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# Antibacterial Property and Bioactive Compounds of Selected Herbal Products

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**Abstract**

Ten herbal products, made up of five powdery and five liquid samples of different brands, were assessed microbiologically for the presence and types of microorganisms. Qualitative and quantitative phytochemical screening of the samples above was also conducted to verify the presence or absence of bioactive components. Ten bacterial species, viz: *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Corynebacterium diptheriae, Streptococcus pyogenes, Enterobacter cloacae, Klebsiella pneumoniae,* and *Clostridium botulinum,* were isolated from these herbal products. The fungi isolated were *Aspergillus Niger, Rhizopus stolonifer, Alternaria alternata, Aspergillus flavus, Fusarium verticillioides, Fusarium oxysporium, and Mucor racemosus.* Results of phytochemical screening revealed the presence of flavonoids, saponins, terpenoids, and tannins. Alkaloid was the most abundant in the samples, with a value of 1070.04 mg/100g in sample F, while the least abundant in sample F was phenol (0.38 mg/100g). The GC-MS analysis revealed the presence of certain compounds such as thiophene, propanoic acid, 2,2-dimethyl-, ethyl ester, pentanoic acid, 2-methyl, toluene, and many others in sample F that exhibited significant antimicrobial effects. These compounds are known to possess antimicrobial properties. Results from this study revealed that, though these herbal products contain bioactive compounds with potential antimicrobial and antioxidant properties, they are contaminated with microorganisms of health importance. Hence, local herbalists preparing these herbal products need to be educated on good manufacturing practices (GMP).

*Keywords:* Herbal; Microbial; Contamination; Antimicrobial; Bioactives.

## 1. Introduction

The increasing resistance of microorganisms to available antimicrobial drugs and the attendant side effects have recently shifted attention to herbal medicine. Herbal drugs are crude preparations made from the leaf, stem, root, flower, seed, or combination of these plant parts used in treating various forms of ailments. Plants and spices have been used in medicine since ancient times [1, 2]. This is because they are more accessible to many people and the raw material is readily available and cheap when compared to synthetic drugs [3, 4]. Herbal medicines are being used by about 80% of the world's population, primarily in developing countries, for primary health care [5–7]. Developed countries are also not left out in the utilization of herbal products for health care [8, 9]. Herbal medicines, however, have shortcomings such as imprecise diagnosis and dosage, a lack of aseptic conditions during production, and a lack of scientific proof of their efficacy [3, 7]. Globally, the herbal medicinal products market has been growing steadily, and issues of their safety have also been of concern [9]. Though the efficacy of some of these herbal products cannot be doubted, some are yet to be tested and their use properly monitored [9]. It is therefore expedient to know the potential side effects of these products [10].

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Common contaminants associated with herbal preparations are heavy metals, pesticides, microbes, and their toxins [11]. Metals like mercury, cadmium, and lead have been reported at levels above the required levels in some herbal medicines [12–14]. Metal toxicity has been linked with the following pathophysiological effects: neurological behavioral effects, cardiac dysfunction, fetal malformations, Alzheimer`s disease, and Parkinson`s disease [15–19]. Moreover, microbial contaminants of health importance have also been reported in herbal medicine. Factors such as unhygienic conditions during preparation, transportation, and marketing are responsible for the contamination of herbal products. Moreover, plants are known to serve as a source of nutrients for the growth and survival of microorganisms [20].

The use of herbal drugs in Nigeria has been on the rise. Most producers of these herbal preparations often attach various health benefits that have not been scientifically proven to their products [3, 7]. The study area is Akure City ($7^{o}$ $15^{'}$ $0^{''}$ N $5^{o}$ $11^{'}$ $42^{''}$) in Akure South Local Government Area of Ondo State, Nigeria. The market for herbal products is booming, with an annual trade fair for herbal products being held in the city. The present study therefore focused on the isolation and identification of microbial contaminants as well as the bioactives present in different herbal products collected from different points of sale in Akure, Ondo State, Nigeria.

## 2. Materials and Methods

### 2.1. Collection of Herbal Samples

Herbal samples, 5 sachets and 5 bottles, were randomly bought at the trade fair center, herbal store, and Motor Park within Akure City, Ondo State, Nigeria. The labels on the herb containers show that they are for the treatment of the following ailments: typhoid fever, sexually transmitted diseases, piles, stomach aches, diabetes, headaches, skin infections, and toothaches, among others.

### 2.2. Microbial Analysis of Herbal Products

Isolation of microorganisms in samples was carried out by serially diluting 1g and 1 mL, respectively, of powder and liquid herbal products in peptone water, and portions (0.1 ml) of the $10^{-4}$ dilution were separately inoculated on nutrient agar and potato dextrose agar plates. The plates were incubated at the appropriate temperature and time for bacteria and fungi. After incubation, colony-forming units on the plates were counted, and discreet colonies were further subcultured to obtain pure colonies. Conventional cultural and colonial characteristics, followed by batteries of biochemical tests, were used for the identification of the isolates [21].

### 2.3. Assessment of Antimicrobial Activity of Herbal medicines

The test bacteria were inoculated into tubes of peptone water separately and incubated for 4 hours. Each of the cultures was then adjusted to the McFarland turbidity standard by diluting with normal saline and inoculating 0.2 ml each onto Mueller-Hinton agar (Oxoid). A cork borer of 6 mm diameter was used to make four (4) wells into the already seeded agar plates, and different concentrations of the herbal preparations (0.5 ml) were introduced into the wells using sterile Pasteur pipettes. The plates were incubated at the appropriate temperature and time. After incubation, zones of inhibition on the plates were measured in mm.

### 2.4. Qualitative and Quantitative Phytochemical Analysis of Herbal Products

The methods of Harbone [22] and Parekh & Chanda [23] were used to determine the presence of phytochemicals in the herbal products.

### 2.5. Identification of Bioactive compounds in Herbal Products

The bioactive components and their percentage of occurrence in the extract of sample F that possess significant antibacterial effects were evaluated using Gas Chromatography-Mass Spectrophotometer (GC-MS).

### 2.6. Statistical analysis

Results of experiments were analyzed by one-way analysis of variance and means compared with Duncan's multiple range tests ($p<0.05$).

## 3. Results

The bacterial load of the herbal product samples ranged from $2.26\times10^{5}$ to $1.40\times10^{5}$ cfu/ml (Table 1), while the fungal load ranged from $3.6\times10^{3}$ to $3.0\times10^{3}$ sfu/ml (Table 2). The microorganisms found in the herbal drug samples were *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Corynebacterium diptheriae, Streptococcus pyogenes, Enterobacter cloacae, Klebsiella pneumoniae, Clostridium*

*botulinum*, *Aspergillus Niger*, *Rhizopus stolonifer*, *Alternaria alternata*, *Aspergillus flavus*, *Fusarium verticillioides*, *Fusarium oxysporium*, and *Mucor racemosus*. The occurrence of the microbial isolates in the different herbal drugs is presented in Tables 3a and 3b. *Shigella dysenteriae* (31.58%) was the highest occurring bacteria in the herbal drug, followed by *Bacillus* cereus (15.79%), while *A. niger*, *A. flavus*, and *Rhizopus stolonifer* were the fungi that had the highest occurrence.

**Table 1. Total viable bacterial count of selected herbal products sold in Akure, Nigeria**

| Sample Code | Bacterial Load (cfu/ml) |
|---|---|
| A | $2.26\times10^5$ |
| B | $7.60\times10^4$ |
| C | $3.0\times10^5$ |
| D | $1.36\times10^5$ |
| E | $2.96\times10^5$ |
| F | $2.40\times10^5$ |
| G | $1.63\times10^5$ |
| H | $1.70\times10^5$ |
| I | $1.33\times10^5$ |
| J | $1.40\times10^5$ |

Key: + = present, - = absent, A = Herbal drug for malaria; B = Herbal drug for typhoid; C = Herbal drug for blood infection; D = Herbal drug for body pain; E = Herbal drug for pile; F = Herbal drug for eye ulcer; G = Herbal drug for blood cleansing (a); H = Herbal drug for inflammation; I = Herbal drug for typhoid; J = Herbal drug for blood cleansing (b)

**Table 2. Total viable fungal count of selected herbal products sold in Akure, Nigeria**

| Sample Code | Fungal Load (sfu/ml) |
|---|---|
| A | $3.6\times10^3$ |
| B | $4.3\times10^3$ |
| C | $4.6\times10^3$ |
| D | $2.0\times10^3$ |
| E | $8.0\times10^3$ |
| F | $6.0\times10^3$ |
| G | $3.0\times10^3$ |
| H | $5.3\times10^3$ |
| I | $4.0\times10^3$ |
| J | $3.0\times10^3$ |

A = Herbal drug for malaria; B = Herbal drug for typhoid; C = Herbal drug for blood infection; D = Herbal drug for body pain; E = Herbal drug for pile; F = Herbal drug for eye ulcer; G = Herbal drug for blood cleansing (a); H = Herbal drug for inflammation; I = Herbal drug for typhoid; J = Herbal drug for blood cleansing (b)

**Table 3a. Occurrence of bacteria isolated from herbal samples**

| Bacterial isolates | A | B | C | D | E | F | G | H | I | J | Frequency (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *B. subtilis* | + | - | - | - | - | - | - | - | - | - | 1(5.26) |
| *S. aureus* | + | - | - | - | - | - | - | + | - | + | 3(15.79) |
| *S. typhi* | - | + | - | - | - | - | - | + | - | - | 2(10.53) |
| *S. dysenteriae* | - | + | + | + | + | + | - | - | + | - | 6(31.58) |
| *P. aeruginosa* | - | - | + | - | - | - | + | - | - | - | 2(10.53) |
| *C. diptheriae* | - | - | - | + | - | - | - | - | - | - | 1(5.26) |
| *S. pyogenes* | - | - | - | - | + | - | - | - | - | - | 1(5.26) |
| *E. cloacae* | - | - | - | - | - | + | - | - | - | - | 1(5.26) |
| *K. pneumoniae* | - | - | - | - | - |  | + | - | - | - | 1(5.26) |
| *C. botulinum* | - | - | - | - | - | - | - | - | - | + | 1(5.26) |

**Table 3b. Occurrence of fungi isolated from herbal samples**

| Fungal isolates | A | B | C | D | E | F | G | H | I | J | Frequency (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. niger* | + | - | - | - | - | - | - | - | + | - | 2(20.00) |
| *R. stolonifer* | - | + | - | - | - | - | - | - | - | + | 2(20.00) |
| *A. alternata* | - | - | + | - | - | - | - | - | - | - | 1(10.00) |
| *A. flavus* | - | - | - | + | - | - | + | - | - | - | 2(20.00) |
| *F. verticillioides* | - | - | - | - | + | - | - | - | - | - | 1(10.00) |
| *F. oxysporium* | - | - | - | - | - | + | - | - | - | - | 1(10.00) |
| *M. racemosus* | - | - | - | - | - | - | + | - | - | - | 1(10.00) |

The isolated microorganisms exhibited varying sensitivity to the herbal samples in the agar well diffusion assay (Tables 4a and 4b). Liquid herbal preparations exhibited inhibitory activity against *Salmonella thyphi* at 25% concentration, except liquid sample D. Other bacteria inhibited by the liquid herbal samples include *Staphylococcus aureus* and *Shigella dysentariaea* at concentrations ranging from 50 to 100%. On the other hand, the reconstituted powdered samples inhibited all the isolates except *Clostridium botulinum*, which was inhibited by samples F and J at 100% concentration. The inhibitory effect of powdered herbal sample F was more pronounced on all the isolates at 100% concentration, with inhibition zones ranging from 4.31mm to 14.06mm. The qualitative phytochemical screening showed that the following compounds: flavonoids, saponins, terpenoids, and tannins are present in the herbal samples (Tables 5a and 5b), and the quantitative result showed that alkaloids were the most abundant in sample F with a value of 1070.04 mg/100 g. GC-MS results also revealed that thiophene, propanoic acid, 2,2-dimethyl-, ethyl ester, pentanoic acid, 2-methyl, toluene, and many other compounds are present in the herbal drug (Sample F) that showed significant antimicrobial effects (Table 6).

**Table 4a. Inhibitory Effect of Liquid Herbal Medicines against Bacterial Isolates**

| Herb Samples (%) | | Mean zone of inhibition (mm) of herbs against bacteria | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *B. subtilis* | *S. aureus* | *S. typhi* | *S. dysenteriae* | *P. aeruginosa* | *C. diptherae* | *S. pyogenes* | *E. cloacae* | *K. pneumoniae* | *C. botulinum* |
| A | 25 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 6.02±0.01$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 7.31±0.33$^{ab}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 7.02±0.04$^{ab}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 100 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 10.10±0.16$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| B | 25 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 10.00±0.00$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 12.01±0.07$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 20.22±0.26$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 100 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 26.00±0.00$^{g}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| C | 25 | 0.00±0.00$^{a}$ | 6.34±0.15$^{c}$ | 11.00±0.00$^{b}$ | 9.06±0.11$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 7.31±0.44$^{c}$ | 13.21±0.14$^{c}$ | 14.00±0.00$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 0.00±0.00$^{a}$ | 11.01±0.06$^{e}$ | 16.04±0.20$^{d}$ | 16.00±0.00$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 100 | 0.00±0.00$^{a}$ | 13.11±0.14 | 20.01±0.10$^{e}$ | 22.11±0.15$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| D | 25 | 0.00±0.00$^{a}$ | 8.03±0.06$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 12.01±0.11$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 0.00±0.00$^{a}$ | 18.11±0.41$^{g}$ | 7.06±0.31$^{ab}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 100 | 0.00±0.00$^{a}$ | 22.02±0.02$^{h}$ | 12.02±0.22$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| E | 25 | 0.00±0.00$^{a}$ | 4.00±0.05$^{b}$ | 7.00±0.00$^{ab}$ | 8.04±0.43$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 9.02±0.13$^{d}$ | 13.11±0.16$^{c}$ | 12.21±0.11$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 0.00±0.00$^{a}$ | 13.00±0.00$^{f}$ | 16.31±0.05$^{d}$ | 16.09±0.31$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 100 | 0.00±0.00$^{a}$ | 18.10±0.04$^{g}$ | 22.04±0.32$^{f}$ | 24.06±0.02$^{f}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Values are presented as mean±SE, values in the same column carrying same superscript are not different significantly according to new Duncan's Multiple Range test at $p<0.05$.

A = Herbal drug for malaria; B = Herbal drug for typhoid; C = Herbal drug for blood infection; D = Herbal drug for body pain; E = Herbal drug for pile; F = Herbal drug for eye ulcer; G = Herbal drug for blood cleansing (a); H = Herbal drug for inflammation; I = Herbal drug for typhoid; J = Herbal drug for blood cleansing (b).

**Table 4b. Inhibitory Effect of Powdered Herbal Medicines against Bacterial Isolates**

| Herb Samples (%) | | Mean zone of inhibition (mm) of herbs against bacteria | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *B. subtilis* | *S. aureus* | *S. typhi* | *S. dysenteriae* | *P. aeruginosa* | *C. diphtheriae* | *S. pyogenes* | *E. cloacae* | *K. pneumoniae* | *C. botulinum* |
| F | 25 | 1.06±0.21$^{b}$ | 4.52±0.17$^{c}$ | 3.42±0.41$^{b}$ | 4.06±0.14$^{c}$ | 1.36±0.11$^{b}$ | 1.00±0.00$^{b}$ | 3.53±0.17$^{c}$ | 2.05±0.16$^{b}$ | 3.31±0.43$^{b}$ | 0.00±0.00$^{a}$ |
| | 50 | 3.00±0.22$^{c}$ | 4.34±0.05$^{c}$ | 6.50±0.32$^{c}$ | 9.00±0.00$^{e}$ | 4.01±0.12$^{c}$ | 3.16±0.01$^{bc}$ | 8.03±0.21$^{d}$ | 4.36±0.05$^{d}$ | 5.00±0.00$^{c}$ | 0.00±0.00$^{a}$ |
| | 75 | 3.00±0.05$^{c}$ | 7.90±0.11$^{e}$ | 10.00±0.00$^{e}$ | 14.15±0.31$^{f}$ | 7.33±0.25$^{d}$ | 4.31±0.17$^{c}$ | 8.16±0.05$^{d}$ | 7.07±0.81$^{e}$ | 9.00±0.00$^{e}$ | 0.00±0.00$^{a}$ |
| | 100 | 4.31±0.33$^{d}$ | 10.05±0.06$^{f}$ | 14.06±0.11$^{g}$ | 15.03±0.07$^{f}$ | 9.18±0.07$^{e}$ | 8.04±0.36$^{d}$ | 10.18±0.04$^{e}$ | 10.00±0.00 | 12.32±0.15$^{f}$ | 10.00±0.00$^{c}$ |
| G | 25 | 0.00±0.00$^{a}$ | 7.44±0.21$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 6.22±0.03$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 11.13±0.44$^{f}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 10.00±0.00$^{f}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 0.00±0.00$^{a}$ | 13.00±0.00$^{g}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 14.14±0.56$^{g}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 100 | 0.00±0.00$^{a}$ | 16.05±0.11$^{i}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 20.06±0.15$^{h}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| H | 25 | 0.00±0.00$^{a}$ | 6.33±0.02$^{d}$ | 2.04±0.05$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 12.26±0.56$^{g}$ | 3.02±0.03$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 0.00±0.00$^{a}$ | 14.17±0.14$^{h}$ | 8.21±0.31$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 100 | 0.00±0.00$^{a}$ | 22.04±0.07$^{j}$ | 10.05±0.04$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| I | 25 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 8.07±0.11$^{d}$ | 2.00±0.00$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 2.99±0.44$^{b}$ | 12.11±0.32$^{f}$ | 6.17±0.02$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 0.00±0.00$^{a}$ | 4.41±0.07$^{c}$ | 14.01±0.07$^{g}$ | 10.08±0.08$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 100 | 0.00±0.00$^{a}$ | 8.27±0.31$^{e}$ | 23.00±0.00$^{h}$ | 14.22±0.21$^{f}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| J | 25 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 4.06±0.06$^{c}$ | 2.20±0.04$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 50 | 0.00±0.00$^{a}$ | 3.16±031$^{b}$ | 9.01±0.12$^{e}$ | 6.00±0.00$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 1.00±0.00$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| | 75 | 4.43±0.21$^{d}$ | 6.05±0.03$^{d}$ | 10.11±0.33$^{e}$ | 10.04±0.21$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 5.03±0.20$^{c}$ | 0.00±0.00$^{a}$ | 4.07±0.04$^{b}$ | 0.00±0.00$^{a}$ |
| | 100 | 7.09±0.06$^{e}$ | 12.07±0.14$^{g}$ | 12.33±0.09$^{f}$ | 10.00±0.00$^{e}$ | 2.00±0.00$^{b}$ | 0.00±0.00$^{a}$ | 7.11±0.25$^{d}$ | 3.31±0.18$^{c}$ | 6.16±0.21$^{d}$ | 2.04±0.11$^{b}$ |

Values are presented as mean±SE, values in the same column carrying same superscript are not different significantly according to new Duncan's Multiple Range test at $p<0.05$. A = Herbal drug for malaria; B = Herbal drug for typhoid; C = Herbal drug for blood infection; D = Herbal drug for body pain; E = Herbal drug for pile; F = Herbal drug for eye ulcer; G = Herbal drug for blood cleansing (a); H = Herbal drug for inflammation; I = Herbal drug for typhoid; J = Herbal drug for blood cleansing (b).

**Table 5a. Qualitative Phytochemical Analysis of Extract of Plant (Sample F)**

| S/N | Component | Results |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavonoids | ++ |
| 3 | Saponins | ++ |
| 4 | Tannins | ++ |
| 5 | Steroids | - |
| 6 | Reducing sugars | + |
| 7 | Phenols | ++ |
| 8 | Cardiac glycosides | + |
| 9 | Terpenoids | ++ |
| 10 | Proteins | - |
| 11 | Anthraquinone | - |

++Strong positive test, + = Weak positive test, − = Negative tests

**Table 5b. Quantitative Phytochemical Analysis of Water Extract of Plant (Sample F)**

| S/N | Component | Value |
|---|---|---|
| 1 | Alkaloids (mg/100 g sample) | 1070.04 |
| 2 | Flavonoids (mg QE/100g) | 6.22 |
| 3 | Saponins (mg DE/100 g extract) | 13.96 |
| 4 | Tannins (mg GAE/100 g extract) | 1.42 |
| 5 | Carotenoids (mg DE/100 g extract) | 4.39 |
| 6 | Total Phenols (mg GAE/100g) | 0.38 |
| 7 | Vitamin C (mg AE/100 g extract) | 9.21 |

**Table 6. Bioactive components present in sample F as revealed by GCMS analysis**

| Peak # | RT | Compounds Name | Molecular Formula | M.W. (AMU) | Peak % area | % Composition | m/z | Structure |
|---|---|---|---|---|---|---|---|---|
| 1 | 2.14 | Toluene | $C_7H_8$ | 92 | 1.27 | 2.74 | 39, 91, 92 |  |
| 2 | 3.08 | Pentanoic acid, 2-methyl | $C_6H_{12}O_6$ | 116 | 4.45 | 2.16 | 43, 75, 116 |  |
| 3 | 3.78 | 2-Hexen-1-ol, 2-ethyl | C8 H16O | 128 | 10.97 | 2.28 | 41, 57, 128 |  |
| 4 | 4.95 | 5-Hydroxy-4-octanone | $C_8H_{16}O_2$ | 144 | 5.09 | 2.34 | 43, 55, 144 |  |
| 5 | 5.47 | Heptane, 2,4-dimethyl- | $C_6H_{12}O_2$ | 128 | 1.75 | 2.88 | 43, 85, 128 |  |
| 6 | 6.82 | Benzene, 1,3-bis(1,1-dimethylethyl)- | $C_{14}H_{22}$ | 190 | 2.23 | 2.42 | 57, 175, 190 |  |
| 7 | 8.71 | 3-O-Methyl-d-glucose | $C_7H_{14}O_6$ | 194 | 17.32 | 36.03 | 43, 51, 194 |  |
| 8 | 8.98 | Propanoic acid, 2,2-dimethyl-, ethyl ester | $C_7H_{14}O_2$ | 130 | 5.09 | 2.03 | 41, 57, 130 |  |
| 9 | 9.42 | Thiophene | $C_4H_4$ | 8 | 4.45 | 2.18 | 45, 58, 84 |  |
| 10 | 10.02 | 2-Ethyl-trans-2-butenal | $C_6H_{10}O$ | 98 | 4.37 | 2.24 | 39, 41, 98 |  |

## 4. Discussion

Herbal drugs have been important to the promotion of the health of individuals, communities, and the nation as a whole. Many individuals rely on these drugs for the treatment of their illnesses [24]. Unknown to many of the users is the fact that most, if not all, of these herbal drugs are contaminated with viable cells of microorganisms, most of which are pathogenic and can be detrimental to human health. This study assessed the microbial loads and types of several herbal products. All the herbal samples collected contained viable cells with a bacterial load that ranged from $2.26\times10^5$ to $1.40\times10^5$ cfu/ml (Table 1) and a fungal load that ranged from $3.6\times10^3$ to $3.0\times10^3$ sfu/ml (Table 2). Kneifel *et al*. [20] had earlier reported that raw materials (plants) used in herbal preparations are also a source of nutrients that facilitate the growth and multiplication of microorganisms. Microorganisms are found in plant parts such as roots, stems, bark, and leaves [25, 26]. It has also been observed that the water used for washing and preparing herbal concoctions is not sterile in most cases. Moreover, during drying, microorganisms from air and water sources can contaminate the plant materials used in preparing herbal remedies.

The microorganisms isolated from the herbal drugs are: Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Corynebacterium diptheriae, Streptococcus pyogenes, Enterobacter cloacae, Klebsiella pneumoniae, Clostridium botulinum, *Aspergillus niger*, *Rhizopus stolonifer*, *Alternaria alternata*, *Aspergillus flavus*, *Fusarium verticillioides*, *Fusarium oxysporium*, and *Mucor racemosus* (Tables 3a and 3b). Several researchers working on herbal medicines have also isolated similar groups of microorganisms [3, 7, 26–30]. These microorganisms might have been acquired during the production and sale of these herbal medicines. Oyetayo [3] had earlier reported that the major shortcoming of herbal medicine is production under unhygienic conditions, which enhances contamination.

The inhibitory effects (Tables 4a and 4b) of these herbal drugs on the microbial isolates ranged from 4.31mm to 14.06 mm. The ability of the herbal drugs to inhibit the microorganisms may be a result of tannins, alkaloids, saponin present in them (Tables 5a and 5b). These phytochemical constituents have been reported as precursors for the synthesis of useful drugs [30]. Extracts of plant parts such as leaves, stems, bark, roots, etc. are known to exhibit antimicrobial activities against pathogenic organisms [31–34]. Alli *et al*. [35] listed the following phytochemicals: tannins, phenols, and reducing sugar as being responsible for the observed antibacterial activity of a medicinal plant, *Bidens pilosa*, and can be used for food preservation. These phytochemical components of herbs, viz: alkaloids, flavonoids, essential oils, terpenes, organic acids, coumarins, and lignans, are known to display potential antimicrobial effects, and herbal medicine formulas (HMFs) usually show stronger antibacterial activity than single herbs [7, 36]. In a recent study, Gancho *et al*. [37] reported that herbal medicine had comparable effects to commercially available drugs. Liang *et al*. [36] further suggested that mechanisms of action of herbs and HMFs as antibacterial agents are by damaging cell membranes and walls, inhibiting nucleic acid and protein synthesis, and increasing intracellular osmotic pressure.

Some of the bioactive compounds, such as thiophene, propanoic acid, 2,2-dimethyl-, ethyl ester, pentanoic acid, 2-methyl, and toluene, found in these herbal drugs (Table 6) are known to have antioxidant and antimicrobial properties [22, 38, 39]. In a recent study, the presence of these bioactive compounds could therefore make these herbal remedies possess other therapeutic effects. Chaughule & Barve [24] reported that multiple alkaloids/compounds that occur naturally (as opposed to single extracts) exhibit synergistic actions such as antiviral, antibacterial, anti-protozoal, and antioxidant.

## 5. Conclusion

Conclusively, herbal products are patronized by many people in Akure metropolis and by Nigerians as a whole. From the data gathered in this study, most of the herbal products sold in Akure are contaminated with microorganisms. However, antimicrobial effects were observed against some microbial isolates, coupled with the presence of bioactive compounds with potential antimicrobial effects in some of the herbal products. It is important to note that the producers and peddlers of these herbal remedies often attach many health benefits to them that have not been scientifically verified. Moreover, the producers do not adhere to good manufacturing practices while they are producing these drugs. Another issue is the post-production handling of these products. In most cases, the products are carelessly displayed during sales and transportation. It is therefore important to educate the masses on the dangers they could be exposed to as a result of consuming some of these unwholesome herbal products. Hence, producers of these herbal remedies are advised to observe a high level of hygiene during production. Moreover, the herbal products must also be kept in an environment that minimizes contamination during transportation and sales. This will ensure that the herbal products will actually be of benefit to the consumers without any detrimental effects. Appropriate regulatory agencies such as the Standard Organization of Nigeria (SON) and the National Agency for Food Administration and Control (NAFDAC) need to be more proactive in safeguarding the health of the uninformed and unsuspecting masses through regular monitoring of the production and sales of these herbal products.

## 6. Declarations

### 6.1. Author Contributions

Conceptualization, V.O.O. and O.A.B.; methodology, V.O.O. and O.A.B.; investigation, V.O.O. and O.A.B.; resources, V.O.O. and O.A.B.; writing—original draft preparation, V.O.O. and O.A.B.; writing—review and editing, V.O.O. and O.A.B. All authors have read and agreed to the published version of the manuscript.

### 6.2. Data Availability Statement

Data sharing is not applicable to this article.

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### 6.4. Institutional Review Board Statement

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### 6.5. Informed Consent Statement

Not applicable.

### 6.6. Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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