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Antibacterial efficacy of essential oil from leaf of *Uvariodendron molundense* (Annonaceae), medicinal plant of Gabon

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Abstract

Increasing bacterial resistance to antibiotics is a serious global problem that has led research to identify new biomolecules with strong antibacterial activity. In nature, essential oils (Eos) contain a wide variety of secondary metabolites that can inhibit or slow down the growth of bacteria in the plant kingdom. The objective of this study is to determine the antibacterial potential of the essential oil of *Uvariodendron molundense* (*U. molundense*) which is a Gabonese plant belonging to the family *Annonacaeae*. The Eos extraction was carried out by hydrodistillation. The antibacterial activity, carried out on 4 bacterial strains: 2 multidrug-resistant hospital strains (MDR) *[Acinetobacter baumannii (A. Baumanni), Escherichia (coli E.]coli)* and 2 reference strains (*Escherichia coli CIP 105182*, *Salmonella typhi ATCC 13311*), was demonstrated by 2 methods. (Diffusion method on agar medium, and the micro dilution method). The results, obtained by the diffusion method, showed that the multidrug-resistant hospital bacterial strains were the most sensitive to Eos of *U. molundense*, on the isolate *A. Baumannii* MDR of 5 to 50% of the Eos concentration with a DIZ of 8.8 to 18.3 mm and on *E. coli* MDR of 25-100% Eos and DZI 8.5 mm -11 mm, than those of reference antibiotics. The best activity was obtained with the essential oil, the DIZ ranging from 23.3 to 27.5 mm with an essential oil concentration of 75% to 100%, v / v, on A. baumannii MDR. Based on the results obtained in this study, we suggest continuing this work on other multidrug-resistant bacterial strains.

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Introduction

Life-threatening infections caused by bacteria that have become resistant to commonly used antibiotics annd have considered the most important world health problem of our time. These infections are more severe, need longer and more complex treatments, but are also more expensive to diagnose and treat (Sydnor and Perl, 2011). Easily treated with various antibiotics if the bacteria involved are sensitive, in recent years, there are only very few or no treatment options available when antibiotic-resistant strains are involved in infections that occur at a worrying rate. Indeed, the increasing resistance of pathogens to antibiotics is a major public threat as it reduces the effectiveness of antibiotic treatment, leading to increased morbidity and mortality (Mulyaningsih *et al.*, 2011). Therefore, it is urgent to find other antimicrobial agents for the treatment of resistant pathogenic microorganisms. The rise in resistance to microbial antibiotics and the harmful effects associated with synthetic antimicrobials have drawn the attention of researchers to natural substances for the treatment of multidrug-resistant infectious diseases (Wright, 2014). Essential oils (EOs) derived from plants seem to be a promising alternative to conventional antibiotics (Kordali *et al.*, 2008). EOs are currently receiving a lot of attention because they have shown activity against antibiotic-resistant pathogens, such as methicillin-resistant golden staphylococci (MRSA), broad-spectrum β-Lactamases (ESBLs) and resistant enterococci vancomycin (VRE) (Tohidpour *et al.*, 2010; Warnke *et al.*, 2013; ObameEngonga *et al.*, 2016; 2017). In consideration of the diversity of molecules present in EOs, the antibacterial activity seems to result from a combination of several modes of action, involving different cellular targets (Djilani et Dicko, 2012; Goetz et Ghedira, 2012).

Many studies in the last years were focused on the beneficial properties of the essential oils, including antibacterial properties. The diversity of the products, the great microbial diversity allowed the publication of many results. However, this area of research is, widely open. In this investigation, we have examined the antimicrobial potential activity of the leaf oil of *Uvariodendron molundense* (Annonaceae), medicinal plant of Gabon against multidrug-resistant Gram-negative MDR hospital strains (*Acinetobacter baumannii* and *Escherichia coli*) and reference strains (*Escherichia coli* CIP 105182 and *Salmonella typhi* ATCC 13311).

Materials and methods

Plant material

Authenticated *Uvariodendron molundense* (Annonaceae) plant material were collected from Mount Sassamongo in Ogooué Ivindo (Gabon) in April 2018. The plant was first identified locally by it local name, ndoundoula (Kota ethnic group) and authenticated by a taxonomist at the Institut de Pharmacopé et de MédecineTraditionnelle (IPHAMETRA). The fresh plant (leaves) was dried in the open air oven and was kept at room temperature until required.

Essential oil obtention

EO was obtained from dried plant materials by hydrodistillation for 3 h, using a Clevenger-type apparatus. Recovered EO was dried using magnesium sulfate then stored at +4°C until tested.

Bacterial strains

A total of 4 bacterial strains were tested in this study, including reference and clinical ones (Table 1). The species are *Acinetobacter baumannii* (MDR), *Escherichia coli* (MDR), *Escherichia coli* CIP 105182 and *Salmonella typhi* ATCC 13311. Clinical strains were collected from patients in therapeutic defeat in a Libreville hospital.

Antibacterial screening of essential oil

The antibacterial screening of the essential oil was carried out using the agar well diffusion method (NCCLS, 2006). The bacteria have grown in nutrient broth at 37°C for 18 h were standardized using normal saline to turbidity of 0.5 Mac Farland standards (10^8cfu/cm^3). Petri dishes (90 cm in diameter) were prepared with 15 ml of a base layer of Müeller-Hinton gelose medium and the test bacteria

were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Six millimeter of sterile paper discs (Whatman No. 3) soaked with 10 µl of the essential oil dilution (A concentration range of the essential oil: 100%, 75%, 50%, 10%, 5%, 3%, 0.5%, was prepared as a percentage of essential oil in DMSO by dilutions of cascade)were placed on the agar in 15 mm of Petri dishes periphery. Paper discs soaked in DMSO without oil were used as negative control while Amoxicillin and Ciprofloxacin were used as positive control. A concentration range of antibiotics (100 µg / ml, 75 µg / ml, 50 µg / ml, 30 µg / ml, 20 µg / ml and 10 µg / ml) was prepared from a stock solution of 5000 µg / ml of antibiotic. The Petri dishes were incubated aerobically at 37°C for 18 to 24 h. All tests were performed in triplicate and antibacterial activity was expressed as the mean of Diameters of Inhibition Zone (DIZ) produced.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A microdilution broth susceptibility assay was used, as recommended by the National Committee for Clinical Laboratory Standards (2006) for the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Briefly, the essential oil was properly prepared, sterilized and transferred in sterile 96 well-plates previously filed with sterile nutrient broth to obtain a twofold serial dilutions ranging from 0.5 µg / mL to 100 µg / mL. Then plates were inoculated with microbial suspensions diluted from the same 0.5 Mac Farland standards to have 5 x 10^5 CFU/ml in each well. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no oil added) and the DMSO inhibitory effect. The final volumes in wells were 200 µl. The plates were incubated aerobically at 37°C and MICs were determined. The MIC was defined as the lowest concentration of the essential oil at which the microorganism tested does not demonstrate visible growth. To determine MBCs, 100 µl of bacterial suspension from subculture demonstrating no visible growth were removed to spread onto Plate Count Agar (PCA) medium plates. Plates were incubated at 37°C for a total period of 48h. The MBC was defined as the lowest concentration of the essential oil at which 99.99 % or more of the initial inoculum was killed.

Statistical analysis

Experimental results were expressed as mean ± standard deviation. All measurements were replicated three times.

Results

As presented in Table 1, the essential oil exhibited considerable inhibitory effects against all bacterial strains and comprends pas ce que tuveuxdireisolates. The essential oil shows that the strong growth inhibitory activity was found from 25% oil on *E. coli* CIP 105182 (24.7 mm), from 5% oil on *S. typhi* ATCC 13311 (16 mm) regarding the reference strains. The best activity on the reference strains is found on *E. coli* with diameters ranging from 24.7 mm (25% oil) to 61.7 mm (100% oil).

Table 1. Antimicrobial activity from essential oil of *Uvariodendron molundense measured in terms of zone of growth inhibition (mm).*

	Concentrations of the essential oil in percentage							Bacteria strains
	100	75	50	25	10	5	3	
Diameter of inhibition zone (mm)	61.7 ± 6	45.3 ± 2.5	44.3 ± 5	24.7 ± 1.5	8.3 ± 1.5	0	0	*E. coli* CIP 105182
	35.7 ± 1.5	30.3 ± 1.5	26.3 ± 2.5	21.7 ± 4.5	22 ± 1	16 ± 1.5	9 ± 3	*S. typhi* ATCC 13311
	27.5 ± 1	23.3 ± 2.5	18.3 ± 4	14.8 ± 1	10.3 ± 0.5	8.8 ± 1.5	0	*A. baumannii* MDR
	11 ± 0.5	10.3 ± 0.5	10 ± 0	8.5 ± 0.5	0	0	0	*E. coli* MDR

The activity varies from sensitive to very sensitive on the multidrug-resistant *A. baumannii* isolate from 5 to 50% of oil concentration (8.8 to 18.3 mm). The multidrug resistant *E. coli* isolate was sensitive to the essential oil of *U. molundense* of from 25 to 100% essential oil (8.5 to 11 mm inhibition diameter).

Table 2. Antimicrobial activity from amoxicillin and ciprofloxacin measured in terms of zone of growth inhibition (mm).

	Antibiotic concentrations in µg / ml						Bacteria strains	Antibiotics
	100	75	50	30	20	10		
Diameter of inhibition zone (mm)	0	0	0	0	0	0	E. coli CIP 105182	Amoxicillin
	13 ± 1	10,7 ± 2,5	11 ± 1	9 ± 2,5	7,7 ± 0,5	0	S. typhi ATCC 13311	
	7,3 ± 1	0	0	0	0	0	A. baumannii MDR	
	9,8 ± 0,25	7,3 ± 1,5	0	0	0	0	E. coli MDR	
	16,3 ± 1,5	12,5 ± 0,5	8 ± 6	9,3 ± 1	8,8 ± 0,25	7,8 ± 0,75	E. coli CIP 105182	Ciprofloxacin
	12,5 ± 5	9,7 ± 3	8,7± 2	7,7 ± 0,5	0	0	S. typhi ATCC 13311	
	9 ± 1	9,3 ± 0,5	0	0	0	0	A. baumannii MDR	
	0	0	0	0	0	0	E. coli MDR	

The best activity was achieved by essential oil, with DZI ranging from 23.3 to 27.5 mm with 75% to 100% concentration of essential oil, v / v, on *A. baumannii* MDR.

To test the antibacterial activity of conventional antibiotics, amoxicillin and ciprofloxacin were used (Table 2). The results of the inhibition diameters obtained varied from 7.3 to 13 mm for amoxicillin. It had no activity below 75 µg / mL on all clinical isolates. Only reference strain *S. typhi* ATCC 13311 exhibited an activity as early as 20 µg / mL (7.7 ± 0.5 mm) whereas *E. coli* CIP 105182 was resistant to amoxicillin. Ciprofloxacin gave inhibition diameters of 7.7 to 16.3 mm on all bacteria. It had no activity on *E. coli* MDR and exhibited activity on *E. coli* CIP 105182 (7.8 ± 0.75 mm), *S. typhi* ATCC 13311 (7.7 ± 0.5 mm) bacteria and *A. baumannii* MDR (9.3 ± 0.5 mm) respectively from concentrations ranging from 10, 30 and 75 µg / mL.

Table 3. Minimum inhibitory concentration, minimum bactericidal concentration data (%).

Bacteria strains	MIC (µg/ml)	MBC (µg/ml)
*E. coli*CIP 105182	30	45
S. typhi ATCC 13311	12	> 100
A. baumannii MDR	75	> 100
E. coli MDR	100	> 100

v/v) of *Uvariodendron molundense* essential oil obtained by microdilution method.

For the dilution method to determine the MIC and MBC values, MICs were found to range from 12 to 100 µg / mL (Table 3). The smallest MICs were those determined on the reference strains.

They were 30 µg / mL and 12 µg / mL respectively for *E. coli* CIP105182 and *S. typhi* ATCC 13311. Apart from *E. coli* CIP 105182, which had a minimum bactericidal concentration of 45 µg / mL, the remaining bacteria tested in this study showed MBCs greater than 100 µg / mL.

Discussion

Increasing bacterial resistance to antibiotics is a serious global problem that has directed/ encouraged research for the identification of new biomolecules with broad antibacterial activity. Plants and their derivatives, such as essential oils (EOs), are often used in folk medicine. Essential oils contain a wide variety of secondary metabolites that can inhibit or slow down the growth of bacteria (Bouyahya *et al.*, 2017).

In our study, the strains showed no resistance to the essential oil tested, since the aromatogram reveals that, in general, the 7 concentrations tested have a sensitive level activity (D ≥ 8 mm), very sensitive (D ≥ 15 mm) and extremely sensitive (D ≥ 20 mm). Nevertheless, resistance of *E. coli*, the reference strain and MDR, to low levels of essential oil (5-3% and 10-3%, respectively) was noted. The antibiogram performed with different concentrations of

Amoxicillin and Ciprofloxacin revealed a low general activity on the bacterial strains tested. Activity levels ranged from resistant; *E. coli* CIP 105182 (Amoxicillin) and *E. coli* MDR (Ciprofloxacin), non-sensitive; with diameters of inhibitions less than 8 mm (*A. baumannii* MDR, Amoxicillin) and sensitive (D ≥ 8 mm). Only Ciprofloxacin at 100 µg / ml indicated a very sensitive level of activity on *E. coli* CIP 105182 (D ≥ 15 mm).

The clinical efficacy of most of the marketed antimicrobials is found to be threatened by the quick emergence of multidrug resistant pathogens which increased need to find alternatives. Recently, many Essentials Oils have been discovered to have significant cytotoxic, antiparasitic and antimicrobial activity against a wide range of pathogens. As a result of this, essentials oils and their components have been used as a source of new antimicrobials in combating for infectious diseases (Taiwo and Adebayo, 2017). This study provided an interesting comparison between the inhibitory activity of sprout growth by two standard antibiotics and the antimicrobial effect of an essential oil. The results obtained showed a very remarkable efficiency, since the oil had very strong activity on all bacteria tested. EOs and their constituents have varied and highly targeted mechanisms of action, affecting in particular the cell membrane and cytoplasm, and in some cases, completely changing cell morphology or even gene expression (Bouyahya *et al.*, 2017). The best results are found with *E. coli* CIP105182 (61.7 mm) and *S. typhi* ATCC 13311 (35.7 mm) strains. These results are in agreement with the work of Alarmal *et al.*, (2012) which showed a strong antibacterial activity of essential oils on different strains at Gram+ and Gram-. Moreover, Other studies have also reported high antibacterial activity of essential oils on *C. albicans*, *E. coli*, and *S. aureus* (SASM, MRSA) (Rosato *et al.*, 2008; Mkaddem *et al.*, 2009).

In the study, the MICs varied from 12 to 100 µg / ml, which meant that this essential oil has a very high antibacterial activity. This statement was confirmed by the studies conducted by Touré *et al.*, (2014) where the MIC of the essential oil of *Chromoleana odorata* and its different fractions were determined by the microdilution methods. The crude essential oil had an MIC ranging from 128 to 256 µg / ml for *Staphylococcus aureus* and *Escherichia coli* strains. Our results were corroborated by the works of El Arch *et al.*, (2003) and Chebaibi *et al.*, (2016), which also proved the antimicrobial activity of essential oils with very low MICs. Our results showed that *U. molundense* essential oil had better antibacterial activity than crude extracts when comparing MICs. Indeed, the extracts gave MICs that varied from 31.25 µg / ml to 500 µg / ml on *E. coli* ATCC 25922 and *S. aureus* ATCC 1103 strains (Ngbolua *et al.*, 2017).

Conclusion

In order to promote Gabonese medicinal plants and search for new antibacterial molecules, we compared the action of the essential oil of *U. molundense* with two reference antibiotics (*Amoxicillin*, *Ciprofloxacin*) on four bacterial strains: 2 multiresistant (*Baumanni*, *E. coli coli*) and reference 2 (*Escherichia coli* CIP 105182, *Salmonella typhi* ATCC 3311). Two methods were used for diffusion and that for dilution.

The results, obtained by the diffusion method, showed that the bacterial strains studied were sensitive to U. molundense HE with inhibition diameters> 8mm. The best activity was obtained with the essential oil, the DIZ ranging from 23.3 to 27.5 mm with an essential oil concentration of 75% to 100%, v / v, on *A. baumannii* MDR. As the results obtained by the dilution method showed that the smallest MICs were those determined on the reference strains: 30 µg / mL and 12 µg / mL respectively for E. coli CIP105182 and for *S. typh*i ATCC 13311. In addition to *E. coli CIP* 105182, which had a minimum bactericidal concentration of 45 µg / mL, the remaining bacteria tested in this study showed MBCs greater than 100 µg / mL.

This study needs to be completed by a number of works such as to:

- test the antibacterial activity of the species on other bacteria

- perform the phytochemical study of the studied species
- Study the antioxidant activity. Perform the phytochemical study of the studied species

Conflict of interest statement

We declare that we have no conflict of interest.

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