Evaluation of antifungal effect of *Parkia biglobosa* and *Vitellaria paradoxa* against selected pathogenic fungi

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Abstract

The aim of this study was to evaluate the antifungal effects of extracts of two plant species namely *Vitellaria paradoxa* and *Parkia biglobosa* against growth of some selected fungal species. Aqueous and ethanolic extracts of these plant species were assessed against *Aspergillus flavus, Candida albican* and *Trichophyton mentagrophyte*. Phytochemical analysis of these plants showed the presence of alkaloids, flavonoids, tannins, saponins and other secondary metabolites. The minimum fungicidal concentration (MFC) of aqueous extract of *P. biglobosa* was 150 mg mL^{-1} against both *C. albican* and *A. flavus*. On the other hand, ethanolic extract of this plant species had MFC of 300 mg mL^{-1} for *A. flavus*, while there was no MFC for *C. albican*. Likewise, aqueous extract of *V. paradoxa* also had same value of MFC against *C. albican* as well as *A. flavus*. Ethanolic extract of *V. paradoxa* had MFC of 150 and 300 mg mL^{-1} against *C. albican* and *A. flavus*, respectively. The combined aqueous extracts of these plant species showed MFC of 300 mg mL^{-1} against both the *C. albican* and *A. flavus*. By contrast, the mixture of ethanolic extracts had MIC of 150 mg mL^{-1} against *C. albican*, and no MFC for *A. flavus*.

Keywords: Antifungal effect, *Parkia biglobosa*, Pathogenic fungi, *Vitellaria paradoxa.*

Introduction

Various modern medicines have been extracted from medicinal plants following the ethnobotanical cues of indigenous remedies associated with traditional medicinal systems (Verma and Singh, 2008). The usage of medicinal plants is a significant resource, a necessity, and a practical substitute for basic healthcare in rural and developing nations (Hayta *et al*., 2014). Since then, 50% of all the medicines in clinical use globally are derived from natural products, providing humans with new treatment choices, of which higher plants account for a total of 25%. Plants were once a significant source of the world's medications (Van and Prinsloo, 2018; Bellwood, 2023). Natural compounds possess a variety of biological activities including antifungal (Shoaib *et al.,* 2018; Naqvi *et al.,* 2023), antibacterial (Ferdosi *et al.,* 2020), antioxidant, anticancer (Khan and Javaid, 2020), antiviral (Naikoo *et al*., 2021) and herbicidal (Javaid and Khan, 2020).

Shea butter tree is a common name for the Sapotaceae family member *Vitellaria paradoxa*. It naturally grows in the open savannah region of West Africa. The Ethiopian mountains' foothills are covered in an abundance of this tree. Many communities in Africa regard *Vitellaria paradoxa* as a sacred tree, and it is a significant plant in various rituals. The kernel of this plant is used to prepare shea butter which is helpful to cure rheumatism, ulcers, rashes, and dermatitis. (Adamu *et al*., 2013). Decoctions made from *V. paradoxa* leaves are used as an eye lotion, a remedy for headaches, migraines, and mouth infections. To cure enteric infections like diarrhea, helminths, skin conditions, and wound infections, this plant's many parts, including the leaves, roots, seeds, and stem bark, are effective (Olasunkanmi *et al*., 2018). *Parkia biglobosa* belongs to the family Mimosaceae. Its stem-bark stem bark has been used in the treatment of a lot of diseases of medical importance (Ahamad and Afolabi, 2010). This study aimed to provide valuable insights into the antifungal potential of *P. biglobosa* and *V. paradoxa*, which could contribute to the development of novel antifungal therapies or natural products for managing fungal infections.

Materials and Methods

Sample collection

The fresh bark of *P. biglobosa* and *V. paradoxa* were obtained in Jos and identified in the Plant Science department, University of Jos, Plateau State.

Preparation of extracts

The plant components were minced, shade-dried for two weeks at room temperature, and ground to a powder with the help of a mortar and pestle. These samples were used in the extraction procedure.

Extracts preparation

Each ground plant material was weighed at 150 g and soaked in 750 mL of 85% ethanol and separately in distilled water and extract was then filtered after the plant material was repeatedly extracted with the two solvents. The ethanolic extract was concentrated at 50 °C and the aqueous extract was concentrated in a water bath. The filtered stock solutions were allowed to air dry for further examination (Al-Hussaini and Al-Mohanna, 2010).

Isolation, identification, and characterization

Three pure isolates of the fungi *Trichophyton mentagrophyte, Aspergillus niger* and *Candida albicans* were obtained from the National Veterinary Research Institute Vom in the Plateau State, and their confirmatory tests were conducted in accordance with the techniques outlined by Jorgensen *et al*. (2015) and Cheesbrough (2009).

Oyeleke and Manga (2008) method was used to standardize the organisms.

Samples were inoculated on Sabouraud dextrose agar (SDA) or potato dextrose agar (PDA). Growth media were incubated (according to require temperature and time depending on the nature of fungi to be recovered (Emmons *et al.*, 1970).

Phytochemical screening

The extracts were analyzed to identify the presence of carbohydrates, tannins, alkaloids, saponins, phenolic anthraquinones and cardiac glycosides (Trease and Evans, 1996; Yadav *et al*., 2014).

Detection of alkaloid

Individual extracts were dissolved in weak hydrochloric acid, filtered, and then subjected to Wagner's reagent (iodine in potassium iodide). Appearance of a brown or reddish precipitate showed the presence of alkaloids.

Detection of glycosides

Glycosides were detected with the help of Keller-Killani test. Each extract was mixed with 2 mL of glacial acetic acid that had been diluted to 5 mL and contained one drop of ferric chloride solution. After that, 1 mL of concentrated H_2SO_4 was applied on top of it. The cardenolide's deoxysugar characteristic can be identified by an interface brown ring. Appearance of a violet ring below a brown ring and a greenish ring in the thin acetic acid layer was the indication of glycosides presence.

Identification of flavonoids

A few drops of NaOH solution were added to the extracts. When diluted acid was added, a vivid yellow color that had formed as an indication of the presence of flavonoids, disappeared.

Identification of saponins

Saponins presence was detected by foam text. Two milliliters of water were shaken with each 0.5 g of the extracts. A foam created that persisted for 10 min, showed that saponins were present.

Identification of tannins

Two milliliters of a 2% solution of $FeCl_3$ were added to the extracts. A blue-green or blue-black color indicated the presence of tannins.

Detection of steroids

Two milliliters of acetic anhydride were mixed with 0.5 g of extract along with 2 mL of H_2SO_4. Transform of violet to blue or green color indicated that steroids were present.

Test for antifungal susceptibility

The Agar well diffusion method was used to evaluate the isolates' susceptibility to *P. biglobosa* and *V. paradoxa* extracts as antifungal agents (Olutiola *et al.*, 2016). Extract concentrations of 50, 100, 200, 250, and 300 mg mL^{-1} were used in the susceptibility test. The spreading technique was used to disseminate the standardized isolates (0.1 mL each) on the surface of the SDA plate. A sterile cork borer was used to drill uniform sized (6 mm) wells into the solidified agar. A micropipette was used to deposit 0.1 mL of the leaf extract at the specified concentration into each well independently. The extract was then well diffused throughout the medium by allowing it to stand for 5 h. The plates were incubated at 37 ^{o}C, for 24 h without being turned over. The positive control was the antifungal medication Amphotericin B. If there was a zone of inhibition surrounding the wells, it showed that the extracts were inhibiting the fungi otherwise, there was no inhibition. Using a properly calibrated meter ruler, the zone of inhibition was then measured to the closest millimeter.

Minimum inhibitory concentration (MIC) determination

The MIC in each case was determined against *T. mentagrophyte, A. niger* and *C. albicans* in the broth medium. Using the conventional procedure, controls were run without the plant extracts. The MIC of the extracts against the test organism was determined using the broth dilution method of Doughari *et al*. (2017). Sterile Mueller Hilton broth (2 mL) was dispensed into the test tubes required for the test. Different concentrations of ethanol and water extracts of *P. biglobosa* and *V. paradoxa* and their mixture were prepared in distilled water (1.4 g

of extract in 5 mL of water to obtain 300 mg mL^{-1}). Two milliliters of each concentration were dispensed into test tubes using a sterile pipette and then serial dilution (double dilution) was carried out as follows; 4.69, 9.38, 18.75, 37.5, 75, 150, and 300 mg mL^{-1}. Inoculum suspensions (10 µL) of microbs were added on individual tubes as follows; negative control, positive control (using Amphotericin B 10 µg), then incubated at room temperature for 72 h. In each example, the MIC was defined as the lowest concentration of plant extracts that still allowed the infected test organism to grow visibly in the broth medium.

Calculation of the minimum fungicidal concentration (MFC)

In order to test for the fungicidal action of the extracts, the contents that did not exhibit any turbidity or apparent fungal growth during the experiment involving the minimal inhibitory concentration were cultivated onto a prepared sabouraud dextrose agar plate. 48 h were spent incubating the test organism-filled plates at 37 °C. The smallest concentration that did not result in any fungal growth on the solid medium was referred to as the minimum fungicidal concentration. The Doughari and Obidah (2008) method was used to calculate the MFC of the extract. In order to determine the MIC, melted, cooled, and prepared SDA was added to a petri dish in an amount equal to the number of culture tubes showing no discernible growth. 0.1 mL from the tubes with no discernible growth was pipetted into the agar on the surface of the agar plates using a sterile pipette. Overnight, the plates were incubated at room temperature and growth was monitored. MFC was determined to be the lowest concentration with no discernible growth.

Results

Phytochemicals identified in the test plants

Table 1 presents the outcome of the phytochemical screening conducted on *P. biglobosa*, *V. paradoxa*, and their combination. The screening identified the presence of saponins, tannins, steroids, alkaloids, flavonoids, cardiac glycosides, and anthraquinone in the tested samples.

Susceptibility test (agar well diffusion assay)

The result of the antifungal assay are presented in Tables 2 and 3. The result indicates an appreciable dose dependent antifungal activity of the plant extracts against the test isolates. Both aqueous and ethanol extracts exhibited a remarkable antifungal activity against the test organisms except *T. mentagrophyte*.

Minimum inhibitory concentration (MIC)

Tables 4 to 6 show the MIC values of various extracts of the two plant species against the target fungal isolated. The least MIC was 75 mg mL^{-1} of the aqueous extract of *P. biglobosa* against *A. flavus*, while the highest MIC was 300 mg mL^{-1} for different solvents of both extracts used. There was no MIC for the mixture of ethanolic extracts *V. paradoxa and P. biglobosa* against *A. flavus.*

Minimum fungicidal concentration (MFC)

The extracts exhibited variable MFC values for different test fungal species. The least MFC was 150 mg mL^{-1} for different extracts, the highest MFC was 300 mg mL^{-1} for different solvents of extracts used while there was no MFC for the ethanolic extract of a mixture of *V. paradoxa* and *P. biglobosa* against *C. albican.* The results for the MFC are depicted in Tables 7 to 9.

Discussion

Alkaloids, saponins, tannins, flavonoids, cardiac glycosides, steroids, and anthraquinone are examples of secondary metabolites that may be present (Shaikh and Patil, 2020). These indicate that *Parkia biglobosa, Vitellaria paradoxa and their* mixture can be used as herbal mixture for the treatment of infections caused by pathogenic fungi and report have shown that several secondary metabolites provide antimicrobial activity (Adebayo and Ishola, 2009) which include alkaloids, saponins, tannins, and flavonoids.

Analysis of the plant extracts revealed the presence of some phytochemicals. Phenolic compounds ranked as one of the largest and most common group of plant metabolites (De-Rio *et al*., 1997), and numerous studies have discussed the antioxidant properties of plants that are rich in phenolic compounds (Parekh and Chanda, 2008).

Both aqueous and ethanolic solvents exhibit a concentration-dependent increase in extract activity. Increasing the extract concentration enhanced their antifungal activity, is consistent with the finding that higher concentrations of antimicrobial substances were effective in inhibiting growth (Prescott *et al*., 2002; Jabeen *et al.,* 2022). The outcome shows that the plant extracts have noticeable, dose-dependent antifungal efficacy against the test isolates. The extracts both aqueous and ethanol exhibited a higher antifungal activity on the test organisms except for *T. mentagrophyte* which does not show activity. Both aqueous and ethanol extracts of the plants show activity on the test organisms except for *T. mentagrophytes* which does not show activity. This disagrees with similar work done by Olusesan and Iye (2021) on the evaluation of different allelopathic plants on the yield of white yam in nematode-infested soil.

The least MIC is at 75 mg mL^{-1} of the aqueous extract of *P. biglobosa* against *A. flavus,* the highest MIC is at 300 mg mL^{-1} which shows for different solvents of both extracts used while there was no MIC for the ethanolic extract of a mixture of

V. paradoxa and *P. biglobosa* on *A. flavus*. The study discovered that whereas antimicrobial compounds with weak activity against an organism have high minimum inhibitory concentrations, medicines with significant antimicrobial potencies have low minimum inhibitory concentrations. This conclusion is supported by the findings of Prescott *et al*. (2002).

The extract was shown to exhibit MFC. The least MFC is at 150 mg mL^{-1} for the differsolventsvent of extracts used, the highest is at 300 mg mL^{-1} for different solvents of extracts used while there was no MFC for the ethanolic extract of a mixture of *V. paradoxa* and *P. biglobosa* on *C. albican*. The result of susceptibility of the fungal isolate to the aqueous and ethanolic extract was comparable to the control (Amphotericin B), which could be used as an alternative antifungal agent for candidiasis and aspergillosis. The plant extracts utilized in this study may be helpful for treating fungus infections as reported by Jeff-Agboola et al. (2012).

Conclusion

At the end of this study, important phytochemical compounds were detected in the stem bark of *V. paradoxa* and *P. biglobosa*. The extracts exhibited significant antifungal activities against clinical isolates of *C. albican* and *A. flavus* except for *Trichophyton mentagrophyte* at varied concentrations. The present of phytochemicals detected in the plant extracts could be responsible for the observed antifungal activities. The outcome of the research analyses has shown that extracts of *P. biglobosa* and *V. paradoxa* leaves were found to possess antifungal activities. This gives credibility to the traditional use of the plant as a medicinal plant and thus, shows that the plant could be exploited as a source of new potent antibiotics in the future.

Conflict of interest

The authors hereby declare no conflict of interest.

Author's contribution

EEE gave concepts and contributed to paper writing. ACN critically evaluated the paper and contributed in the writing. CEO and JOE supervised the work. AOO, HWN, and EGO helped in data curation and analysis. ODC critically reviewed and finalized the paper.

Table 1: Phytochemical constituents of *Parkia biglobosa and Vitellaria paradoxa* and their mixture.

Secondary metabolites	*Parkia biglobosa*	*Vitellaria paradoxa*	Mixture of extracts
Saponins	+	+	+
Tannins	+	+	+
Steroids	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Cardiac glycosides	+	+	+
Anthraquinone	−	+	+

+ = Present, − = Absent

Table 2: Antifungal activity of aqueous and ethanolic extract of *Parkia biglobosa* on test organisms.

Test organism	Aqueous extract Zones of Inhibition Concentrations (mg mL^{-1})					Ethanolic Extract Zones of Inhibition Concentrations (mg mL^{-1})					Amphotericin B (mg mL^{-1})
	300	250	200	100	50	300	250	200	100	50	
Candida albican	12	10	8	−	−	10	9	−	−	−	12
Aspergillus flavus	12	12	10	−	−	13	11	−	−	−	10
Trichophyton mentagrophytes	−	−	−	−	−	−	−	−	−	−	12

Reference drug = Amphotericin B, positive control drug for fungi.
− = No zone of inhibition

Table 3: Antifungal activity of aqueous and ethanolic extract of *Vitellaria paradoxa* on test organisms.

Test organism	Aqueous extract Zones of Inhibition Concentrations (mg mL^{-1})					Ethanolic extract Zones of Inhibition Concentrations (mg mL^{-1})					Amphotericin B (mg mL^{-1})
	300	250	200	100	50	300	250	200	100	50	
Candida albican	13	11	9	9	−	12	10	9	−	−	12
Aspergillus flavus	15	12	12	−	−	11	−	−	−	−	10
Trichophyton mentagrophytes	−	−	−	−	−	−	−	−	−	−	12

Reference drug = Amphotericin B, positive control drug for fungi.
− = No zone of inhibition

Table 4: Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extract of *Parkia biglobosa against* the test organisms.

Test organism	Concentrations (mg mL^{-1})							MIC
	300	150	75	37.5	18.75	9.375	4.6875	
Aqueous extract								
Candida albican	−	−	+	+	+	+	+	150
Aspergillus flavus	−	−	−	+	+	+	+	75
Ethanolic Extract								
Candida albican	−	+	+	+	+	+	+	300
Aspergillus flavus	−	−	+	+	+	+	+	150

+ = Fungal growth appeared, − = No fungal growth

Table 5: Minimum inhibitory concentration (MIC) of aqueous and ethanolic extract of *Vitellaria paradoxa against* the test organisms**.**

Test organism	Concentrations (mg mL^{-1})							MIC
	300	150	75	37.5	18.75	9.375	4.6875	
Aqueous extract								
Candida albican	−	−	+	+	+	+	+	150
Aspergillus flavus	−	−	+	+	+	+	+	150
Ethanolic extract								
Candida albican	−	−	+	+	+	+	+	150
Aspergillus flavus	−	+	+	+	+	+	+	300

+ = Fungal growth appeared, − = No fungal growth

Table 6: Minimum inhibitory concentration (MIC) of aqueous and ethanolic extract of mixture of *Parkia biglobosa* and *Vitellaria paradoxa* against the test organisms.

Test Organism	Concentrations (mg mL^{-1})							MIC
	300	150	75	37.5	18.75	9.375	4.6875	
Aqueous extract								150
Candida albican	−	−	+	+	+	+	+	
Aspergillus flavus	−	+	+	+	+	+	+	300
Ethanolic extract								150
Candida albican	−	−	+	+	+	+	+	
Aspergillus flavus	+	+	+	+	+	+	+	0

+ = Fungal growth appeared, − = No fungal growth

Table 7: Minimum Fungicidal Concentration (MFC) of aqueous and ethanolic extract *of Parkia biglobosa* against test
Organisms

Test organism	Concentrations (mg mL^{-1})							MFC (mg mL^{-1})
	300	150	75	37.5	18.75	9.375	4.6875	
Aqueous extract								
Candida albican	−	−	+	+	+	+	+	150
Aspergillus flavus	−	−	+	+	+	+	+	150
Ethanolic extract								
Candida albican	+	+	+	+	+	+	+	0
Aspergillus flavus	−	+	+	+	+	+	+	300

+ = Fungal growth appeared, − = No fungal growth

Table 8: Minimum fungicidal concentration (MFC) of aqueous extract of *Vitellaria paradoxa* against the test organisms.

Test Organism	Concentrations (mg mL^{-1})							MFC
	300	150	75	37.5	18.75	9.375	4.6875	
Aqueous extract								
Candida albican	−	+	+	+	+	+	+	300
Aspergillus flavus	−	+	+	+	+	+	+	300
Ethanolic extract								
Candida albican	−	−	+	+	+	+	+	150
Aspergillus flavus	−	+	+	+	+	+	+	300

+ = Fungal growth appeared, − = No fungal growth

Table 9: Minimum fungicidal concentration (MFC) of aqueous extract of mixture of *Parkia biglobosa* and *Vitellaria paradoxa* against the test organisms.

Test organism	Concentrations (mg mL^{-1})							MFC
	300	150	75	37.5	18.75	9.375	4.6875	
Aqueous extract								
Candida albican	−	+	+	+	+	+	+	300
Aspergillus flavus	−	+	+	+	+	+	+	300
Ethanolic extract								
Candida albican	−	−	+	+	+	+	+	150
Aspergillus flavus	+	+	+	+	+	+	+	−

+ = Fungal growth appeared, − = No fungal growth

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