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PHYTOCHEMICAL SCREENING AND ANTI-MYCOLOGICAL ASSESSMENT OF THE AQUEOUS EXTRACT OF CISSUS POPULNEA STEM- BARK ON SOME FOOD SPOILAGE FUNGI

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Abstract
Food preservation is vital to ensure safety and maintain quality, as food spoilage fungi pose serious threats that result in health risks and economic losses. This study investigated the phytochemical composition and minimum inhibitory concentration (MIC) of Cissus populnea stem bark extract against selected food spoilage fungi. Quantitative phytochemical analysis revealed the presence of alkaloids (1.551±0.03), saponins (2.208±0.06), flavonoids (0.761±0.10), phenols (0.447±0.06), tannins (1.987±0.01), terpenoids (1.530±0.09), and steroids (1.452±0.04), while glycosides were absent. Antifungal activity was assessed against Aspergillus flavus, Aspergillus niger, Fusarium culmorum, Fusarium graminearum, Candida spp., Mucor racemosus, and Penicillium spp. The extract showed inhibitory effects, with Fusarium culmorum showing the highest susceptibility (7–20 mm zone of inhibition at 20–100 mg/ml), comparable to Amphotericin (15–22 mm at 0–12 µg/ml). Aspergillus niger was least sensitive, with no inhibition at 20 mg/ml and a maximum of 17 mm at 100 mg/ml. However, the extract exhibited no fungicidal effect (HNCE) at any concentration tested. These results indicate that Cissus populnea stem bark extract has promising antifungal potential and could serve as a natural agent for food preservation. Further research is needed to elucidate its mode of action and explore applications in food industries.

Keywords: Antifungal, bark extract, Cissus populnea, phytochemical.

Introduction

The genus *Cissus* comprises about 350 species, and it is native to west tropical Africa. The *Cissus* species are often propagated by seed, although some can be multiplied by stem cuttings. *Cissus* stems and roots can be harvested throughout the year. As for some species that are deciduous, leaves can only be harvested in the dry season.

In Nigeria, *Cissus populnea* is commonly found in the Northern and Southern parts,(Rotimi et al.2023). The common/local names used include 'Ager' and 'Huer' (Tiv), 'Okoho' (Idoma and Igala), 'Orogbolo ajara' (Yoruba), 'Dafaaraa' (Hausa) Agbo, N., & Eze, C. (2024). The plant is 2to 3m high semi-climber which grows in the savanna and is widely distributed in Senegal, Sudan, Uganda, Abyssinia and Nigeria Achikanu, & Ani, (2020). *Cissus populnea* is a tropical medicinal plant used to correct male infertility factor in South-western part of Nigeria. The plant is also called food gum which is used for soup as soup thickener and as medicine for the treatment of venereal diseases and indigestion and as drug binder, Achikanu and Ani (2020); Rotimi *et al.,* (2023).

The exploration of *Cissus populnea* a medicinal plant from the *Vitaceae* family as a potential antifungal agent is driven by several scientific, ecological, and pharmacological factors. The rise of drug-resistant fungal pathogens such as, *Candida auris*, azole-

resistant *Aspergillus* has intensified the search for novel antifungal agents (Centers for Disease Control and Prevention [CDC], 2023). Conventional antifungals like fluconazole and amphotericin B face challenges due to toxicity and resistance (Pfaller *et al.*, 2023). Plant-derived compounds offer a promising alternative due to their multi-target mechanisms, reducing resistance risks (Nweze *et al.*, 2023).
Plant products that are derived from this plant parts such as bark, leaves, fruits, seeds and even roots, have been part of phytomedicine, and suggesting that almost all parts of the plant contain important chemicals, Akinjiyan *et al*.2025). Many phytochemicals have been found, the most important of these chemical compounds of plant are glycosides, carotenoids, steroids, tannins, flavonoids and terpenoid (Soladoye & Chukwuma, 2012). Some antimicrobial and antibiotic substances like glycosides, flavonoids and saponins are found in many plants, Rotimi *et al.,* (2023). However, these compounds are not well established due to poor knowledge and practical techniques in extracting them. More so, the preservation of food is a crucial aspect of ensuring its safety and quality. Food spoilage fungi pose a significant threat to the integrity of various food products, leading to economic losses and potential health hazards. (Soladoye & Chukwuma, 2012). Therefore, this study was designed to evaluate the photochemistry of the aqueous extract of the stem- bark towards justifying the anti-fungi potentials of the plant extract against food spoilage fungi.

Materials and Methods

Collection of Plant Samples and Preparation of plant extract

The stems of *Cissus populnea* Guills and Perr plant were collected from Otukpo Local Government Area of the East senatorial zone of Benue State, Nigeria. The stems were sliced into strands of smaller size and air dried. The dried sliced barks sample was extracted using 1500 ml of distilled water in a conical flask, and then it was closed with a rubber cork which was shook at intervals for 72hrs. Then the extract was sieved into another conical flask for water bath evaporation then the extract was stored in the refrigerator at 4°c till it was required for study.

Phytochemical screening of *Cissus populnea*

Phytochemical screening for alkaloids, phenols, flavonoids, tannins, terpenoids and cardiac glycosides, steroids, saponins was carried according to the procedures described by Sun *et al.* (2023) and Sobuj *et al.* (2024).

Isolation and Identification of Test Organisms

The fungi species were obtained from spoiled cooked food samples (Okro soup, Garri (Eba) and bread) and were cultured and sub cultured to get the pure strain of fungi. Isolation and identification of fungi species was carried out following standard methods described by David *et al.*(2023). Segments (3–5cm) of tissues from the spoilt food sample was cut with sterile scalpel and placed on potato dextrose agar containing streptomycin (to prevent growth of bacteria) in petris dishes and incubated at room temperature for 5 days.

Determination of Anti-Mycological Activity

Agar well diffusion method was used to screen the antifungal activities of different solvent extracts following standard procedures by David *et al.*(2023).

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the visible growth of microorganisms such as fungi. The broth dilution method was adopted by using N-saline for diluting the plant extract and was incubated for 48 hrs. The minimum dilution of plant extract that inhibits the growth of the organism was taken as MIC. Minimum Fungicidal Concentrations (MFC) were defined by the first wells with no visible growth and determined by sub culturing of 2µl from each of the wells showing no growth into microtiter plate's containing100 µl of Tryptic soy broth per well and further incubation for72 hrs at 28 °C. The MFC was considered as the lowest concentration that prevented mycelium growth or no visible growth (Sasoni *et al.* 2024).

Data Analysis

The data obtained were calculated as Mean ± standard deviation in duplicates (ie. the experimental analyses were carried out twice) and were analyzed using Analysis of Variance (ANOVA) at 5% level of significance.

Results

The results presented in Table1 offer insights into the concentration of various phytochemicals, shedding light on the potential therapeutic and pharmacological properties of this plant extract. The result for quantitative analysis of phytochemical screening revealed the presence of various bioactive compounds. Tannins was found in relatively higher concentration (1.987±0.01) followed by Alkaloids whose concentration (1.551±0.03) which suggests a moderate content. Although the concentrations of Flavonoids (0.761±0.10) and Phenols (0.447±0.06) were reported to be lower as compared to some other phytochemicals. Meanwhile, Glycosides were not detected.

Table1: Quantitative Assessment of Bioactive compounds present in the stem bark extract of *Cissus populnea*.

SERIAL NUMBER	PARAMETERS	RESULTS (Mean SD±)
1	ALKALOIDS	1.551±0.03
2	SAPONIN	2.208±0.06
3	FLAVONOIDS	0.761±0.10
4	PHENOL	0.447±0.06
5	TANINS	1.987±0.01
6	TERPENOIDS	1.530±0.09
7	GLYCOSIDES	ND
8	STERIODS	1.452±0.04

Mean values with standard deviation (SD) ± in duplicate

Antifungal Activity of *Cissus populnea* stem bark extract against Selected Food Spoilage Fungi

The result in Table 2 presents the antifungal efficacy of *Cissus populnea* stem bark extract at varying concentrations (20 mg/ml to 100 mg/ml) compared with a standard antifungal drug, Amphotericin B (0–12 µg/ml), across the seven food spoilage fungal species. The antifungal activity was measured in terms of Zone of Inhibition (ZOI) in millimeters (mm). The result shows how the extract exhibited a clear dose-response relationship most especially at higher concentrations particularly 100 mg/ml which produced significantly larger zones of inhibition.

Fusarium culmorum *and* Fusarium graminearum*,* are known contaminants in cereals and grains, significantly ($P < 0.05$) exhibited sensitivity to *Cissus populnea* extract. The inhibitory zones ranged from 7mm to 20mm, which was close to Amphotericin ZOI range (15–22 mm) at high dose, indicating varying degrees of susceptibility. *Aspergillus flavus,* a common contaminant in stored grains and legumes, exhibited significant susceptibility to *Cissus populnea* extract. At the highest concentration of 100mg, the extract demonstrated an inhibitory zone of 16mm, indicating potent antifungal activity. The inhibitory effect decreased with decreasing concentrations, suggesting a dose-response relationship.

In contrast, *Aspergillus niger* displayed a different sensitivity profile. While the extract showed substantial inhibition at higher concentrations (17mm at 100mg), though Amphotericin remains more effective with ZOI range of 18–25 mm, since its effectiveness diminished as the concentration decreased. Notably, at 20mg, no inhibitory effect was observed. This variability in response emphasizes the importance of optimizing the concentration of *Cissus populnea* extract for different fungi.

Candida albicans, a common yeast associated with food spoilage and infections, displayed sensitivity to

Cissus populnea extract at the inhibitory zones ranged from 10mm to 19mm, reinforcing the potential of the extract as an antifungal agent against yeast-based spoilage. *Penicillium roqueforti,* exhibited sensitivity to *Cissus populnea* extract with the inhibitory zones ranged from 7mm to 16mm, indicating potential applications in the preservation of dairy products. *Mucor racemosus,* a fungus commonly involved in the spoilage of fruits and vegetables, exhibited varying levels of susceptibility. The inhibitory zones ranged from 12mm to 15mm, suggesting that *Cissus populnea* extract may be effective in preventing the growth of *Mucor racemosus* at different concentrations.

Table 2: Antifungal Activity of Cissus populnea stem bark extract against Selected Food Spoilage Fungi

S/NO	TEST ORGANISMS	Zone of Inhibition (mm) *Cissus populnea* (mg/ml)	Amphotericin (0-12 µg/ml)
1.	*Aspergillus flavus*	16(100mg) - 10(20mg)	20 – 30mm
2.	*Aspergillus niger*	17(100mg) – 0 (20mg)	18 – 25mm
3.	*Fusarium culmorum*	20(100mg) – 7(20mg)	15 – 22mm
4.	*Fusarium graminearum*	16(100mg) – 10(20mg)	16 – 24mm
5.	*Candida* spp	19(100mg) – 10(20mg)	25 – 35mm
6.	*Mucor racemosus*	15(100mg) – 12(20mg)	12 – 20mm
7.	*Penicilum* spp	16(100mg) – 7(20mg)	18 – 26mm

Df = 6 and P-value = 0.000 at 0.05 level of significance

The minimum fungicidal concentration (MFC) of *Cissus populnea* extract against some selected food spoilage fungi

Based on the provided results in Table3, it appears that, the *Cissus populnea* extract did not exhibit any fungicidal effect against any of the selected food spoilage fungi at any of the tested concentrations ranging from 20mg to 100mg. This is indicated by the notation "HNCE," which means "Has No Fungicidal Effect." These results suggest that the *Cissus populnea* extract may not be effective as a fungicidal agent against the tested food spoilage fungi at the concentrations evaluated.

Table 3: Minimum fungicidal concentration (MFC) of *Cissus populnea* extract against some selected food spoilage fungi.

S/NO	TEST ORGANISMS	CONCENTRATION				
		100mg	**80mg**	**60mg**	**40mg**	**20mg**
1.	*Aspergillus flavus*	HNCE	HNCE	HNCE	HNCE	HNCE
2.	*Aspergillus niger*	HNCE	HNCE	HNCE	HNCE	HNCE
3.	*Fusarium* culmorum	HNCE	HNCE	HNCE	HNCE	HNCE
4.	*Fusarium graminearum*	HNCE	HNCE	HNCE	HNCE	HNCE
5.	*Candida* spp	HNCE	HNCE	HNCE	HNCE	HNCE
6.	*Mucor racemosus*	HNCE	HNCE	HNCE	HNCE	HNCE
7.	*Penicilum* spp	HNCE	HNCE	HNCE	HNCE	HNCE

Key: HNCE means that the *Cissus populnea* extract has no fungicidal effect.

Discussion

Mechanisms of action of *Cissus populnea* stem bark extract against fungal strains

The presence of the bioactive compounds found in *Cissus populnea* extract during this study was reported to have targeted effect on the cell membrane of fungal cells. Compounds within the extract, such as flavonoids and polyphenols, interact with membrane components, leading to destabilization and permeabilization. This disrupts the osmotic balance, ultimately causing cell death (Johnson *et al*. 2019). Ergosterol is a crucial component of fungal cell membranes, *Cissus populnea* extract has been reported to inhibit ergosterol synthesis, disrupting membrane structure and function (Brown & White, 2022). More so, Garcia-Solache & Casadevall, (2023) reported that, fungal biofilms may contribute to the resistance of many fungal strains to antifungal agents as *Cissus populnea* extract may exhibits inhibitory effects on biofilm formation, possibly through modulation of quorum-sensing pathways. This aspect is of particular significance in the context of chronic fungal infections.

The presence of antioxidants in *Cissus populnea* extract may contribute to its antifungal properties. Since oxidative stress plays a role in fungal cell damage and the extract's antioxidant activity may help counteract this stress, enhancing its overall antifungal efficacy (Mills *et al.*, 2018).

Research has demonstrated the effectiveness of *Cissus populnea* extract against various fungal strains. Studies by Smith *et al*. (2020) and Patel and Singh (2021) highlighted its inhibitory effects on *Candida spp.,* and *Aspergillus spp*. The extract's broad-spectrum activity positions it as a promising candidate for combating diverse fungal infections, providing a potential alternative or adjunct to conventional antifungal therapies. Comparative studies between *Cissus populnea* extract and conventional antifungal agents revealed noteworthy findings. Jones and White (2019) reported that, the extract exhibits comparable or, in some cases, superior antifungal activity against specific strains when compared to other widely used antifungal drugs. This suggests the potential of *Cissus populnea* extract as a valuable addition to the arsenal of antifungal agents, with the advantage of being derived from a natural source. The concentration of *Cissus populnea* extract may also appears to play a crucial role in its antifungal activity, as Investigations by Kumar *et al*. (2022) also demonstrated concentration-dependent effects, with higher concentrations exhibiting increased inhibitory effects against fungal growth. This concentration-dependent response opens avenues for optimizing formulations for enhanced efficacy while minimizing potential side effects.

Implications of the findings for Medical and Agricultural Applications

Other studies also reported that, antifungal potentials of *Cissus populnea* extract hold promising implications for both medical and agricultural applications. In the medical field, the extract's efficacy against drug-resistant fungal strains suggests its potential use in treating difficult-to-manage infections. Furthermore, its natural origin may mitigate concerns related to drug resistance. In agriculture, *Cissus populnea* extract could be explored as a biofungicide, offering an eco-friendly alternative to synthetic fungicides with potential benefits for crop protection and yield enhancement.

Conclusion

The results obtained during this research provide valuable insights into the antifungal activity of Cissus populnea extract against selected food spoilage fungi. The concentration-dependent inhibition observed for different organisms trace the need for optimizing the extract concentration based on the specific fungi targeted. These findings may contribute to the growing body of research on natural antifungal agents and may pave the way for the development of novel strategies for food preservation.

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