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Evaluation of antibacterial activity of methanolic extracts and cassanediterpenoids isolated from the stem and the root barks of *Erythrophleumsuaveolens* (Guill. and Perr.) Brenan (Fabaceae)

Kabran Aka Faustin[1*], KablanAhmont Landry Claude[2], Ballo Daouda[1], Konan Dibi Jacques[1], AttiouaKoffi Barthélemy[1], Ahoua Angora Rémi Constant[3], AdikoN'dri Marcelline[4], Sissouma Drissa[1]

1Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire
2Département de Mathématiques, Physique et Chimie, UFR des Sciences Biologiques, Université Peleforo Gon Coulibaly, BP 1328 Korhogo, Côte d'Ivoire
3Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, BP 1303 Abidjan 01, Côte d'Ivoire
4Laboratoire de Pharmacognosie, Botanique, Biologie végétale et Cryptogamie, UFR des Sciences Pharmaceutiques et Biologiques, Université Félix Houphouët-Boigny, 22 BP 714 Abidjan 22, Côte d'Ivoire
**Corresponding author. Kabran Aka Faustin*
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Abstract: Our research work focused on Erythrophleumsuaveolens (Fabaceae), a plant of Ivorian flora used externally in the treatment of Buruli ulcer. In order to provide a scientific basis for this use, we carried out in vitro evaluation of antibacterial activity of methanolic extracts (stem and root barks) and 6 cassanediterpenoids (nitrogenous and non-nitrogenous) isolated from this plant in our previous work. Methanolic extracts of stem and root barks, and isolated compounds from E. suaveolens were evaluated on Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli at the final concentration of 1.5 mg/mL. The methanolic extract of stem bark was active on all the strains studied (Staphyloccocus aureus ATTC, Pseudomonas aeruginosa CIP, Staphyloccocus aureus Sensible, Escherichia coli CIP, Staphyloccocus aureus CIP) but the methanolic extract of root bark was active only on two strains (Staphyloccocus aureus Sensible, Staphyloccocus aureus CIP).In addition, only nor-cassamide (cassanediterpenoid amide) showed activity on Staphyloccocus aureus Sensitive strain. These preliminary results could justify the use of E. suaveolens in traditional medicine.
Key Words: Fabaceae, Erythrophleumsuaveolens, antibacterial activity, Staphyloccocus aureus.

I. INTRODUCTION

Fabaceae is the most common familyfound in tropical rainforests and in dry forests in the Americas, Asia and Africa. It contains certain species particularly used in traditional medicine for varied applications. *E. suaveolens* is widely used in traditional African medicine. The stem and root barks are used against malaria, heart problem, hemorrhages and migraines, edemas, gangrenous wounds, rheumatism, arthritis, chickenpox, parasitic infection, eye treatment and measles [1]. The different parts of the plant are used as emetic, purgative, anesthetic and anthelmintic [2-4].*E. suaveolens* is used in western Côte d'Ivoire to treat Buruli ulcer [5]. This pathology is a chronic and debilitating skin infection caused by *Mycobacterium ulcerans*, a mycobacterium from the same family as that responsible for tuberculosis and leprosy. Côte d'Ivoire is the first country to make the fight against this condition a public health problem [6]. The disease is expanding in Côte d'Ivoire with more than 17.861 cases accumulated in 2005 [7]. It occurs mainly in tropical rural areas with a hot and humid climate [6]. The ulcerative wound caused by this germ then becomes the site of a bacterial superinfection with opportunistic germs such as *E. coli*, *Klebsiella sp*., *Vibrionaceae*, *Pseudomonas aeruginosa* and the subculture, mainly the genus *Pseudomonas* (59.5%), then the *Vibrionaceae* (40.5%) [8].Recent work carried out using this plant made it possible to isolate secondary metabolites belonging to cassanediterpenoids [9-12]. Before carrying out tests on *Mycobacterium ulcerans*, we carried out tests on the opportunistic germs of Buruli ulcer. To contribute to the search for new therapies accessible to all, we initiated a research project aimed at finding extracts or compounds with antibacterial activities.

II. MATERIAL AND METHODS

II.1. Material

II.1.1. Plant material

The stem and root barks of *E. suaveolens* were collected in Toumodi (Center of Côte d'Ivoire) in December 2014. They were identified and authenticated by Professor IpouIpou Joseph of the Centre National de Floristique (CNF). The different specimens (n° 10 DIBI ES-ET-2014 and n° 10 DIBI ES-ER-2014) were deposited in the herbarium of the CNF of Félix Houphouët-Boigny University (Abidjan- Côte d'Ivoire).

II.1.2. Biological Activity

The antibacterial activity was evaluated on *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (CIP) 4.83, *Pseudomonas aeruginosa* (CIP) 103467, *Escherichia coli* (CIP) 54127AF and *Staphylococcus aureus* sensitive to penicillin according to the protocol used by Ahoua et *al.*[13].These strains from the Institut Pasteur of Côte d'Ivoire and the National Laboratory of Public Health of Côte d'Ivoire were provided by the Microbiology Laboratory of Centre Suisse de RecherchesScientifiques in Côte d'Ivoire.

II.2. Methods

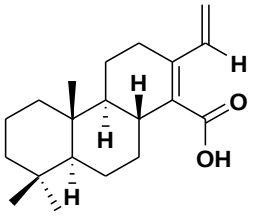
II.2.1. Extraction, isolation, purification and structural determination methods

II.2.1.1. Extracts

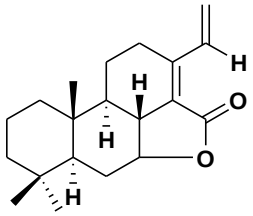
The dried stem bark (ES.ET) and root bark (ES.ER) of *E. suaveolens* were reduced to powder. The various powders (10 g) were extracted with 100 mL of methanol. The obtained solutions were each concentrated under reduced pressure using the rotavapor at 40 °C in semi-solid mass. The semi-solid mass was further dried under vacuum to give a hardened amorphous mass, which was kept in the refrigerator until necessary for use (ES.ET: 1.2 g; ES.ER: 1.3 g).

II.2.1.2. Isolated compounds

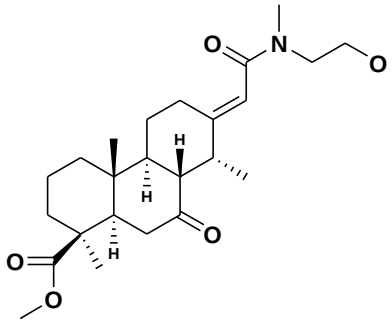
Recent studies by our research team have isolated cassanediterpenoids. The Methods of extraction, isolation, purification and structural determination are described by [5, 9-12].Six cassanediterpenoids (**Figure 1**) identified as acid cassan-13,15-diene-17-oic (**1**), cassan-15-ene-[7,17]-γ-lactone (**2**), *nor*-cassamide (**3**), 6*α*-hydroxy-*nor*-cassamide(**4**), *nor*-cassamine (**5**) and 6α-hydroxy-*nor*-cassamine (**6**), isolated from the dried powdered root bark of *E. suaveolens*[5, 9-12] were used in this study.



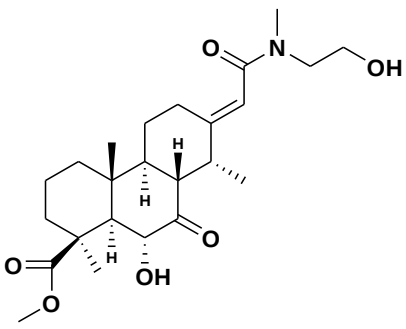
Acid cassan-13,15-diene-17-oic (**1**)



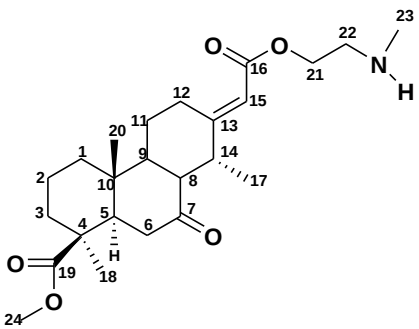
Cassan-15-ene-[7,17]-γ-lactone (**2**)



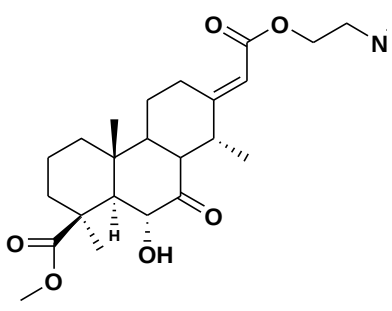
nor-cassamide (**3**)



6*α*-hydroxy-*nor*-cassamide(**4**)



nor-cassamine (**5**)



6*α*-hydroxy-*nor*-cassamine (**6**)

Figure 1: Cassanediterpenoids isolated from the root bark of *E. suaveolens*

II.2.2. Antimicrobial activity

II.2.2.1. Sensitivity test

Mueller-Hinton agar in Petri dishes with a thickness of 4 mm were soaked with an inoculum equivalent to 0.5 of McFarland. After drying, wells with a diameter of 6 mm were made in the agar using sterile Pasteur pipette. Fifty microliters (50 µL) of extract at 1500 µg/mL in DMSO or antibiotic at 25 µg/mL in distilled water was poured in the wells. Plates were left at ambient laboratory temperature for 15 to 30 min for a pre-diffusion of the solutions, and then incubated at 37 °C for 18 h. After incubation, the diameters (mm) of inhibition zones were measured. The tests were carried out twice.

II.2.2.2. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) were determined by using broth microdilution method in 96-wells microplates. The plant extracts were solubilized in DMSO (30 mg/mL) and serially diluted in Mueller-Hinton medium, from 1500 to 1.5 µg/mL. The final concentrations were 50 to 0.05 µg/mL for antibiotics. All the tested bacteria were used with an initial inoculum of 3×10^6 bacteria/mL. The microplates were incubated at 37 °C for 18 h.

II.2.2.3. Statistical analysis

Statistical tests were carried out with STATISTICA 7.1 software. The ANOVA test against a classification criterion was used to compare the means of the inhibition diameters with each other. When there is a difference between the means, the ANOVA test is supplemented with the Tukey HSD test to determine extracts whose activities differ from each other.

III. RESULTS AND DISCUSSION

Antibacterial screening was carried out on methanolic extracts from stem and root barks, and 6 cassanediterpenoids isolated from *E. suaveolens*. The inhibition diameters are between 0 and 11.5 mm (**Tables 1** and **2**). The methanolic extract from stem bark (ES.ET) was active against *Staphyloccocus aureus* ATTC, *Pseudomonas aeruginosa* CIP, *Staphyloccocus aureus* Sensible, *Escherichia coli* CIP and *Staphyloccocus aureus* CIP with inhibition diameters between 10 and 11.5 mm (**Table 1**). In contrast, the methanolic extract from the root bark (ES.ER) was active only against the *Staphyloccocus* strain (*Staphyloccocus aureus* Sensible and *Staphyloccocus aureus* CIP) with inhibition diameters of 9 mm. As for the isolated compounds, the inhibition diameters are presented in **Table 2**. Thus, *nor*-cassamide (**3**) was active on *Staphyloccocus aureus* Sensitive with an inhibition diameter of 9 mm.

Table 1: Diameters of the zones of inhibition of the extracts in solid medium on the different strains

	Inhibition zone diameter (mm) at 1.5 mg/mL			
	ES.ET	**ES.ER**	**Tétra.**	**Gen.**
S. a. ATTC	11.5±1.00	0	27	27.25
S. a. CIP	11±1.00	9±1.00	24.5	26.5
S. a. Sensible	10±1.00	9±1.00	25	26.25
P. a. CIP	10.5±1.00	0	24.5	26.0±1.00
E. c. CIP	10±1.00	0	25	25.0±1.00

ES.ET: *E. suaveolens* stem bark methanolic extract; **ES.ER**: *E. suaveolens* root bark methanolic extract; **S.a.ATTC**: *Staphyloccocus aureus* ATTC; **P.a.CIP**: *Pseudomonas aeruginosa* CIP; **S.a. Sensitive**: *Staphyloccocus aureus* Sensitive; **E.c.CIP**: *Escherichia coli* CIP; **S.a.CIP**: *Staphyloccocus aureus* CIP; **ATCC**: American Type Culture Collection; **CIP**: Collection of the Institut Pasteur; **Gen.**: Gentamicin; **Tetra.**: Tetracycline

It is found that the activity of cassanediterpenoids with amide function is better than that of cassanediterpenoids with amine function. These latter are more active than the non-nitrogenous cassane type diterpenoids. The presence of nitrogen increases the antibacterial activity of our different molecules. Only *nor*-cassamide (**3**) showed activity on *Staphyloccocus aureus* Sensitive. We can deduce that the hydroxyl group in position 6 of 6*α*-hydroxy-*nor*-cassamide (**4**) would decrease the activity of the compound, unlike *nor*-cassamide which has a methylene group in position 6. We can conclude that the activity could be due to the presence of the amide function in the structure.

Table 2: Diameters of the zones of inhibition of cassanediterpenoids (**1-6**) in solid medium on the different strains

	Inhibition zone diameter (mm) at 1.5 mg/mL							
	1	**2**	**3**	**4**	**5**	**6**	**Tétra.**	**Gen.**
S. a. ATTC	0	0	0	0	0	0	27	27.25
S. a. CIP	7.0±0.5	7.0±0.5	8.0±0.5	7.0±0.5	7.0±0.5	7.0±0.5	24.5	26.5
S. a. Sensible	7.25±0.5	7.0±0.5	9.0±0.5	8.0±0.5	8.0±0.5	0	25	26.25
P. a. CIP	0	0	0	0	0	0	24.5	26.0±1.00
E. c. CIP	0	0	0	0	0	0	25	25.0±1.00

The minimum inhibitory concentrations (MIC) obtained are recorded in **Table 3**. Analysis of this table reveals that the MICs obtained on the strains of *Staphylococcus aureus* ATTC, *Staphylococcus aureus* CIP, *Staphylococcus aureus* Sensible, *Escherichia coli* CIP and *Pseudomonas aeruginosa* CIP vary from 0.023 µg/mL to 1.5 µg/mL. Gentamicin and tetracycline (25 µg/mL) used as references, showed activity on all strains with inhibition diameters between 23 and 28 mm, which is considerably greater compared to those obtained for our extracts and isolated compounds.

Table 3: Minimum inhibitory concentration (MIC) of extracts and compound **3** in liquid medium on the different strains

	MIC (µg/mL)				
	ES.ET	**ES.ER**	**3**	**Tétra.**	**Gen.**
S. aureus ATTC	15	-	-	$1.9.10^4$	$3.125.10^4$
S. aureus CIP	0.023	15	15	500.10^4	500.10^4
S. aureus Sensible	0.75	15	15	500.10^4	500.10^4
P. aeruginosa	1.5	-	-	$3.125.10^4$	$1.56.10^4$
E. coli CIP	15	-	-	$1.9.10^4$	$1.56.10^4$

The isolated compounds, Acid cassan-13, 15-diene-17-oic (**1**), cassan-15-ene-[7, 17]-γ-lactone (**2**), *nor*-cassamide (**3**), 6*α*-hydroxy-*nor*- cassamide(**4**), *nor*-cassamine (**5**) and 6α-hydroxy-*nor*-cassamine (**6**) as well as the methanolic extract of the root bark (ESER) did not show any sensitivity on the bacterial strains. Only the methanolic extract from the stem bark (ESET) showed moderate activity on *Staphyloccocus aureus* CIP with a MIC of 0.023 µg/mL (**Table 3**).

IV. CONCLUSION

The study carried out on the stem and the root barks of *E. suaveolens*, a plant species used in traditional Ivorian medicine, had the specific objectives of evaluating the anti-bacterial activity of the extracts and isolated compounds. At the biological level, the results are promising. Indeed, the evaluation of the antibacterial activity gives the best result with the methanolic extracts of the stem and the root barks as well as *nor*-cassamide on *Staphyloccocus aureus* sensitive.

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