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Antimicrobial Efficacy of *Vitellaria paradoxa* fractions and compounds on some wood Fungi and Bacteria

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Abstract
This study examined antifungal efficacy of *Vitellaria paradoxa* fractions and compounds in the control of some wood degrading fungi. Stem bark and heartwood parts of *Vitellaria paradoxa* were collected, dried, pulverised and macerated sequentially in n- hexane, methanol and ethyl acetate solvents. The mixtures were filtered, evaporated and the dried samples were mixed and run over silica gel in column chromatography with a mixture of n -hexane and ethyl acetate solvents to obtain fractions. The fractions collected were evaporated and those with white needles were subjected to Magnetic Resonance (NMR) spectroscopic analysis. Spinasterol was isolated and characterised from the heartwood fraction while the stem bark fractions were fatty. *Vitellaria paradoxa* fractions were active against *Serpula lacrymans, Sclerotium rolfsii, Aspergillus fumigatus, Fomitopsis pinicoca, Phaeolus schweinitzii, Rhizopus* sp., *Coniophora puteana, Gloeophyllum sepiarium, and Fibroporia vaillantii* at zones of inhibition (ZOI) of 18 mm - 24 mm. Although the antibiotics were active (25 – 31 mm), they were found inactive against the *Fomitopsis pinicoca* fungus which was sensitive to all the *V. paradoxa* fractions at zones of inhibition of 18 - 24 mm. The minimum inhibitory concentrations (MIC) of the *V. paradoxa* fractions were active at 50 µg/mL against all test fungi. At minimum fungicidal concentration (MFC) of between 50 - 200 µg/mL, all the test fungi were killed. Based on the ZOI, MIC and MFC, the *V. paradoxa* stem bark heartwood fractions have been proven to be very efficient in inhibiting the growth of test wood rot fungi; hence the species could be explored as a potential source of bioactive fungicides.

Keywords: Bacteria, Compound, Fraction, Fungi, Spinasterol, *Vitellaria paradoxa*
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Introduction

Vitellaria paradoxa (C. F. Gaertn) formally referred to as *Butyrospermum parkii* and *Butyrospermum paradoxa* belongs to the family Sapotaceae. It is commonly known as Shea tree or Shea butter tree (IPGRI, INIA. 2006). In English, it is called Shea butter tree; Shea nut tree; karate in French (Teketay et al., 2003); *Ka'danya, Kadanya, Mankade* in Hausa; *Okwuma, Osisi* in Igbo (Audu & Awulu, 2017), *Ori, Emi* in Yoruba (Animasaun et al., 2019), and *Chamegh* in Tiv (Shomkegh et al., 2016). The Shea butter tree is of average height of 10 - 15 m with dense spreading canopy, thick, dark and fissured bark (ICRAF, 2000). This species is known both as a fruit tree and as an oilseed crop (Ugese et al., 2008).

The species originated in African and is found in areas with 400 -1800 mm rainfall per year (IPGRI, INIA. 2006). It either grows naturally or is farmed as a tree crop in the arid Savanna region of West Africa countries (Audu & Awulu, 2017). It is believed to have spread from Senegal in West Africa to Uganda in East Africa till Adamaoua Province in Cameroon which is in North-South

Africa (IPGRI, INIA. 2006). *Vitellaria paradoxa* grows in the wild area of dry savannah region of West Africa from Senegal in the west to Sudan in the east to the foothills of Ethiopian highlands. This species also grows in nineteen countries of Africa. Namely Burkina Faso**,** Ghana, Chad, Cameroon, Central African Republic, Ethiopia, Niger, Ivory Coast, Mali, Nigeria, Sierra Leone, Guinea Bissau, Togo Uganda, Zaire, Senegal, Guinea, Benin and Sudan (Warra, 2011).

Vitellaria paradoxa is multipurpose agroforestry indigenous tree species that contribute immensely to the livelihood of rural communities for income generation and as source of raw material for many industries. The ripe fruits are eaten as a snack and as famine food. Shea butter, or Shea oil, is a raw material used in factories to manufacture margarine, baking fat, cocoa butter substitutes as well as different pharmaceutical and moisturising beauty products (IPGRI, INIA. 2006). Shea butter leaves are good forage for animal feeding, soil improvement (Ziblim et al., 2015). The species has the capacity to improve nutrition, increase healthcare, decrease rural poverty and aid sustainable land care (Moore, 2008).The wooden stem is resistant to termites and a good timber used for various constructing purposes.

Several authors have reported the use of Shea butter in medicine (Prescott et al*.,* 2002; El-Mahmoud et al., 2008; Ahmed and Sani, 2013; Ahmed et al., 2012; Fodouop et al., 2015). It is used in the treatment of rheumatic and joint pains and healing of wounds, managing swellings and bruises, dermatitis and other skin (Fodouop et al*.,* 2015). Some people have been reported to consume extract of *V. paradoxa* for treatment of various bacterial and fungal infections (Kalgo et al., 2019). The stem back extract possesses immunomodulatory properties which have effect on human neutrophils viability and function (Kalgo et al., 2019) and contain antioxidant constituents with antimicrobial activities (Olasunkanmi et al., 2017). Shea tree bark, if purified, could be used to produce an antiseptic agent capable of treating skin infections caused by groups of fungi (Ahmed et al., 2009).

Vitellaria paradoxa has been researched as an effective medicinal plant (Prescott et al., 2002). It has proved to be active against bacterial and fungal diseases (El-Mahmoud et al., 2008; Ahmed and Sani, 2013). The barks, leaves and roots extracts of *V. paradoxa* contain phytochemical constituents inhibits growth of some dermatophytes (Boyejo et al., 2019) and could be used as a potential source of antibiotic substance for a drug development (Ajijolakewu and Awarun, 2015). *Vitellaria paradoxa* may be used as potent sources of bioactive substances in the production of drugs against diseases caused by superficial and enteric organisms (Ajijolakewu and Awarun, 2015). The leaf, stem bark, and seed of shea tree contain a host of bioactive compounds that can be utilized in the control of infections caused by *T. mentagrophyte, A. fumigatus, E. flocossum, T. rubrum,* and *M. Audouinii* (Ahmed et al., 2012).

Wood is versatile and can be used for a very long time but it is subject to biodeterioration. Wood deterioration is a very essential process in the environment that recycles complex organic materials and it is a fundamental component of life (Blanchette, 2000). Primarily, fungi, inserts, ants and bacteria are some of the agents of deterioration. Wood decay fungi are classified into two major groups: white- and brown-rot fungi (Blanchette, 2000). White rot fungi can degrade all cell wall components, including lignin. They cause bleaching of common wood colouration, metabolize large amounts of lignin in wood is unique among microorganisms, degrade cells and reduce the strength properties of wood in the late stages of decay. On other hand, brown-rot fungi depolymerise cellulose quickly during initial stages of wood colonization. Substantial losses in wood strength start very early in the decay process, frequently before decay evidence is visually shown (Blanchette, 2000). Bacteria are known to have the ability to decompose wood cellulose although their influence on wood decay is restricted. Different bacteria from woodland soil contain enzymes used in the breakdown of cellulose and cellulose products (Llado´ et al., 2015).

Fungi and bacteria play a major role in the well being, diversity, and productivity of forest ecosystems. Fungi feed on woody products and serve to recycle nutrients. As a result, they physically and chemically break down wood products thereby causing lots of economic hazards (Marcot, 2007). Despite the many studies

that have been done on the medicinal and antimicrobial properties of *V. paradoxa,* not much has been done on the effect of *V. paradoxa* activity on wood degrading fungi and bacteria. Therefore, this study was undertaken to evaluate the compounds present in the stem bark and heartwood fractions of *V. paradoxa* and assess their effect on selected wood decay fungi and bacteria.

Materials and Methods

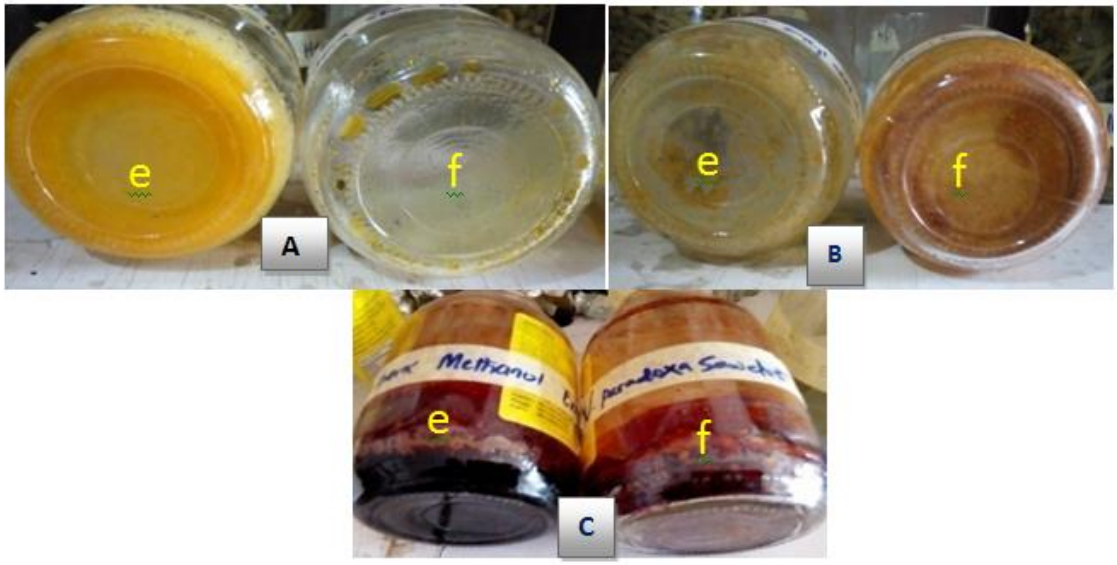
Plant parts Collection and preparation

Stem bark of *V. paradoxa* was collected from the wild at the Federal University of Agriculture, Makurdi campus, located between longitudes 8° 21˙ and 9° E and latitudes 7° 21˙ and 8° N in Benue State, Nigeria, within the southern guinea savannah ecological zone (Seibert, 2007). The stem bark was air- dried during harmattan season and pulverised. The heartwood was sawn at Makurdi Head Bridge Timber Shed to collect its sawdust which was also dried to avoid being damp.

Plate 1: Stem and canopy of *Vitellaria paradoxa* tree at College of Animal Science, FUAM

Extraction of plant materials

Extraction of *V. paradoxa* stem bark and heartwood was done successively by dissolving 1,000 g and 600 g of stem bark and heartwood, respectively, into 1,000 mL (w/v) of n-hexane for 24 hours, followed by filtration of the n' hexane extract. The residue left from the hexane extraction was again macerated with ethyl acetate and methanol, respectively, for 24 hours each. The mixtures were filtered with Whatman No.1 filter papers into well labelled glass bottles as n -hexane, ethyl acetate and methanol extracts, respectively. The filtrates were evaporated to obtain dried extracts (Plate 1).



Key: [A] N' Hexane extracts, [B] Ethyl acetate extracts [C] Methanol extracts [e] Dried filtrate of stem bark extracts [f], Dried filtrate of sawdust extracts

Plate 1: Dried samples of *V. paradoxa* heartwood (sawdust) and Stem Bark Extracts

Column Chromatography and Nuclear Magnetic Resonance (NMR) spectroscopic analysis

Dried crude mixture of n-hexane and ethyl acetate extracts was run over silica gel with solvent mixtures of successive increasing polarity of hexane and ethyl acetate in the ratio of 95:5 - 0:100, respectively, in column to produce pure compounds or fractions according to Ekhuemelo et al. (2018). The fractions of the pure compounds were collected in well- labelled vials and allowed to dry (Plate 2). Nuclear Magnetic Resonance (NMR) spectroscopic analysis was done on selected fractions which contained white needle-shaped crystals. NMR data was acquired on a Bruker-Avance 500MHz spectrometer. The NMR data was processed using MestreNova® 12 software. The characterization was done using the ^{1}H NMR data.

Plate 2: Collected *V. paradoxa* Fractions in Vials from Column Chromatography

Standardization of the test bacteria and fungi

Standardized inoculums of each test fungus (*Serpula lacrymans Sclerotium rolfsii, Aspergillus fumigatus, Fomitopsis pinicoca, Phaeolus schweinitzii, Rhizopus* sp., *Coniophora puteana, Gloeophyllum sepiarium, and Fibroporia vaillantii*) and each test bacterium (*Acidobacterium*

capsulatum, Actinobacteria sp. Agrobacterium tumefaciens, Bacillus subtilis Ralstonia solanacearum, Enterococcus faecium, Pseudomonas syringae, Escherichia coli, Proteus mirabilis and *Pseudomonas aeruginosa*) were achieved by preparing their respective suspensions up to 0.5 McFarland standards. The growth of the actively growing culture in the broth was altered with sterile saline or broth to obtain turbidity that was clearly comparable to that of the 0.5 McFarland standards according to Lalitha, (2004); Kuta et al. (2017).

Determination of antimicrobial activity of Vitellaria paradoxa fractions

The activity of *V. paradoxa* fractions on test and bacteria and fungi samples was investigated using agar diffusion method. Concentrations of 200 µg/mL and 100 µg/mL were prepared from each fraction and antibiotics respectively. The antibiotics used as control were Ketoconazole, fluconazole and Fulcin for fungi; and ciprofloxacin, sparfloxacin and cefuroxime for bacteria according to Ekhuemelo *et al.* (2018). The Muller-Hinton and Sabouraud Dextrose agar were used in the culture of bacteria and fungi, respectively. The incubation of test fungi was made at 30 ^{o}C for 7 days and at 37 ^{o}C for 24 hours for bacteria. During the period plates of the media were observed for inhibition of growth. Zones of inhibition were measured and results recorded in millimetres (mm).

Minimum inhibitory concentration (MIC)/ Minimum Fungicidal concentration (MFC)

The MIC of the antifungal fractions and compounds was determined by broth dilution methods. Different concentrations (200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL) of fractions were added to media in the Petri dishes and inoculated with the test fungi. The mixture was incubated and examined for growth according to Ekhuemelo et al. (2018). The lowest concentration of the fraction that inhibits visible growth of the test fungi was recorded as the MIC.

The MFC of the fraction was determined by sub-culturing the contents of the Petri dishes that showed inhibition on Muller-Hinton and Sabouraud Dextrose agar plates for bacteria and fungi, respectively, while the absence of growth on incubation was recorded as MFC.

Results

Characterization of VPH22 as Spinasterol

Vitellaria paradoxa heartwood fraction (VPH22) was obtained as white needles. It's proton nuclear magnetic resonance spectrum (^{1}H-NMR) gave the following data (Figure 1) below: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.19 – 5.13 (m, 2H), 5.03 (dd, *J* = 15.1, 8.6 Hz, 1H), 3.60 (tt, *J* = 10.7, 4.4 Hz, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.09 – 1.96 (m, 6H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.88 (s, 2H), 0.86 (s, 3H), 0.84 (s, 3H), 0.82 (s, 1H), 0.81 (s, 2H), 0.80 (s, 8H), 0.55 (s, 2H). The signals at δ 5.19 – 5.13 (m, 2H) and 5.03 (dd, *J* = 15.1, 8.6 Hz, 1H), indicate the presence of olefinic bonds (Alexandri et al., 2017), while that at 3.60 (tt, *J* = 10.7, 4.4 Hz, 1H) indicate an oxymethine proton (Sun and Yasukawa, 2008); signals at 2.34 (t, *J* = 7.5 Hz, 2H) and 2.09 – 1.96 (m, 6H) are representative of methine and methylene protons. The signals at 1.03 (d, *J* = 6.6 Hz, 3H), 0.88 (s, 2H), 0.86 (s, 3H), 0.84 (s, 3H), 0.81 (s, 3H) and 0.55 (s, 3H) are due to methyl protons (Cheung, and Williamson, 1969). The data acquired was reminiscent of that for sterols and triterpenes. A literature look up revealed the data to be unambiguously identical to that of spinasterol (Villaseñor and Domingo, 2008). *Vitellaria paradoxa* heartwood fraction (VPH22) was characterised as Spinasterol (Figure 2).

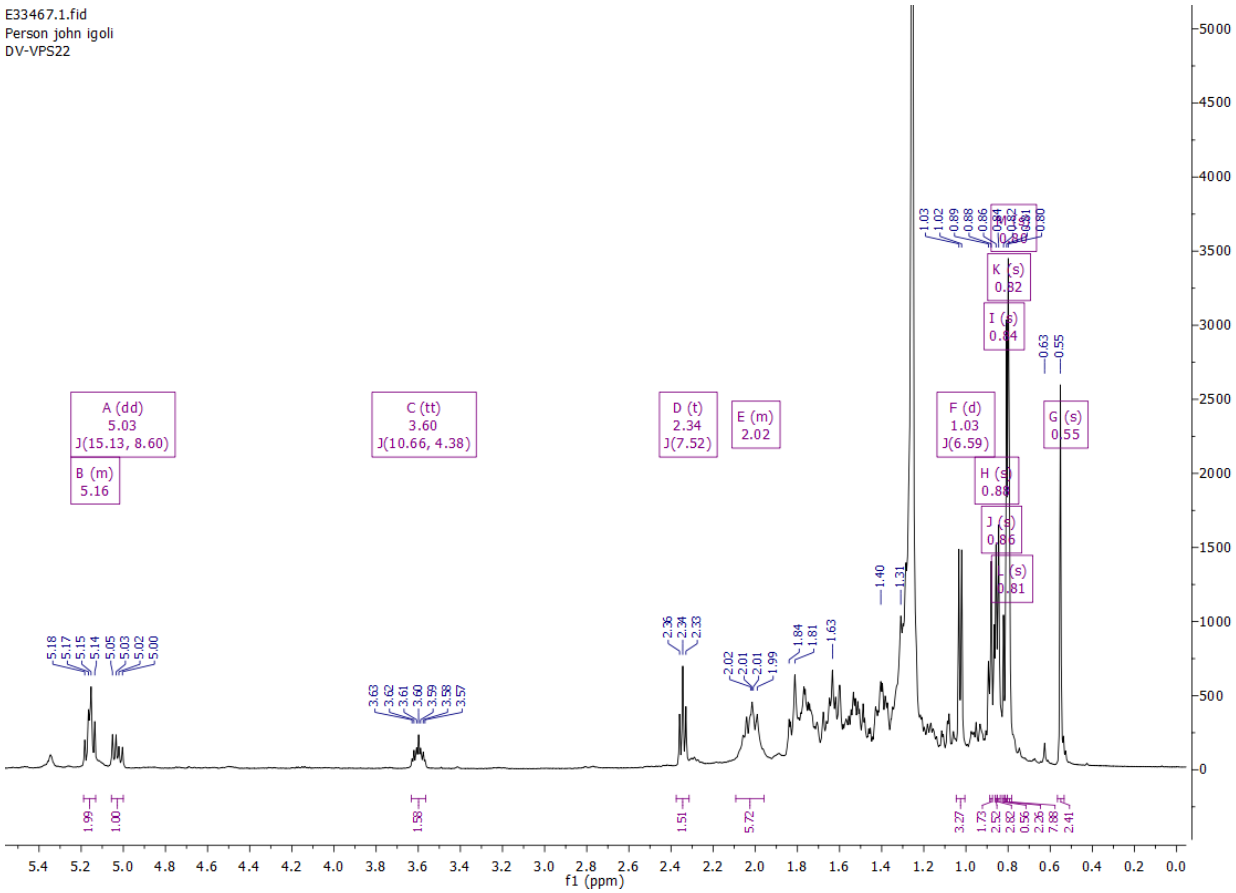


Figure 1: Proton NMR Spectrum of *Vitellaria paradoxa* heartwood fraction (VPH22)

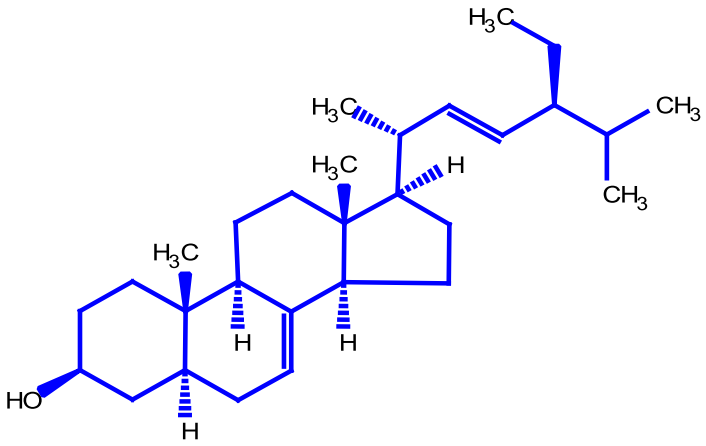


Figure 2: Structure of VPH22 (Spinasterol)

Effect of Antifungal activities of V. paradoxa fractions and Antibiotics against test fungi

The test fungi were sensitive to all the *V. paradoxa* fractions (VP17, VP28, VP34, VP47, VP23, VP134, and VPH22) at zones of inhibition (ZOI) of between 18 and 24 mm (Table 1). *Aspergillus fumigatus, Coniophora puteana, Fomitopsis pinicoca, Gloeophyllum sepiarium, Phaeolus schweinitzii, Rhizopus* sp. and *Serpula lacrymans* were sensitive to *V. paradoxa* stem bark fraction (VP17) with ZOI of between 20 mm to 24 mm. It was also observed that *Coniophora puteana, Fibroporia vaillantii, Fomitopsis pinicoca*, *Gloeophyllum sepiarium, Rhizopus* sp., *Serpula lacrymans and Sclerotium rolfsii* were sensitive to *V. paradoxa* stem

bark fraction (VP28) with ZOI ranging between 20 mm and 24 mm while the antibiotics were active on all test fungi at ZOI of 25 – 31 mm except *F. pinicoca* which was resistant.

Effect of Minimum inhibition concentration (MIC) of V. paradoxa Fraction against test fungi

At the minimum inhibition concentration (MIC) of 50 µg/mL, VP23 fraction prevented the growth of *Aspergillus fumigatus, Phaeolus schweinitzii, Rhizopus* sp. and *Serpula lacrymans* test pathogens (Table 2). Also, at 50 µg/mL*, V. paradoxa* stem bark fraction (VP34) stopped the growth of *Aspergillus fumigatus, Fibroporia vaillantii, Fomitopsis pinicoca, Phaeolus schweinitzii,, Rhizopus* sp. and *Serpula lacrymans* test pathogens while, *V. paradoxa* heartwood fraction **(**VP22) only inhibited *Coniophora puteana, Fibroporia vaillantii, Rhizopus* sp. and *Serpula lacrymans* test pathogens. However, at100 µg/mL*, Fibroporia vaillantii* and *Fomitopsis pinicoca* growth was prevented by VP23 isolate while *Coniophora puteana and Fibroporia vaillantii* growths were inhibited by *V. paradoxa* stem bark fraction **(**VP134).

Effect of Minimum Fungicidal concentration of V. paradoxa fractions on test Fungi

Table 2 presents the MFC of *V. paradoxa* fractions of VP17, VP28, VP34, VP47, VP23, VPH22 and VP134 against test fungi. VP134 fraction was the most active fraction compared to the rest as it completely inhibited the growths of *Fomitopsis pinicoca, Rhizopus* sp. and *Serpula lacrymans* at MFC of 50 µg/mL. At 100 µg/mL, VPH22 fraction killed *Fomitopsis pinicoca* while VP134 fraction completely stopped the growth of *Coniophora puteana* and *Fibroporia vaillantii.* At 200 µg/mL rest fractions killed all the test fungi.

Table 1: Sensitivity of *Vitellaria paradoxa* Fractions and Standard Antibiotics against Test Fungi

S/No	Test fungi	Zone of Inhibition (mm) of *Vitellaria paradoxa* Fractions (200 µg/mL)							Zone of Inhibition (mm) of Antibiotic (100 µg/mL)		
		VP17	VP28	VP34	VP47	VP23	VP134	VPH22	Fluconazole	Fulcin	Ketoconazole
1	*S. rolfsii*	0	21	24	21	0	0	0	0	0	25
2	*S. lacrymans*	21	20	23	20	21	23	20	0	30	30
4	*Rhizopus* sp.	20	23	0	24	23	21	22	0	29	27
3	*P. schweinitzii,*	20	0	20	23	20	0	21	0	25	0
5	*G. sepiarium*	24	21	21	0	0	0	0	29	0	28
6	*F. vaillantii*	23	20	22	0	19	20	23	0	28	0
7	*F. pinicoca*	0	24	0	0	18	19	21	0	0	0
8	*C. puteana*	21	23	20	23	0	18	0	0	31	0
9	*A. fumigatus*	20	0	20	0	21	0	20	0	29	25

Key: R = Resistance, VP = *Vitellaria paradoxa* stem bark, VPH= *Vitellaria paradoxa* heartwood, ZOI < 10 mm is inactive; 10 -13 mm is partially active; 14 -19 mm is active, and >19 mm is very active.

Table 2: Minimum Inhibition Concentration and Minimum Fungicidal Concentration of *Vitellaria paradoxa* against Test Fungi

S/ No	Test Fungi	VP17 (µg/mL)		VP28 (µg/mL)		VP34 (µg/mL)		VP47 (µg/mL)		VP23 (µg/mL)		VPH 22 (µg/mL)		VP 134 (µg/mL)	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1	*S. rolfsii*	R	R	50	200	50	100	50	200	R	R	R	R	R	R
2	*S. lacrymans*	50	200	50	200	50	100	50	200	50	200	50	200	50	50
4	*Rhizopus* sp.	50	200	50	200	R	R	50	100	50	200	50	200	50	50
3	*P. schweinitzii,*	50	200	R	R	50	200	50	100	50	200	50	200	R	R
5	*G. sepiarium*	50	100	50	200	50	200	R	R	R	R	R	R	R	R
6	*F. vaillantii*	R	R	50	100	R	R	R	R	100	200	50	200	100	100
7	*F. pinicoca*	50	100	50	200	50	200	R	R	100	200	50	100	50	50
8	*C. puteana*	50	200	50	100	50	200	50	100	R	R	R	R	100	100
9	*A. fumigatus*	50	200	R	R	50	200	R	R	50	200	50	200	R	R

Key: VP = *Vitellaria paradoxa* stem bark, **VPH**= *Vitellaria paradoxa* heartwood; **R** = Resistance; **MIC** = Minimum Inhibitory Concentration, **MFC =** Minimum Fungicidal Concentration

Effect of V. Paradoxa fractions and Antibiotics against test bacterial

The antibacterial activities and zones of inhibition (ZOI) of *V. paradoxa* fractions (VP17, VP28, VP34, VP47, VP23, VP134 and VPH31) indicated that all the test bacteria were sensitive to *V. paradoxa* fractions at ZOI ranging from 20 - 32 mm while the three antibiotics were active at zones of inhibition of 29 - 39 mm for all the bacteria except *P. aeruginosa* (Table 3). *Vitellaria paradoxa* stem bark fractions were more active at ZOI of 20 – 32 mm compared to the heartwood fraction which was active at ZOI of between 24 – 28 mm.

Effect of minimum inhibition concentration and minimum bactericidal concentration (MBC) of V. paradoxa Fractions against Test Bacteria

The minimum inhibition concentration of *V. paradoxa* fractions against the test bacteria showed that stem bark fraction (VP 23) was the most active with MIC of 25 µg/mL while the remaining fractions inhibited the test bacterial growths between MIC of 25 – 50 µg/mL. Similarly, the stem bark fraction (VP 23) of *V. paradoxa* had the least MBC of 50 µg/mL. The remaining fractions obtained from both stem bark and heartwood had MBC of between 100 – 200 µg/mL. *Pseudomonas aeruginosa* was the most resistant bacteria. Although it was resistant to the three antibiotics, its growth was inhibited by only VP134 at MIC 50 µg/mL and killed at MBC of 100 µg/mL.

Table 3: Sensitivity of *V. paradoxa* Fractions and Standard Antibiotics against Test Bacteria

S/No.	Test Bacteria	Zone of Inhibition (mm) of *V. paradoxa* Fractions (100 µg/mL)							Antibiotics (100 µg/mL)		
		VP17	VP28	VP34	VP47	VP23	VP134	VPH31	Ciprofloxacin	Sparfloxacin	Cefuroxime
1	*A. capsulatum*	27	0	23	23	28	0	0	0	32	0
2	*Actinobacterium sp.*	0	24	0	0	30	29	0	0	30	0
3	*A. tumefaciens*	23	23	30	30	0	24	25	32	0	34
4	*B. subtilis*	23	25	25	26	31	0	26	28	35	0
5	*R. solanacearum*	24	24	26	23	32	0	23	0	30	31
6	*E. faecium*	24	23	25	24	0	0	27	26	0	30
7	*E. coli*	0	0	27	24	28	25	0	34	29	39
8	*P. syringae*	20	24	0	26	27	0	28	0	31	0

9	*P. aeruginosa*	0	0	0	0	0	27	0	0	0	0
10	*P. mirabilis*	0	25	23	24	0	24	0	32	33	31

Zone of Inhibition < 10 mm is inactive; 10 -13 mm is partially active; 14 -19 mm is active, and >19 mm is very active.

S/No.	Test Bacteria	VP17 (µg/mL)		VP28 (µg/mL)		VP34 (µg/mL)		VP47 (µg/mL)		VP 23 (µg/mL)		VP134 (µg/mL)		VPH 31 (µg/mL)	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	*A. capsulatum*	25	100	R	R	50	100	50	200	25	50	R	R	R	R
2	*Actinobacteria sp.*	R	R	50	100	R	R	R	R	25	50	25	50	R	R
3	*A. tumefaciens*	50	200	50	200	25	50	25	50	R	R	50	100	50	100
4	*B. subtilis*	50	200	50	100	50	100	50	100	25	50	R	R	50	100
5	*R. solanacearum*	50	200	50	100	50	100	50	200	25	50	R	R	50	100
6	*E. faecium*	50	200	50	100	50	100	50	100	R	R	R	R	25	50
7	*E. coli*	R	R	R	R	25	50	50	100	25	50	50	100	R	R
8	*P. syringae*	50	200	50	100	R	R	50	100	25	50	R	R	25	50
9	*P. aeruginosa*	R	R	R	R	R	R	R	R	R	R	25	50	R	R
10	*P. mirabilis*	R	R	50	100	50	100	50	50	R	R	50	100	R	R

Table 4: Minimum Inhibition Concentration and minimum Bactericidal concentration (MBC) of *V. paradoxa* Fractions against Test Bacteria

Key: VP = *Vitellaria paradoxa* tem bark; VPH = *Vitellaria paradoxa* heartwood; R = Resistance; MIC =Minimum Inhibitory Concentration, MBC = minimum bactericidal concentration

Discussion

Vitellaria paradoxa heartwood fraction (VPH22) was characterised as spinasterol while the other fractions were fatty. Pohl *et al.* (2011) noted that fatty acids contain antibacterial, antimalarial and antifungal activity. It was observed that though they may not be as efficient as chemical fungicides, the environmental risks are less. Meneses-Sagrero (2017) reported spinasterol in the methanol extract of *Stegnosperma halimifolium* to have anti-proliferative (a substance used to prevent or retard the spread of cells) properties. El Kharrasi *et al.* (2014) in their studies observed that spinasterol obtained from argan oil and cactus pear seed oil had inhibitory activity on the cellular growth of microglial murine cells. Meneses-Sagrero *et al.* (2017) reported spinasterol compounds isolated from methanol extract of *Pueraria mirifica* roots to exhibit strong anti-proliferative effects against breast cancer and cervical cells. Meneses-Sagrero *et al.* (2017) also reported that spinasterol isolated from the n-hexane fraction and methanol extracts of *S. halimifolium* stem had anti-proliferative effects on cervical cancer and murine macrophage cancer cells. Csupor-Loaffer *et al.* (2011) observed that spinasterol isolated from *Conyza canadensis* plant species had anti-proliferative activity against breast cancer cells (MCF-7), cervical cancer (HeLa) cell line, non-cancerous human fetal fibroblast (MRC-5) cell line and epidermoid carcinoma (A431) cell lines. Kuiate *et al.* (2009) reported spinasterol as one the most active compounds of *Microglossa angolensis* with active antibacterial and antifungal principles. The study of antifungal activity by Johann *et al.* (2011) on five species of polygala (*P. campestris, P. cyparissias, P. paniculata, P. pulchella* and *P. sabulosa*) revealed that spinasterol compound showed a wide activity against the test microbes. Garba and Salihu, (2011) had isolated ketone (2), 1-phenyl-1, 4-pentanedione and ester (1), 2-O-butyl-1-O-(2'-ethylhexyl) benzene-1, 8-dicarboxylate from *Vitellaria paradoxa.* Fatty acids with their derivatives are promising great potential as environmentally friendly antifungal agents that can lead to novel antifungal drugs (Pohl *et al.,* 2011).

The zones of inhibition of *V. paradoxa* fractions (18 - 24 mm) were at the same range of 25 mm – 31 mm recorded for the three antibiotics. *Fomitopsis pinicoca* fungus was resistant to all antibiotics but was sensitive to all fractions of *V. paradoxa* at zones of inhibition of 18 - 24 mm.

These values are within the very active (>19 mm) range described by Guevara, (2005), as a standard zone of inhibition of antibiotics. Boyejo *et al.* (2019) reported zones of inhibition of *V. paradoxa* ethanol crude bark extract on dermatophytes as 20.5mm *(Microsporum audouinii),* 19.5 mm (*Microsporum ferrugenum)* and 17.5mm (*Trichophyton rubrum*) while the acetone bark extract were 19 mm (*Microsporum audouinii),* 17.5 mm (*Microsporum ferrugenum*) and 16.5 mm (*Trichophyton rubrum*) at concentration of 250 mg/mL.

The zone of inhibition of *V. paradoxa* fractions on all test bacteria ranged from 20 - 32 mm while the antibiotics were between 29 - 39 mm. Although ZOI recorded for *V. paradoxa* fractions were slightly lower than the ones for the three antibiotics, they both were very active according to Guevara, (2005) who reported ZOI greater than 19 mm to be very active. Garba and Salihu, (2011) observed that ketone (2), 1-phenyl-1,4-pentanedione and ester (1), 2-O-butyl-1-O-(2'-ethylhexyl) benzene-1,8-dicarboxylate compounds isolated from *V. paradoxa* recorded zones of inhibition of 25 - 28 mm against *S. aureus* and *B. subtilis* (gram positive bacteria) and *E. coli, S. typhi* and *P. aeruginosa* (gram negative) at the concentration of 7x102 µg cm^{-3}.

Minimum inhibitory concentrations (MIC) of *V. paradoxa* fractions were active at 50 µg/mL against all test fungi. At minimum fungicidal concentration (MFC) of between 50 - 200 µg/mL*,* all test fungi were killed. This performance of *V. paradoxa* may be due to the presence of spinasterol compounds*.* Freire *et al.* (2005) reported the presence of spinasterol as natural sources of bioactive sterols from the wood and bark of the Acacia species (*A. longifolia. A. dealbata, A. melanoxylon* and *A. retinodes)*. Spinasterol and a-Spinasterol have been reported by Ravikumar *et al.* (2010) to show antiproliferative effects that inhibit cell growth. Spinasterol has also been demonstrated to be a powerful inhibitor of glomerular mesangial cell multiplication (Ravikumar et al., 2010). Villasenor and Domingo (2000) reported result of in vivo studies that proved the efficacy of anti-tumorigenic activity of spinasterol to skin tumours without co-tumour or co-carcinogen promoter activities as well as possessing active anti-proliferative effect on gynaecological cancer cells.

The MIC of *V. paradoxa* on all the test bacteria was between 25 – 50 µg/mL while the MBC was between 50 - 200 µg/mL. Least MIC (25 µg/mL) and MBC (50 µg/mL) were recorded for the stem bark. This implies that fractions from the stem bark were more potent on the test bacteria than on the fractions from the heartwood. Kuete *et al.* (2009) reported MIC of 625 µg/mL for *Citrobacter freundii*, 156 - 625 µg/mL for *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa*; 312 – 625 for µg/mL *Klebsiella pneumoniae* 312 µg/mL for *Salmonella typhi*; 39 - 312 µg/mL for *Staphylococcus aureus* and 156 – 312 µg/mL for *Streptococcus faecalis* from *Ficus ovate* fractions. Kuete *et al.* (2009) also recorded MBC of >625 µg/mL for *Citrobacter freundii*; 156 - 625 µg/mL *Escherichia coli*; 625 - > 625 µg/mL for *Pseudomonas aeruginosa* and *Klebsiella pneumonia*; 625 µg/mL *Salmonella typhi* and *Streptococcus faecalis*; 312 - > 625 µg/mL *Bacillus cereus* and 78 - 625 µg/mL *Staphylococcus aureus*, respectively from *Ficus ovate* fractions.

This study revealed that *V. paradoxa* heartwood fractions possess spinasterol. The stem bark was more active on wood fungi compared to the heartwood fraction. This agrees with Boyejo *et al.* (2019) who reported that stem bark extract of *V. paradoxa* was most active compared to the leaves and roots extracts. The presence of spinasterol in the heartwood could be due to the deposition and accumulation of extractives in the heartwood of the tree over a period of time.

Conclusion

Spinasterol compound was isolated from the heartwood of *Vitellaria paradoxa.* All fractions exhibited high antifungal and antibacterial activity as it controlled all test wood fungi and bacteria. Although *Fomitopsis pinicoca* fungus and *Pseudomonas aeruginosa* bacteria were resistant to all antibiotics, they were sensitive to fractions of *V. paradoxa* probably because of its spinasterol compound which is a natural source of bioactive sterols. The stem bark was more active on wood fungi compared to the heartwood fraction. The results of ZOI, MIC and MFC showed that *V. paradoxa* stem bark heartwood fractions were

very efficient in inhibiting the growth of test wood rot fungi and wood colonising bacteria; hence the species should be explored as a potential source of bioactive fungicides.

Reference

Ahmed, R.N., Abdulrahaman, A. A. and Sani, A. (2012). In vitro evaluation of antifungal potentials of methanolic extracts of three organs of *Vitellaria paradoxa* (Shea plant). J. Sci. Tech. Math. & Edu. 8(2): 8 – 15.

Ahmed R. N., and Sani A. (2013). Antimycotic activity and toxicological effects of stem bark extract of *Vitellaria paradoxa* in wistar rats. Sci. Inter. (Lahore), 25(1):91-102.

Ahmed R.N., Sani A., and Igunnugbemi O. O. (2009). Antifungal profiles of extracts of *Vitellaria paradoxa* (Shea-butter) Bark. *Ethnobot Leaflets*, 13: 679-688.

Ajijolakewu, K. A. and Awarun F. J. (2015). Comparative Antibacterial Efficacy of *Vitellaria paradoxa* (Shea Butter Tree) Extracts Against Some Clinical Bacterial Isolates. Not. Sci. Biolo. 7(3):264 – 268.

Alexandri, E., Ahmed, R., Siddiqui, H., Choudhary, M. I., Tsiafoulis, C. G., and Gerothanassis, I. P. (2017). High resolution NMR spectroscopy as a structural and analytical tool for unsaturated lipids in solution. *Molecules*, *22*(10), 1663.

Animasaun, D. A. Oyedeji, S., Olorunmaiye, K. S., Azeez, M. A., Tijani, I. A. and Morakinyo, J. A. (2019). Morpho-chemical divergence and fatty acid profile of shea tree seeds (*Vitellaria paradoxa*) collected from different locations in Kwara State, Nigeria. Act. Bot. Croa. 78 (1), 17–24

Audu, J. and Awulu, J.O. (2017). Effect of extraction methods on some food and biodiesel properties of shea-nut oil (*Vitellaria paradoxa*). J. Posthar. Tech. 5(1): 17-26.

Blanchette, R. A. (2000). A review of microbial deterioration found in archaeological wood from different environments. Inter. Biodeter. & Biodegra. 46: 189–204.

Boyejo A.O., Azeez I.A., Owolabi S.L., and Issah A.O. (2019). Antifungal and Phytochemical Screening of Extract from *Vitellaria Paradoxa* (Shea Butter Tree) Leaves, Barks and Roots on Dermatophytes. Inter. J. Scient. & Res. Publi. 9(6): 884 – 891.

Cheung, H. T., and Williamson, D. G. (1969). NMR signals of methyl groups of triterpenes with oxygen functions at positions 2, 3 and 23. *Tetrahedron*, 25(1), 119-128.
Csupor-Loaffer, B., Hajdu, Z., Zupko, I., Molnar, J., Forgo, P., Vasas, A., Kele, Z., Hohmann, J., 2011. Antiproliferative constituents of the roots of Conyza canadensis. Plan. Medi. 77, 1183–1188.

Ekhuemelo, D. O., Agbidye, F. S., Anyam, J. V. and Ugba, R. B. (2018). Antimicrobial effect of isolated compound of *Anadelphia afzeliana* (Rendle) Stapf on selected wood fungi and bacteria in Makurdi, Nigeria. Nig. J. Biotech. 35(2): 108-120.

El Kharrasi, Y., Samadi, M., Lopez, T., Nury, T., el Kebbaj, R., Andreoletti, P., El Hajj, H., Vamecq, J., Moustaid, K., Latruffe, N., El Kebbaj, L., Masson, D., Lizard, G., Nasser, B., and Cherkaoui-Malki, M., (2014). Biological activities of schottenol and spinasterol, two natural phytosterols present in argan oil and in cactus pear seed oil, on murine miroglial BV2 cells. Bioch. & Biophy. Res. Comm. 446, 798–804.

El-Mahmood A. M., Doughari J. H., Ladan N. (2008). Antimicrobial screening of stem bark extract of *Vitelleria paradoxa* against some enteric pathogenic microorganisms. Afri. J. Pharm. & Pharmaco. 2(5):089-094.

Freire, C. S. R., Coelho, D. S. C., Santos, N. M., Silvestre, A. J. D., and Pascoal Neto, C. (2005). Identification of Δ7 phytosterols and phytosteryl glucosides in the wood and bark of several Acacia species phytosterols and

phytosteryl glucosides in the wood and bark of several *Acacia* species. Lipi. 40(3): 317–322.

Fodouop, S. P. C., Gatsing, D., Tangue, B. T., Tagne, R. S., Tala, S. D., Tchoumboué, J., and Kuiate, J. R. (2015). Effect of *Salmonella typhimurium* infection on rat's cell oxidation and in vivo antioxidant activity of *Vitellaria paradoxa* and *Ludwigia abyssinica* aqueous extract. Asian Pacif. J. Trop. Dis. 5(1), 38–46.

Garba S. and Salihu L. (2011). Antibacterial Activities of 2-O-butyl-1-O-(2'-ethylhexyl) benzene-1,8-dicarboxylate and 1-phenyl-1,4-pentanedione Isolated from *Vitellaria paradoxa* Root Bark. Asian J. of Scient. Res. 4 (2): 149-157.

Guevara, B. Q. (2005). A Guidebook to Plant Screening: Phytochemical and Biological, Revised Edition, UST Publishing House, Manila. Pp 156.

ICRAF (2000). International Centre for Research in Agroforestry. Agroforestree Database 2000
IPGRI, INIA (2006). Descriptors for Shea tree (*Vitellaria paradoxa*). International Plant Genetic Resources Institute, Rome, Italy; Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain. P 63.

Johann, S. Mendes, B.G., Missau, F.C., de-Resende, M.A., and Pizzolatti, M.G. (2011). Antifungal activity of five species of Polygala. Braz. J. Microb. 42: 1065-1075.

Kalgo, M.U., Hamid, K.M., Muhammad, U. A., Balarabe, A., Yeldu, M. H., Yahaya, I.S., Kalgo, Z.M. Aliyu, B. and Y. G. (2019). Bala Effects of aqueous stem bark extract of *Vitellaria paradoxa* on human neutrophil function and viability. Intern. J. Biolog. & Med. Res., 10(3):6782-6787.

Kuete V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. (2009). Antimicrobial activity of the crude extract, fractions and compounds from stem bark of *Ficus ovata* (Moraceae). J. Ethnopharm., 124,556 - 561.

Kuiate, J., Tene, M., Tane, P., and Tamokou, J. (2009). Antimicrobial clerodane diterpenoids from *Microglossa angolensis* Oliv. et Hiern. Ind. J. Pharmaco. 41(2), 60.

Kuta, F. A., Oyedum U., Garba S. A., Bala, J. D. and Adedeji, S. A. (2017). Antibacterial Activity of *Vitellaria paradoxa* on some Enteric Bacteria. Nig. J. Microb. 31(1): 3882-3892.

Lladó, S., Žifčáková, L., Větrovský, T., Eichlerová, I., and Baldrian, P. (2015). Functional screening of abundant bacteria from acidic forest soil indicates the metabolic potential of Acidobacteria subdivision 1 for polysaccharide decomposition. Bio. & Fert. Soi. 52(2), 251–260.

Marcot B. G. (2007). A Review of the Role of Fungi in Wood Decay of Forest Ecosystems. U.S. Department of Agriculture. Research note PNW-RN-575. Pp 1- 31.

Meneses-Sagrero, S. E., Navarro-Navarro, M., Ruiz-Bustos, E., Del-Toro-Sánchez, C. L., Jiménez-Estrada, M., and Robles-Zepeda, R. E. (2017). Antiproliferative activity of spinasterol isolated of *Stegnosperma halimifolium* (Benth, 1844). Sau. Pharm. J., 25(8), 1137–1143.

Moore, S., (2008). The role of *Vitellaria paradoxa* in poverty reduction and food security in the Upper East region of Ghana. Ear. & Environ. 3, 209–245.

Olasunkanmi, O.O., Akinpelu, D. A., Adeniyi, P. O., Ajayi, O. F., Omololu-Aso J. and Olorunmola F. O. (2017).Investigations into Antibacterial, Phytochemical and Antioxidant Properties of *Vitellaria paradoxa* (Gaertn.) Stem Bark Extracts. J. Pharma. Res. Intern. 20(5): 1-17, 2017.

Pohl C. H., Kock J. L. F. and Thibane V. S. (2011). Antifungal free fatty acids: A Review. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.). Pp61 – 71.

Prescott M. L, Harley P. J. and Klein A. D. (2002). Microbiology. 7th edition. McGraw Hill Inc.

Ravikumar, Y. S., Mahadevan, K. M., Manjunatha, H., and Satyanarayana, N. D. (2010). Antiproliferative, apoptotic and antimutagenic activity of isolates from Polyalthiacerasoides seeds. Phytomed. 17(7), 513–518.

Seibert, U. (2007). Languages of Benue state, Nigeria, B. Ed project Report, Department of Languages department of languages and linguistics, University of Jos; 86p.

Shomkegh, S. A., Mbakwe, R. and Sale F. A. (2016). Ethnobotanical Survey of Wild Plants Utilized for Craft Making and Local Construction among the Tiv People of Benue State, Nigeria. J. Agric. & Ecol. Res. Intern. 9(3): 1-11.

Sun, Y., and Yasukawa, K. (2008). New anti-inflammatory ergostane-type ecdysteroids from the sclerotium of Polyporus umbellatus. *Bioorganic & medicinal chemistry letters*, *18*(11), 3417-3420.

Teketay, D. Gurmu, D. and Bekele, T. (2003). *Vitellaria paradoxa:* a multipurpose industrial oilseed tree. Wal. 23: 3-23.

Ugese, F. D., Baiyeri, P. K. and Mbah, B. N. (2008). Nutritional composition of shea (*Vitellaria paradoxa*) fruit pulp across its major distribution zones in Nigeria. *Fruits:* Th. Intern. J.of Trop. & Subtrop. Horticu. 63 (3) 163 – 170.

Villaseñor, I. M., and Domingo, A. P. (2000). Anticarcinogenicity potential of spinasterol isolated from squash flowers. *Teratogenesis, carcinogenesis, and mutagenesis*, *20*(3), 99-105.

Warra, A.A. (2011). Cosmetic Potentials of African Shea Nut (*Vitellaria paradoxa*) Butter. Cur. Res. Chem. 3: 80-86.

Ziblim, A. I., Abdul-Rasheed, S. and Aikins, T. K. (2015). Forage species used by livestock in the Kumbungu District of the Northern Region, Ghana. UDS Intern. J. Devel. [UDSIJD], 1(1): 18 – 29.