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Differential Antimicrobial Effects of Conventional and Ethnobotanical Extracts from *Vitellaria paradoxa* Roots, Barks and Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: To determine the antimicrobial potentials of leaves, roots and barks of *Vitellaria paradoxa* "Shea-nut tree" used in traditional medicine for treatment of stomach ache and control of diarrhea.
Place and Duration of Study: Department of Microbiology, Federal University of Agriculture Abeokuta, Ogun State, P.M.B 2240, Nigeria, between August 2010 and May 2011.
Methodology: The leaves, roots and leaves were extracted by four solvents: methanol, omidun, sterile-omidun and aqueous. These extracts were tested for antimicrobial activities at different concentrations (100 mg/ml, 50 mg/ml and 25 mg/ml respectively) against some gastroenteric organisms using the disc diffusion assay. The test organisms used are typed culture *E. coli* ATCC 25922 and clinically isolated Enteropathogenic *E. coli*, Enterohaermohargic *E. coli*, *Salmonella typhi* and *Shigella flexneri.* The extractants served as negative control while loperamide antibiotics served as positive control.
Results: Generally, all the extracts exhibited varying antimicrobial activities against the test organisms with most of them exhibiting low Minimum Inhibitory Concentration (MIC). There was significant difference ($p<0.05$) in the effects of each of the extracts when used at different concentrations. The higher the concentration of extract, the wider the zone of inhibition. Methanol

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extracts showed the highest potency (19.0 mm for bark and 6.67 mm for root) followed by omidun extracts (17.33 for bark and 4.67 mm for root). Aqueous and sterile omidun extracts produced low inhibition at high concentration and no inhibition at low concentrations. At 100 mg/ml methanol extraction, the bark of Shea butter tree gave the highest potency (13.67–19.00 mm) followed by extracts of leaves (11.33-16.70 mm) while the extracts of roots had the lowest potency (6.67-17.00 mm).

Conclusion: Extracts of this plant parts have antimicrobial effects on the tested enteric bacteria, hence serve as potential therapeutic agent against diarrhea.

Keywords: Shea butter tree; methanol extract; omidun extract; inhibition zone.

1. INTRODUCTION

Antibiotic chemotherapy is widely practiced for the treatment of various microbiological infections; however, Fleming warned that the misuse of antibiotics could lead to the emergence of resistant forms of bacteria which pose a great threat to the global public health [1,2]. Aetiological agents especially bacteria have already developed resistance to most of the commonly employed antibiotics [3]. For example, antimicrobial resistance in *Enterobacteriaceae* poses a critical public health threat, especially in the developing countries [4].

The increase in antibiotic resistant strains of clinically important pathogens have led to the emergence of new bacterial strains that are multi drug resistant [5,6]. Non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality [7] which requires a feasible way to combat the problem by the development of new antimicrobial agents that are cheaper, and possess better bioactive potential and less side effects [8]. hence, the need for herbal medicine, which represents an effective alternative to avoid drug resistance [9].

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Medicinal plants have been used for years in daily life to treat diseases all over the world [10]. In Africa, one of such plants which is claimed to have anti-diarrhea property is *Vitellaria paradoxa* (the shea-nut tree). The shea-nut tree is known as Kandayi, Osisi and Emi among the Hausa, Igbo and Yoruba people of Nigeria. Almost all parts of the Shea nut tree have some practical use. The supernatant obtained from soaking the bark of *Vitellaria paradoxa* (Shea- nut tree) has been claimed locally by indigenes in West Africa to be active against gastrointestinal diseases like “Diarrhea”. Hence, this plant is locally used by local indigenous people in the treatment of diarrhea, dysentery and stomach disorder.

The conventional method to extract plant materials is to use methanol, ethanol, acetone and so on as extracting solvents, but the ethno-botanical approach like the use of Palmwine, “Omidun/ekan-ogi” (the water derived from three days fermented milled maize), local gin as extracting solvent and ways in which they are prepared locally, has received less attention. “Omidun” has been used traditionally to prepare several medicinal herbs in Nigeria. It is used to soak bark of root of some plants to treat fever and malaria. It is also popularly used as solvent for herbal extraction [6].The type of solvents and methods of preparation affect antimicrobial activity of plants [11].

“Omidun/omi-ogi”, the supernatant of “ogi” an acid fermented cereal mash made from maize or corn has been used in the extraction of antimicrobial agents from some leaves such as *Bryophyllum pinnatum* and *Kalanchoe crenata*. The extracts were found inhibitory against some Gram-negative organisms [6]. Some microorganisms associated with the supernatant solution of fermented cereal mash have been found to posses therapeutic properties against some diarrhea causing microorganisms [12].

Report have shown research work had been done on Shea-nut tree but nothing on the comparative antimicrobial effect of the different tree parts extracted using the conventional and ethno-botanical approach, hence this research to investigate the potential therapeutic effects of the different parts of Shea- nut tree extracted using Methanol, Omidun, Sterile-omidun and aqueous on some enterobacteria.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh leaves, roots and barks of *Vitellaria paradoxa* were collected from Onipako village, Ilorin Kwara State, Nigeria. The plant was identified by trained plant taxonomists at the Federal University of Agriculture Abeokuta, Nigeria. The collected Plant materials were washed with sterile water and dried under shade to prevent direct effect of sun, which might affect the chemical constituents in the extract.

2.2 Sample Preparation and Extraction Procedure

Two hundred grams of each plant were cut into small pieces with a surface-sterilized scalpel before milling with milling machine. A quantity (100g) of the fine powder each of the leaves, roots and bark was weighed and suspended into a 2500ml-capacity conical flask, after which, 1000ml of 95% methanol, sterile distilled water, sterile omidun and non- sterile omidun respectively and allowed to stand for 48 hours with constant shaking at regular intervals at room temperature. The percolates were then filtered and the solvents (methanol, omidun and water) were evaporated using a rotary evaporator (Stuart, Barloworld model RE 300) to obtain the methanol, omidun and aqueous extracts of each of the plant parts respectively. These were stored in sterile air-tight containers and stored in the refrigerator at 4ºC until needed for analysis.

2.3 Preparations of Dilutions of Crude Extract for Antibacterial Assay

For this, the method of Akujobi et al. [13] was used. In brief, the crude extracts (2.1g) were separately dissolved in 4ml 30% dimethyl sulphoxide (DMSO) and further diluted to obtain 100 mg/ml, 50mg/ml and 25 mg/ml concentrations. These were stored at 15ºC until required.

2.4 Test Microorganisms

The organisms *Escherichia coli* ATCC 25922, EPEC, EHEC, *Salmonella typhi* and *Shigella flexneri* were obtained from Sacred Heart Hospital Lantoro, Abeokuta and National Institute of Medical Research Yaba Lagos. They were re-isolated and the pure cultures were sub cultured on Nutrient agar slants. They were stored at 40ºC until required for the study.

2.5 Evaluation of Antimicrobial Activity

Antimicrobial activity was tested using a modified disc diffusion method originally described by Ncube et al. [14]. The filter paper discs (Whatman No.1, 6.3mm in diameter) were impregnated with each of the extracting solvents (omidun, sterile omidun, methanol and sterile distilled water) at different concentrations. The extracting solvents were used as negative control whereas a reference antibiotic (loperamide-1.0mg/disc) was used as positive control. One milliliter (equivalent to 10^6 CFU/ml) of overnight broth culture of each of the test organisms was transferred into sterile Petri-dishes (different organism per plate) using sterile syringe, overlaid with 20ml molten Mueller Hinton agar (MHA Difco, France).

The seeded plates were slowly rotated and allowed to set on a flat surface after a uniform distribution of the bacterial isolate. Filter paper discs containing each extract were arranged on the surface of the inoculated plates and kept in the refrigerator for 1 hour to allow the extracts to diffuse into the agar [15] before incubation for 24 h at 37ºC. The experiment was performed in triplicates after which the average diameter of zone of inhibition (mm) was measured and recorded.

2.6 Statistical Analysis

The data obtained from the study were analyzed statistically using the Analysis of Variance (ANOVA).

3. RESULTS AND DISCUSSION

The results obtained in this study indicated that the extracts inhibited the growth of the test organisms.

Results of the minimum inhibitory concentrations of the extracts on the test organisms are shown in Tables 1, 2, 3 and 4. The lowest minimum inhibitory concentration (MIC) of 3.13mg/ml was produced by bark on *Salmonella typh*i, whereas highest concentration of 50 mg/ml was observed against EPEC, *Shigella flexneri* and Salmonella *typhi* for all the plant parts. However, the MICs for the root were nil for *Sh. flexneri* and *E.coli* ATCC 25922.

Table 1. MIC for "Omidun" leaf, bark and root extracts of *V. paradoxa*

Test orgs.	MIC at different concentrations (mg/ml)		
	Leaf	Bark	Root
EHEC	12.5	6.25	12.5
EPEC	50	25	50
Sa. typhi	6.25	3.13	6.25
Sh. Flexneri	12.5	25.0	12.5
E. coli ATCC 25922	12.5	25	6.25

The antibacterial activities observed in the various plant parts may support their use in traditional medicine, since extracts of the plant have been reported to be used in the treatment of stomachache and reported that extracts of the plant are used in the treatment of stomach ache and in the control of diarrhoea and dysentery [16]. The low MIC exhibited by some of the extract on the test organisms is of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infections due to these microorganisms, especially as they frequently develop resistance to known antibiotics [17]. Their use also will reduce the cost of obtaining health care.

3.2 Antimicrobial Activities of Different Concentrations of Extracts

As shown in Tables 5, 6 and 7; there was significant difference ($p<0.05$) in the effects of the extracts when used at different concentrations. However, higher inhibition zone was produced when larger concentration of the extracts (100mg/ml) was used. Methanol extracts of the leaves, roots and barks produced the highest inhibition zones, followed by omidun extracts and the lowest inhibition zone was produced by aqueous and sterile omidun extracts used at high concentration. No inhibition was produced when they were used at low concentrations.

At 100mg/ml methanol extraction, the bark of Shea butter tree gave the highest potency (13.67 – 19.00mm) followed by extracts of leaves (11.33-16.70mm) while the extracts of roots had the lowest potency (6.67-17.00mm).

Increase in concentration of extracts increased the zone of inhibition of the test microorganisms. Other research works have also shown that extracts of plants inhibit the growth of various microorganisms at different concentrations [18,19,20]). The large zones of inhibition exhibited by the methanol extracts of the bark on *E. Coli* justified their use by traditional medical practitioners in the treatment of diarrhea and dysentery. *E. coli* is the common cause of travelers' diarrhea and other diarrheagenic infections in humans [21].

Table 2. MIC for Sterile "Omidun extracts of leaf, bark and root of *V. paradoxa*

Test orgs.	MIC at different concentrations (mg/ml)		
	Leaf	Bark	Root
EHEC	12.5	12.5	12.5
EPEC	12.5	50	50
Sa. typhi	25	12.5	25
Sh. Flexneri	25	12.5	50
E. coli ATCC 25922	25	12.5	12.5

Table 3. MIC for methanol extracts of leaf bark and root of *V. paradoxa*

Test orgs.	MIC at different concentrations (mg/ml)		
	Leaf	Bark	Root
EHEC	25	6.25	6.25
EPEC	12.5	12.5	6.25
Sa. typhi	50	12.5	12.5
Sh. Flexneri	50	25	25
E. coli ATCC 25922	12.5	6.25	25

Table 4. MIC for aqueous extracts of" leaf, bark and root of *V. paradoxa*

Test orgs.	MIC at different concentrations (mg/ml)		
	Leaf	Bark	Root
EHEC	25	12.5	50
EPEC	50	50	25
Sa. typhi	25	12.5	25
Sh. Flexneri	25	6.25	-
E. coli ATCC 25922	12.5	-	-

No growt

The inability of the sterile omidun and aqueous extracts to inhibit some organisms at low concentrations may be that they possess insignificant active components needed to inhibit the growth of the test organisms. Since plants possess various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of plants [22].

Other research works have also shown that extracts of plants inhibit the growth of various microorganisms at different concentrations [13,19].

Table 5. Antibacterial activities of the different concentrations of bark extract (mm)

		Organism				
		EHEC	**EPEC**	***S. typhi***	***Sh. flexneri***	***E. coli* ATCC 25922**
Concentration (mg/ml) 100	Methanol	13.67±1.53	15.00±1.00	15.67±2.08	18.67±0.57	19.00±1.00
	Sterile omidun	6.00±1.00	6.33±0.58	6.67±0.57	Xxx	Xxx
	Omidun	11.67±0.58	13.00±1.00	12.33±1.52	15.67±0.58	17.33±1.53
	Aqueous	2.33±4.04	2.00±3.46	3.67±3.51	4.00±3.61	2.00±3.46
Concentration (mg/ml) 50	Methanol	9.33±1.53	11.00±1.00	11.00±1.00	11.00±1.00	6.67±2.08
	Sterile omidun	Xxx	Xxx	Xxx	Xxx	Xxx
	Omidun	8.00±1.00	9.33±0.58	10.11±1.16	9.33±0.58	6.00±2.00
	Aqueous	2.00±3.46	2.00±3.46	1.67±2.08	3.00±2.65	1.00±1.73
Concentration (mg/ml) 25	Methanol	7.00±1.00	7.33±0.58	5.00±1.00	3.33±0.58	4.67±0.58
	Sterile omidun	Xxx	Xxx	Xxx	Xxx	Xxx
	Omidun	5.00±1.00	6.67±0.58	5.00±2.65	2.67±0.58	3.33±0.58
	Aqueous	Xxx	Xxx	Xxx	Xxx	Xxx

xxx- No inhibition zone

Table 6. Antibacterial activity of the different concentrations of methanol, omidun, sterile omidun and aqueous extract of root (mm)

		Organism				
		EHEC	**EPEC**	***S. typhi***	***Sh. flexneri***	***E. coli* ATCC 25922**
Concentration (mg/ml) 100	Methanol	12.67±1.16	6.67±2.08	17.00±1.00	14.67±1.16	15.00±1.00
	Sterile omidun	5.33±0.58	5.33±0.58	6.67±1.16	Xxx	Xxx
	Omidun	11.00±1.00	4.67±1.16	12.33±1.53	10.33±0.58	12.67±1.53
	Aqueous	5.33±0.58	5.33±0.58	5.67±1.16	4.67±3.06	5.33±3.51
Concentration (mg/ml) 50	Methanol	8.00±1.00	4.67±1.16	13.33±4.93	13.33±4.93	9.33±0.58
	Sterile omidun	Xxx	Xxx	Xxx	Xxx	Xxx
	Omidun	7.00±1.00	4.67±0.58	9.33±0.58	9.33±0.58	11.00±1.00
	Aqueous	Xxx	Xxx	1.67±2.89	1.33±2.31	Xxx
Concentration (mg/ml) 25	Methanol	6.33±1.16	3.00±1.00	2.67±1.16	3.00±1.00	4.67±0.58
	Sterile omidun	Xxx	Xxx	Xxx	Xxx	Xxx
	Omidun	4.67±0.58	2.67±0.58	2.67±0.58	2.33±0.58	3.67±0.58
	Aqueous	Xxx	Xxx	Xxx	Xxx	Xxx

Xxx- No Inhibition zone

Table 7. Antibacterial activity of the different concentrations of methanol, omidun, sterile omidun and aqueous extract of leaf (mm)

		Organism				
		EHEC	**EPEC**	***S. typhi***	***Sh. flexneri***	***E. coli* ATCC 25922**
Concentration (mg/ml) 100	Methanol	11.33±1.53	12.67±1.53	13.33±1.16	15.67±0.58	16.67±0.58
	Sterile omidun	5.00±1.00	5.00±1.00	5.67±0.58	Xxx	Xxx
	Omidun	12.67±3.79	10.00±1.00	11.67±2.08	12.00±1.00	13.67±2.52
	Aqueous	5.00±3.61	4.67±3.79	3.33±3.06	3.00±2.65	2.00±3.46
Concentration (mg/ml) 50	Methanol	4.67±1.16	8.67±1.53	11.00±1.00	11.00±1.00	9.33±0.58
	Sterile omidun	Xxx	Xxx	Xxx	Xxx	Xxx
	Omidun	4.00±1.00	7.67±1.53	9.67±1.16	9.33±0.58	7.33±0.58
	Aqueous	Xxx	Xxx	Xxx	Xxx	Xxx
Concentration (mg/ml) 25	Methanol	3.00±1.00	4.33±1.53	5.00±1.00	3.33±0.58	6.67±1.16
	Sterile omidun	Xxx	Xxx	Xxx	Xxx	Xxx
	Omidun	2.67±0.58	3.67±1.16	4.67±0.58	3.33±1.16	3.33±0.58
	Aqueous	Xxx	Xxx	Xxx	Xxx	Xxx

Xxx- No Inhibition zone

4. CONCLUSION

It was also observed from this work that methanol and unsterile omidun were better for extraction than water. This agrees with the report of Boer et al. 2005 who stated that active components of plants are more soluble in organic solvent and Falana et al. 2011 who reported the presence of some microorganisms with anti diarrhea properties in omidun.

The high potency exhibited by methanol extract may be because of its chemical composition, this agrees with previous report by Chatha et al. [23], that maximum extract yield from rice bran was obtained with methanol. However, the potency of omidun extracts can be attributed to its microbial compositon, *Lactobacillus plantaum* [24]. *L. plantarum* has also been reported to produce metabolic products which are with antimicrobial activities [25].

Also, the antibacterial activities observed in the various plant parts confirm their use in traditional medicine. As reported that extracts of the plant are used in the treatment of stomach ache and in the control of diarrhoea and dysentery [16].

The study has also shown that the observed *in vitro* antibacterial effect of *the various parts of Vitellaria paradoxa* on the bacterial isolates appear interesting and promising. Therefore, the *in vivo* antimicrobial effects of these plant parts will be of interest for further study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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