INNOVARE JOURNAL OF LIFE SCIENCE

Vol 11, 2023

ISSN - 2321-550X

Research Article

EFFECT OF SUBMERGED FERMENTATION ON THE ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF AQUEOUS EXTRACTS OBTAINED FROM *VOACANGA AFRICANA* STAPF SEED

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Received: 08 June 2023, Revised and Accepted: 27 June 2023

ABSTRACT

Voacanga africana Stapf which belongs to the family, *Apocynaceae* is among useful medicinal plants of West Africa. The present study investigates the antibacterial and antioxidant properties of aqueous extracts obtained from raw and fermented *V. africana*. One portion of *V. africana* was subjected to submerged fermentation for 7 days. Aqueous extracts of the raw and fermented samples of *V. africana* were obtained using standard methods. Antibacterial effect of the aqueous exracts was assessed by agar well diffusion while a battery of antioxidant test which include DPPH scavenging, ABTS, and Fe^{2+} chelation was employed. The bioactive compounds present in the extract were assessed using gas chromatography-mass spectrophotometer. Extract obtained from the fermented seed of *V. africana* had good antimicrobial effect with zones of inhibition ranging from 4.67 to 22.00 mm. Aqueous extract obtained from fermented *V. africana* exhibited better antioxidant capacity with DPPH scavenging effect being the highest (27.93) when compared with extract obtained from raw *V. africana.* Bioactive compounds such as Eicosane, 1,14-Dibromotetradecane, 7-Oxodehydroabietic acid, oleic acid and so on were present in the aqueous extract obtained from fermented *V. africana.* Data gathered from this study revealed that fermentation enhances the antimicrobial and anoxidant properties of *V. africana.* The presence of bioactive compounds in the aqueous extract of *V. africana* indicates that it could be a good source of natural antimicrobial and antioxidant compounds for the improvement of human health.

Keywords: *Voacanga africana*, Fermentation, Aqueous, Extract, Antioxidant, Antimicrobial.

 DOI: http://dx.doi.org/10.22159/ijls.2023v11i4.48556. Journal homepage: https://innovareacademics.in/journals/index.php/ijls

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 in 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. Voacangine, 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, diarrhea, infant convulsion, and heart arches, 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 matured 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 powered *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 was 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, terpernoids, 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 10^6–10^8 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 milliliters. 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 – picryhydrazyl (DPPH) was determined using [17] method. The extract (1 mL) was mixed with 1 mL of 0.4M 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 Puntel *et al*. [18]. Freshly prepared 500 µmol L^{-1} $FeSO_4$ was added to a solution containing 168 µL of 0.1 molL^{-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 phlobatinin 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*

Phytochemical	Raw	Fermented
Saponin	+	+
Tannin	+	+
Phlobatinnin	-	-
Flavonoid	+	+
Steroid	-	-
Terpenoid	+	+
Alkaloid	+	+
Anthraquinone	-	-
Cardiac glycosides	+	+

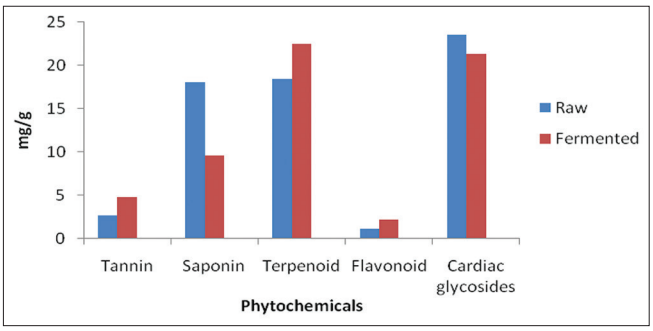

Fig. 1: Quantitative phytochemical constituents of raw and fermented *Voacanga africana*

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 (8.00 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, Eicosane, Butyl-9-tetradecanoate, 2-Methyl-z,z-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 Onyedineke [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

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

Test organisms	Control (ciprofloxacin)	Fermented seed extract	Raw seed extract
Staphylococcus aureus ATCC 23235	27.00±0.10^{c}	12.00±1.00^{b}	5.67±0.90^{a}
Staphylococcus aureus (Clinical)	22.00±0.01^{c}	10.67±0.58^{b}	4.67±0.50^{a}
Bacillus cereus ATCC 14579	27.00±0.11^{c}	22.00±1.00^{b}	18.67±0.60^{a}
Bacillus cereus (Clinical)	22.00±0.01^{c}	16.00±1.00^{b}	14.00±0.98^{a}
Escherichia coli ATCC 25922	22.00±0.10^{b}	21.67±0.60^{b}	16.00±1.00^{a}
Escherichia coli (Clinical)	12.00±0.02^{c}	4.67±0.52^{a}	10.67±0.80^{b}
Salmonella typhi ATCC 6539	27.00±0.01^{c}	11.00±0.98^{b}	4.00±1.00^{a}
Salmonella typhi (Clinical)	27.00±0.10^{c}	8.00±1.00^{b}	0.00±0.00^{a}

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

Constituents	Retention time	Peak
Butanoic acid	4.060	1
1,3-propandienol	4.454	2
Undecanoic acid	7.384	4
N-Cyclodecylacetamide	7.609	5
n-Decanoic acid	8.116	7
12-Bromodacanoic acid	8.736	8
Furo[2,3:4,5]thiazolo[3,2-g] purine-8-methanol	8.877	9
Nonanoic acid	9.559	10
9-Bromomonoanoic acid	9.778	11
7-Oxodehydroabietic acid	9.891	12
Phenol	10.313	13
n-Hexadecanoic acid	10.736	14
2-Heptanal	10.877	15
4-Fluoro-2-nitroaniline	10.961	16
Senecionacium	11.271	17
Diethyl Phthalate	12.511	18
1,14-Dibromotetradecane	14.285	21
1,2-Benzisothiazole	14.511	22
Cyclopropaneoctanal	14.595	23
Pentadec-7-ene	14.933	24
p-Meth-8 (10)-en-9-ol	15.356	25
Oleic acid	15.553	26
Eicosane	16.483	28
Butyl-9-tetradecanoate	17.609	31
2-Methyl-z, z-3,13-octadecadienol	17.778	32

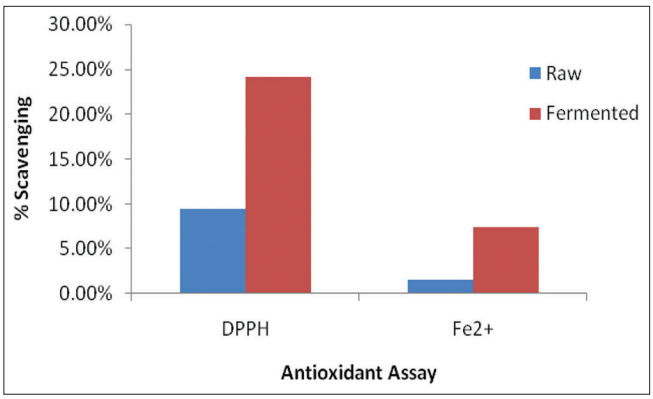

Fig. 2: Antioxidant effect of raw and fermented extracts of *Voacanga africana*

present in fermented substrates. Nazarni *et al.* [21] reported an increase in total phenolic and flavonoid content of tigarun flower while total tannin content decreased. Moreover, Haile and Kang [22] also reported significantly higher flavonoid content in fermented coffee beans than the unfermented coffee beans, while fermentation significantly decreased the tannin content compared to that in unfermented coffee. Fermentation process is known to release bound phenolic compounds from complexes [23,24]. This could explain the increase in the levels of tannin and flavonoid contents of fermented *V. africana* seed compared to the unfermented seeds.

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 Fe^{2+} 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-Dibromotetradecane, Cyclopropaneoctanal 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, vincanol, 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 propertied 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.

ACKNOWLEDGMENTS

None.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FUNDING

This research received no external funding.

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