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Research Article

In vitro Antifungal Activity of the Soap Formulation of the Hexane Leaf Extract of *Morinda morindoides* (Morinda; Rubiaceae)

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Abstract

Purpose: *The aim of this study was to formulate the hexane extract of the leaves of Morinda morindoides (Baker) Milne-Redh (Rubiaceae) as soap and evaluate its antifungal activity against fungal isolates of human origin.*

Method: *The hexane extract of Morinda morindoides was incorporated as an antifungal agent in soap (SMM) and tested against 4 strains of dermatophytes (Candida albicans, Trichophyton rubrum, Trichophyton mentagrophytes and Aspergillus fumigatus) using basic soap (BS) as control. Agar dilution method at serial concentrations ranging from 125 mg/ml to 3.9 mg/ml was used for the determination of the antimicrobial parameters - MIC (minimum inhibitory concentration) and IC_{50} (concentration producing 50 % inhibition) for these strains.*

Result: *The growth of all fungal strains tested was inhibited by the Morinda morindoides extract soap (SMM) at MIC of 31.25 mg/ml. On the other hand, basic soap (control) inhibited Candida albicans at MIC of 125 mg/ml and at MIC of 62.50 mg/ml for the other 3 strains tested. Thus, SMM showed stronger antifungal activity against the strains tested than the control (basic soap).*

Conclusion: *The hexane extract of Morinda morindoides leaves incorporated in soap exerted antifungal activity against the fungal strains tested. Thus, this soap formulation may find use in the treatment of dermatomycoses.*

Keywords: *Morinda morindoides; Antifungal activity; Hexane extract; Soap; Dermatophytes*

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INTRODUCTION

The rapid development of multiresistant bacterial and fungal strains of clinically important pathogens has spurred scientists to attempt to develop newer broad spectrum antimicrobial agents [1]. High cost of new generation antibiotics has directed a searchlight on less costly sources of effective antimicrobial substances such as plants. A number of herbs with significant antimicrobial activity have been reported in the literature [2]. The importance of African traditional medicine in the management of diseases has long been established [3]. In Ivory Coast, traditional medicines are increasingly sought from tradomedical practitioners and herbalists for the treatment of various diseases. Among the remedies used, plant drugs constitute an important part. A number of scientific investigations have highlighted the importance and contribution of many medicinal plants [4].

Morinda morindoides (Baker) Milne-Redh (Rubiaceae) is found at the borders of tropical forests. In the Democratic Republic of Congo, *M. morindoides* has long been used in villages and towns in the treatment of some parasitic diseases; the leaf extracts of the plant have been shown to possess antiprotozoal activity particularly against *Entamoeba histolytica* as well as antirheumatic properties [5]. The decoction of the leaves has also been used for the treatment of malaria, intestinal worms, and amoebiasis [6]. *M. morindoides* is well known in the traditional medical practice of the west central part of Ivory Coast. It is commonly called Zêlékelé in the local language of 'Bété' where it is used as an antimicrobial agent where the leaves are employed in traditional medicine to treat diarrheoa [7].

Ten flavonoids, identified as quercetin, quercetin-7, 4-dimethylether, quercetin-3-O-rutinoside, quercetin-3-*O*rhamnoside, kaempferol-3-*O*-rhamnoside, kaempferol-3-O-rutinoside, kaempferol-7-*O*rhamnosylsophoroside, chrysoeriol-7-*O*neohesperidoside, apigenin-7-*O*-glucoside, and luteolin-7-*O*-glucoside [8], have been isolated from the butanol and ethyl acetate fractions of the plant leaf. In addition, a total of eight iridoid glycosides, among which are gaertneroside, gaertneric acid, methoxy-gaertneroside and epoxygaertneroside, were also isolated from the same fractions by Cimanga *et al* [5].

The aim of this study was to formulate the hexane extract of the leaves of *Morinda morindoides* (Baker) Milne-Redh (Rubiaceae) as soap and to evaluate the *in vitro* antifungal activity of the soap against fungal isolates of human origin, namely, *Candida albicans, Trichophyton rubrum, Trichophyton mentagrophytes* and *Aspergillus fumigatus*.

EXPERIMENTAL

Plant material

The leaves of *Morinda morindoides* (Rubiaceae) were collected from Daloa (central west region of Ivory Coast) in June 2006. The plant was identified and authenticated by Pr AKE ASSI, of the Department of Botany, University of Cocody. A voucher specimen (no. 17710) of the plant was deposited in the herbarium of the National Floristique Center of the University of Cocody-Abidjan.

Preparation of hexane extract

The leaves of *M. morindoides* were cleaned of extraneous matter, air-dried at room temperature for 7 days and ground into a fine powder. The powdered material (100 g) was extracted with 250 ml of hexane (Merck, Darmstadt, Germany) for 24 h using a Soxhlet extractor. The extract was filtered with Whatman filter paper no. 1, and the filtrate was evaporated under vacuum in a rotary evaporator (Buchi) at 55 °C. The extract obtained was used as antifungal agent to make soap.

Preparation of soap

Soap containing the extract was prepared by incorporating the extract into basic soap prepared as follows. 60 g of sodium hydroxide was dissolved in 170 ml of water and allowed to cool to 35 – 40 °C. Also, 450 g of a mixture (w/w) of coconut oil (Sicor, Ivory Coast) and palm oil (Blohorn, Ivory Coast) were heated in a water bath at 55 °C. Both the aqueous and oily phases were mixed together. To 90 g of the soap formed, 10 g of the extract was added and gently stirred to form a viscous gel. The remainder of the soap (590 g) was also gently stirred to form a viscous gel which served as control. The two soap formulations were poured into separate soap moulds. Two days later, the two solidified products were removed from the moulds and used for the *in vitro* antifungal tests.

Fungal cultures

The antifungal activity of the soap containing the extract was evaluated against 4 strains of fungi provided by the Medical Sciences Department of Cocody University, Abidjan, Ivory Coast. These strains were clinical isolates of the following dermatophyte species: *Candida albicans* 3076/PV/06/04/00, *Trichophyton rubrum* 2630/D/24/07/99, *Trichophyton mentagrophytes* 13801/D/19/06/99 and *Aspergillus fumigatus* 896/AB/18/01/00.

Antifungal assay

Antifungal activity was assessed according to the agar dilution method [9] on Sabouraud agar (ref 695771, Dardilly Admy 42310, Oxoid Ltd). The extract soap and the basic soap (control) were incorporated into the growth medium in tubes to give serial two-fold dilutions. The resulting concentrations ranged from 125.0 to 3.9 mg/ml. A tube, containing nutrient broth only, seeded with test organism was served as growth control. The tubes were inoculated with a microorganism suspension with a density of 10^5 cells/ml and then incubated at 30 °C for 5 days. All experiments were performed in triplicate. Antimicrobial parameters (MIC and IC_{50}) were determined after counting the colony of fungi in the tubes for each series. The total score of colony of the control tube was considered as 100 %. MIC (minimal inhibitory concentration) was defined as the lowest concentration that produced no visible fungal growth after the incubation period while IC_{50} was defined as the concentration that produced 50 % inhibition. IC_{50} values were computed from the survival curves for fungi strains using of Prism® version 5.01 (GraphPad, USA).

Statistical analysis

The data were analyzed by one-way ANOVA followed by Dunnett's t-test using Instat® (Graph Pad software, U.S.A). Results were considered statistically significant at 95 % confidence interval ($p < 0.05$).

RESULTS

The antifungal effects of test soap and control on the four fungi strains are presented in Table 1. The values were used for the determination of MIC and IC_{50}, and the data for these parameters are recorded in Table 2. The MIC data indicate that *C. albicans* was inhibited at a concentration of 125 mg/ml for the basic soap (control) compared with 31.25 mg/ml for the test soap (SMM) containing the extract. The other three fungi *(T. rubrum, T. mentagrophytes* and *A. fumigatus)* were each also inhibited at a concentration of 31.25 mg/ml (SMM) but at a higher concentration of 62.5 mg/ml by the control (BS). *C. albicans* presented the highest IC_{50} of 10.41±0.01 for BS and 6.5±0.01 for SMM while the other fungal strains showed lower IC_{50} values ranging from 2.44±0.01 to 2.78±0.01 for SMM and 3.25±0.01 to 6.50±0.01 for BS.

Table1: Comparative antifungal activities of the soap containing *Morinda morindoides* (SMM) extract and basic soap (BS, control) against four fungal strains (mean ± SEM, n = 3, p < 0.05

Soaps concentrations (mg/ml)								
Fungus	**Soaps**	**0**	**3.90**	**7.81**	**15.62**	**31.25**	**62.5**	**125**
Candida albicans	BS	100.0±1.7	90.0±1.7	60.0±1.7	30.0±0.6	5.0±0.6	2.0±0.6	0
	SMM	100.0±1.0	70.0±1.2	40.0±0.6	6.0±0.6	0		
Trichophyton rubrum	BS	100.0±2.0	40±1.7	30.0±2.1	10.0±0.6	5.0±0.6	0	
	SMM	100.0±1.7	30.0±1.2	10.±1.2	3.0±0.6	0		
Trichophyton mentagrophytes	BS	100.0±1.2	30.0±0.6	20.0±0.6	5.0±0.6	2.0±0.6	0	
	SMM	100.0±1.7	20.0±1.2	15.0±1.7	3.0±1.2	0		
Aspergillus fumigatus	BS	100.0±2.1	70.0±0.6	30.0±1.2	15.0±1.2	5.0±0.6	2.0±0.6	0
	SMM	100.0±1.0	30.0±0.6	10.0±1.2	2.0±0.6	0		

Table 2: Antifungal parameters for the test soap containing *Morinda morindoides* extract (SMM) and basic soap (BS, control) against four fungi strains (mean±SEM)

Fungus	**BS**		**SMM**	
	IC_{50} (mg/ml)	**MIC (mg/ml)**	**IC_{50} (mg/ml)**	**MIC (mg/ml)**
Candida albicans	10.41±0.01	125	6.50±0.01	31.25
Trichophyton rubrum	3.25±0.01	62.50	2.78±0.01	31.25
Trichophyton mentagrophytes	2.78±0.01	62.50	2.44±0.01	31.25
Aspergillus fumigatus	6.50±0.01	62.50	2.78±0.01	31.25

DISCUSSION

To evaluate the antifungal activity of the basic soap formulation of the hexane extract of *Morinda morindoides*, we compared its activity with that of basic soap (BS) alone which served as control. For each type of soap, the results showed a decrease in the number of colonies with increase in the extract concentration of the soap. The results showed that *T. mentagrophytes* was the most sensitive strain while *C. albicans* was the most resistant, irrespective of the soap type. The other two strains, *T. rubrum* and *A. fumigatus*, presented intermediate sensitivity in relation to the other two strains. The MIC and IC_{50} data also indicate that of the two soap formulations, the one containing the plant extract (SMM) was more active and inhibited more effectively the growth of the test strains.

To the best of our knowledge, this is the first time the antifungal activity of the hexane extract of *M. morindoides* has been reported. This observation may be attributed to the nature of its biologically active contents. The antifungal activity may be due mostly to the lypophilic metabolites in the extract since a lipophilic solvent was used for the extraction. Phytochemical screening of *M. morindoides* by Cimanga *et al* [8] yielded ten flavonoïds, namely, quercetin, quercetin-7, 4-dimethylether, quercetin-3-O-rutinoside, quercetin-3-*O*rhamnoside, kaempferol-3-*O*-rhamnoside, kaempferol-3-O-rutinoside, kaempferol-7-*O*rhamnosylsophoroside, chrysoeriol-7-*O*neohesperidoside, apigenin-7-*O*-glucoside, and luteolin-7-*O*-glucoside), amongst other compounds. These flavonoïds are likely to have played a major role in the antifungal activity of the extract since they are lipophilic due to the presence of a phenyl chain. The lipophilic nature of these

compounds would facilitate induction of antifungal activity by interaction with the cell membranes of the fungi. In addition, flavonoids exhibit antioxidant properties [9] which are believed to be responsible for the inhibitory effects exerted on several enzymes. Thus, the characteristics of the constituents, as outlined in the foregoing, might have been responsible for the antifungal activity of the soaps containing the plant extract.

CONCLUSION

The fungal strains used in this study are mostly responsible for superficial mycoses. However, treatments of these infections are sometimes ineffective. This study has shown that incorporation of the hexane extract of *M. morindoides* leaves in soap exerted antifungal activity against dermatophytes, some of which are the most frequent species implicated in dermatomycoses in Ivory Coast. Further research is needed to determine the toxicological profile of this soap formulation and as well as develop it further for subsequent clinical evaluation.

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