

Antimicrobial potential of shea butter production waste : *In vitro* antifungal activity of shea hull and press cake methanolic extracts.

Abstract :

Fungal infections represent a major health concern, particularly due to the emergence of strains with reduced sensitivity to commonly used antifungal treatments. In this context, exploring new natural substances offers a promising avenue. This study aimed to evaluate *in vitro* the antifungal activity of methanolic extracts from shea (*Vitellaria paradoxa*) shells and seed cakes against *Candida albicans*, *Aspergillus flavus*, and *Aspergillus fumigatus*. The selection of shea is justified by its importance in traditional uses and the presence of bioactive compounds in its various parts, notably in the shells and seed cakes derived from kernel processing. Therefore, "agar diffusion" method were carried out by immersing sterile microbiological discs in shea hulls/press cakes macerats (50 mg/mL) and placing them on Sabouraud Agar which were pre-impregnated with microbial suspensions (10^{-3} UFC). Inhibition halos around the discs were measured after incubation. Minimum inhibitory concentration (MIC) was also determined. Phytochemical screening was also performed to identify the main classes of compounds present in the extracts.

The results obtained show that the three tested fungal strains are sensitive to the shea shell and seed cake extracts, with inhibition zones ranging from 13 to 17 mm. The MICs were 1.56 mg/mL for *A. flavus* and *A. fumigatus*, compared to 6.25 mg/mL for *C. albicans*. Phytochemical screening revealed the presence of polyphenols, flavonoids, tannins, saponins, steroids, and triterpenes in both extracts. These findings highlight the promising antifungal potential of shea shells and seed cakes, suggesting that these co-products could serve as a valuable source of bioactive substances. They also open up new avenues for the improved valorization of residues from shea processing.

Keywords : *Vitellaria paradoxa*; shea shells; shea seed cakes ; antifungal activity;

Introduction

Infections caused by fungi (*Aspergillus*) or yeasts (*Candida*), also known as mycoses, are increasingly difficult to inactivate, with a rapidly growing frequency, thus causing a real public health problem (Azim and Ahmed, 2024, Esmaeili et al., 2025).

Candida sp and *Aspergillus* are occasional but often dangerous parasites (Azim and Ahmed, 2024, Stemler et al., 2024). *Aspergillus* is a filamentous species of opportunistic pathogenic fungus, but very virulent in the event of a drop in immunity, particularly in HIV-positive patients. It causes cutaneous and systemic infections, particularly bronchopulmonary infections, through its vegetative form or through the spores that it disseminates in the air (Tan et al., 2022, Stemler et al., 2024). As for *Candida* sp, it induces several types of infections ranging from a simple skin infection to more serious cases such as oral and sexual infections. It should also be noted that *Candida* sp and *Aspergillus* cause cases of food poisoning (Lass-Flörl et al., 2024). Despite the availability of antibiotics and appropriate modern drugs, a resurgence of these infections is observed. The appearance of strains resistant to the usual antibiotics and the emergence of the pathogenicity of strains that are usually saprophytic in our environment are the main factors of this strong resurgence (Vitiello, 2023). The increase in fungal resistance to conventional drugs and the fact that most antifungal drugs only have fungistatic activity, justify the search for new strategies to fight fungal infections (Dong et al., 2023, Esmaeili et al., 2025). Thus, several works have highlighted the biological activities (antifungal, antibacterial, anti-inflammatory, antioxidant, etc.) of several aromatic and medicinal plants; in particular the inhibitory power of their extracts on the growth and toxinogenesis of several bacteria and fungi responsible for food and skin infections (Dong et al., 2023, Jadimurthy et al., 2023, Esmaeili et al., 2025). These considerations led us to consider the possibilities of using shea to treat various infections involving fungal and bacterial agents.

Indeed, shea is a multipurpose tree where all its parts are used by rural populations ; the leaves, bark, flowers, latex, roots and shea butter are widely used in traditional pharmacopoeia (Ojo et al., 2021). However, the biological activity of shea cakes and hulls (waste products from shea butter production) has not been studied to date.

The objective of this study is therefore to investigate the *in vitro* antifungal activity of methanolic extracts of shea hulls and cakes on two fungal strains and one yeast, with the aim of searching for active natural substances for a possible biological control.

60

61 **MATERIALS AND METHODS**

62 **Plant material**

63 Organic material for this study consisted in shea hulls and press cakes which constitute waste
64 products from the shea butter process of (Megnanou et al. 2007). Shea nuts collected in
65 m'bahiakro, town located in the central east of Ivory Cost.

66 **Microorganisms used**

67 Antifungal activity of shea hulls and press cakes extracts was tested against two fungal strains
68 (*Aspergillus fumigatus*, *Aspergillus flavus*) and one yeast (*Candida albicans*). These strains
69 were supplied by the National Polyclinic Institute Houphouët Boigny of Yamoussoukro ; they
70 are known to be cause several infectuions.

71 **METHODS**

72 **Shea hulls and press cake hydromethanolic extracts setting**

73 Method described by Guede-Guina et al. (1997) was used for methanolic extraction of shea
74 hulls and press cakes.

75 100 g of shea hulls/press cakes powder were homogenised under magnetic agitation in 1 L of
76 70% methanolic for 48 hours. After filtering of the mixture on hydrophilic cotton and
77 wattman paper, solvent was evaporated at 40°C using a rotavapor. The extracts obtained
78 (SHE and SPCE, hulls and press cakes, respectively) were stored at 4°C. After recovery of the
79 extracts, a solution (50 mg/ml) of each extract was prepared and autoclaved at 121°C for 15
80 minutes to perform the antifungal test. The sterility of the extract solutions was checked by
81 sowing aliquots of each solution onto Sabouraud agar and incubated at 30°C for 24 hours.

82 **Phytochemical screening of shea hulls and press cakes extracts**

83 Phytochemical screening of shea hulls and press cakes extracts consisted in detecting their the
84 active constituents, namely, as far as polyphenols, flavonoids, saponins, steroids, tannins and
85 triterpennic alchools are concerned. Therefore, methods described by Martinez et al. (2003),
86 Sofowra (1993) and Wall et al. (1952) were carried.

87 **Antimicrobial test**

88 **Preparation of microbial suspensions**

Before performing the antimicrobial essays, sprouts were cultured in microbiological nutrient broth at 37°C for 24 hours and then transplanted on Sabouraud agar. An isolated colony of each strain were used to inoculate 10 mL of microbiological nutrient broth. The suspension was homogenized and 1 mL aliquot was used to prepare 10⁻³ diluted suspension. 10 µL of the previous suspension were spread on Sabouraud agar. Agar plates were incubated at 30°C for 1 to 7 days and then, the colonies were counted to determine the microbial load De Souza et al. (1995).

Antimicrobial assay

Antimicrobial activity was determined by the disc diffusion method (NCCLS, 1997). 100 µL of the previously prepared suspension was spread on petri dishes containing Sabouraud agar. Sterile 6 mm diameter discs were separately impregnated with 20 µL of our extracts and placed on the agar inoculated with the selected microorganisms. Blank discs were used as negative controls. Petri dishes containing microorganisms and discs were stored at 4°C for 1 h and incubated for 48 h at 30°C. Antimicrobial power of extracts was matérialized by an inhibition zone which diameter was measured to evaluate extracts efficiency in vitro, against each strain. Strains were classifie either sensitive, highly sensitive, extremely sensitive or resistant Hamidi (2013) as fonction to the diameter of inhibition zone. Hence :

- Non-sensitive (-) or resistant: diameter < 8 mm.
- Sensitive (+): diameter between 9 and 14 mm.
- Very sensitive (++) : diameter between 15 and 19 mm.
- Extremely sensitive (++) : diameter > 20 mm.

Determination of minimum inhibitory concentration (MIC)

Andrews (2001) agar dilution method was adopted in this study with little modification. The agar dilution method was used to determine the concentration of extract which would inhibit the growth of fungi seeded on Sabouraud agar. Therefor, extracts serial dilutions were performed in ordre to get successive concentrations (50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg/ml). The antimicrobial test described above was performed for each concentration. The Minimum Inhibitory Concentration (MIC) has been determined (to the naked eye), and corresponds to the lowest concentration of the extract for which no halo is visible around the impregnated disc.

120

121 **Statistical Analysis**

122 Analysis test were carried out in triplicate and the data were expressed as mean $\pm$ SD.
123 Statistical was performed utilizing Statistica version 7.1, One-way analysis of variance
124 (ANOVA) followed by the LSD Post Hoc test. The P values more than 0.05, less than ≤ 0.05
125 and $\leq$ less than 0.01 were considered as not significant, significant and highly significant
126 values respectively.

127 **Results and discussion**

128 Resistance of microorganisms to synthetic antibiotics has led researchers to search for new
129 natural molecules by increasingly turning to extracts from various plant organs such as leaves,
130 seeds and flowers. Thus, in this study, antimicrobial activity of methanolic extracts of shea
131 hulls and cakes against infection-causing strains was evaluated by determining the inhibition
132 zone diameters and MICs (Tables II and III). Analysis of the results showed that all the strains
133 tested were susceptible to both extracts. Indeed, inhibition zone diameters were between 13
134 and 17 while the MICs were 1.56 mg/mL for *Aspergillus* strains and 6.25 mg/mL for *Candida*
135 *albicans*. These findings align with those of Kouakou and Megnanou (2021), who also
136 demonstrated the antifungal activity of methanolic extracts from *Vitellaria paradoxa* shells
137 and seed cakes. However, while their study focused on *Candida albicans*, *Trichophyton*
138 *rubrum*, and *Microsporum canis*, our work extends these findings to include *Aspergillus*
139 *flavus* and *Aspergillus fumigatus*. The inhibition power of the shea hull and oilcake extracts
140 can be correlated to the presence of phenolic compounds in our extracts insofar as several
141 works carried out and allowed to attribute the antimicrobial activity of several plants to their
142 phenolic fraction. This hypothesis is further supported by the work of Kouakou et al. (2021),
143 who demonstrated the presence of several phenolic compounds in shea shells and seed cakes.
144 More broadly, recent studies show that various plant secondary metabolites can contribute to
145 the observed antifungal activity against *Aspergillus* and *Candida* species (Tan et al., 2022;
146 Khan et al., 2023). Indeed, according to the literature, the antimicrobial activity of plants
147 depends on the nature and structure of phenolic compounds; and more precisely to its
148 hydroxyl function. This functional group binds to soluble extracellular proteins as well as to
149 the cell walls of microorganisms to inhibit their proliferation (Tan et al., 2022; Khan et al.,
150 2023).

Furthermore, phytochemical screening of methanolic extracts of shea hulls and oil cakes revealed the presence of two major families of molecules, namely phenolic compounds (tannins, flavonoids and saponins) and terpenes (sterols and triterpene alcohols) (Table I). The presence of these metabolites could contribute to the antimicrobial activity observed in the present study. This interpretation is consistent with the work of Kouakou et al. (2021), who reported the presence of phenolic compounds and flavonoids in shea hulls and press cakes. Recent studies have further reported antifungal activities associated with plant tannins and flavonoids. These compounds may interfere with fungal growth through several mechanisms, including alterations of membrane integrity and interference with cellular processes (Tan et al., 2022; Khan et al., 2023; Savu and Stefan, 2024). Several recent studies have also reported antifungal activities associated with plant phenolic compounds and flavonoids, supporting the possible contribution of these metabolites to the activity observed in the present study (Tan et al., 2022 ; Khan et al., 2023; Dong et al., 2023).

The inhibitory effect of the methanolic extracts of shea hull and oilcake against the fungal strains tested highlights their potential as sources of bioactive compounds. However, further studies involving the isolation and characterization of the active compounds, as well as toxicity and in vivo evaluations, are necessary before considering therapeutic applications.

Table I : Phytochemical screening of shea hulls and press cake extract

	Polyphenols	Flavonoids	Tannins	Saponin	Steroids	Triterpenes
SHE	+	+	+	+	+	+
SPCE	+	+	+	+	+	+

(-) = Absent (+) = Presence

SHE : Shea Hull Extract ; SPCE : Shea Press Cake Extract

Table II : Shea hulls and press cake inhibition halo diameter

Microorganisms	SHE	SPCE
<i>Candida albicans</i>	15	13
<i>Aspergillus flavus</i>	15	17
<i>Aspergillus fumigatus</i>	15	16

Table II : Minimum inhibitory concentrations of shea hulls and press cakes extracts against various fungal strains

Microorganisms	Minimum inhibitory concentration (mg/mL)	
	SHE	SPCE
<i>Candida albicans</i>	6.25	6.25
<i>Aspergillus flavus</i>	1.56	1.56
<i>Aspergillus fumigatus</i>	1.56	1.56

Conclusion

The present study demonstrates the antifungal potential of methanolic extracts from shea (*Vitellaria paradoxa*) shells and seed cakes against *Candida albicans*, *Aspergillus flavus*, and *Aspergillus fumigatus*. The results show that all three tested strains are susceptible to the extracts, with inhibition zone diameters ranging from 13 to 17 mm. The minimum inhibitory concentrations of 1.56 mg/mL obtained for *A. flavus* and *A. fumigatus*, and 6.25 mg/mL for *C. albicans*, reflect a significant antifungal activity. Furthermore, phytochemical screening revealed the presence of several classes of secondary metabolites, notably polyphenols, flavonoids, tannins, saponins, steroids, and triterpenes. The presence of these compounds could contribute to the observed activity, although their individual involvement was not established within the scope of this study. Overall, these findings underscore the value of shea shells and seed cakes as potential sources of bioactive substances. They also offer a promising outlook for the valorization of these often-underutilized co-products of the shea industry.

References

- Andrews, J. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, 48 (1): 5-16.
- Azim, A. & Ahmed, A. (2024). Diagnosis and management of invasive fungal diseases in non-neutropenic ICU patients, with focus on candidiasis and aspergillosis: a comprehensive review. *Frontiers in Cellular and Infection Microbiology*, 14: 1256158. <https://doi.org/10.3389/fcimb.2024.1256158>.
- De Souza, C., Koumaglo, K. & Gbeassor, M. (1995). Evaluation des propriétés antimicrobiennes des extraits totaux de quelques plantes médicinales. *Pharmacopée et médecine traditionnelle africaine*: 103-112.
- Dong, F.-R., Gao, L., Wang, L., Jiang, Y.-Y. & Jin, Y.-S. (2023). Natural products as antifungal agents against invasive fungi. *Current Topics in Medicinal Chemistry*, 23(19): 1859–1917. <https://doi.org/10.2174/1568026623666230417105227>.
- Esmaeili, A., Saleh, I. & Abu-Dieyeh, M. H. (2025). Antifungal potential of plant-based extracts against *Candida* species: values, safety concerns, and possible applications. *Phytochemistry Reviews*, 24: 5801–5844. <https://doi.org/10.1007/s11101-025-10093-x>.
- Guede-Guina, F., Kra, A. M., Vangah-Manda, M., Bonga, G. M. & De Souza, C. (1997). Inhibition par MISCA-F2 de la croissance de *Aspergillus fumigatus* ; *Candida albicans* et *Cryptococcus neoformans*, 3 germes fongiques opportunistes au cours du SIDA. *J Afr Biomed*, 2: 11-16.
- Hamidi, A. (2013). Etude phytochimique et activité biologique de la plante *Limoniastrum guyonianum*. Thèse de doctorat, Université Kasdi Merbah, Ouargla, Algérie, 86.
- Jadimurthy, R., Jagadish, S., Nayak, S. C., Kumar, S., Mohan, C. D. & Rangappa, K. S. (2023). Phytochemicals as invaluable sources of potent antimicrobial agents to combat antibiotic resistance. *Life*, 13(4): 948. <https://doi.org/10.3390/life13040948>.
- Khan, A., Moni, S. S., Ali, M., Mohan, S., Jan, H., Rasool, S., Kamal, M. A., Alshahrani, S., Halawi, M. & Alhazmi, H. A. (2023). Antifungal activity of plant secondary metabolites on *Candida albicans*: an updated review. *Current Molecular Pharmacology*, 16(1): 15–42. <https://doi.org/10.2174/1874467215666220304143332>.

Kouakou, A. B. & Megnanou, R.-M. (2021). Potential treatment of mycosic dermatoses by shea (*Vitellaria paradoxa*) nutshells and press cakes: in vitro efficacy of their methanolic extracts. *Arabian Journal of Medicinal & Aromatic Plants*, 7(3): 341–351.

Kouakou, A. B., Doué, G. G., Mégnanou, R.-M. & Djoman, A. E. S. (2021). Shea shells and press-cake as new sources of bioactive phenolic compounds: GC-MS profile and in vitro antioxidant activity. *European Journal of Biology and Biotechnology*, 2(5): 53–59.
<https://doi.org/10.24018/ejbio.2021.2.5.252>.

Lass-Flörl, C., Kanj, S. S., Govender, N. P., Thompson, G. R. III, Ostrosky-Zeichner, L. & Govrins, M. A. (2024). Invasive candidiasis. *Nature Reviews Disease Primers*, 10: 20.
<https://doi.org/10.1038/s41572-024-00503-3>.

Martinez, A., Valencia, G. & Marcha, F. (2003). Phytochemical screening methods. In *Manual de Practicas de Farmacognosia y Fitoquimica*, edited by Medellín, Universidad de Antioquia, pp. 59-65.

Megnanou, R.-M., Niamke, S. & Diopoh, J. (2007). Physicochemical and microbiological characteristics of optimized and traditional shea butters from Côte d'Ivoire. *African Journal of Biochemistry Research*, 1 (4): 41-47.

National Committee for Clinical Laboratory Standards. (1997). Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A. Wayne, PA: NCCLS.

Ojo, O., Kengne, M. H. K., Fotsing, M. C., Mmutlane, E. M. & Ndinteh, D. T. (2021). Traditional uses, phytochemistry, pharmacology and other potential applications of *Vitellaria paradoxa* Gaertn. (Sapotaceae): a review. *Arabian Journal of Chemistry*, 14: 103213.
<https://doi.org/10.1016/j.arabjc.2021.103213>.

Savu, M. & Stefan, M. (2024). Anti-Candida activity of flavonoids - an overview. *Journal of Experimental and Molecular Biology*, 25(1): 67–84. <https://doi.org/10.47743/jemb-2024-126>.

Sofowra, A. (1993). *Medicinal plants and traditional medicines in Africa*. New York, 256.

Stemler, J., Nacov, J. A., Sprute, R., Cornely, O. A., Joest, M., Becker, S. & Klimek, L. (2024). *Aspergillus*-associated diseases from an infectious diseases and allergological perspective. *Allergo Journal International*, 33: 140–152. <https://doi.org/10.1007/s40629-024-00286-9>.

Tan, L. F., Yap, V. L., Rajagopal, M., Wiart, C., Selvaraja, M., Leong, M. Y. & Tan, P. L. (2022). Plant as an alternative source of antifungals against *Aspergillus* infections: a review. *Plants*, 11(22): 3009. <https://doi.org/10.3390/plants11223009>.

Vitiello, A., Ferrara, F., Boccellino, M., Ponzo, A., Cimmino, C., Comberiati, E., Zovi, A., Clemente, S. & Sabbatucci, M. (2023). Antifungal drug resistance: an emergent health threat. *Biomedicines*, 11(4): 1063. <https://doi.org/10.3390/biomedicines11041063>.

Wall, M. E., McClenna, M. L. & Klump, M. E. (1952). Detection and estimation of steroid and sapogenins in plant tissue. *Analytical Chemistry*, 24: 1337-1342.

UNDER PEER REVIEW IN IJAR