Unregulated Promotion and Sale of Herbal Remedies: A Safety and Efficacy Evaluation of Twelve Such Commercial Products Claimed to be Beneficial and Patronised for a Variety of Ailments in Nigeria

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Abstract
Preparation, distribution, advertisement, and usage of phythomedicinal products in Nigeria are weakly regulated raising genuine concern for public safety. In this study, twelve highly patronised commercial herbal products (S1-S12) claimed to be beneficial in the management of either diabetes mellitus, diarrhoea, or hypertension were procured from herbal vendors in Nigeria and evaluated against their claims of efficacy and safety using established whole animal and in vitro models. S1, S2, and S3 were tested for anti-hyperglycaemic activity in normoglycemic and diabetic rats and only S1 and S2 showed some reductions in blood glucose at some time points but S3 produced no reduction in blood sugar at all the time points. Three samples, S4, S5, and S6 significantly (P ≤ 0.05) inhibited the frequency of diarrhoea drops in rats at a dose of 250 mg/kg and caused minimal (P>0.05) reduction in intestinal transit of a charcoal meal in mice. Infusion of aqueous extract of samples (S7-S12) up to 1 mg/kg, showed that only the mean arterial blood pressure of cannulated cat was reduced by S7 and S8 but samples S9, S10, S11, and S12 did not cause any reduction in the arterial blood pressure. At a dose of 1 mg/kg, S7 and S8 caused a BP reduction of 150 and 76.92% relative to the reduction produced by ACh (1 µg/kg). The safety of these samples was assessed by determining the acute toxicity of these preparations and checking the nature of contaminating organisms and the levels of such contamination (bio-load). Acute toxicity studies in mice showed that at doses above 5000 mg/kg, per os, all the herbal samples did not cause death or produced signs of acute intoxication in mice. The samples were found to be contaminated with a variety of microorganisms, although their bio-loads were largely within pharmacopoeia specification. S7 is the most heavily contaminated; S3, S4, and S12 are contaminated with Gram negative organisms that may be potentially pathogenic. The outcome of this preliminary investigations demonstrates the need for stricter regulation and registration of commercial herbal products. Post-marketing surveillance, pharmacovigilance, and random screening of herbal products should be entrenched in the regulatory framework to quickly dictate any possible adverse effect and ensure consistency in the quality of distributed herbal medicines.

Keywords: Commercial herbal products; Phytoedicine; Preclinical screening; Pharmacovigilance

Introduction
For many centuries, medicinal plants and herbs have been part of healthcare of many countries and constitute alternative sources of therapeutic substances. Herbal drugs are increasingly used worldwide during the last few decades as seen in the rapidly growing global and national markets of herbal drugs. According to a World Health Organization (WHO) estimate, the demand for medicinal plants by the year 2050 would be about US $5 trillion annually. This rising trend in the use of herbs to treat diseases is a global phenomenon seen in both developing and developed countries [1]. Many of the orthodox medications in use today, such as the opium, aspirin, digitalis, and quinine, have a long history of use as herbal remedies or were derived from knowledge obtained from their use in traditional medicine. The current gold standard drugs in the treatment of malaria (artesiminin based combination therapies, ACT) were developed from the discovery and isolation of artemisinin from Artemisia annua L., a plant used in China for almost 2000 years. The WHO estimation is that in some Asian and African countries, 80% of the population depend on traditional medicine for primary health care and in many developed countries, 70-80% of the population had used some form of alternative or complementary medicine (e.g. acupuncture) [2]. In Germany, it has been reported that about 600-700 plant-based medicines are available and are prescribed by some 70% of German physicians and in the last 2 decades in the United States, public dissatisfaction with the cost of prescription medications, combined with an interest in returning to ‘natural’, ‘green’ or ‘organic’ remedies, has led to an increase in herbal medicines use [3].

Unfortunately, the number of reports of people experiencing adverse effects caused by herbal drugs is also increasing. Counterfeit, poor quality or adulterated herbal products in the markets are serious patient safety threats [2]. Despite the invaluable contribution of herbal therapy in healthcare, there have been many controversies with regulation, safety, and standardisation. At one end of the divide, some herbalists maintain that traditional remedies have a long history of use and are completely safe and as such do not require the same level of safety testing as orthodox medications. On the other hand are opinions in favour of legally enforced quality standards, safety testing, and prescription by qualified practitioners. There are not many countries with national policies for traditional medicine. Regulating traditional medicines use [3].

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medicine products, practices, and practitioners is made more difficult because of variations in the classification of traditional therapies by different countries. A single herbal product may be categorized as either a food, a dietary supplement or as herbal medicine, depending on the country. However, there are no controversies on the need to protect the health of the consuming populace.

The increased awareness, surge in public interest, and the huge patronage of alternative medicines have been associated with increased commercial interests and some attendant safety concerns. This is due largely to unethical promotion and sale of all sorts of remedies with unverified bogus claims of efficacies to the unsuspecting public. For instance, there has been a recent phenomenal surge in the number of commercial advertisement, promotion, and trado-medical fairs on herb-based products globally and particularly in Nigeria. These phytomedicinal products are often promoted to the public as being “natural” and completely ‘safe’. Majority of the lay public believe that because medicines are herbal (natural) or traditional they are safe and cannot cause harm. However, it is known that traditional medicines and practices can cause harmful reactions if the product or therapy is of poor quality, or it is taken inappropriately or in conjunction with other medicines [2].

Furthermore, adulteration, inappropriate formulation, or lack of understanding of plant, and drug interactions have led to adverse reactions that are sometimes life-threatening or lethal [4]. With the rising incidence of liver and kidney disorders, cancers, and unexplained deaths, it is probable that adverse reactions due to herbal products are under-reported. It is well known that patients are reluctant to tell their doctors that they are taking herbal products [5] and communication between patients and traditional healthcare providers is generally poor leading to high risk in the practice. As a result, it has become exceedingly important to create the conditions for the correct and proper use of traditional medicines.

These facts and a concern for public safety informed the present study, conceived to check the safety and efficacy of some samples of commercial herbal products promoted in some trado-medical fairs in Nigeria in relation to their labelled claims. The safety evaluation of these herbal products is important since the popular belief that herbal therapies are without untoward effects have often been proven incorrect [6]. Some medicinal plants widely assumed to be safe have been proven to be potentially toxic [7]. More so, inadvertent contamination by microbial or chemical agents during any of the production steps can also affect the quality, safety, and efficacy of these products. The efficacy of the herbal preparations was determined using whole animal and in vitro pharmacological activities screening models relevant to their claimed therapeutic uses. The relative safety of the samples was assessed by evaluating the oral acute toxicity profiles of the herbal preparations in mice and by assessing the nature and level of microbial contamination.

Materials and Methods

Test products

The study was conducted on twelve commercial herbal preparations procured from herbal vendors in Nigeria. These herbal preparations were selected on the strength of their popularity, availability, affordability and the claims of efficacy. These samples were ultimately

<table>
<thead>
<tr>
<th>Sample</th>
<th>Source of sample</th>
<th>Organoleptic properties, packaging and, storage</th>
<th>Labelled indication/Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 (S1)</td>
<td>Sourced from a herbal vendor in Awka, Anambra State; Nigeria. Marketed as ‘Okaka Powder Mixture’</td>
<td>Packaged in a small plastic container and placed on the shelf in the herbal shop. S1 is a black solid powder with a coarse texture and slightly bitter to taste.</td>
<td>S1 is promoted and sold as anti-diabetic medication. Contains dried paw-paw leaf powder (Carica papaya) and other unspecified substances.</td>
</tr>
<tr>
<td>Sample 2 (S2)</td>
<td>Sourced from a vendor in Awka, Anambra State. Marketed as ‘Prince Truth Herbal Tonic’</td>
<td>A brown liquid sample, with a slightly bitter taste and a foul odour. It was stored in plastic bottles and placed on the shelf.</td>
<td>S2 contains paw-paw (Carica papaya) extract, cashew (Anacardium occidentale) leaf extract, and other unspecified ingredients. It is generally marketed as anti-diabetic.</td>
</tr>
<tr>
<td>Sample 3 (S3)</td>
<td>Procured from a herbalist from the South-west Nigeria. S3 is marketed as ‘Baba Ogun Powder’</td>
<td>S3 appears as a light brown solid powder that is smooth to touch. It is stored in small on the shelf.</td>
<td>Personal interview with the vendor revealed that S3 contains Moringaoleifera leaf extract and other unspecified ingredients. S3 is promoted and used as anti-diabetic medication.</td>
</tr>
<tr>
<td>Sample 4 (S4)</td>
<td>Procured from a herbalist situated at Nnobi Community, Anambra State, Nigeria.</td>
<td>S4 is a brown powder with a salty taste, packaged in small plastic container placed on the shelf.</td>
<td>S4 is promoted and sold for the treatment of diarrhoea.</td>
</tr>
<tr>
<td>Sample 5 (S5)</td>
<td>S5 was procured from a herbal vendor in Onitsha, Anambra State, Nigeria.</td>
<td>S5 is a dark coloured liquid preparation with a slightly bitter taste, packaged in a white plastic bottle of 250 ml capacity.</td>
<td>S5 is promoted and sold for the treatment of diarrhoea.</td>
</tr>
<tr>
<td>Sample 6 (S6)</td>
<td>Procured from a herbalist from the South-west Nigeria in a Trado-medical Trade Fair/ exhibition in Nsukka, Enugu state, Nigeria. S6 is a dark coloured liquid preparation with a slightly bitter taste, packaged in an amber coloured bottle of 250 ml capacity.</td>
<td>Personal interview with the vendor revealed that S6 contains Aspidosperma depressum anti-diabetic and other unspecified ingredients.</td>
<td>S6 is promoted and sold for the treatment of diarrhoea.</td>
</tr>
<tr>
<td>Sample 7 (S7)</td>
<td>S7 was obtained from a herbalist in a Trado-Medical Trade Fair/exhibition in Nsukka, Enugu State, Nigeria.</td>
<td>It is an ash coloured bulk powder with slight bitter taste.</td>
<td>S7 is promoted and used for the management of hypertension.</td>
</tr>
<tr>
<td>Sample 8 (S8)</td>
<td>Procured from a herbal vendor in a Trado-Medical Trade Fair/shibition in Nsukka, Enugu State, Nigeria.</td>
<td>Cream coloured powder stored in small plastic containers with a bland taste.</td>
<td>Promoted and used for the management of hypertension.</td>
</tr>
<tr>
<td>Sample 9 (S9)</td>
<td>Sourced from a herbal vendor in a Trado-Medical Trade Fair (Nsukka, Enugu State, Nigeria).</td>
<td>Coarse cream coloured bulk powder dispersed in aliquots</td>
<td>S9 is promoted and used for the management of hypertension.</td>
</tr>
<tr>
<td>Sample 10 (S10)</td>
<td>S10 was procured from a herbal vendor in a Trado-Medical Trade Fair (Nsukka, Enugu State, Nigeria).</td>
<td>Ash coloured fine powder package in small plastic bags. S10 tastes sour.</td>
<td>Promoted and used for the management of hypertension.</td>
</tr>
<tr>
<td>Sample 11 (S11)</td>
<td>S11 was procured from a herbalist in a Trado-Medical Trade Fair in Nsukka, Enugu State, Nigeria.</td>
<td>White bitter tasting powder package in small amber coloured bottles.</td>
<td>Promoted and used for the management of hypertension.</td>
</tr>
<tr>
<td>Sample 12 (S12)</td>
<td>S12 was procured from a herbalist in a Trado-Medical Trade Fair in Nsukka, Enugu State, Nigeria.</td>
<td>S12 is a slight orange coloured liquid preparation with a slight bitter taste.</td>
<td>Promoted and used for the management of hypertension.</td>
</tr>
</tbody>
</table>

Table 1: Identity, source, and indication of herbal drug samples used in the study.
selected for this pilot study based on high patronage and usage, which stimulated concerns for public safety in the communities where they are patronised. In the study, the herbal product samples selected were identified serially from sample 1 to sample 12 (S1 - S12) (Table 1).

Animals

White albino mice (18-25 g), albino rats (180-210 g), guinea pigs (weighing 280-320 g), rabbits (3.6-4.1 kg), adult cats (2.5-3.2 kg) were observed for the number of deaths in 24 h. The death pattern samples were suspended in 10% tragacanth slurry. The treated mice and given (per os) were randomly divided into three groups (n=3) for each sample by Lorke [11]. The test was divided into two stages. In the first stage, the cultures isolated from the samples were also determined by Gram examination of the colonies on nutrient agar. The staining patterns of were characterised using the physical appearance and macroscopic activity in alloxan-induced diabetic rats. Twenty-five normal healthy albino rats were weighed and randomly divided into five groups (n=5). The rats were fasted overnight but had unrestricted access to drinking water before and throughout the duration of the test. At the end of the fast, blood samples were withdrawn from the tail and the blood glucose level was determined with a glucometer (Accuchek active®). The rats in group 1 served as the negative control group and were given only vehicle (0.5 ml of 10% tragacanth; per os); Group 2 rats received glibenclamide (10 mg/kg, per os) as reference drug.

The third to fifth groups received 250 mg/kg (per os) of S1, S2, and S3, respectively. Blood samples were withdrawn from the tail vein at 2, 4, 8, and 24 h. Blood glucose levels were determined using glucometer and percentage reductions in blood glucose levels were calculated using the formula:

\[ \text{Reduction in blood glucose (\%) = } \frac{G_i - G_t}{G_i} \times 100 \]

where \( G_i = \) blood glucose level at time interval and \( G_t = \) initial blood glucose level.

Anti-hyperglycaemic activities of S1, S2, and S3 in alloxan-induced diabetic rats

Samples S1, S2, and S3 were also tested for anti-hyperglycaemic activity in alloxan-induced diabetic albino rats. Twenty-five normal healthy albino rats were weighed, fasted overnight (but with access to drinking water prior to and throughout the duration of the experiment) and bled from the tail for fasting blood glucose (FBS) determination. Thereafter, diabetes was induced by a single intraperitoneal injection of 140 mg alloxan/kg in distilled water to the fasted rats. After five days of treatment with alloxan, during which the animals had free access to food and water, the FBS was determined for each rat. Rats showing FBS ≥ 150 mg/dl were considered diabetic and were selected for the study. For the investigation, previously confirmed diabetic rats were randomised into five groups (n=5), fasted overnight and treated as follows:

Group 1- The negative control group received only vehicle (0.5 ml of 10% tragacanth; per os).
Group 2- The positive control group received glibenclamide (10 mg/kg, per os) suspended in the vehicle reference treatment.
Group 3-5 were administered with S1, S2, and S3 respectively at a dose of 250 mg/kg (per os)

Blood samples were withdrawn from the tail vein at 1, 3, 6, and 24 h time intervals. Blood glucose levels were determined with Accuchek active® glucometer. Percentage reductions in blood glucose levels were calculated by the formula:

\[ \text{Reduction in blood glucose (\%) = } \left( \frac{G_i - G_t}{G_i} \right) \times 100 \]

where

The effect of S4, S5, and S6 on gastrointestinal motility in mice

Since some of the studied herbal products are promoted and sold as anti-diarrhoea therapy, the effect of oral administration of these herbal samples (S4, S5, and S6) on peristalsis induced by charcoal meal was studied [12]. Adult albino mice of either sex were randomised into five groups (n=5) and were starved overnight prior to the experiment. The first group received 0.2 ml/mouse of 1% tragacanth slurry to serve as the control; the second group received 2.5 mg/kg atropine sulphate and served as the positive control. The last three groups were administered 250 mg/kg (per os) of S4, S5, and S6, respectively. Twenty minutes later, 0.5 ml of charcoal meal (a 5% activated charcoal suspension in 10% aqueous slurry of tragacanth) was administered by gavage to the mice. The animals were sacrificed 15 min later, dissected and the small intestine removed and straightened on a dissecting board. The distance travelled by the charcoal plug was measured from the pylorus to the caecum and expressed as a percentage of the total length of the intestine.

The effect of S4, S5, and S6 on castor oil-induced diarrhoea in rats

Further investigation on the efficacy of the herbal samples (S4, S5, and S6) was carried out using castor oil-induced diarrhoea in rats. This particular test has often been taken as a standard and adequate test for evaluating the anti-diarrhoeal activity of any agent. This is because the castor oil by its polyvalent nature of action encompasses most of the common aetiological factors of diarrhoea. Castor oil alters intestinal motility, increases mucus secretion, produces gastroenteritis, and decreases sodium transport motility, increases mucus secretion, produces gastroenteritis, and decreases sodium transport in vivo [13]. S4, S5, and S6 were investigated for anti-diarrhoea properties using a modified model of the castor oil induced diarrhoea previously described [14]. Rats were fasted for 12 hr prior to the experiment and randomly divided into five groups (n=5). The rats in the first group served as the negative control and were given 0.2 ml/rat of 1% tragacanth using orogastric cannula. The second group received atropine sulphate (3 mg/kg), a standard anti-diarrhoea agent, as reference treatment. The last three groups were the test groups and were treated with S4, S5, and S6 (250 mg/kg per os). After 45 min of the different treatment, castor oil 1.0 ml/rat was administered orally to each rat and thereafter the rats were separated into single cages for observation. The time of diarrhoea onset was recorded for each animal in the group, which is the time interval between castor oil administration and the appearance of the first faecal drop. The mean number of defecation and the number of wet faeces were also recorded for the test group (Df) and for the control (Dc). Observation for defecation continued up to 6 h on pre-weighted (Wf) filter paper placed beneath the individual rat cages. The filter paper was replaced at hourly intervals and re-weighted (Wf) with the wet faeces. The fresh weight of the faecal droppings was determined (Wf-Wp) g. The fluid content of the faeces was also determined by drying the filter paper to a constant weight and then re-weighted (Wf). The water content was estimated as (Mf-Mw). G. Inhibition of diarrhoeic drop was calculated by the formula:

\[
\text{Inhibition of diarrhoeic drop (%) } = \left( \frac{Dc - Df}{Dc} \right) \times 100
\]

In vitro study of S4, S5, and S6 on isolated guinea pig ileum

The effects of S4, S5, and S6 on the isolated intestinal smooth muscle of guinea pig were investigated. Segments of ileum (2 cm) isolated from freshly sacrificed guinea pig were suspended in 50 ml organ bath containing solution of the following composition (g/L): NaCl-8.0, KCl-0.2, CaCl2-0.2, NaHCO3-1, NaH2PO4-1, MgCl2-0.1, glucose-2. The solution was maintained at 37°C and aerated with air. The tissue was allowed to equilibrate for 30 min under resting tension of 0.5 g before exposure to drugs. The effect of the samples on the isolated tissue was studied by adding increasing concentration of the samples (5 µg/ml - 5 mg/ml) into the organ bath. The effects of the samples on sub-maximal contractions evoked by acetylcholine (10 µg/ml), histamine (10 µg/ml), and nicotine (50 µg/ml) were also determined.

Screening of samples S7-S12 for anti-hypertensive activity using anaesthetized cat

Six (S7, S8, S9, S10, S11, and S12) of the twelve herbal products that are studied were promoted and sold as anti-hypertensive remedies were investigated for any anti-hypertensive effect using anaesthetized cat model. Cat was anaesthetized with 50 mg pentobarbitone sodium/kg (i.p.) and fixed in supine position on a dissecting table. A small mid-tracheal incision (approx. 1 cm) was made to expose the trachea and the left carotid artery and another incision on the left thigh to expose the femoral vein. Three cannulas were inserted at the femoral vein, the carotid artery, and the trachea respectively. The exposed surface for cannulation was covered with a piece of gauze moistened in warm saline. Cat was injected with 0.2 ml of heparinized saline (0.9% NaCl) to prevent blood clotting. Body temperature of the animal was maintained at 37°C using overhead lamp. All drug administrations and treatments were accomplished through the femoral vein cannula. The carotid artery cannula was connected to a blood pressure transducer, through which changes in blood pressure were recorded on double channel recorder (Ugo Basile Gemini 7070, Italy). The cat was oxygenated through the tracheal cannulation. After 30 min period of equilibration, control responses to acetylcholine (1 µg/kg) and noradrenaline (1 µg/kg) were obtained before the administration of the test samples. Graded doses (1-1000 µg/kg) of each test sample were injected intravenously through the femoral vein. Arterial BP was allowed to return to resting level between injections and flushed on each occasion with 0.2 ml of heparinized normal saline. Changes in BP were recognized as difference between the steady state values before and the peak readings after injection of each treatment. The blood pressure changes were measured and compared to the mean changes in arterial blood pressure of anaesthetized cannulated cat produced by ACh (1 µg/kg).

In vitro screening of samples S7-S12 on isolated smooth muscles of rabbit jejunum

The effects of the samples (S7-S12) on the isolated rabbit jejunum were investigated. Segments of the rabbit jejunum (2-3 cm piece) isolated from freshly sacrificed local breed of rabbit were suspended in 50 ml organ bath containing Tyrode solution at 37°C aerated with air. The tissue was allowed to equilibrate for 30 minutes under resting tension of 0.5 g before exposure to test samples. The effects of increasing concentrations (5-800 µg/ml) of samples on the isolated tissue were determined. The effect of the samples on submaximal relaxations evoked by noradrenaline (1 µg/ml) was also determined. Responses were recorded through a lever on double channel recorder (Ugo Basile Gemini 7070).

Statistical analysis

Results are presented as mean and standard error of the mean (SEM). To demonstrate statistical significance of data, a One-way Analysis of Variance (ANOVA) using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA) was performed followed by
During the 24 h observation period in the two stages of the test. Thus, not result in any lethality and caused no signs of acute intoxication on organisms (Table 3).

Sample S3, S4 and S12 were contaminated with Gram negative bacteria (S8, S10), chains of rods (S2, S3, S5, S6), chains and bunch of cocci (S6, S7, S11). Sample S3, S4 and S12 were contaminated with Gram negative rods singly dispersed (Table 2).

Secondary metabolites were found present in varying amount in some of the samples (Table 2).

<table>
<thead>
<tr>
<th>Herbal product</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
<th>S9</th>
<th>S10</th>
<th>S11</th>
<th>S12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>_</td>
<td>++</td>
<td>+</td>
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<td>+</td>
<td>++</td>
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<tr>
<td>Alkaloids</td>
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<tr>
<td>Acidic compound</td>
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<td>+++</td>
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<tr>
<td>Resins</td>
<td>+</td>
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<td>Flavonoids</td>
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<tr>
<td>Saponins</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Reducing sugar</td>
<td>+++</td>
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<td>Fats and oils</td>
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<td>Steroids</td>
<td>+++</td>
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<tr>
<td>Terpenoids</td>
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<td>Carbohydrates</td>
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<tr>
<td>Proteins</td>
<td>+++</td>
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</tbody>
</table>

(Where: -=absent, ++=Present in low concentration, +++=moderately present and ++++=Present in high concentration).

Table 2: Result of phytochemical studies on the different herbal samples screened.

Table 3: Bio-load and gram staining characteristics of contaminants.

Dunnett’s posthoc test. Generally, differences between test and control treatments were considered significant at P ≤ 0.05.

Results

Preliminary phytochemical characterisation of samples

Preliminary phytochemical analysis of the samples showed positive reaction for the presence of a wide range of bioactive phytoconstituents such as tannins, resins, glycosides, carbohydrates, acidic compounds, saponins, reducing sugars, steroids, terpenoids, and proteins. These secondary metabolites were found present in varying amount in some of the samples (Table 2).

Bio-load and microbiological characterization of contaminating microorganisms

Sample S7 was the most heavily contaminated of all the samples with a viable cell count of 6100 CFU/g while S1, S8, and S10 are the least contaminated with viable cell count of 10 cfu/g each (Table 3).

In mean blood glucose levels at 8 h compared to the mean blood glucose in the negative treatment group (Figure 2). Samples S1 and S3 did not reduce the mean blood sugar level, but rather caused an increase in mean blood glucose levels at all the time points (Figure 1). The group of rats that received sample S2 showed a significant reduction (P<0.05) of about 7.5% in mean blood glucose levels at 8 h compared to the mean blood glucose in the negative treatment group (Figure 2). Samples S1 and S3 did not reduce the mean blood sugar level, but rather caused an increase in mean blood glucose levels at all the time points. Rats that received glibenclamide (10 mg/kg) as a standard oral hypoglycaemic agent showed a significant reduction (p<0.05) in the mean blood glucose level at all the time points (Figure 1).

The effects of S1, S2, and S3 on blood glucose levels in normoglycemic rats

In normoglycaemic rats, oral administration of the three herbal test samples (S1, S2, and S3) at 250 mg/kg did not lower the glucose levels at 2, 4, and 24 h time points (Figure 1). The group of rats that received sample S2 showed a significant reduction (P<0.05) of about 7.5% in mean blood glucose levels at 8 h compared to the mean blood glucose in the negative treatment group (Figure 2). Samples S1 and S3 did not reduce the mean blood sugar level, but rather caused an increase in mean blood glucose levels at all the time points. Rats that received glibenclamide (10 mg/kg) as a standard oral hypoglycaemic agent showed a significant reduction (p<0.05) in the mean blood glucose level at all the time points (Figure 1).

The effects of S1, S2, and S3 on blood glucose levels in alloxan-induced diabetic rats

Oral administration of S1, S2, and S3 (250 mg/kg) did not reduce the mean blood glucose level in alloxan-induced diabetic rats at 1 h (Figure 3). In the groups that received S1 and S3, there was an increase in mean blood glucose levels at 3 and 6 h time points (Figure 3). The groups that received S2 showed a significant reduction (P<0.05) in mean blood glucose level at 36.1% and 79.1% at 3 h and 6 h time points, respectively (Figure 3). At 24 h, S1 was shown to cause a significant decrease in mean blood glucose level of 16.6%. Sample S3 did not show any reduction in blood glucose level at all the time points assayed (Figure 3).
The effect of S4, S5, and S6 on charcoal meal gastrointestinal motility in mice

In the gastrointestinal transit experiment, the three herbal samples (S4, S5, and S6) inhibited gastrointestinal transit to varying extents, but these inhibitions were not significant when compared to the negative (P>0.05). The percentage inhibitions were S4 = 25.48%; S5 = 27.45% and S6 = 29.14%. These values were not significantly different from the untreated control (P>0.05). Atropine sulphate (2.5 mg/kg) used as the positive control produced a significant inhibition of intestinal transit by as much as 61.65% (Figure 2).

The effect of S4, S5, and S6 on castor oil-induced diarrhoea in rats

The three samples (S4, S5, and S6) significantly (P ≤ 0.05) inhibited the frequency of watery faeces drops in rats at a dose of 250 mg/kg. Sample S4 produced the highest inhibition of the frequency of watery faeces (69.38%); comparable to the effect of Atropine sulphate used as a standard drug. Samples S5 and S6 have a percentage inhibition of diarrhoeic drop of 46.94% and 61.22%, respectively (Table 4). Samples S5 and S6, but not S4, prolonged the onset of diarrhoea onset after induction with castor oil. The fresh weight and the water content of the faecal matter were also reduced in rats that received S4, S5, and S6.

The effect of S4, S5, and S6 on isolated guinea-pig ileum

The effect of S4, S5, and S6 on the isolated guinea-pig ileum preparation was determined in vitro. The three herbal samples did not evoke the contraction of guinea pig ileum when applied alone. Pre-treatment with S4, S5, and S6 did not influence contractile responses induced by acetylcholine, histamine, and nicotine on the guinea-pig ileum.

The effect of herbal extracts S7-S12 on arterial blood pressure of anaesthetized

Infusion of aqueous extract of the herbal samples at doses up to 1000 µg/kg, showed that the arterial blood pressure of anaesthetized cats was reduced only by S7 and S8; S9, S10, S11, and S12 did not produce any reduction (Figure 4). At a dose of 1 mg/kg, S7 and S8 caused a BP reduction of 150 and 76.92% relative to the reduction produced by ACh (1 µg/Kg).

The effect of herbal extracts S7 and S8 on isolated rabbit jejunum

In vitro, samples S7-S12 have no effect on the intrinsic contraction of rabbit jejunum preparation. However, S7 and S8 at concentration up to 200 µg/ml attenuated submaximal relaxations evoked by noradrenaline (1 µg/ml).

Discussion

Natural medicinal products are gaining increasing worldwide acceptance and usage and are promoted as Complementary and Alternative Medicines (CAM) [15]. Numerous reasons have been adduced to explain this trend. It has been partly attributed to the abundance and readily available natural raw materials for making these medicines. An estimated record of 10²⁶-²⁷ beneficial and potentially medicinal substances has been cited [16]. Secondly, about one-third of the world's population still lacks access to essential drugs and the figure is believed to be higher and is over 50% in the poorest parts of Africa and Asia [17]. Particularly remarkable is the assumption that herbal medicines and other CAMs are natural and, as such, entirely safe and free from adverse effects seen with orthodox medications. This belief is popular, global, and widely held in both uninform and educated urban populations; but has been shown to be misleading
Only S2 (250 mg/kg) caused a significant reduction (P<0.05) in blood glucose levels at 8 h. S1 and S3 did not reduce blood glucose but rather produced anti-hyperglycemic activities. The only exception being the apparent reduction in blood glucose of 16.6% recorded at 24 h for S1. Paradoxically, group of rats treated with 250 mg/kg of S1 and S3 showed remarkable increase in blood glucose levels at various time points measured. However, S2 showed reduction in blood glucose levels at 3, 6 and 24 h time points.

Although, the study was not designed to investigate the possible mechanism of anti-hyperglycemic effect, it has been reported previously studies that herbal preparations and extracts could cause anti-hyperglycaemic effects by promoting the regeneration of β-cells or by protecting these cells from destruction, by restricting glucose load, as well as by promoting unrestricted endogenous insulin action. Anti-hyperglycaemic effect can also be caused by insulin-secreting effect of the extract on β-cells or due to activation of insulin receptors leading to increased peripheral glucose utilization [23]. It is expected that any herbal product claiming anti-diabetic efficacy will have any of these properties and invariably causing anti-hyperglycemic effects in either normoglycemic or in alloxanized animal models. Of the three herbal samples promoted as anti-diabetic remedies and tested in this study, only S2 showed significant anti-hyperglycemic activities. However, the study did not address other potential pharmacological activities which can be beneficial to diabetic patients such as lipid-lowering, weight loss, and anti-oxidant effects etc.
All the three samples (S4, S5, and S6) promoted and marketed as anti-diarrhoeal therapies showed significant (P ≤ 0.05) inhibition of castor oil-induced diarrhoea in rats, but did not show significant inhibition of intestinal transit in mice. Castor oil-induced diarrhoea has been associated with increased intraluminal fluid accumulation caused by ricinoleic acid on the rat intestine, followed by chemical gastroenteritis [24]. It has been suggested that cytomorphological changes and increased intestinal epithelial permeability is responsible for the intraluminal fluid accumulation in response to ricinoleic acid [25,26]. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins, which stimulate motility and secretion [26]. In vitro, S4, S5, and S6 samples did not produce any significant effect on acetylcholine, histamine, and nicotine-evoked contractions of guinea pig ileum preparations suggesting that the anti-diarrheal effect of these herbal samples are not mediated by a direct antagonism of muscarinic, histaminergic or nicotinic receptors. Previously, the anti-diarrheal properties of medicinal plants have been associated with the presence of some plants metabolites such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids [27-29]. Preliminary phytochemical investigation showed the presence of some of these phytoconstituents in these samples.

Six herbal samples S7-S12 that are promoted and patronised as anti-hypertensive medications were tested for anti-hypertensive activities using anaesthesied cat model. The study revealed that only S7 and S8 showed potential anti-hypertensive activities and, in vitro, attenuated submaximal relaxations evoked by noradrenaline in rabbit jejunum. Infusion of four of the herbal samples, S9, S10, S11, and S12 did not reduce the arterial blood pressure in canulated cats. Hypertension is a major risk factor for stroke, heart attacks (myocardial infarction), congestive heart failure, arterial aneurysms, peripheral arterial disease, and a major cause of chronic kidney disease [30]. Even moderate elevation of arterial blood pressure is associated with a shortened life expectancy. For this reasons, the high patronage and use of these herbal products of equivocal benefits by hypertensive patients is troubling and calls for action and enlightenment. Some of the patients patronising these herbal products are uninformed and have absolute confidence in these products to help reduce their elevated blood pressure and may not even have access to medical facilities for periodic monitoring of their blood pressure. For patients relying entirely on these herbal products with doubtful efficacies, life-threatening conditions associated with prolonged elevated arterial blood pressure may lead to morbidity complications or sudden death syndrome.

In the acute toxicity study, oral administration of all the herbal samples at doses up to 5000 mg/kg did not cause lethality in mice and produced no signs of acute intoxication indicating that for all practical purposes, the herbal samples are reasonable safe [11]. However, this study did not cover possible subchronic and chronic toxicity effects of the herbal products. Although safety studies in animal models may often be extrapolated to humans, it does not necessarily follow that these herbal products will be judged to be completely safe in humans. Therefore, actual controlled clinical trials on human subjects will offer more definitive evidence on their safety.

Herbal medicinal products usually contain bacteria and moulds from soil and atmosphere, but there are limits of contamination that could be tolerated. For example, the European Pharmacopoeial limits of bacterial contamination are: total aerobic bacteria (10<sup>3</sup> CFU/g), enterobacteria and other Gram negative organisms (10<sup>5</sup> CFU/g). Pathogenic enterobacteria such as <i>Escherichia coli</i> and <i>Salmonella</i> should always be absent [31]. In this study, the herbal samples were shown to be contaminated by a variety of bacteria and fungi which could be as a result of unhygienic preparation method or poor storage condition. Apart from causing diseases, these micro-organisms could lead to rapid deterioration of the products thus invariably affecting their safety and efficacy. Previous studies on herbal products have shown wide variety of microbial contaminants [32,33]. Microbial contamination of herbs and/or products can result from improper handling during production and packaging. The most likely sources of contamination are microbes from the ground and processing facilities (contaminated air, microbes of human origin). Cross contamination is also possible from handling materials such as plastics, glass, and other materials that come in contact with medicinal herbs, herbal preparations or products. World Health Organization (WHO) contaminant guidelines proposed that contamination should be avoided and controlled through quality assurance measures that will include good agricultural and collection practices (GACP) for medicinal plants [15], and good manufacturing practices (GMP) for herbal medicines [34,35].

The microbial load and gram characters of contaminating microorganisms the herbal products are considerably varied. The herbal products studied met the pharmacopoeia specifications on total microbial count, but contained gram-negative organisms. There is a great chances that they could be contaminated with <i>E. coli</i> and/or <i>Salmonella</i> species. Of utmost concern is the level of these potentially pathogenic gram negative contaminants in the products [36,37] which suggests faecal contamination. Although WHO has developed guidelines to ensure quality of herbal remedies which includes a details on the techniques and measures needed for proper cultivation, collection, processing, and preparation of medicinal herbs [15,34]. Despite the existence of the guideline, there is still a gap between the available knowledge and implementation. Most of the farmers and herbalist who are involved in the cultivation, preparation, and handling of these products are either uninformed or not trained in the use of these guidelines in their practice.

Regulation of herbal products varies from country to country [38]. In some countries, herbal remedies are well-established, whereas in others they are regarded as food and therapeutic claims are not allowed. There are many traditionally used herbs and so much folk-knowledge about these herbal medicines in developing countries such as Nigeria, but scientific evidence from tests done to check their safety and effectiveness are either lacking or limited. However, there are weaklegislations and loose regulatory environment which have been taken advantage of by commercial vendors of herbal therapies. The public is at great risk in unregulated commercial promotion and sale of herbal remedies. The vulnerability of the consuming public is made worse by the general, but wrong perception of herbal medicines as entirely natural and safe.

**Conclusion**

These studies on twelve herbal product samples which are promoted and patronised for diabetes, hypertension, and diarrhoea showed that although some of the samples produced some levels of efficacy against claimed benefits, others did not show any of the claimed effects in the preclinical screening. The outcome of this preliminary evaluation demonstrates the need for stricter regulation and registration of commercial phytomedicinal products which will include scientific scrutiny of claimed efficacy and safety of these products. Since the safety, effectiveness, and quality of finished herbal medicinal products

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depend on the quality of their raw materials and how these materials are handled through production processes, the Ministry of Health should adopt active documentation and training of herbalist to sanitize their practices. Post-marketing surveillance, pharmacovigilance, and random screening of herbal products should be entrenched in the regulatory framework to quickly dictate any possible adverse effect and to make sure there is consistency in the quality of distributed herbal medicines.

**Conflict of Interest**

The authors declare that they have no competing interests. The authors alone are responsible for conducting and reporting this research.

**References**