INTRODUCTION

The search for cheap, safe and effective bioactive compounds to solve myriads of health challenges faced by man had been on for years. Attention has been on plants as source of these novel bioactive compounds. The idea that certain plants had healing potential and contained compounds currently characterize as antimicrobial principles, was well accepted [1]. Medicinal plants are therefore important sources of new chemical substances that have potential therapeutic effects [2]. They have been utilized in traditional medicine and modern medicine [3]. In developing countries, most people depend completely on traditional medical practices for their health care needs and various plants are used in such traditional medicine [1, 4].

Voacanga africana is one of such useful medicinal plant found in tropical Africa. It belongs to the family Apocynaceae. Voacanga spp. consist of small trees inhabiting the tropical and subtropical regions of Africa, South East Asia, Australia and New Guinea [5]. V. africana contains several important phytochemicals. Vocaangine, a major alkaloid present in V. africana has been used as precursor in the semi-synthesis of ibogaine, an anti-addiction medication [5].

The different parts of the plants had been used for various medicinal purposes throughout its distribution area [6,7]. The leaf is used in the treatment of anaemia and other blood disorders, malaria, diarrhoea, infant convulsion, and heart archeus, relieve shortness of breath as well as fatigue and leprosy [8-10]. The root decoction is drunk 3 times daily to treat post-partum pains and hernia [11]. Tan et al. [8] also reported that dried and powdered root mixed with porridge is used to treat kidney and menstrual pain in women while the fruit bark and leaf extracts are used to treat cases of orchitis, ectopic testis and gonorrhea. Extracts obtained from the bark of V. africana possess broad spectrum antimicrobial effect [12].

V. africana is a well known medicinal plant in Nigeria as affirmed by traditional medical practitioners. However, there is still scanty information on the effect of fermentation on some phytochemical and some medicinal properties of this plant. The present study is therefore aimed at assessing the effect of fermentation on the phytochemical, antibacterial and antioxidant properties of V. africana seed.

METHODS

Source of V. africana

The mature fruits of V. africana were harvested where it was growing naturally in Akure, Ondo State, Nigeria (Lat.7.25256°N, Long. 5.19312°E). The plant fruit was identified and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure (FUTA) by a plant Taxonomist.

Source of microbial isolates

Clinical and typed microorganisms were sourced from Department of Microbiology, FUTA and Nigeria Institute of Medical Research Lagos. The isolates were maintained on agar slant and stored in the refrigerator at 5°C until use.

Fermentation of V. africana seed

Dried and powdered V. africana seed (200 g) was divided into two portions. One portion (100 g) was not fermented while the other portion was subjected to submerged fermentation in water (400 mL) at room temperature (27±2°C) following the methods of Abiola and Oyetayo [13].

Preparation of raw and fermented extracts of V. africana seed

Raw and fermented V. africana seed were dried and pulverized. The pulverized V. africana seeds were kept in air-tight cellophane bag until required. Powdered V. africana seed (100 g) was placed in 500 mL of water in a conical flask, each was shaken in a rotary shaker at 121 rpm for 24 h. After 24 h, the suspension was filtered with a double layer muslin cloth and whatman No. 1 filter paper. The resulting filtrate was concentrated under reduced pressure in rotary evaporator (52A; Union Laboratory, England) at 40°C.
Qualitative and quantitative phytochemical analysis of raw and fermented *V. africana* seed

Qualitative and quantitative phytochemical screening of the crude extracts of the raw and fermented *V. africana* seed was performed using standard procedures as described by Harborne [14,15]. The quantity of the following phytochemicals viz; saponin, tannin, flavonoid, terpenoids, Phlobatinnin, Anthroquinone, cardiac glycosides and alkaloids were assessed in the extracts obtained from the raw and fermented *V. africana* seed.

**Determination of antibacterial effect of raw and fermented *V. africana* seed**

Antimicrobial activity of raw and fermented *V. africana* seed extracts was determined by the agar well diffusion method [16]. Bacterial isolates were cultivated on nutrient broth at 37°C for 24 h to a turbidity that equals that of 0.5 Mc Farland standards which is the equivalent of 10^5–10^6 cfu/mL. Cooled (45°C) molten Mueller Hinton agar (20 mL) was poured into sterile Petri-dishes and was left to solidify. A portion of the cultures were spread on the surface of the solidified agar plates. About 8 mm wells were bored in the agar with sterile cork borer (8 mm). Concentrations (100mg/mL) of extracts were prepared respectively for raw and fermented *V. africana* seed extracts. The extracts were filter sterilized through 0.22 μm membrane and were introduced into the wells already seeded with the microbial isolates used as test organisms. The plates were incubated at 37°C for 24 h. Ciprofloxacin was used as positive control. The diameter of the inhibition zones were measured in millimeters. Inhibition zones were measured in triplicates (three plates per indicator organism).

**Antioxidant assay**

The following antioxidant assays were performed on the stem bark and leaf extracts obtained from raw and fermented *V. africana* seed extracts.

**Scavenging ability of raw and fermented *V. africana* seed extracts on DPPH radicals**

The free radical scavenging ability of the extract against 1,1-diphenyl - 2 - picrylhydrazyl (DPPH) was determined using [17] method. The extract (1 mL) was mixed with 1 mL of 0.4 M methanolic solution of the DPPH. The mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

**Ferrous ion chelating ability of raw and fermented *V. africana* seed extracts**

The Fe^2+ chelating ability of raw and fermented *V. africana* seed extracts was determined by employing a modified method of Punnet et al. [18]. Freshly prepared 500 μmol L^-1 FeSO_4 was added to a solution containing 168 μL of 0.1 mol L^-1 Tris-HCl (pH 7.4), together with 218 μL of saline and an ethanol extract (1–5 mg/mL). The solution was incubated for 5 min, followed by addition of 13 μL of 0.25, 1, 10% phenantroline (w/v). Absorbance was read at 510 nm. Fe^2+ chelating ability was expressed as percentage inhibition.

**Statistical analysis**

Experiments were carried out in replicates and data obtained were analyzed by one way analysis of variance and means were separated by Duncan multiple range test (SPSS 17.0 version). Differences were considered significant at p≤0.05.

**RESULTS**

The phytochemical content of raw and fermented extracts of *V. africana* seed is presented in Table 1. Saponin, tannin, flavonoid, terpenoid, alkaloids and cardiac glycosides were present in both raw and fermented extracts of *V. africana* seed while phlobatinnin and anthraquinone were absent. Quantitative phytochemical screening also revealed that the tannin, terpenoid and flavonoid contents of fermented extracts of *V. africana* seed was higher than what was obtained in raw extract (Fig. 1). However, the saponin and cardiac glycosides of the raw extracts of *V. africana* seed was higher than what was obtained in fermented seed extract.

**Table 1: Qualitative phytochemical properties of raw and fermented *V. africana***

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Raw</th>
<th>Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Extract obtained from fermented seed of *V. africana* exhibited higher antioxidant activity than the raw extract (Fig. 2). The DPPH scavenging (24.10%) and Fe^2+ chelating effects (7.38%) of aqueous extract obtained from fermented seed of *V. africana* was higher than what was obtained in raw seed extract.

Fermented seed of *V. africana* extract also displayed higher and significantly different (p≤0.05) antibacterial effect on all test organisms except *Escherichia coli* (Clinical) that the raw extract inhibited better than the fermented extract (Table 2). *Shigella typhi* (Clinical) that was not inhibited by the raw extract was however inhibited by the fermented seed extract (500 mm). Moreover, the extracts and ciprofloxacin (Positive control) exhibited higher antibacterial effects on typed organisms than the clinical organisms.

Table 3 revealed the bioactive compounds present in the fermented seed extract of *V. africana*. Some of the major compounds identified are: Cyclopropaneoctanal, Pentadec-7-ene, p-Meth-8(10)-en-9-ol, Oleic acid, Ecosane, Butyl-9-tetradecanoate, 2-Methyl-2z,3,13-octadecadienol.

**DISCUSSION**

The use of plant for prophylactic and therapeutic purposes by man had been recorded from time immemorial [3]. Over 4 decades ago, it was stated that about 3.5–4 billion people in the world rely on plants as sources of drugs [19]. The search for bioactive components of plants had been a major focus of researchers in the field of drug discovery. Hence, plant parts such as leaf, stem bark, root, seed, and flower had been screened for their potential pharmaceutical uses.

In the present study, raw and fermented seed of *V. africana* seed was found to contain major phytochemicals such as saponin, tannin, flavonoid, terpenoid, alkaloids and cardiac glycosides. The presence of these phytochemicals in *V. africana* seed had earlier been reported by Duru and Onyededike [20]. However, tannin, terpenoid and flavonoid contents of fermented extracts of *V. africana* seed were higher than what was obtained in raw extract. The increase in flavonoid is in line with earlier report on the effect of fermentation on some phytochemicals.
Retention
Control (ciprofloxacin)
Staphylococcus aureus ATCC 23235
27.00±0.10
c
Staphylococcus aureus (Clinical)
22.00±0.01
c
Bacillus cereus ATCC 14579
27.00±0.11
c
Bacillus cereus (Clinical)
22.00±0.01
c
Escherichia coli ATCC 25922
22.00±0.10
b
Escherichia coli (Clinical)
12.00±0.02
b
Salmonella typhi ATCC 6539
27.00±0.01
b
Salmonella typhi (Clinical)
27.00±0.10
b

Table 2: Antibacterial property of raw and fermented V. africana extracts

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Retention time</th>
<th>Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanoic acid</td>
<td>4.060</td>
<td>1</td>
</tr>
<tr>
<td>1,3-propanediol</td>
<td>4.454</td>
<td>2</td>
</tr>
<tr>
<td>Undecanoic acid</td>
<td>7.384</td>
<td>4</td>
</tr>
<tr>
<td>N-Cyclohexylacetamide</td>
<td>7.609</td>
<td>5</td>
</tr>
<tr>
<td>n-Decanoic acid</td>
<td>8.116</td>
<td>7</td>
</tr>
<tr>
<td>12-Bromododecanoic acid</td>
<td>8.736</td>
<td>8</td>
</tr>
<tr>
<td>Furo[2,3-4][thiazolo[3,2-g]purine-8-methanol</td>
<td>8.877</td>
<td>9</td>
</tr>
<tr>
<td>Nonanoic acid</td>
<td>9.559</td>
<td>10</td>
</tr>
<tr>
<td>9-Bromonoanoic acid</td>
<td>9.778</td>
<td>11</td>
</tr>
<tr>
<td>7-Oxodehydroabietic acid</td>
<td>9.891</td>
<td>12</td>
</tr>
<tr>
<td>Phenol</td>
<td>10.131</td>
<td>13</td>
</tr>
<tr>
<td>n-Hexadecanoic acid</td>
<td>10.736</td>
<td>14</td>
</tr>
<tr>
<td>2-Heptanal</td>
<td>10.877</td>
<td>15</td>
</tr>
<tr>
<td>4-Fluoro-2-nitroaniline</td>
<td>10.961</td>
<td>16</td>
</tr>
<tr>
<td>Selenocianic acid</td>
<td>11.271</td>
<td>17</td>
</tr>
<tr>
<td>Diethyl Phthalate</td>
<td>12.511</td>
<td>18</td>
</tr>
<tr>
<td>1,14-Dibromotetracaine</td>
<td>14.285</td>
<td>21</td>
</tr>
<tr>
<td>1,2-Benzisothiazole</td>
<td>14.511</td>
<td>22</td>
</tr>
<tr>
<td>Cyclopropanoetral</td>
<td>14.595</td>
<td>23</td>
</tr>
<tr>
<td>Pentadec-7-ene</td>
<td>14.933</td>
<td>24</td>
</tr>
<tr>
<td>p-Meth-8 (10)-en-9-ol</td>
<td>15.356</td>
<td>25</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>15.553</td>
<td>26</td>
</tr>
<tr>
<td>Eicosane</td>
<td>16.483</td>
<td>28</td>
</tr>
<tr>
<td>Butyl-9-tetradecanoate</td>
<td>17.609</td>
<td>31</td>
</tr>
<tr>
<td>2-Methyl-2,3,31-3-octadecadienol</td>
<td>17.778</td>
<td>32</td>
</tr>
</tbody>
</table>

Values (means±standard error) with different superscript along rows are significantly different (p≤0.05)

Table 3: Bioactive components obtained from fermented V. africana seed extract

Fig. 2: Antioxidant effect of raw and fermented extracts of Voaanga africana

The fermented extract of V. africana seed also exhibited better and higher antioxidant properties than the non-fermented seed extract in terms of DPPH scavenging and Fe²⁺ chelation activities. This could be attributed to the higher flavonoid and tannin contents in fermented extracts of V. africana seed. Phenolic compounds such as flavonoid and tannin had been reported as potent antioxidant compounds [25]. The chemical nature of phenolics made it possible to scavenge for free radicals and chelate metal ions [26,27]. It has been reported that the higher the phenolic content of a sample the higher its antioxidant capacity [28]. Phenolic compounds are also known to possess anti-inflammatory, antimicrobial and anticancer activities [29-31].

The result of antimicrobial test also revealed that fermented extract obtained from V. africana seed also exhibited higher and significantly different antimicrobial effects than extract obtained from raw V. africana seed. Duru et al. [12] had earlier reported the antimicrobial effects of hot water, hot and cold ethanolic extracts of the bark of V. africana on some selected bacteria and fungi. Fermented extract obtained from the V. africana seed was found to be active against all the test bacteria used in this study (Table 2). Submerged fermentation usually yields organic acids, enzymes and other metabolites that possess antimicrobial property [32]. This might have contributed to the higher antimicrobial activity of the fermented extract. Moreover, bioactive compounds found in the extract of fermented V. africana seed such as; Oleic acid, Eicosane, Diethyl Phthalate, 1,14-Dibromotetracaine, Cyclopropanoetral and so on are known to possess antimicrobial property [33-35]. The medicinal properties of V. africana had been attributed to more than 100 phytochemicals (indole alkaloids, ibogain, tabernanthin, ibogamine, vincaneol, vincamone etc.) found in different parts of the plant [5].

Bacteria obtained from clinical sources were also less susceptible to the antibacterial effects of the extracts when compared with the typed bacterial. This may be due to previous exposure of the clinical bacteria obtained from clinical sources to antibiotics during the treatment of patients. Large amounts of antibiotics used for human therapy, as well as for farm animals and even for fish in aquaculture, resulted in the selection of pathogenic bacteria resistant to multiple drugs [36].

The results obtained from this study indicate that extracts obtained from fermented V. africana seed had higher antioxidant and antimicrobial properties than the extract obtained from raw V. africana seed. Submerged fermentation thus enhanced the antimicrobial effect of V. africana seed. V. africana seed could be a source of natural antioxidant and antimicrobial compounds for pharmaceutical exploitation and applications.

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

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