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Food Safety Implications: Assessing the Potential of *Desmodium velutinum* Leaves Extracts to Control the Most Predominant Fungal Contamination in Ready-To-Eat Fried Chicken

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Abstract

This study investigated the antifungal properties of *Desmodium velutinum* leaves extracts against *Aspergillus flavus*, the most prevalent fungus isolated from ready-to-eat fried chicken sold in Ihiala, Anambra State. A total of 21 samples were collected from street vendors and fast-food establishments and cultured on Sabouraud Dextrose Agar. The fungal isolates were identified based on colony morphology and microscopic characteristics. The leaf extracts of *D. velutinum* were analyzed for phytochemical constituents using spectrophotometry, revealing the presence of alkaloids, saponins, flavonoids, steroids, cardiac glycosides, phenolics, and tannins. The antifungal activity of the extracts was evaluated using agar-well diffusion and tube dilution methods. The ethanolic extract exhibited greater antifungal activity (16.00 mm) compared to the aqueous extract (14.00 mm), although both were significantly ($p \leq 0.05$) different from ketoconazole (21.50 mm). The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values demonstrated the inhibitory and cidal effects of the extracts. The study suggests that *D. velutinum* could be a potential alternative therapy for diseases associated with *A. flavus* and emphasizes the importance of personal and environmental hygiene in preventing fungal contamination of ready-to-eat foods. The findings highlight the potential of plant-based extracts in addressing food-borne fungal infections and promoting public health.

Keywords: *Phytochemical, Antifungal, Ketoconazole, Cidal, Aspergillus.*

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1. Introduction

Fungal contamination of food is a significant concern globally, posing health risks to humans and animals. Fungi such as mold and yeast can spoil food, producing toxins that lead to allergic reactions, central nervous system difficulties, and organ damage (Kennedy *et al.*, 2007). These microorganisms thrive in various environments, including acidic foods with low water content, and can grow on any food left for an extended period. *Aspergillus flavus*, a common soil fungus, can infest a wide range of agricultural products and produce aflatoxins, which are carcinogenic toxins (FAO/WHO, 2007). Aflatoxins have been linked to liver cancer in laboratory animals, and their presence in food is a significant public health concern. The

Joint Expert Committee on Food Additives (JECFA) has evaluated the risks associated with aflatoxin intake, highlighting the need for effective measures to prevent fungal contamination and mycotoxin production (FAO/WHO, 2007). Exposure to aflatoxins through contaminated food can lead to various health problems, including diarrhea, vomiting, abdominal pain, and in severe cases, convulsions, coma, and death (Scheidegger and Payne, 2003). *Aspergillus flavus* is also a leading cause of invasive aspergillosis, a potentially life-threatening disease. The treatment of fungal infections often involves antifungal agents, but these can be expensive, adulterated, or have toxic side effects (Kexianyi *et al*., 1998; Kennedy *et al*., 2007).

In many regions, particularly in Africa, traditional medicine and plant-based remedies are commonly used to treat various ailments, including diarrhea. *Desmodium velutinum*, a medicinal plant used in traditional medicine, has shown promise in treating diarrhea and other health issues (Anowi *et al*., 2012). The use of natural plants as medicinal agents has gained attention in recent years, with many studies exploring their potential benefits and efficacy (Sharma *et al*., 2001).

This study aims to evaluate the effect of *Desmodium velutinum* leaves extracts on *Aspergillus flavus*, a fungus commonly isolated from ready-to-eat fried chicken sold in Ihiala L.G.A., Anambra State. The findings of this research may contribute to the development of natural and effective remedies for fungal infections and food-borne illnesses.

2. Materials and Methods

Sample Collection: The fresh leaves of *Desmodium velutinum* were collected from Umuabani village Neni, Anaocha Local Government Area, Anambra State.

Plate 1: Leaves of *Desmodium velutinum*

Preparation of Samples for Extraction: The leaves of *Desmodium velutinum* were plucked off and dried under shade at room temperature for 14 days. The dried samples were pulverized using electric grinder, weighed and kept ready for the extraction of active ingredients (Ekesiobi 2025a; Ekesiobi *et al*., 2017).

Extraction Procedure: A 20 g portion of the sample was extracted by maceration in 200 ml of ethanol and water respectively for 3 days. The resulting extracts were subsequently filtered using Whatman No. 1 filter paper. The extracts were evaporated to dryness at room temperature in a steady air current (Ekesiobi *et al*., 2025b; Ekesiobi *et al*., 2025c).

Preparation of Test Sample: In this study, concentration of 400mg/ml of the extracts was used to screen for the antimicrobial activity. This was done by using the modified method of NCCLS (2000). Here, 2.5 g of the extract was dissolved in each of the extracting solvents.

Isolation and Identification of Test Organism: A total of 21 fried chicken samples were collected aseptically from different street hawkers (9) and fast food joints (12) in Ihiala L.G.A., Anambra State, using sterile aluminum foil to prevent contamination. The samples were transferred to microbiology laboratory within 1 h of collection. One gram (1 g) of each sample was weighed from each of the whole chicken samples and introduced into a test tube containing 10 ml of peptone water, also the whole chicken samples were washed using peptone water and the samples were seeded on Sabouroud dextrose agar (SDA) and potato dextrose agar (PDA) plate and incubated at room temperature (28-30 ℃) for 5 days. The organism obtained was aseptically subcultured on SDA plate and incubated for 5 days at room temperature. The pure culture of the test organism was characterized and identified using their colonial descriptions, microscopic characteristics and Fungal atlas (Iheukwumere *et al*., 2012; Ekesiobi *et al*., 2025d; Ekesiobi *et al*., 2025e; Ekesiobi *et al*., 2025f).

Lacto phenol cotton blue test: The identification of molds is based on the shape, method of production, and arrangement of spores (conidial ontogeny). Lactophenol blue solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal elements are stained intensely blue. The preparation has three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls. The procedure involves placing a clean glass slide on a sheet of white paper, followed by a small drop of lactophenol cotton blue in the center of the slide. Using a stick broken into half to produce a rough tapered edge, remove a fragment of a fungal colony (approximately 1-2 mm from the periphery) with the broken stick and place in the lactophenol cotton blue. Gently tease the fragment until it has been separated. Gently lower a coverslip over it. Do not tap or push down as this may dislodge conidia from conidiophores. Examine microscopically for presence of fungal elements. For a permanent preparation, rim the cover slip with clear nail polish.

Maintenance of Test Organism: The isolated organism was used for the antifungal sensitivity testing. Prior to the test, the organism was sub cultured on SDA at room temperature (28-32℃) for 5 days. Then the culture was transferred into peptone water and incubated at room temperature for 5 days (Iheukwumere and Umedum 2013).

Sensitivity Testing Using Agar-Well Diffusion Method: This was carried out using the modified method of (Iheukwumere and Umedum 2013). Each labeled plate was uniformly inoculated with the test organism using pour plating method. A sterile cork borer of 5 mm diameter was used to make wells on the medium. One tenth milliliter (0.1 ml) of

various concentrations of the extracts was dropped into each labeled well and then incubated at room temperature for 3 days. Antifungal activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation. Ketoconazole (500mg/ml) was used as control.

Determination of Minimum Inhibitory Concentration (MIC): This was carried out using the modified method of Iheukwumere and Umedum 2013). Here, various concentrations of the test extracts were obtained using double-fold serial dilution. Each dilution was assayed against the test organism using tube dilution method. One milliliter (1 ml) of the test organism was added into each dilution and incubated at room temperature for 7 days. The MIC was defined as the lowest concentration able to inhibit any visible fungal growth. This was determined and recorded.

Determination of Minimum Fungicidal Concentration: This was determined using the modified method of (Iheukwumere and Umedum 2013). Here, equal volumes of various concentrations of those tubes that did not produce any visible growth from MIC were sub cultured on sterile pour plate and incubated at room temperature for 3 days. The lowest concentration of the extracts that killed the test organism was taken as the MFC.

Statistical Analysis: The results of the data generated were expressed as mean, percentage and Table; Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test as described in the study published by Abiodun *et al*. (2024a), Abiodun *et al*. (2024b) and Abiodun *et al*. (2024c), Iheukwumere *et al*. (2025g), and Iheukwumere *et al*. (2025h).

3. Results

The result of the phytochemical constituents of the leaf extract of *Desmodium velutinum* is shown in table 1. The result revealed the presence of alkaloids, cardiac glycosides, flavonoids, tannins, saponins, steroids and phenolics. The phytochemicals constituents may be responsible for the activity of the leaf extracts of *Desmodium velutinum*.Table 2 shows the number and percentage of fungal isolates from ready-to-eat fried chicken samples. Fungi isolated includes *Aspergillus flavus, Aspergillus niger, Cunninghamela elegans and Chrysosporium* species, of which *A. flavus* is the predominant fungi species isolated from the fried chicken samples having a total number and total percentage value of 21 (50.00%) respectively.Table 3 shows the characterization and identities of the isolates. The following organisms were isolated; *Aspergillus flavus*, *Aspergillus niger*, *Cunninghamella elegans* and *Chrysosporium* spp. from ready-to-eat fried chicken using potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) media, characterized and identified based on their colonial description, microscopic characteristics and the help of fungal atlas.The mean diameter zones of inhibition (mm) of the aqueous and ethanolic extracts of *Desmodium velutinum* against *A. flavus* were shown in table 4. The study revealed that the extracts inhibited *A. flavus*. The ethanolic extract inhibited the test organism more than the aqueous extract and their inhibitory activities differed significantly ($P< 0.05$) from that of ketoconazole (control). The absolute ethanol (0.1 ml) and distilled water (0.1 ml) used in this study as extracting solvents had no effect on the tested organism as shown in table 4. The results of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the ethanolic and aqueous extracts of *Desmodium velutinum* are shown in table 5. The result revealed that the ethanolic extract exhibited more pronounced activity than the aqueous extract. The ethanolic and aqueous leaf extracts of *D. velutinum* exhibited similar MICs compared to ketoconazole (control) which had more pronounced activity. The ethanolic leaf extract of *D. velutinum* and ketoconazole (control) showed similar fungicidal activity compared to aqueous leaf extract of *D. velutinum* which had no fungicidal activity against *A. flavus*.

Table 1: Phytochemical constituents of *Desmodium velutinum* extracts

Phytochemicals	Amount (g/100g)
Alkaloids	3.45
Cardiac glycosides	0.52
Flavonoids	2.94
Tannins	2.14
Saponins	1.34
Steroids	0.64
Phenolics	1.24

Table 2: Prevalence of the fungal isolates from ready-to-eat fried chicken samples

Isolates	**Street vended fried chicken (%)**	**Fast food joints fried chicken (%)**	**Total (%)**
Aspergillus flavus	14 (66.67)	7 (33.33)	21 (50.00)
Aspergillus niger	9 (69.23)	4 (30.77)	13 (30.95)
Cunninghamella elegans	4 (66.67)	2 (33.33)	6 (14.29)
Chrysosporium species	2 (50.00)	2 (50.00)	4 (9.52)
Total	29 (65.91)	15 (34.19)	42 (100.00)

Table 3: Characteristics and identities of the isolates

Isolates	Colonial description	Microscopic examination
Aspergillus flavus	The colonies are granular to wooly, Yellow green, downy to powdery, and have some shade of yellow or yellow-brown.	The conidiophores are long (400-800µm), the vesicle are 25-45µm in diameter, phiallides are three fouth or the entire circumference of the vesicle, may be uniserated, or biserated, conidia may be spherical, smooth or slightly roughened.
Aspergillus niger	Initially white, turning yellow becoming dark to deep brown jet black. The underside of the colony is buff or yellow-grey.	The vesicles are globose, they bear a double row of phiallides, conidiation is extremely profuce, has spherical, black conidia that become roughened with maturity.
Cunninghamellaelegans	*Cunninghamella* colonies are rapidly growing, cottony, and white to tannish-gray in color. The reverse is pale, produces purely gray colonies	Nonseptate or sparsely septate broad hyphae, terminal vesicles, Sporangiophores are erect and form short lateral branches each of which terminates in a swollen vesicle. The vesicle has spine-like denticles on its surface. Sporangioles are round to oval in shape, one-spored, and are formed on these denticles. Sporangiospores are one-celled, solitary, and globose to ovoid in shape. The walls of the spores often have needle-like crystals. Zygospores have tuberculate projections and may form only after appropriate mating studies.
***Chrysosporium* species**	*Chrysosporium* colonies grow moderately rapidly. They may be granular, woolly, or cottony and flat, or raised and folded in appearance. From the front, the color is white cream, yellow or tan to pale brown. The reverse is white to brown.	Hyphae are septate while the conidia are hyaline, broad-based, one-celled, and smooth- or rough-walled. These conidia are broader than the vegetative hyphae and occur terminally on pedicels, along the sides of the hyphae, or in intercalary positions. Arthroconidia are abundant and larger than their parent hyphae in diameter.

Table 4: Diameter zone of inhibition of *Desmodium velutinum* extract against *A.flavus* using 5mm cock borer

Extract (400mg/ml)	*A. Flavus* ($\bar{x}$± SD)mm
EED	16.00 ± 2.92
AED	14.00 ± 0.82
Ketoconazole	21.50 ± 0.50
Absolute ethanol (0.1ml)	0.00 ± 0.00
Distilled water (0.1 ml)	0.00 ± 0.00

Key: EED = Ethanolic extract of *D. velutinum*, AED = Aqueous extract of *D. velutinum*, Ketoconazole= Standard

Table 5: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration of the extract against *A. flavus*

Extract	MIC (mg/ml)	MFC (mg/ml)
EED	400	400
AED	400	Nil
Ketoconazole	200	400

4. Discussion

In the present study, *Aspergilus flavus*, *Aspergilus nigel*, *Cunninghamella elegans* and *Chrysosporium species* were the fungal organisms isolated from ready-to-eat fried chicken samples, of which *A. flavus* was the most predominant species. This correlates with the findings of other researchers (Ekesiobi

et al., 2025g; Ekesiobi *et al*., 2025h; Iheukwumere *et al*., 2025a; Iheukwumere *et al*., 2025b, Iheukwumere *et al*., 2025c). The higher incidence of moulds in the fried chicken samples may be attributed to the use of different untreated food additives, which may be the main source of mould contamination in fried chicken (Ekesiobi *et al*., 2025g). Ready-to-eat (RTE) foods can be described as food that was meant for immediate consumption at the point of sale. It could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Ekesiobi *et al*., 2025h). Different terms have been used to describe such ready- to- eat foods: These include convenient, ready, instant and fast foods. Examples of such ready to eat foods include pastries, meat pie, sausage, rolls, burger, moin-moin, salad or coleslaw, fried meat, fried chicken, milk and milk products (Iheukwumere *et al*., 2025a). WHO, (2002) reported that food borne illnesses of microbial origin are a major health problem associated with street- foods as well as fast food centers and this is as a result of the traditional processing methods that are used in preparation, inappropriate holding temperatures, prolong exposure to the atmosphere and poor personal hygiene of food handlers which are some of the main causes of contamination of street-vended food. Fungi contamination of street vented items as well as in fast food restaurants among other contaminants poses major health problems to consumers. Among the problems includes diarrhea as a result of improper handling of food items (Iheukwumere *et al*., 2025c). Therefore, the present study was carried out to evaluate the effect of *Desmodium velutinum* leaf extracts on the most predominant fungus isolated from ready-to-eat fried chicken sold in Ihiala L.G.A., Anambra State.

The results of the present study revealed that the leaf extracts of *Desmodium velutinum* showed pronounced activity against *Asperguilus flavus*, which was the most predominant fungus isolated from ready-to-eat fried chicken sold in Ihiala L.G.A., Anambra State. The result of the phytochemical analysis revealed the presence of alkaloids, cardiac glycosides, flavonoids, tannins, saponins, steroids and phenolics (table 1). The presence of these phytochemicals may be responsible for the antifungal activity exhibited by the leaf extracts of *D. velutinum*. Similar findings were made by different researchers (Iheukwumere *et al.*, 2012; Iheukwumere *et al*., 2025d; Iheukwumere *et al*., 2025e).

The result of the diameter zone of inhibitions of the ethanolic and aqueous leaf extracts of *D. velutinum* against *A. flavus* clearly revealed the pronounced activities of the extracts against the tested organism. The ethanolic extract showed more activity against *A. flavus* than the aqueous extract. This showed that the active phytochemical constituents of the leaf had more ability to dissolve in ethanol (organic solvent) than/in water (inorganic solvent). Similar conclusion was drawn by different researchers (Iheukwumere *et al*. 2012; Iheukwumere *et al*., 2025f). Though aqueous extract produced higher amount of extract but exhibited relatively lower activity than the ethanolic extract which was obtained in lower quantity. This indicates that the amount of yield did not always influence the inhibition of microbial growth but the active ingredients found in the extract play the major role. Similar observation was made by Iheukwumere *et al*., (2012). The study further highlighted that ethanol was able to extract more of the phytochemical constituents because ethanol is an organic and polar solvent and most of the phytochemical constituents are organic in nature. This observation suggested that the organic solvent extraction is suitable to verify the antifungal properties of medicinal plants (Iheukwumere *et al.*, 2012; Iheukwumere *et al*., 2025d; Iheukwumere *et al*., 2025e). Some phytochemical work by intercalating with DNA of the organism (e.g. alkaloid), interferes with protein synthesis and disrupt cell membrane (e.g. saponins) while others interfere signal transduction pathway, metabolic processes, damage metabolic and cellular enzymes, disrupt proton motive force, electron flow, coagulation of cell component and modulation of gene expression (Kotzekidou *et al.*, 2008).

No studies have been carried out to evaluate the antifungal activity of *D. velutinum* leaf extract, but similar studies using the stem and leaf extract of *D. velutinum* have been documented. Anowi *et al*., (2012) reported the anti- pyretic activity of *Desmodium velutinum* leaf extract in albino wistar rats. The extract showed significant anti-pyretic activity on experimental rats used in the study (Anowi *et al*., 2012). The gastrointestinal antimotility and gastroprotective properties of the leaf and stem extracts of *D. velutinum* against castor oil-induced diarrhea in rats was reported by Ezike *et al*., (2013). Nwankwo, (2013) also reported the cardio protective effect of N-hexane extract of *Desmodium velutinum* stem on albino wistar rat. The study showed that N-hexane extract of *Desmodium velutinum* stem posse's cardio protective effect on albino wistar rats than the known drug (vasoprin) if only one can increase the dosage of the extract for a period of time (Nwankwo, 2013).

The result of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the leaf extracts of *D. velutinum* showed that ethanolic and aqueous leaf extracts of *D. velutinum* exhibited similar activity in their MICs compared to ketoconazole (control) which had more pronounced activity. Also the ethanolic leaf extract exhibited similar fungicidal activity with ketoconazole (control). This means that infections caused by *A.flavus* could be managed effectively using the single dose of this leaf extract. Also, further research involving *in vivo* assays will be needed to establish the relationship between the MICs and MBCs obtained in this study and the effective dosage that should be administered in ethnomedical practice.

5. Conclusion

This study highlights the health risks associated with consuming street-vended foods contaminated with pathogens like *A. flavus*, which can cause diarrhea. The findings demonstrate the antifungal activity of *Desmodium velutinum* leaf extract against *A. flavus*, supporting its traditional use in treating diarrhea and suggesting a potential safe and economical alternative to conventional treatments

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