

Original Research Article

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Antibacterial Activity of Soap containing Hexane Extract of Leaves of *Morinda morindoides* (Morinda; Rubiaceae) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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The objective of this study was to perform antibacterial activity of soap containing hexane extract of leaves of *Morinda morindoides* (Baker) Milne-Redh (Rubiaceae) codified "MORINDA" against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This soap was developed by incorporation hexane extract of *M. morindoides* as antimicrobial agent in formula of basic soap (soap without extract). Minimum inhibitory and bactericidal concentrations (MIC and MBC) of soap "MORINDA" and basic soap against the two above bacteria strains were determined by agar both dilution and plate methods. The results showed high bactericidal activity of soap "MORINDA" against two strains tested with same values of MIC and MBC of 31.25 mg/mL. Basic soap presented the lower bactericidal effect with MIC of 62.50 mg/mL for the two strains tested and MBC of 125.00 mg/mL and 62.50 mg/mL respectively for *S. aureus* and *P. aeruginosa*. Comparatively to control soap, hexane extract of *M. morindoides* gave to soap "MORINDA" effectiveness bactericidal power against these two bacteria involved in cutaneous infections. This study corroborate our previous investigations which showed antifungal activity of this soap and high antibacterial activity of hexane extract of *M. morindoides*.

Introduction

Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the

usage of antibacterial agent, host characteristics and environmental factors (Langlois-Klassen *et al.*, 2007; Patwardhan *et al.*, 2005). This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they

produce adverse effects compared to plant derived drugs (Abiramasundari *et al.*, 2011; Parrales *et al.*, 2012). These antimicrobial 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 (Abdel Rahman *et al.*, 2011). In this perspective *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 (Zirihi *et al.*, 2005; Cimanga *et al.*, 2006). The decoction of leaves of *M. morindoides* is used for treatment of malaria and amoebiasis (Kambu *et al.*, 1990). Also different extracts (aqueous, ethanol, ethyl acetate, hexane) of leaves of plant are showed some interesting biologicals activities (Zihiri *et al.*, 2005; Bagre *et al.*, 2007; Moroh *et al.*, 2008; Meite *et al.*, 2009; Cimanga *et al.*, 2010). In previous study, hexane extract of leaves of this plant revealed *in vitro* bactericidal properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Ouattara *et al.*, 2013). In addition, this extract incorporated in formula of basic soap presents interesting antifungal activity (Toure *et al.*, 2010). The purpose of this study consist to evaluated *in vitro* antibacterial activity of this soap against *S. aureus* and *P. aeruginosa*, two bacteria implicated in some cutaneous infections.

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, 100 g of dry powdered plant material was extracted with 250 ml of hexane (Merck, Darmstadt, Germany) for 24 h using a Soxhlet extractor. Extract was filtered with Whatman filter paper no.1, and filtrate was evaporated under vacuum in a rotary evaporator (Buchi) at 55°C. A greenish paste obtained with a yield of 9.24±0.18% is hexane extract.

Preparation of soap

The soap codified "MORINDA" 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 "MORINDA", 10 g of hexane 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 hexane extract (basic soap). After 24 hours, two types of soaps are obtained: A soap with greenish-colored containing hexane extract of *M. morindoides* which is the soap "MORINDA" and a soap with no extract of *M. morindoides* which is basic soap (Caubergs, 2008; Oladele *et al.*, 2010).

Bacterial strains

The bacteria used in this study were *Staphylococcus aureus* (587/10) and *Pseudomonas aeruginosa* (602/10). These hospital strains were all identified following NCCLS recommendations by Bacteriological Laboratory of Pasteur Institute of Côte d'Ivoire.

Antibacterial assay

Antibacterial activity of soap containing hexane extract of *M. morindoides* (soap "MORINDA") and soap without extract (basic soap) 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 1.95 to 125 mg/ml. A tube and Petri dish 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. The lowest concentration of the plate, which did not show any visible growth after incubation, was considered as minimal inhibitory concentration (MIC). To determine the minimal bactericidal concentration (MBC) for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar by streaking. Plates inoculated with bacteria were then incubated at 37°C for 24 hours. After incubation the concentration at which no visible growth was noted as MBC (Soussy *et al.*, 2010; Adeshina *et al.*, 2012).

Statistical analysis

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

Results and Discussion

Results of colonies counting from dose-response of soap "MORINDA" and control soap on *in vitro* growth of *S. aureus* and *P. aeruginosa* are recorded respectively in Tables 1 and 2. These data made it possible to determine antibacterial parameters of soaps against *S. aureus* (Table 3) and *P. aeruginosa* (Table 4). The results showed significantly ($p < 0.05$) high bactericidal activity of soap

"MORINDA" against two strains tested with same values of MIC and MBC of 31.25 mg/mL. Control soap presented the lower bactericidal effect with MIC of 62.50 mg/mL for the two strains tested and MBC of 125.00 mg/mL and 62.50 mg/mL respectively for *S. aureus* and *P. aeruginosa*. According to MBC values, soap "MORINDA" is 4 times more effective than control soap against *S. aureus* and 2 times with *P. aeruginosa*.

Comparatively to control soap, hexane extract of *M. morindoides* gave to soap "MORINDA" effectiveness bactericidal power against the two bacteria tested. This interesting bactericidal activity of soap "MORINDA" corroborate our previous investigations which showed antifungal activity of this soap (Toure *et al.*, 2010) and high antibacterial activity of hexane extract of *M. morindoides* (Ouattara *et al.*, 2013). In fact hexane extract of *M. morindoides* incorporated to 10 % as active agent in formulation of anti-infectious soap expresses its antimicrobial properties.

Table.1 Results of colony counting from dose-response of soaps on in vitro growth of *Staphylococcus aureus*

Soaps concentrations (mg/mL)	Control soap	Soap "MORINDA"
0.00	100 ± 1.2	100 ± 0.14
3.90	42 ± 0.6	26 ± 1.2
7.81	22 ± 0.4**	17 ± 1.4**
15.62	9 ± 0.4	5 ± 0.2
31.25	4 ± 0.6**	0 ± 0.00
62.50	0 ± 0.00	0 ± 0.00
125.00	0 ± 0.00	0 ± 0.00

Values are the mean ± SEM (n = 3).

** Mean values with the same superscript within a row do not differ significantly (p < 0.05).

Table.2 Results of colony counting from the dose-response of soaps on the in vitro growth of *P. aeruginosa*

Soaps concentrations (mg/mL)	Control soap	Soap "MORINDA"
0.00	100 ± 1.4	100 ± 1.14
3.90	38 ± 1.2	19 ± 0.22
7.81	17 ± 0.6**	8 ± 0.6**
15.62	6 ± 0.8	2 ± 0.12
31.25	3 ± 0.4	0 ± 0.00
62.50	0 ± 0.00	0 ± 0.00
125.00	0 ± 0.00	0 ± 0.00

Values are the mean ± SEM (n = 3).

** Mean values with the same superscript within a row do not differ significantly (p < 0.05).

Table.3 Antibacterial parameters (mg/mL) of soaps against *Staphylococcus aureus*

Soaps	IC ₅₀	MIC	MBC	MBC/MIC	Interpretation
Control soap	3.36±1.36	62.50	125.00	2	Bactericidal
Soap "MORINDA"	2.64±0.86	31.25	62.50	1	Bactericidal

Mean±SEM, n = 3, p < 0.05

Table.4 Antibacterial parameters (mg/mL) of soaps against *Pseudomonas aeruginosa*

Soaps	IC ₅₀	MIC	MBC	MBC/MIC	Interpretation
Control soap	3.15±1.14	62.50	62.50	1	Bactericidal
Soap "MORINDA"	2.41±0.57	31.25	62.50	1	Bactericidal

Mean±SEM, n = 3, p < 0.05

In addition similar work on *Aloe vera* and *Ageratum conyzoides* (Moody *et al.*, 2004), *Tithonia diversifolia*, *Aloe secundiflora* and *Azadirachta indica* (Kareru *et al.*, 2010) and *Senna alata* (Oladele *et al.*, 2010) has also shown that these extract express their antimicrobial virtue when they used as active compound in anti-infectious soaps. The results of this study provide a basis for prevention and effective treatment of microbial cutaneous infections. In perspectives we plan to evaluate toxicity of soap "MORINDA" and carry out clinical tests for treatment of cutaneous infections.

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