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RESEARCH ARTICLE

Action Spectrum of *Terminalia Mantaly* on the *In Vitro* Growth of *Pseudomonas Aeruginosa*

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ABSTRACT

Given the resistance of bacteria against molecules available for the treatment of infectious diseases, the search for new molecules is required. The answer to this call has led to the evaluation of the antibacterial activity of *Terminalia Mantaly* on the *in vitro* growth of *Pseudomonas aeruginosa* ATCC 27863 and hospital isolate of *Pseudomonas aeruginosa*. In fact, eight extracts are prepared from this specie plant: two crude extracts (aqueous and hydro-ethanol) and six extracts from liquid/ liquid partition of the hydro-ethanol extract. Those extracts were tested. Studies by the diffusion method in solid medium and the method of double dilution in liquid medium revealed that the extracts have an inhibitory activity on the germs studied. Of all extracts, M_{12} aqueous phase of the hexane-water partition presented the best activity. The diameters are 19 ± 0.57 mm inhibitions against *Pseudomonas aeruginosa* ATCC 27863 and 14 ± 1 mm on the clinical isolate. The minimum inhibitory concentrations (MIC) are 312.5 µg / ml and 1250 µg / ml respectively on these bacterial germs.

KEYWORDS

Terminalia Mantaly, *Pseudomonas Aeruginosa*, Antibacterial Activity

INTRODUCTION

Opportunistic bacterium, *Pseudomonas aeruginosa* is the most remote from the hospital pathogens.

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This bacterium is responsible for many infections in immunocompromised patients (diabetes, cystic fibrosis, cancer, HIV). She is also involved in nosocomial infections, including respiratory, for which mortality remains high. *Pseudomonas aeruginosa* is naturally resistant to most antibiotics active on Gram-negative bacilli such as: aminopenicillins cephalosporins, tetracyclines. In addition, this

bacterium has the distinction of developing resistance to almost all available antibiotics in therapeutic. The poor sensitivity of the bacteria to antibiotics therefore makes treatment difficult.[1,2,3,4]

This reality leads us to orient ourselves to the medicinal plants in the search for new active compound. *Terminalia Mantaly* H. Perrier was selected after ethnobotanical survey. This plant is traditionally used for *post-partum* care, against diarrhea, oral candidiasis and gastrointestinal. Other studies have shown that *Terminalia Mantaly* exhibits antibacterial activity and antifungal activity. In order to broaden the spectrum of this activity, several extracts were prepared and tested in the *in vitro* growth of strain of *Pseudomonas aeruginosa*.[5,6,7,8,9]

MATERIALS AND METHOD

Materials

For this study we used the stem bark of *Terminalia Mantaly*. This specie has been identified by the National floristic Center of University of Felix Houphouet-Boigny. The barks were dried at the shade and were crushed.

For these tests, *Pseudomonas aeruginosa* ATCC 27863 and the isolate hospital were used. It was supplied by the National Laboratory of public earth (LNSP) of Côte d'Ivoire. The profiles of these bacteria are show in Table 1.

Method

Preparation of Extracts

The extracts were prepared according to the method described by Zihiri and Kra. For the preparation of crude extracts (aqueous and ethanolic 70 %), 100 g of plant powder was extracted in blender with one liter (1 L) of distilled water or a mixture of ethanol-water (70/30) (v/v). The homogenate obtained in each case was centrifuged in a square of fabric, and then filtered on absorbent cotton and on Whatman paper 3mm respectively. The aqueous and hydro-alcoholic filtrates were concentrated under a vaccum at 70°C. The concentrates were evaporated at 50°C for 48 hours. The extracts obtained from the crude aqueous and ethanolic extracts 70 % were coded LYTERaq and LYTEReth respectively. [10]

Besides from the LYTEReth extract, 6 other extracts were prepared. Indeed, 3 portions of 10 g are subjected to a liquid / liquid partition in 500 mL of 3 different mixtures of solvents (hexane-water, ethyl acetate-water and butanol-water; v/v: 50/50).

Table 1: Bacterial Germs Studied

Souches	Origine	Profil Antibactérien
Pseudomonas aeruginosa ATCC 27853	Collection	TZP^S TIC^S TCC^S ATM^S CAZ^S CFS^S SSS^S IPM^S GM^S TM^S AN^S NET^S CIP^S CS^S FOS^S C^S RA^S TE^S
Pseudomonas aeruginosa 100	Fibroid	TZP^S TIC^S TCC^R ATM^S CAZ^S CFS^S SSS^S IPM^R GM^S TM^S AN^S NET^S CIP^S CS^S FOS^S C^R RA^R TE^R

ATCC: American Type Culture Collection, R: resistant, S: sensitive

TZP: Piperacillin, ICT Ticarcillin, TCC: Ticarcillin + Clavulanic Acid, ATM: Aztreonam, CAZ: ceftazidime, CFS: Cefsulodin, SSS: Sulfamide, IPM: Imipenem, GM: Gentamycin, MT: Tobramycin, AN: Amikacin, NET netilmicin, CIP: Ciprofloxacin CS: Colistin, FOS: Fosfomycin, C: Chloremphenicol, RA: Rifampicin, TE: Tetracyclin.

After decantation the various phases were separated and were concentrated under a vacuum. The following extracts were obtained: M_{11}: the hexane phase (M_{11}); the aqueous phase of the partition hexane-water (M_{12}), the acetate phase (M_{21}), the aqueous phase of the partition ethyl acétate-water (M_{22}), the butanol phase (M_{31}); the aqueous phase of the partition butanol-water (M_{32}).

It's exempt from solvent as after the extract cannot still contain solvent. After, all the obtained extracts were tested for biological assays.

Antibacterial Assays

The agar diffusion Muller-Hinton in agar plates was used to evaluate the activity of different obtained extracts. Culture medium has been in contact with inocula prepared from the fresh colony (18 to 24 hours of incubation) density of 0.5 McFarland approximately 10^6 CFU / mL for 5 minutes. The petri dishes were then dried for 15 minutes at 37°C. With sterilized object wells of 5 mm in diameter were punched in the agar, 6 wells maximum for each petri dish. 100 mg / ml extract solutions were prepared by diluting 100 mg of extracts in 1 ml of distilled water. Forewother, 50 µL of each extract solution were distributed into the wells. The plates are then incubated at 37°C for 18 to 24 hours. This method allows to verify the activity antibacterial of this plant.

To evalue the antibacterial activity, the microdilution in microplate method was used. The Muller-Hinton broth is introduced in the wells (12 columns of 8 wells). Forewother, the concentration of extracts were prepared. These concentrations were range of 10000 µg / mL to 195 µg / mL. 100 µL of plant extract is introduced into the wells of each column according to the range of prepared concentration. Then 100 µL of concentrated broth twice already contained the fresh colony of germ (24 hours at 37°C) was added to each well. Two wells were used for each column for sterility control (containing 200 µL of MH broth) and one cup for growth control containing 200 µL of broth.[11]

The plates thus prepared were incubated at 37 °C for 18 hours. The minimum inhibitory concentration (MIC) was determined for using an indicator of bacterial growth, p-iodonitrotetrazolium (INT). After 18 hours, 40 µL of this solution concentrated of 0.2 mg / mL were added in different wells of micro plates. The plates were then incubated at 37 °C for 30 minutes. The MIC is determined in wells containing the concentration at which there is no staining violet so any bacterial activity. For the determination of the MBC, a broth is collected from the well without visible growth and seeded new Muller-Hinton agar in petri dishes. After 24 hours of incubation, the concentration at which no visible growth was observed corresponded to the minimum bactericidal concentration (MBC).[12]

RESULTS DISCUSSION

Agar Diffusion Method

Method of technical wells on agar revealed that *Terminalia Mantaly* exhibits antibacterial activity against strains of *Pseudomonas aeruginosa*. This activity is represented by the inhibition diameters ranging from 8.33 ± 0.88 to 19 ± 0.57 mm (Table 2).

Table 2: Diameters of Inhibition Zones of Extracts

Terminalia mantaly	***Pseudomonas aeruginosa* ATCC 27853** Diameter (mm)	***Pseudomonas aeruginosa* clinical isolate** Diameter (mm)
Lyther aq	12.33 ± 0.33	8.33 ± 0.88
Lyther eth	15 ± 0.57	11 ± 0.57
M_{11}	ND	ND
M_{12}	19 ± 0.57	14 ± 1
M_{21}	ND	ND
M_{22}	15.33 ± 0.33	11 ± 0.57
M_{31}	ND	ND
M_{32}	17.33 ± 0.33	13.33 ± 0.66

ND : Not Determined

Table 3: Parameters Antibacterial of Extracts

Extracts	*Pseudomonas aeruginosa* ATCC 27853		*Pseudomonas aeruginosa* clinical strain	
	MIC (µg /mL)	MBC (µg /mL)	MIC (µg /mL)	MBC (µg /mL)
LYTHER aq	1250	1250	5000	5000
LYTHEReth	625	625	2500	2500
M_{11}	>10000	>10000	>10000	>10000
M_{12}	312.5	312.5	1250	1250
M_{21}	>10000	>10000	>10000	>10000
M_{22}	625	625	2500	2500
M_{31}	>10000	>10000	>10000	>10000
M_{32}	312.5	625	1250	2500

Method of Double Dilution in Liquid Medium

The larger diameters are obtained with the M_{12} extract (hexane-water partition) on all strains. The method of double dilution in liquid medium was used to determine the antibacterial parameters (MIC and MBC) of different extracts (Table 3).

Water being the most used solvent in traditional medicine; it was used to check the antibacterial activity of this species (*Terminalia mantaly*). Thus the aqueous extract has an interesting antibacterial activity against strains of *Pseudomonas aeruginosa*. Given the results obtained with this extract, we wanted to improve this activity given work Ackah *et al.*, 2008 and Yayé *et al.* in 2012 using the solvent mixture of ethanol/water (70/30).[8,13]

The analysis of the results in Table 2 and 3 shows that LYTEReth (MBC of 625 µg/mL of *Pseudomonas aeruginosa* ATCC 27853 and 2500 µg/mL on the clinical strain) extract is 2 times more active than LYTERaq (MBC of 1250 µg/mL and 5000 µg/mL) respectively on the same bacterial germs. Thus, the ethanol-water (70/30) is a solvent that best concentrates the active principles of *Terminalia mantaly*.

Comparing this performance to those of other studies, it has revealed that LYTERaq and LYTEReth extracts are better than those obtained by Katerere and Ellof in 2005, antibacterial activity. In fact, the aqueous extract of *Sutherlandia frutescens* (10 mg/mL) is less active than LYTERaq extract on the *in vitro* growth of *Pseudomonas aeruginosa*. Moreover, LYTERaq and LYTEReth extracts are more active than the aqueous extracts (5mg/mL and 10 mg/mL) and ethanol (2.5 mg/mL and 5 mg/mL) respectively of *Terminalia catappa* and *Terminalia glaucescens* on the *in vitro* growth of *Pseudomonas aeruginosa* (Bolou *et al.*, 2011). Indeed, in 2004 those of Biyiti *et al.*, have shown that LYTEReth is 3 to 15 times and 30 to 120 times more active than the hydro-ethanolic extracts of *Harrissonia abyssinica* (MBC = 9.37 mg/mL) and *Cissus petiolata* (MBC = 75 mg/mL) of *Pseudomonas aeruginosa*.[14,15,16]

Therefore from the analysis of the results of extracts partitions M_{11}, M_{21} and M_{31} have showed no activity on the *in vitro* growth of those germs. The most active extracts of these bacterial organisms are M_{12}, M_{22} and M_{32}. However, extracts M_{12} and M_{32} (MIC = 312.5 µg/mL) are two times more active than M_{22}

(MIC = 2500 µg/mL). The comparison made based on MBC value indicated that M_{12} is two times more active than M_{32} (MBC = 625 µg/mL of *Pseudomonas aeuginosa* ATCC 27853 and MBC = 2500 µg/mL of the clinical isolate).

Moreover, the comparaison based on the MBC values shows that M_{12} extract is 2 times more active than LYTEReth and 4 times more active than LYTERaq.

The mixture solvent of hexane-water is the best solvent which concentrates the active principles of *Terminalia mantaly*. This has been demonstrated by the work of Ackah *et al.* in 2008 and Ahon *et al.* in 2012. According to these authors, extracts of *Terminalia catappa* and *Terminalia superba* from the hexane-water partition showed the best activities on the in vitro growth of *Cryptococcus neoformans*, *Candida albicans* and *Aspergillus fumigatus*. In addition, the comparison of the results of those Katerere and Eloff shows that the M_{12} (312.5 µg/mL) extract is 8 to 32 times more active than the hexane extract of *Sutherlandia frutescens* (10 mg/mL) on the in vitro growth of *Pseudomonas aeuginosa*.[13,14,17]

CONCLUSION

This study shows that *Terminalia mantaly* H.Perrier exhibits antibacterial activity on all strains in varying concentrations. The extract M_{12} from the hexane-water partition is more active than all extract of this plant. The extraction method used is better way to concentrate the active compounds of this species. Further study sorting phytochemical coupled column chromatography would improve benefit this activity and have an idea of the chemical nature of the active ingredient.

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REFERENCES

1. Bentzmann S, & Plesiat P. (2011). *Pseudomonas aeruginosa*: Une virulence complexe, Revue Francophone des Laboratoires, 435, 73-81.
2. Sligl, W., Taylor, G., & Brindley, P. G. (2006). Five years of nosocomial Gram-negative bacteremia in a general intensive care unit: epidemiology, antimicrobial susceptibility patterns, and outcomes. *International Journal of Infectious Diseases*, *10*(4), 320-325.
3. Faure K, Kipnis E, Guery B. (2008). Prise en charge des pneumonies liees a *Pseudomonas aeruginosa*. *Review Tun Infectiology, 2(1),* 1-8.
4. Rossolini, G. M., & Mantengoli, E. (2005). Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. *Clinical Microbiology and Infection*, *11*(s4), 17-32.
5. River, C., Nicolas, J. P., Caradec, M. L., Desire, O., & Schmitt, A. (2005). Medicinal plants in the northern region of Madagascar: ethnopharmacological approach. *Bulletin of the French Society of Ethnopharmacology*, *36*, 36-49.
6. Andriantsoa M, Andriantsiferana R. (1983). Contribution a l'etude des propriétés antibactériennes de *Terminalia mantaly*, *Archive du Centre National de Recherche Pharmaceutique, 2,* 184-187.
7. Yaye, Y. G., Kra, A. K. M., Ackah, J. A. A. B., & Djaman, A. J. (2011). Évaluation de l'activité antifongique et essai de purification des principes actifs des extraits de terminalia mantaly (h. perrier), une combretacée, sur la croissance in vitro DE Candida albicans. Assessment of the antifungal activity and test of active principle purification of excerpt of terminalia mantaly (h. Perrier), a combretaceae, on the In Vitro Growth of Candida albicans. *Bulletin de la Société Royale des Sciences de Liège*, *80*, 953-964.

8. Yayé, Y. G., Ackah, J. A., Kra, A. K. M., & Djaman, J. A. (2012). Anti-Fungal activity of Different Extracts of Terminalia Mantaly H. Perrier on the in Vitro Growth of *Aspergillus fumigatus*. *European Journal of Scientific Research*, *82*(1), 132-138.
9. Bognan, A. J. A. A., Guillaume, Y. Y., Felix, Y. H., Mathieu, K. A. K., & Joseph, D. A. Antifungal activity of Terminalia mantaly on the in vitro growth of Cryptococcus neoformans. *International Journal of Biochemistry Research & Review, 3(1),* 63-73.
10. Zirihi, G. N, Kra, A. M., Guédé-Guina, F., (2003). Évaluation de l'activité antifongique de Microglossa pyrifolia (Lamarck) O. Kunze (*Asteraceae*) sur la croissance in vitro de *Candida albicans*, Revue Médecine et Pharmacie d'Afrique, 17, 11-18.
11. Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, *64*(8), 711-713.
12. Doughari, J. H. (2007). Antimicrobial activity of Tamarindus indica Linn. *Tropical Journal of Pharmaceutical Research*, *5*(2), 597-603.
13. Ackah, J. A. A. B, Kra, A. K. M, Zirihi, G. N, & Guédé-Guina, F. (2008). Evaluation et essais d'optimisation de l'activité anticandidosique de *Terminalia catappa* Linn ($TEKAM_3$), un extrait de *Combretaceae* de la pharmacopée ivoirienne, *Bulletin de la Société Royale des Sciences de Liège, , 77*, 120-136.
14. Katerere, D. R., & Eloff, J. N. (2005). Antibacterial and antioxidant activity of Sutherlandia frutescens (Fabaceae), a reputed Anti-HIV/AIDS phytomedicine. *Phytotherapy Research*, *19*(9), 779-781.
15. Bolou, G. E. K., Bagré, I., Ouattara, K., & Djaman, A. J. (2011). Evaluation of the antibacterial activity of 14 medicinal plants in côte d'ivoire. *Tropical Journal of Pharmaceutical Research*, *10*(3), 335-340.
16. Biyiti, L. F., Meko'o, D. J. L., Tamzc, V., & Amvam, Z. P. H. (2004). Recherche de l'activité antibactérienne de quatre plantes médicinales camerounaises. *Pharm. Med Trad Afr*, *13*, 11-20.
17. Ahon, M. G., Akapo-Akue, J. M., Kra, M. A., Ackah, J. B., Zirihi, N. G., & Djaman, J. A. (2011). Antifungal activity of the aqueous and hydro-alcoholic extracts of Terminalia superba Engl. on the in vitro growth of clinical isolates of pathogenic fungi. *Agriculture & Biology Journal of North America*, *2*(2), 250-257.

