



Article Info

Received: 11th December 2018

Revised: 5th February 2019

Accepted: 18th February 2019

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Cite this: *CaJoST*, 2019, 1, 1-5

Comparative Phytochemical and Antifungal Activities of Stem and Root Bark Extracts of *Vitellaria paradoxa* (Shea Butter) Tree

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Vitellaria paradoxa is used in traditional medicine for treatment of fungal infections. This research was designed to comparatively investigate the phytochemicals and the antifungal potentials of the stem and root bark extracts of *V. paradoxa*. The powdered stem and root barks were extracted successively by maceration using n-hexane, dichloromethane and methanol. Crude methanol extracts were also obtained by extraction of fresh powdered samples with methanol using maceration. Antifungal profiles of the extracts were examined against clinical isolates of *Aspergillus niger* and *Candida albican* using standard procedure. The crude extract and fractions of stem showed higher activity against *Candida albican* with zones of inhibition ranging from 10-25 mm. *Aspergillus niger* was more susceptible to the root extracts with zones of inhibition range of 9-23 mm. For both stem and root, the crude methanol extracts showed more activity than their corresponding fractions. For the fractions, methanol showed highest activity followed by n-hexane. The test organisms were resistant to dichloromethane extract of root. *A. niger* was resistant to dichloromethane extract. The result also showed that activity of all the extracts is concentration dependent. Fluconazole powder was used as control which showed higher activity than all the extracts at concentration of 5 mg/mL. The preliminary phytochemical screening was carried out using standard procedures on both stem and root and it revealed the presence of steroids/triterpenes, alkaloids, tannins, flavonoids, saponins, carbohydrates and proteins. The antifungal activity may be due to the presence of these phytochemicals. Therefore, *V. paradoxa* could be used as a potential source of antifungal substance for a drug development.

Keywords: *Vitellaria paradoxa*, Phytochemicals, Antifungal activity, *Aspergillus niger*, *Candida albican*

1. Introduction

The development of resistance to antifungal drugs has become increasingly apparent, especially in patients who require long-term treatment or who are receiving antifungal prophylaxis (Loeffler and Stevens, 2013). It is estimated that fungal species particularly *Candida*, *Aspergillus*, *Pneumocystis*, and *Cryptococcus* spp. cause at least 1.4 million deaths worldwide per year (Brown *et al.*, 2012), mostly in people with weakened immune system (HIV/AIDS patients). Oladele *et al.* (2013) reported that 50% of the over 155 million Nigerian population are children and 38 million are women between the ages of 15 to 50 years. 1.5 million Nigerian women get recurrent vaginal thrush. Local estimates of *Tinea capitis* exceed 20% of school age children, suggesting that over 15.5 million children have *Tinea capitis*. Despite the constant introduction of new and effective

synthetic drugs into the market, medicinal plants, which are the historical basis of therapeutic health care, represent an alternative that is economical, accessible, and applicable to various pathologies, particularly in developing countries (Ashcroft *et al.*, 1999). For these reasons, people turn to herbal medicine for treatment of many diseases because they are found to be effective and are free from undesirable side effects (Philomena, 2011). In an effort to expand the spectrum of antifungal agents from natural resources, *Vitellaria paradoxa*, commonly known as shea butter tree, belonging to *Sapotaceae* family, the only species in genus *Vitellaria* (Byakagaba, 2011), was selected for this research. The shea tree is a traditional African food plant. It has been claimed to have potential to improve nutrition, boost food supply in the "annual hungry season" (Masters *et al.*, 2010) foster rural development, and support sustainable land care. Poulsen (1981) cited in Hall *et al.* (1996) reported three decades

ago that *V. paradoxa* is the second most important oil crop in Africa after oil palm and is the most economically and culturally important tree species in the Sudano Sahelian region of Africa where oil palm does not grow. The bark is used for traditional medicines and the latex is used for making glue. Shea trees provided fodder for 70% of surveyed households in Nyankpala, northern Ghana (Poudyal, 2011).

A large number of Plants extracts have been experimented on different fungal strains for screening antifungal activity. The results of these experiments revealed that the activity is due to several secondary metabolites. It was revealed by Taddeo and Claude (2011), that screening plant extracts against the test organisms normally shows that fungi vary widely in the degree of their susceptibility to antifungal agents. Some plant extracts have antifungal agents that are fungistatic at lower concentrations while becoming fungicidal at higher concentrations (Taddeo and Claude, 2011).

Based on the available literature, there are no reports of researches comparing the antifungal activities of stem and root bark extracts of *V. paradoxa*. This research is designed to comparatively investigate the phytochemicals and antifungal potentials of the stem and root bark extracts of *V. paradoxa*. The objectives are to extract the samples with methanol and successively with n-hexane, dichloromethane and methanol, conduct preliminary phytochemicals analysis of the extracts and test for antifungal activities of the extracts.

2. Materials and Methods

2.1 Sampling and Sample Treatment

The stem and root barks of *Vitellaria paradoxa* were collected by first removing the hard surface and then inner reddish part of the bark was taken as the sample. The leaves and fruits were also collected for the purpose of identification. Sample collection was done at Maiyama town, Maiyama Local Government Area of Kebbi State, Nigeria in November 2016. The plant sample was identified at the Herbarium Section of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, with voucher number (UDUH/ANS/0114). The fresh samples were sun-dried for two weeks, ground into a fine powder and stored in a plastic container until required for further use.

2.2 Extraction and Determination of Phytoconstituents.

The powdered samples of the stem and root (70 g) barks were extracted by maceration in two different air-tight jars using 350 mL of methanol in order to obtain crude methanol extracts. The successive extraction was carried out starting with 200 g of the powdered stem and root barks where 1000 cm³ of n-hexane was used (1:5 w/v). This was allowed to macerate for 24h. The mixtures were then decanted, filtered with muslin cloth and then with Whatman No. 1 filter paper. The residues were washed with fresh solvents until the solution obtained turn colourless. The residues were air-dried, weighed and then macerated with dichloromethane (1:5 w/v). The procedure above was repeated to obtain the second fraction and the third using methanol. The filtrates from each round of extractions were combined and evaporated to dryness. These were then packed in separate clean dry bottles and stored at room temperature until required for use. The extracts were screened for the presence of steroids/triterpenes, alkaloids, tannins, flavonoids, saponins, carbohydrates and proteins as described by Evans (1996).

2.3 Test Organisms

Clinical isolates of *Aspergillus niger* and *Candida albican* were obtained from the Department of Veterinary Microbiology, Usmanu Danfodiyo University Sokoto, Nigeria.

2.4 Preparation of the Medium

The culture media was prepared according to the manufacturer's instructions as follows: Saboraud dextrose agar medium (38 g) was weighed and mixed with 585 mL of sterile distilled water and the medium was then sterilized by autoclaving at 121 °C for 15 min under 1 bar pressure which was then dispensed into Petri-dishes to yield a uniform depth. The medium was allowed to cool at room temperature, undisturbed, until the culture medium hardened. It was then left in an incubator at 37 °C for 24 h in an inverted position to test for sterility. Saboraud dextrose agar plates were then stored at 4 to 8 °C and wrapped in plastic bags.

2.5 Determination of Antifungal Activities (Agar Well Diffusion Method)

The standard inoculum (0.1 mL, 0.5 McFarland turbidity standard = 1.0 x 10⁸ cfu/mL) of each test fungus was spread into sterile Saboraud Dextrose Agar plates so as to achieve even

growth. The plates were allowed to dry and a sterile cork borer (8.0 mm diameter) was used to bore wells aseptically in the agar plates. The extracts were prepared and serially diluted using 10% dimethylsulphoxide (DMSO) to achieve different concentrations of 25, 50, 100 and 200 mg/mL respectively for each extract. Subsequently, 200 µL of each concentration of the extracts was introduced into the wells earlier bored in the Agar plates. The extracts were allowed to diffuse into the medium (kept for 1 h on the bench before incubation) at 37 °C for 48 h. Fluconazole was used as a positive control (5 mg/mL was prepared by dissolving 50 mg of the drug powder in 10 mL of DMSO and introduced the single hole at the centre of each petri dish). Antifungal activity of the extracts was determined by measurement of zones of inhibition produced

around the wells (meter rule was placed in 3 different directions an average was calculated). The diameter of the zones indicated the degree of susceptibility of the test fungi (Lino and Deogracious, 2006).

3. Results and Discussion

The plant has recently been a research focus as potential source for drug development due to its antibacterial (El-Mahmood *et al.*, 2008) and antifungal (Ahmed and Sani, 2013) activities. The extracts revealed the presence of some metabolites as presented in Tables 1 and 2. Also, as shown in Tables 3 and 4, the extracts exhibited antifungal activities against *Aspergillus niger* and *Candida albicans*.

Table 1. Phytochemical composition of the stem bark extracts of *V. paradoxa*

Test	n-Hexane	DCM	MeOH	Crude MeOH
Steroids/Triterpenes	+	+	+	+
Alkaloids	-	+	-	+
tannins	-	-	+	+
Flavonoids	-	-	+	+
Saponins	-	-	+	+
Proteins	-	-	+	+
Carbohydrates	-	-	+	+

Key: + = detected, - = not detected, DCM= dichloromethane, MeOH= methanol

Table 2. Phytochemical composition of the root bark extracts of *V. paradoxa*

Test	n-Hexane	DCM	MeOH	Crude MeOH
Steroids/Triterpenes	+	+	+	+
Alkaloids	-	+	-	+
tannins	-	-	+	+
Flavonoids	-	-	+	+
Saponins	-	-	+	+
Proteins	-	-	+	+
Carbohydrates	-	-	+	+

Key: + = detected, - = not detected, DCM= dichloromethane, MeOH= methanol

Table 3. Antifungal Activity of the Stem Bark Extracts

Conc. (mg/mL)	Zones of Inhibition (mm)								
	CME		MF		DF		HF		
	<i>A. niger</i>	<i>C. albican</i>	<i>A. niger</i>	<i>C. albican</i>	<i>A. niger</i>	<i>C. albican</i>	<i>A. niger</i>	<i>C. albican</i>	
200	22.0	25.0	15.0	23.0	R	11.0	12.0	13.0	
100	18.0	22.0	14.0	21.0	R	10.0	11.0	12.0	
50	16.0	18.0	13.0	18.0	R	9.0	10.0	10.0	
25	14.0	15.0	11.0	16.0	R	R	R	R	
Fluconazole (5 mg/mL)	10.0	15.0	10.0	15.0	10.0	15.0	10.0	15.0	

R= resistant, crude methanol extract (CME), methanol fraction (MF), dichloromethane fraction (DF) hexane fraction (HF)

Table 4. Antifungal Activity of the Root Bark Extracts

Conc. (mg/mL)	Zones of Inhibition (mm)							
	CME		MF		DF		HF	
	<i>A. niger</i>	<i>C. albican</i>	<i>A. niger</i>	<i>C. albican</i>	<i>A. niger</i>	<i>C. albican</i>	<i>A. niger</i>	<i>C. albican</i>
200	23.0	18.0	17.0	16.0	R	R	13.0	R
100	19.0	16.0	16.0	15.0	R	R	11.0	R
50	18.0	15.0	15.0	14.0	R	R	10.0	R
25	12.0	12.0	14.0	12.0	R	R	9.0	R
Fluconazole (5 mg/mL)	10.0	15.0	10.0	15.0	10.0	15.0	10.0	15.0

R= resistant, crude methanol extract (CME), methanol fraction (MF), dichloromethane fraction (DF) hexane fraction (HF)

The results of the phytochemical screening carried out on the stem and root barks of *V. paradoxa* showed that both contain steroids, triterpenes, tannins, flavonoids, saponins, alkaloids, proteins and carbohydrates (Tables 1 and 2). These compounds have been reported to be responsible for the antimicrobial activity of medicinal plants (Mathias *et al.*, 2007). These constituents were found to be similar to those reported for most medicinal plants. The presence of saponins in this plant could be responsible for the traditional use of shea butter in the relaxation of muscles and in treatment of sprains, wounds and colds as practiced generally in Nigeria (Isah *et al.*, 2007). However, n-hexane fractions of both stem and root bark shows the presence of only steroids and triterpenes. The result obtained is similar to that by Isah *et al.*, (2007) and Kamoldeen and Fola (2015) for the stem bark and root barks of *V. paradoxa*. Crude methanol extract of both samples showed the presence of all the phytochemicals tested.

The antifungal activity demonstrated by the stem and root bark extracts of *V. paradoxa* (Tables 3 and 4) could be attributed to the presence of the metabolites determined in the preliminary qualitative phytochemicals analysis (Tables 1 and 2). Terpenoids and alkaloids have been proved to have antifungal activity (Ghoshal *et al.*, 2006; Taddeo and Claude, 2011). This agrees with what was reported by Ghoshal *et al.* (2006) that alkaloids, terpenoids and lactones are responsible for antifungal activity. Both crude extract and the fractions (n-hexane, dichloromethane and methanol) of stem bark showed greater activity against *Candida albican* than *Aspergillus niger*. While the root extracts showed greater activity against *Aspergillus niger*. Crude methanol extract showed more activity than the fractions in both stem and root. This may be as a result of the synergistic behaviour of the plant extracts. Methanol extract showed the highest activity followed by n-hexane extract

for both stem and root. The differences in polarity among various solvents have been reported to be accountable for the differences in solubility of plant active components, hence variation in degree of activity (El-Mahmood *et al.*, 2008). In the stem extracts, total resistance was observed only on *A. niger* to dichloromethane, while for the root, total resistance was observed on both *A. niger* and *C. Albican* to dichloromethane and on *C. Albican* to methanol. This could be as a result of synergy. The result also showed that the activity demonstrated by all the extracts was concentration dependent (the higher the concentration the greater the activity). Similar results have been reported by several researchers including El-Mahmood *et al.* (2008) and Kamoldeen and Fola (2015). Fluconazole was used as control. The standard showed average inhibition diameter of 15.0 mm on *C. Albican* and 10 mm on *A. niger* at 5 mg/mL. Thus, the control drug showed higher activity than all the extracts. This is because the antifungal drug is in pure state (Prescott *et al.*, 2002).

4. Conclusion

The various extracts of both stem and root bark of *Vitellaria paradoxa* contain steroids, triterpene, flavonoids, saponins, tannins, alkaloids, carbohydrates and proteins. The extracts of both stem and root bark of *Vitellaria paradoxa* demonstrated antifungal activity against the tested clinical isolates (*Aspergillus niger* and *Candida albican*), thus justifying its use in traditional medicine for treating different diseases associated with the tested isolates, as it also could serve as a new and cheaper alternative for antifungal drugs.

Acknowledgement

The authors expressed their sincere appreciation to Abdulmalik of the Department of Veterinary

Microbiology, Usmanu Danfodiyo University Sokoto for his technical assistance.

Conflict of interest

The authors declare no conflict of interest.

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