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DEVELOPMENT AND ANTIMICROBIAL POTENTIAL OF SOAP BASED ON AQUEOUS EXTRACT OF LEAVES OF *MORINDA MORINDOIDES* (MORINDA, RUBIACEAE) AGAINST CUTANEOUS PATHOGENS.

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ABSTRACT

Different extracts of leaves of *Morinda morindoides* (Baker) Milne-Redh (Rubiaceae) showed interesting *in vitro* antimicrobial activities against pathogenics involved in cutaneous infections. The purpose of this investigations was to formulated soap based on aqueous extract of leaves of the plant as antimicrobial agent and tested it activity against 2 bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and 4 fungi (*Candida albicans*, *Candida tropicalis*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*). The method of study was to developed soap (Soap S1) by incorporation of aqueous extract of *M. morindoides* as antimicrobial agent in formula of basic soap (Soap S0). Antimicrobial parameters (MIC and IC_{50}) of 2 soaps (S0 and S1) against the above microorganisms strains were determined by agar both dilution and plate methods. All strains tested were inhibited by both types of soaps with values of MIC ranging between 62.50 – 31.25 mg/mL and 125.00 – 62.50 mg/mL respectively for soap S1 and soap S0. *C. albicans* was the least strain whereas *T. mentagrophytes* was most sensitive in the presence of 2 soaps. In comparison with control soap (S0), these results showed that aqueous extract of *M. morindoides* gave to soap S1 an effective antimicrobial power on tested microorganisms. This investigation is a part of valorization of previous work of our research team on extracts of *M. morindoides.* Thus this finding is a real hope in development of products to effectively prevent or fight microbial cutaneous infections.

KEYWORDS: *Morinda morindoides*, aqueous extract, soap, antimicrobial potential, valorization, cutaneous infections.

INTRODUCTION

Morinda morindoides has been subject of several research studies. This plant is well known in traditional medicine in Côte d'Ivoire and Democratic Republic of Congo for treatment of diarrhea and some parasitic diseases.[1; 2] The decoction of leaves of *M. morindoides* is used for treatment of malaria and amoebiasis.[3] Also different extracts of leaves of plant are showed some interesting biologicals activities.[2; 4; 5; 6] These investigations were justified by microbial resistance to synthetic pharmaceutical products and their high cost.[7; 8] Plant substances are of natural origin and it is thought that their influences on the environment are few and can be used as biological control agents.[9] In previous study, four extracts (aqueous, ethanol, hexane and ethyl acetate) of leaves of *M. morindoides* revealed *in vitro* antifungal and antibacterial properties.[10; 11] In addition, hexane extract incorporated in formula of basic soap presents interesting antifungal activity.[12] In order to enhance results of previous research, our team is committed to development of plant derived products to prevent and treat effectively microbial skin infections. The objective of this study consist to developed soap based on aqueous extract of leaves *M. morindoides* and evaluated it *in vitro* antimicrobial activity of against two bacteria and four fungi implicated in cutaneous diseases.

MATERIALS AND METHODS

Collection of plant material

Leaves of *M. morindoides* (Rubiaceae) were collected from Daloa (central west region of Ivory Coast). The plant was identified and authenticated with voucher specimen no. 17710 in herbarium of National Floristique Center of University Felix Houphouët-Boigny (Côte d'Ivoire).

Preparation of extract

Leaves of *M. morindoides* were cleaned of extraneous matter, air-dried at room temperature for 7 days and ground into a fine powder. For each extraction, forty grams (40 g) of fine powder of *M. morindoides* was mixed with one liter of distilled water for 24 h with constant stirring at 80°C. The extract was filtered twice through cotton wool, then through Whatman filter paper (no. 1). The filtrate was evaporated to dryness using under vacuum in a rotary evaporator (Buchi) at 60°C. We obtained around 4.52 g (11.31±0.32%) of brown powder denoted aqueous extract of *M. morindoides*.

Preparation of soap

The soap codified "S1" was obtained with the cold method by adding 2 mixtures A and B. Mixture A was obtained by dissolving 16.135 g of sodium hydroxide crystals in 59.58 g of distilled water. To sodium hydroxide solution obtained after 24 hours were added 1.5 g of sodium chloride and 1.2 g of sodium bicarbonate at the time of use. The mixture B which will be used as fat in preparation of soap consists of 50 g of coconut oil and 50 g of palm oil. Mixture A was gradually added with stirring to mixture B. The mixture (A + B) obtained was homogenized until a viscous mass (tracing) was formed. To obtain soap S1, 10 g of aqueous extract of *M. morindoides* was added to 90 g of soap mass obtained (basic soap) and then homogenized. Homogeneous mass obtained was poured into the molds. In parallel, control soap was prepared according to same method but without aqueous extract (soap S0). After 24 hours, two types of soaps are obtained: a soap with brown colored containing aqueous extract of *M. morindoides* which is the soap "S1" and a soap with no extract of *M. morindoides* which is basic soap.[13; 14]

Microorganism strains

The microorganisms used in this study are composed of 2 bacteria *Staphylococcus aureus* (587/10) and *Pseudomonas aeruginosa* (602/10), 2 yeasts *Candida albicans* (3076/PV) and *Candida tropicalis* (13763/D) and 2 molds *Trichophyton rubrum* (14301/D) and *Trichophyton mentagrophytes* (13801/D). The bacteria are hospital strains identified following NCCLS recommendations by Bacteriological Laboratory of Pasteur Institute of Côte d'Ivoire. The fungi are also hospital strains provided by Mycology Laboratory of Medical Sciences Faculty of University Felix Houphouët-Boigny (Côte d'Ivoire).

Antimicrobial assay

Antifungal activities was assessed according to agar dilution method on Sabouraud agar (Scharlau).[15] The fungi cultures were inoculated in Sabouraud agar (Scharlau) and incubated for 48 h at 30.0±0.1°C. Each soap was incorporated into growth medium to give serial two-fold dilutions. The resulting concentrations ranged from 125 to 3.90 mg/mL. A medium containing nutrient broth only seeded with the test organisms was served as control of growth. The counts of fungi cultures were adjusted to yield 10^5 to 10^6 mL^{-1}, respectively, using the standard McFarland counting method. The test microorganisms (0.1 mL) were inoculated with a sterile swab on the surface of appropriate solid medium in tubes. The cultures were incubated for 2 to 5 days at 30.0±0.1°C.

Antibacterial activities of soap containing aqueous extract of *M. morindoides* (soap S1) and soap without extract (soap S0) against *S. aureus* and *P. aeruginosa* were performed by Mueller Hinton growth medium with broth dilution agar method coupled and seeding on agar plate. Each test soap was incorporated into growth medium in tubes and Petri dishes to give serial two fold dilutions. The resulting concentrations ranged from 3.90 to 125 mg/ml. A tube and Petri dishe containing nutrient broth only, seeded with test organism was served as growth control. Bacterial cell suspensions were inoculated on the tubes and plates using a bacterial planter (0.3 ml for *S. aureus* and 0.1 ml for *P. aeruginosa*). All the inoculated tubes and plates were then incubated at 37°C±2°C for 18 h.[16; 17]

All experiments were performed in triplicate. The antimicrobial parameters-minimum inhibitory concentration (MIC) and concentration producing 50% inhibition (IC_{50}) were determined after counting the colony of microorganisms of each series. The total score of colony of the control tube was considered as 100%. The MIC is defined as the lowest concentration that produced no visible microbial growth after the incubation time. Values of IC_{50} were determined on the survival curves of microorganisms strains established with Graph Pad software, U.S.A.

STATISTICAL ANALYSIS

All the data were analyzed by one-way ANOVA and differences between the means were assessed with Dunnet/Turkey's multiple comparison tests using Graph Pad software, version 5.01 (USA). Differences were considered significant at $p < 0.05$.

RESULTS

Inhibitory effects of soaps (soap S1 & soap S0) are shown in Table 1 whose values were used to represent survival curves (Fig.1 & Fig.2) for determination of MIC and IC_{50} (Table 2). The results showed that these soaps were significantly ($p < 0.05$) effective against all tested microorganisms strains. The high activity was demonstrated by soap S1 with MIC values of 62.50 – 31.25 mg/mL against 125.0 – 62.50 mg/mL for soap 2. Among fungi tested, *C. albicans* was presented the lowest sensitivity to the both soap at 62.50 mg/mL and 125.0 mg/mL respectively with soap S1 and soap S0. While *T. mentagrophytes* was the most sensitive fungi strain. According to bacteria strains, *S. aureus* showed significantly ($p < 0.05$) the most resistance with IC_{50} values of 2.87±1.18 mg/mL (soap S1) and 3.36±0.68 mg/mL (soap S0) against 2.79±1.11 mg/mL (soap S1) and 3.15±1.24 mg/mL (soap S0) for *P. aeruginosa.*

In comparison with control soap (S0), these results showed that aqueous extract of *M. morindoides* gave to soap S1 an effective antimicrobial power on tested microorganisms. This finding corroborated with early work of Toure *et al*.[12] about antifungal activity of soap whose hexane extract of *M. morindoides* was active compound. This soap was active against some of fungal strains of this study (*C. albicans*, *T. rubrum* and *T. mentagrophytes*) with a MIC of 31.25 mg/mL. This soap (soap with hexane extract) is more active than soap S1 (soap with aqueous extract) on *C. albicans*. Other researchers had also studied antimicrobial activities of soaps based on different active compounds. Soap containing aqueous extract of leaf of *Senna alata* (L.) Roxb tested at concentration of 100 µg/mL by Aminuddin *et al*.[18] was no active against *P. aeruginosa* ATCC-27853 and *S. aureus* ATCC-29213. These authors were conclude that the lack of activity against bacteria tested was due to low concentration of extract of *Senna alata*. Antimicrobial activities of commercial medicated soaps (Crusader and Antigal) were evaluated by Obi.[19] against *S. aureus*. This study revealed that the soaps Crusader and Antigal whose antimicrobial agents are synthetic compounds (trichlocarban and tricloson) had respective MICs of 62.5 mg/mL and 500 mg/mL against *S. aureus*. According to these results our soap S1 containing naturel active compound have best antimicrobial potential to be used as medicated soap.

Table 1: Comparative inhibitory effects of soaps on *in vitro* growth of microorganisms tested.

		Soaps concentrations (mg/mL)						
Microorganisms	Soaps	0.00	3.90	7.81	15.62	31.25	62.50	125.00
Candida albicans	S0	100±1.70	90±1.70**	60±1.70	30±0.60**	5±0.60	2±0.0	0±0.0
	S1	100±0.56	88±1.20**	51±1.20	25±1.20**	5±1.16	0±0.0	0±0.0
Candida tropicalis	S0	100±1.53	93±0.33	85±2.03	63±0.88	21±1.20**	0±0.0	0±0.0
	S1	100±1.53	84±1.16**	70±0.88	42±1.45	0±0.00	0±0.0	0±0.0
Trichophyton rubrum	S0	100±2.00	40±1.70**	30±2.10**	10±0.60**	5±0.60	0±0.0	0±0.0
	S1	100±1.16	35±0.33**	23±1.20	6±0.33	0±0.0	0±0.0	0±0.0
Trichophyton mentagrophytes	S0	100±1.20	30±0.60	20±0.60**	5±0.60	2±0.00**	0±0.0	0±0.0
	S1	100±0.00	22±0.58	14±0.88**	3±0.00	0±0.0	0±0.0	0±0.0
Staphylococcus aureus	S0	100±1.2	42±0.6	22±0.4**	9±0.4	5±0.6**	0±0.0	0±0.0
	S1	100±1.16	32±1.20	16±0.58	6±0.88	0±0.0	0±0.0	0±0.0
Pseudomonas aeruginosa	S0	100±1.4	38±1.2	17±0.6**	6±0.8	3±0.4	0±0.0	0±0.0
	S1	100±1.16	30±1.10	10±0.38	2±0.58**	0±0.0	0±0.0	0±0.0

Keys: Mean ± SEM (n = 3) S0 : Control soap S1 : Soap with aqueous extract of *M. morindoides*
** Mean values with the same superscript within a row do not differ significantly ($p < 0.05$).

Table 2: Antimicrobial parameters of soaps against microbial strains (Mean±SEM, n = 3, $p < 0.05$)

Microorganisms	Soaps	IC_{50} (mg/mL)	MIC (mg/mL)
Candida albicans	S0	10.41±0.01	125.00
	S1	6.11±1.20	62.50
Candida tropicalis	S0	20.45±0.98	62.50
	S1	10.04±0.63	31.25
Trichophyton rubrum	S0	3.25±1.75	62.50
	S1	3.00±1.77	31.25
Trichophyton mentagrophytes	S0	2.78±0.77	62.50
	S1	2.50±0.37	31.25
Staphylococcus aureus	S0	3.36±0.68	62.50
	S1	2.87±1.18	31.25
Pseudomonas aeruginosa	S0	3.15±1.24	62.50
	S1	2.79±1.11	31.25

Keys: S0 : Control soap S1 : Soap with aqueous extract of *M. morindoides*

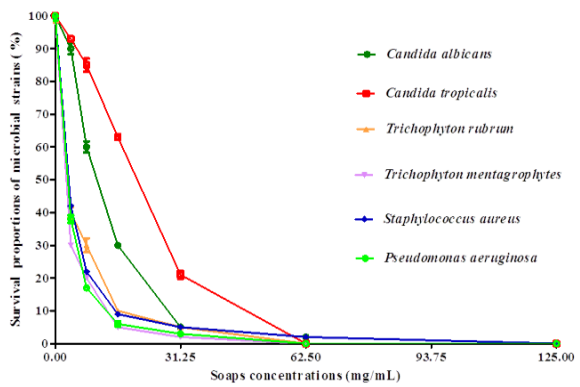

Figure 1: Survival curve of microorganisms tested against soap S0

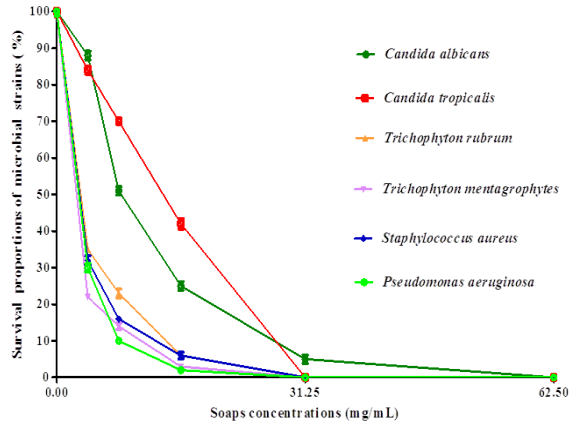

Figure 2: Survival curve of microorganisms tested against soap S1

CONCLUSION

The result of this study indicated that, soap containing aqueous extract of *M. morindoides* exhibited a real antimicrobial potential against pathogens implicated in skin infections. This soap with natural antimicrobial agent can constitute a real hope in development of medicated product to prevent and cure microbial cutaneous infections. This investigation is a part of valorization of previous work of our research team on extracts of *M. morindoides*. We plan to test other extracts of the plant as antimicrobial agents to determine which one leads to the best medicated soap. Also we make skin and eye irritation tests to ensure the safety of the soap before making clinical trials against some microbial cutaneous diseases.

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