8	3.74 ± 0.06^{cde}	0.30 ± 0.16^{a}	4.13 ± 0.12^{d}	6.89 ± 0.01^{g}	0.74 ± 0.03^{c}
HM_9	4.42 ± 0.61^{fgh}	0.75 ± 0.11^{h}	3.16 ± 0.01^{a}	7.79 ± 0.01^{j}	1.58 ± 0.02^{h}
HM_{10}	3.40 ± 0.26^{bc}	0.95 ± 0.06^{j}	8.41 ± 0.05^{i}	7.21 ± 0.01^{h}	1.27 ± 0.07^{e}
HM_{11}	1.94 ± 0.10^{a}	0.63 ± 0.07^{d}	10.73 ± 0.03^{k}	6.14 ± 0.02^{d}	0.86 ± 0.02^{d}
HM_{12}	4.75 ± 0.15^{gh}	1.06 ± 0.16^{k}	6.13 ± 0.03^{f}	4.24 ± 0.01^{a}	1.25 ± 0.01^{e}
HM_{13}	3.58 ± 0.11^{bcd}	0.72 ± 0.08^{g6}	3.21 ± 0.01^{a}	6.37 ± 0.01^{e}	0.61 ± 0.01^{b}
HM_{14}	6.15 ± 0.11^{b}	4.22 ± 0.46^{b}	1.87 ± 0.03^{a}	4.25 ± 0.03^{a}	1.34 ± 0.02^{a}
HM_{15}	8.12 ± 0.16^{e}	3.65 ± 0.06^{a}	24.63 ± 0.03^{f}	35.30 ± 0.40^{d}	4.73 ± 2.51^{b}
HM_{16}	11.37 ± 0.21^{g}	5.18 ± 0.21^{d}	20.90 ± 0.02^{e}	40.73 ± 0.37^{e}	12.62 ± 0.04^{c}
HM_{17}	6.18 ± 0.31^{c}	7.46 ± 0.14^{g}	15.40 ± 0.02^{c}	28.38 ± 0.02^{b}	4.81 ± 0.05^{b}
HM_{18}	5.77 ± 0.22^{a}	5.70 ± 0.16^{e}	18.64 ± 0.02^{d}	32.38 ± 0.12^{c}	6.37 ± 0.02^{b}
HM_{19}	6.70 ± 0.23^{d}	4.96 ± 0.27^{c}	30.03 ± 0.03^{g}	66.23 ± 0.20^{f}	22.79 ± 0.04^{d}
HM_{20}	8.77 ± 0.21^{f}	7.44 ± 0.17^{f}	6.13 ± 0.03^{b}	4.25 ± 0.01^{a}	1.25 ± 0.01^{a}
WHO Standard (2018)	NE	50.00	500.00	50.00	10.00

NE: Not Established

4.1.4 The selected physicochemical parameters of the herbal samples

Table 4.4 represents the results obtained for pH, viscosities, bulk densities, and electrical conductivities of the herbal samples. The values ranged from 4.21±0.15 to 7.44±0.25, 0.53±0.11 to 23.45±0.46, 0.45±0.00 to 0.67±0.00 g/cm^3, and 2.06±0.01 to 39.08±0.32 µs/cm respectively.

Table 4.4: Selected physicochemical parameters of the herbal samples

Samples	pH	Viscosity	Bulk density g/cm^3	Electrical conduct (µS/cm)
HM_1	6.07±0.01^{i}	0.53±0.11^{a}	ND	5.72±0.15^{f}
HM_2	4.31±0.05^{b}	0.57±0.06^{ab}	ND	4.08±0.06^{c}
HM_3	5.23±0.15^{f}	0.59±0.09^{abc}	ND	6.37±0.12^{h}
HM_4	4.21±0.15^{a}	23.45±0.46^{f}	ND	12.50±0.01^{j}
HM_5	4.70±0.06^{c}	0.55±0.01^{ab}	ND	2.81±0.15^{b}
HM_6	4.75±0.17^{d}	0.67±0.03^{de}	ND	5.47±0.12^{e}
HM_7	5.71±0.15^{g}	0.61±0.12^{bc}	ND	2.06±0.01^{a}
HM_8	5.03±0.21^{e}	0.70±0.17^{e}	ND	6.04±0.15^{g}
HM_9	4.77±0.26^{d}	0.56±0.11^{ab}	ND	4.06±0.13^{c}
HM_{10}	6.37±0.21^{j}	0.63±0.06^{cd}	ND	23.43±0.13^{l}
HM_{11}	5.89±0.25^{h}	0.55±0.07^{ab}	ND	21.75±0.35^{k}
HM_{12}	7.26±0.12^{k}	0.69±0.11^{de}	ND	5.19±0.19^{d}
HM_{13}	7.41±0.25^{l}	0.57±0.08^{ab}	ND	6.88±0.33^{l}
HM_{14}	5.27±0.02^{b}	ND	0.53±0.01^{d}	33.45±0.21^{f}
HM_{15}	5.03±0.02^{a}	ND	0.67±0.00^{f}	9.31±0.33^{a}
HM_{16}	5.74±0.03^{c}	ND	0.56±0.01^{e}	13.57±0.23^{b}
HM_{17}	5.78±0.12^{c}	ND	0.46±0.01^{b}	39.08±0.32^{g}
HM_{18}	6.62±0.02^{e}	ND	0.45±0.00^{a}	26.48±0.36^{e}
HM_{19}	7.44±0.01^{f}	ND	0.50±0.01^{c}	25.26±0.37^{d}
HM_{20}	6.37±0.02^{d}	ND	0.45±0.01^{a}	24.34±0.15^{c}
WHO Standard (2019)	4.00 – 7.50	NE	IP(0.35)	NE

ND = Not Determined NE = Not Established P = International Pharmacopoeia

The pH values of the liquid ranged from 4.21±0.15 to 7.41±0.25 for liquid samples and 5.03±0.02 for powdered samples. Odoh and Ajiboye (2019) recorded a pH range of 12.30-1.05. Plant extract may act as effective antioxidant at acidic pH level close to neutral (Ayobami and Sylvester, 2019). The pH values obtained for the analyzed herbal samples are within the permissible limits as recommended by WHO i.e are within the range of 4.0-7.5, which indicate that these samples will work maximally at these range of values because they are neither highly acidic nor alkaline.

Viscosity values of liquid samples range from 0.53±0.11 to 23.45±0.46 for the liquid samples. Statistically, these samples show lesser degree of similarities and are more significantly different from sample to sample. The samples have a good viscosity values except for HM_4 that is very viscous (23.45±0.46) this will slow the rate of flow of the active ingredients to target organs and thus delay the therapeutic effects.

Bulk density values for powdered samples range from 0.45±0.00 to 0.67±0.00 g/cm^3. These values are significantly different from sample to sample ($p \leq 0.05$). For the powdered samples, the bulk density values are not below the limit, as recommended by the international pharmacopoeia (0.35) which means that all these samples can be compounded in tablet forms for better therapeutic effects.

The electrical conductivity values for liquid herbal samples range from 2.06±0.01 to 23.43±0.13µs/cm and 9.31±0.33 to 39.08±0.32µs/cm for powdered samples. Rasha *et al*. (2015) recorded the highest electrical conductivity to be 0.116 µs/cm. A good antioxidant property of herbal medicine contains a good amount of electrical conductivity (Rasha *et al*, 2015). Electrical conductivity measures the concentration of

electrolytes released by the plant products, which is a parameter used to determine the quality of herbal medicine (Paulo *et al*., 2017). Low value of electrical conductivity indicates that the herbal product has high quality while high value indicates low quality herbal product. The electrical conductivity values obtained show the good quality of the samples but the degree of their qualities varies because the less the value the more the quality. HM_5 and HM_7 are the most quality herbal samples with values as; 2.81±0.15 and 2.06±0.01 while HM_{14} as the least quality value, 33.45±0.21.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the results obtained on the analysis of thirteen (13) liquid and seven (7) powdered herbal samples, the physicochemical parameters tested for showed a level of safety and good quality for the samples since their pH, viscosities, bulk densities and electrical conductivities had values below their respective maximum permissible values. In addition, the branded medicinal products had phytochemical substances required for their medicinal efficacy. For instance, the traditional uses of some of these branded medicinal products for the treatment of hypertension, haemolytic, inflammatory, bacterial, fungal, viral, insects and even cancer problems can be traced to the presence of such phytochemicals as the alkaloids and saponins which were found in high quantities for the liquid and solid samples respectively.

However, some of these phytochemicals could also act as anti-nutrients when consumed in large quantities since they are known to interact with essential microelements. The nitrates, nitrites, and phytate content of both liquid and powder samples are the permissible limits.

These are known to chelate calcium, iron and zinc and are appreciable to exert an adverse effect when used in overdose. However, they are also known to have some positive effects like antioxidant, hypo-cholesterolemic and hypo-lipidemic properties.

In all the analyzed herbal samples, lead was not detected and cadmium was below the maximum permissible limit for both liquid and powdered samples. These herbal

products could be good sources of the selected microelements analyzed in this study such as copper, iron, manganese and zinc.

5.2 Recommendations

The following recommendations were made from the results obtained in this research carried out on the selected branded herbal medicines sold in Minna:

I. Though herbal medicines could be contaminated by heavy metals from the surrounding, the contents of phytochemicals are good enough to neutralized the harmful effects of the heavy metals since they can chelate these heavy metals.

II. Other chemical parameter and the anti-nutritional factors determined in these herbal medicines proved that such medicines could be good and safe for human consumption.

III. Since this study established the fact that the selected brands of herbal medicines from Minna metropolis possess the selected parameters analyzed hence revealing their nutritional and possible medicinal importance, their use as therapeutic substances could probably not pose any health dangers especially when used according to their prescription.

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