Potential treatment of mycosic dermatoses by shea (*Vitellariaparadoxa*) nutshulls and press cakes: *in vitro* efficacy of their methanolic extracts

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Abstract : Shea (*Vitellariaparadoxa*) nutshulls and press cakes, are generally considered as waste or just partially exploited as fertilizer or fuel. However, the present study demontrated *in vitro,* the antifungal activity of thesesub-products macerats, against strains of *Candida albicans*, *Tricophytonrubrum* and *Microsporum canis*. Therefore, "*agar diffusion*" mehod were carried out by immersing sterile microbiological discs in shea hulls/press cakes macerats(50 mg/mL)and lying them down on Sabouraud Agar which were pre-impregnated with microbial suspensions (10^{-3} UFC). Inhibition halo around discs were measured days after incubation. Minimum inhibitory concentration (MIC) was also determined. Results revealed significant ($p < 0.01$) inhibition power of both macerats on all the tested strains ; the latent showed variable sensibility. Indeed inhibition halo of *Tricophytonrubrum, Microsporum canis*, *Candida albicans*18887, and *Candida albicans*18702, measured 13.5±1 and 14±1.5 ; 13±0.6 and 15±0.9 ; 13±1.3 and 15±1.2 ; 17 ± 3.1 and 11±1.4 mm, respectively for shea hulls and press cakes macerats. MIC also varied signficantly (3.125 to 12.5 mg/mL). The best antimicrobial efficiency was recorded by hulls against *Candida albicans*18702 and by press cakes against *Microsporum canis*. About *Tricophytonrubrum* and *Candida albicans*18887,MIC value was6.25 mg/mL with both macerats. This antimicrobial power would be linked to flavonoids, tannins, saponosides, steroids and triterpens, detected in both macerats.

With such constituents and ability, sheahulls and press cakes might bevalorized as powerfull organic ressources of active biomolecules for pharmaceutical and cosmetics formulations under to preventor/andto heal fungal infections.

Keywords:Active biomolecules, antifungal, minimal inhibitory concentration, sheahulls and press cakes

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Introduction

According to studies carried out by the World Health Organization (WHO) in developing countries, fungal diseases nowplay an important role in infectious pathology, with a prevalence ranging from 21 to 87% (OMS 2005). The frequency, recurrence and the severity of these diseases have been increasing in recent decades in West Africa in general and in Côte d'Ivoire in particular (Dosso et al. 1986, De Souza et al. 1995). Despite the existence of antibiotics and appropriate modern drugs, a resurgence of fungal diseases has been observed, caused by the advent of several factors including HIV-AIDS, that leads to an increase in the frequency of opportunistic fungal infections. Skin infections of mycotic types have thus become a real public health problem (Eholié 1993, Dupont 1987, Kra 2001). There are several antifungal molecules used in the treatment of mycoses, their effect would be to eliminate the germs involved or to stop their growth. However, some of these molecules often prove to be highly toxic to human cells(Aubry and Gaüzère 2015), while others have lost their efficiency due to resistance and mutation phenomena(Dupont et al. 1996, Feuilhade De Chauvin 1998). In addition to these various difficulties, there is the high cost of active drugs, which is therefore, out of modest incomes peuple reach(Dupont et al. 1997, Loren et al. 1992). These situations have led scientific community to search new bioactive molecules by focusing more and more on biological sources, namely on medicinal plants. Thus, several studies have been undertaken in order to detect the antibacterial and/or antifungal activity of different parts (root, stem, bark, leaves, etc.) of several medicinal plants (Akakpo-Akue 2006, Guede-Guina et al. 1997, Ouattara et al. 2016). Among these medicinal plants, is shea (*Vitellariaparadoxa*) tree, which has several virtues. Indeed, all parts of the tree are traditionally used to treat several health disturbences(Dubut 2012, Amougou Marie 2009). Fat (shea butter) extracted from shea fruits kernels is also widely exploited for several medicinal purposes. During its production (Bonkoungou 1987, Pesquet 1992, Hall et al. 1996)hulls and press cake are discarded, because they are considered as rebus, and often cause environmental pollution problems. In some areas, they are just exploited as fuel or animal feed(Nkouam 2007, Tchakala et al. 2019), even if Kitamura et al. (2003)demonstrated the ability of shea kernels pigment to be ingused as food pigment. According to these authors, this pigment would be linked to phenolic compounds. Moreover, some studies revealed the presence of bioactive molecules (vitamins, phytohormons, phenolic compounds, etc.) in the hulls of cereals

and oleaginous seeds ; this would justified heir use in animals feeling. Such information about seeds hulls and shea kernels would suggest their exploitation as active bimolecular matrix for pharmacological purposes in order to benefit from their potential properties.Hence,in the interest of demonstrating the therapeutic potential of shea hulls and press cake the present study aimed to *in vitro*, the antifungal efficiency of their extracts against strains responsible for mycosis.

MATERIALS AND METHODS

Plant material

Organic material for this study consisted in sheahulls and press cakes. All the material was kindly provided by the *Unit of Pedagogy and Research in Biotechnology*, located at the University Félix Houphouët-Boigny (Côte d'Ivoire). Shea hulls and press cakes for the present study, constituted rejects from the process of Megnanou et al. (2007).

Microorganisms used

Strains of yeasts (*Candida albicans* 18702, *Candida albicans*18887) and fungis (*Trichophyton rubrum* and *Microsporum Canis*) were used for antimicrobial test. These strains were supplied by the National Polyclinic Institute Houphouët Boigny of Yamoussoukro; they are known to cause several mycosis.

METHODS

Sheahulls and press cake hydromethanolic extracts setting

Extraction process consisted in macerating heahulls/press cakes powders in hydromethanolic mixture, as described by Guede-Guina et al. (1997).

A suspension of 100 g of sheahulls/press cakes powder in 1 L of 70 % (v/v), were homogenized under magnetic agitation for 48 hours. The mixture was filtered on hydrophilic cotton and Wattman paper, and methanol was evaporated at 40°C using a rotavapor, until obtaining a dried macerat-powder. The resulting powders (SHE and SPCE, hulls and press cakes, respectively) were stored in sterile vials at 4°C.For antimicrobial test, fresh solutions (50mg/ml) of each extract were prepared by dissolving 2.5 g in 50 mL of solvent and autoclaved at 121°C for 15 min. The sterility of the extract solutions was checked by sowing aliquots of each solution on agar Sabouraud and incubated at 30°C for 24 hours.

Phytochemical screening of sheahulls and press cakes extracts

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

Antimicrobial test

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 prepare10^{-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

Disc diffusion method has been chosen for antibacterial assay (Mukhtar and Ghori 2012). Duplicates of sterile6 mm diameter discs (Wattman N°1 paper - Selecta, Germany), soaked with 20 µl of sheahulls/press cakes extracts and then placed on Sabouraud agar which was previously contaminated by spreading and flooding microbial suspensions on the surface Petri dishes containing contaminated agar and impregned discs were then, incubated for 1 to 7 days at 30°C. Antimicrobial power of extracts was materialized by an inhibition zone which diameter was measured to evaluate extracts efficiency *in vitro*, against each strain. Discs impregnated with sterile distilled water were used as negative control. 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. Therefore, extracts serial dilutions were performed in order 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.

StatisticalAnalysis

Analysis test were carried out in triplicate and the data were expressed as mean ± SD. Statistical was performed utilizing Statistic a version 7.1, One-way analysis of variance (ANOVA) followed by the LSD Post Hoc test. The P values more than 0.05, less than ≤0.05 and ≤ less than 0.01 were considered as not significant, significant and highly significant values respectively.

RESULTS

Phytochemical screening

Phytochemical screening carried out on the extracts of sheahull and cake revealed the presence of two families of compounds, namely phenolic compounds and terpenoids. As for phenolic compounds, the presence of compounds such as tannins, flavonoids and saponins could be demonstrated. As for the so-called terpene compounds, the presence of sterols and triterpene alcohols was proven (Table 1).

Table 1:Phytochemical screening of sheahulls and press cake extract

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

(-) = Absent (+) = Presence

SHE : Shea Hull Extract ; SPCE : SheaPress Cake Extract

Antimicrobial activity of sheahulls and pres scakes extracts

Results of sheahulls and press cakes extracts efficiency against fungal strains are resumed in Table2. A significant difference was observed between hulls and press cakes activities. However, at 50 mg/ml of concentration both extracts induced inhibition halo with the whole studied strains. These inhibition halos diameters ranged from13 to 17 mm and from11 to 15mmfor hulls and press cake, respectively. Hence, all the tested strains were either sensible (9 to 14 mm) or very sensible (15 to 19 mm) to both sheahulls and press cakes extracts. More precisely *Candida albicans* 18702wasvery sensible to sheahulls extract while *Trichophyton rubrum*,*Microsporum canis* and *Candida albicans*18887 were just sensible. With shea press cakes extracts, the whole tested strains were very sensible except for *Candida albicans*18887 (11±1.4 mm).

Table 2:Sheahulls and press cake inhibition halo diameter

Microorganismes	**Inhibition halo diameter (mm)**		
	SHE	**SPCE**	**SDW**
***Candida albicans*18702**	17±3.1	11±1.4	0
***Candida albicans*18887**	13±1.3	15±1.2	0
Trichophyton rubrum	13.5±1	14±1.5	0
Microsporum canis	13±0.6	15±0.9	0

SHE : Shea Hull Extract; SPCE : SheaPress Cake Extract; SDW : Sterile Distielled Water

Minimal inhibitory concentration of sheahulls and press cakes extracts

Minimum inhibitory concentrations of the sheahulls and press cakes extracts against the whole tested strains ranged from 3.125 to 12.5 mg/mL, and varied from a strain to another (Tables 3and4). Considering sheahulls extract, its MIC was the lowest (3.125 mg/mL) against *Candida albicans* 18702 strain, and the highest (12.5 mg/mL) against *Microsporum canis. S*hea press cakes alsohad the lowest MIC (3.125 mg/mL) against *Microsporum canis* strain;but the highest value (12.5 mg/mL) was recorder against *Candida albicans* 18702.

Table 3: Minimum inhibitory concentrations of sheahulls extracts against various fungal strains

Conc. (mg/mL)	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.5625**	**0.78125**
C. albicans 18702	-	-	-	-	-	+	+

C. albicans 18887	-	-	-	-	+	+	+
T. rubrum	-	-	-	-	+	+	+
M. canis	-	-	-	+	+	+	+

Conc. = Concentration;(-) = Growth inhibited; (+) = Growth

Table 4: Minimum inhibitory concentrations of sheapress cakes extracts against four fungal strains

Conc. (mg/mL)	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.5625**	**0.78125**
C. albicans 18702	-	-	-	+	+	+	+
C. albicans 18887	-	-	-	-	+	+	+
T. rubrum	-	-	-	-	+	+	+
M. canis	-	-	-	-	-	+	+

Conc. = Concentration;(-) = Growth inhibited; (+) = Growth

DISCUSSION

Drugs against microbial diseases consistmost of time, in antimicrobial molecules which would be generally synthetic (Aubry and Gaüzère 2015). Some of these molecules would be microbe's secondary metabolites. Both sources were adopted in substitution to vegetal resource, for biodiversity preservation. However, the present study proposes a vegetal source of antimicrobial molecules, which would not only preserve vegetal biodiversity but would also contribute to reduce the amount of environmental polluents in shea butter producing areas. Indeed, in these regions, sheahulls and press cakes constitute producing rejects. or a few parts is used either as fuel or as animal feed (Nkouam 2007, Tchakala et al. 2019). Results of the present study showed that extracts of sheahulls and press cakes have (*in vitro*) a significant antifungal power against germs inducing several mycoses. This property can be correlated to the inhibition power of phenolic and terpenenoic compounds detected in their hydromethanolic extracts. Indeed, similar works have been carried out on plant parts (leaves roots, etc.), and the antimicrobial activity was attributed to their phenolic and terpene fraction (Balinado and Chanb 2018, Salwa et al. 2018). Appropriately, can be cited the work of Cafarchia et al. (1999)whoshowedthat the antifungal activity of propolis against dermatophytes and *Candida sp*was due to its high flavonoid content. As well, those of Stern et al. (1996) and Min et al. (2008) who showed that tannins would possess

toxic activity against filamentous fungi, yeasts and bacteria, then also be cited. Furthermore, the presumed presence of tannins and flavonoids in sheapress cakes confirms the results obtained by Kitamura et al. (2003)regarding the extraction of food pigment from shea kernel.

The inhibitory effect of the hydromethanolic extracts of sheahulls and press cake against the pathogenic tested microbial strains thus, places them as potential candidates for the development of drugs for the treatment of mycoses caused by these strains. Moreover, their efficiency Bergman at relatively low concentration against current mycoses germs like *Candida albicans* 18702, *Candida albicans*18887, *Trichophyton rubrum* and *Microsporum Canis*. It worth noting here that *Trichophyton rubrum* would cause mycoses such as athletic foot. As for *Candida albicans* 18702 and *Candida albicans*18887, they would induce many moth, skin and sexual infections. About *Microsporum Canis*, it woud cause ringworm(Aubry and Gaüzère 2015).Many of these infections would need association of several molecules from various sources. Hence, instead of constituting an environment polluent (rejects) or being exclusively used as fuel/animal food, sheahulls and press cakes can be considered as organic sources (matrix) of efficient active molecules with antimicrobial and other pharmacologic abilities.

Conclusion

Skin diseases are nowadays an important part of infectious pathology, despite the existence of appropriate modern antibiotics. The objective of this study, which aims to effectively combat skin infections, was to evaluate the antimicrobial activity of sheahulls and press cakes, by-products of shea butter processing. This study revealed that their hydromethanolic extracts contain phenolic and terpenoic compounds which would confer to them, antifungal power with relatively low minimum inhibitory concentration. It would therefore be very appropriate to consider their use in the treatment of mycotic diseases rather than reducing them to a waste product or only to animal feed.

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Competing interests:
The authors have no conflicts of interest relevant to the ideas and/or contents of the manuscript.

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