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Assessment of Antifungal Activity of an African Medicinal Herb *Thonningia sanguinea* Against *Cryptococcus neoformans*

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Abstract: The treatment of infectious diseases with herbs is a traditional practice in many societies. Among medicinal herbs, *Thonningia sanguinea* (THOS) is used in treatment of diarrhoea, asthma and mycoses in certain African populations in Ivory Coast, Togo and Ghana. Despite the marked reductions in rates of AIDS-associated fungal infections, such as cryptococcosis, in United States, the burden of these diseases in developing countries is large and increasing. In this study, we have evaluated the antimicrobial activity of aqueous extracts of *Thonningia sanguinea* on *Escherichia coli*, *Salmonella enteritidis*, *Shigella sonnei*, with emphasis on *Cryptococcus neoformans*. Microorganims were cultured for 48 hours on agar in presence of increasing concentration of aqueous extract of THOS to determine the minimum inhibitory concentration (MIC) and the concentration that inhibits growth by 50% (IC_{50}). All microorganisms tested show different sensitivity (dose-response) to aqueous extract. We chose the most sensitive microbe to improve the extraction. We show that an extract prepared in ethanol (96%) reduces dramatically the IC_{50} on *Cryptococcus neoformans*; suggesting that the antimicrobial active principle of THOS may be soluble in an organic solvent.

Key words: *Thonningia sanguinea* · *Cryptococcus neoformans* · antifungal activity

INTRODUCTION

According to World Health Organization [1], 80% of the population in developing countries are unable to afford pharmaceutical drugs and rely only on plant based traditional medicines to sustain their primary health care needs. There was a renewed interest for phytomedicine during last decade and nowadays many medicinal plant species are being screened [2]. *Thonningia sanguinea* Vahl (family Balanophoraceae) [3, 4] also called N'goudro or Kouwleni in Djimini (a vernacular language in Ivory Coast) is one such important medicinal plant, which is used against several pathologies including bronchial asthma, skin diseases and dysentery [5]. Given its interesting therapeutic properties, *Thonningia sanguinea* (THOS) is studied for pharmacological activities. In this respect, it has been demonstrated that THOS is a potent antioxidant [6, 7]. Only a few reports exist regarding the antimicrobial properties of THOS [8]. Therefore, the spectrum of this plant remains to be defined.

In this context, the present work was designed to investigate the antimicrobial activity of THOS on three enterobacteria (*Escherichia coli*, *Salmonella enteritidis* and *Shigella sonnei*) and one fungus (*Cryptococcus neoformans*).

MATERIALS AND METHODS

Preparation of medicinal plant extract: Inflorescences and roots of THOS were collected in 2002 in the savanna of Sokala-Sobara, a village of the department of DABAKALA, northern region of Ivory Coast. These materials were washed and dried at room temperature; then ground with a crusher IKA-MAG/type MFC/JANKE & KUNKEL. The extracts were prepared as described by KRA [9]. Briefly, after weighing 200g, the powder was put into 2 L of distilled water or ethanol 96%; the mixture was stirred for 48 hours at room temperature. This mixture was double filtered on absorbent cotton and on Whatman paper 3 mm, successively. The filtrate was evaporated with reduced pressure in a Rotavapor at 70°C to obtain aqueous or ethanol extract according to the extraction solvent.

Microorganisms: The isolates of *Escherichia coli*, *Salmonella enteritidis*, *Shigella sonnei* and

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Cryptococcus neoformans were a gift from the Faculty of Medicine of Cocody University of Ivory Coast.

***In vitro* assays:** The growth of the strains was evaluated on selective agar medium (Drigalski, Salmonelle/Shigelle (S/S) and Sabouraud, containing increasing concentration of THOS extract. The plates were used as controls. One for media sterility containing no microbe and one to assess microbes growth; both plates did not contain THOS extract. All tubes and plates were previously sterilized at 121°C for 15 min and the pH was ajasted [10]. We inoculated 10µl of a 10^{-1} dilution of the inoculum on THOS treated plates and non-treated plates and incubated them for 48 hours at optimum temperature (37°C for enterobacteria and 30°C for the yeast). The colonies were counted and compared with control which was considered as 100% of growth. Testing known concentrations of extracts in agar media allowed quantification of activity and determination of MIC. The surface of the corresponding MIC plates were brushed and grown in new media. Each extract was tested in triplicate.

RESULTS

All tested strains were sensitive to the aqueous extracts of THOS. *C. neoformans* was the least resistant to the antimicrobial activity of this extract (Table 1). The dose-response effect was observed with the aqueous extract (Fig. 1 A) as well as with the ethanol extract (Fig. 1 B) on *C. neoformans*. When we switched from the aqueous extract to the ethanol extract, the IC_{50} and the MFC were considerably reduced (Fig. 1 A and B). Indeed, by making the ratio MFC_{aq}/MFC_{eth} (6.25/0.098), we obtained an antifungal effect 63 times higher for the ethanol extract. The ethanol extract showed a reduction of the IC_{50} from 1. 5 mg ml^{-1} to 0.06 mg ml^{-1}.

DISCUSSION

The purpose of this study was to evaluate the antimicrobial activity of THOS on four microorganisms. The antibiograms carried out from the percentages of survival allowed determination of the concentrations

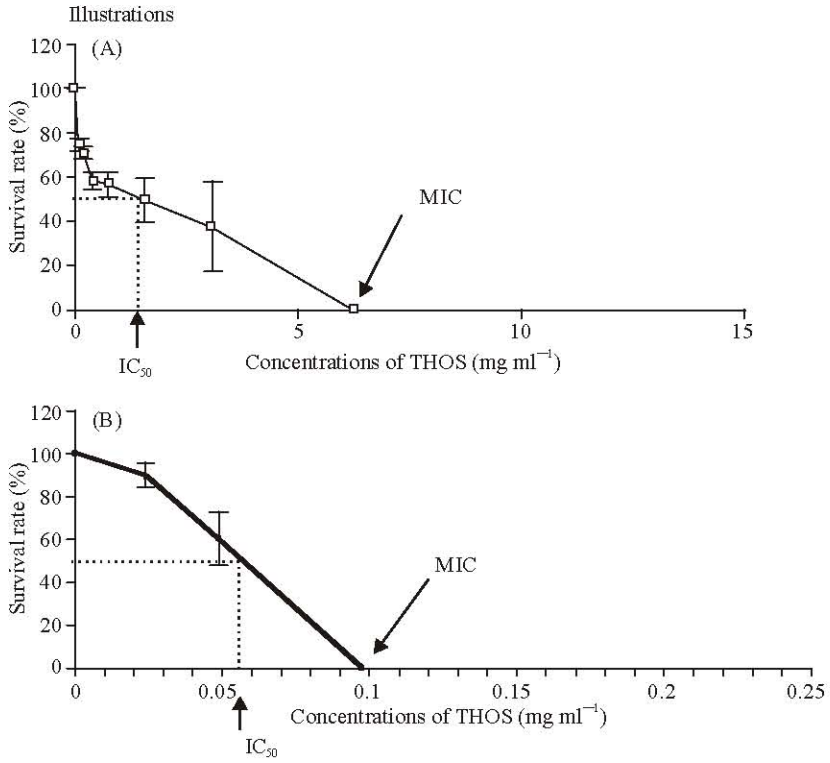

Fig. 1: Sensitivity of Cryptococcus neoformans to two extracts of THOS after 48 H of incubation
A: Dose-response effect of aqueous extract on the growth of Cryptococcus neoformans. Increasing concentrations of THOS extract reduced progressively the survival rate of this microbe. The MIC (minimum inhibitory concentration) is reached at 6.25 mg/ml. B : Effect of ethanol extract on the growth of Cryptococcus neoforman after 48 hours of culture. A dose-response effect is also observed, with a MIC at 0.098 mg/ml. IC50aq/ IC50eth = 1.5/0.06 = 25

Table1: Antimicrobial activity of aqueous extract of THOS
The aqueous extract of THOS inhibits the *in vitro* growth of *Escherichia coli*, *Salmonella enteritidis*, *Shigella sonnei* and *Cryptococcus neoformans*. The IC_{50} (concentration inhibiting 50% of growth) for each microbe is indicated

Microorganisms	IC_{50}
Escherichia coli	93.1 mg ml^{-1}
Shigella sonnei	47.4 mg ml^{-1}
Salmonella enteritidis	35.5 mg ml^{-1}
Cryptococcus neoformans	1.5 mg ml^{-1}

necessary to inhibit growth by 50%. The values of aqueous extract are presented in Table 1. We have demonstrated that the MIC of THOS for *C. neoformans* can be reduced from 6.25 mg ml^{-1} to 0.098 mg ml^{-1} using ethanol instead of water (Fig. 1A and B). In addition, the ethanol extract reduced the IC_{50} of THOS activity on *C.neoformans* by a factor of 25 (Fig. 1A and B). These results suggest that *C. neoformans* is the most sensitive strain to *Thonningia sanguinea* and that ethanol extraction is more appropriate.

All the strains tested affect human health, but cryptococcosis is a life-threatening opportunistic fungal infection in both HIV-positive and –negative patients [11]. Despite major advances in the treatment of HIV infection, cryptococcosis is still a major problem for AIDS patients [12]. The incidence of cryptococcosis in patients with negative serology for HIV ranges from 0.2 to 0.9 per 100 000 inhabitants [13] and the mortality rate remains around 15-20% in HIV- negative and HIV-positive patients in countries where highly active antiretroviral therapy (HAART) is available [14]; however, it is much higher in some countries of Africa and southeast Asia [15-17], thus calling for improvement of therapeutic management [18, 19]. Cryptococcosis can be caused by two varieties of encapsulated yeast *C. neoformans*: *C. neoformans* var. *neoformans* and *C. neoformans* var. *gatti*; the former being more prevalent [18]. *C. neoformans*: *C. neoformans* var. *neoformans* has strong tropism for the central nervous system and most of the infections involve the brain [20] causing higher morbidity and mortality. Its ecological niche is in the immediate environment of human. In fact, it has been associated with the feces of pigeons [20]. Hence, we focused our study on this fungus. To verify whether MIC corresponded to minimal fungicidal concentrations (MFC), we brushed the plates containing THOS that showed no growth and inoculated new plates containing no THOS. We observed no growth in these new media after 48 hours, indicating that the MIC corresponds to MFC. This suggests that aqueous extract as well as ethanol extract of THOS can have fungicidal effects on this fungus. However, ethanol extract multiplied this effect by a factor of 63 if we consider the MFC values (MFC_{aq}/MFC_{eth}). Our results point in the same direction of Ohiri and Uzodinma who have shown that the methanol extraction was more efficient for this plant [8]. Taken together, the results suggest that active principles of THOS are organic solvent soluble. The broad spectrum action of *Thonningia sanguinea* (*Apergillus niger*, *Candida albicans*, *Bacillus subtilis*, *Klebisiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* [8], *Salmonella enteritidis, Shigella sonnei* and *Cryptococcus neoforman* may sustain its traditional use against several infections diseases.

On the basis of results obtained *in vitro*, ethanol extract of THOS could be used for the identification of bioactive molecules to treat cryptococcosis and other bacterial infections.

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