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Antimicrobial anthraquinones and triterpenoid isolated from *Morinda geminata* DC (Rubiaceae)

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Abstract: *Morinda geminata* DC is a plant of the traditional Senegalese pharmacopoeia, the leaves of which are used in the treatment of various diseases. The phytochemical investigation of its leaves and roots resulted in the isolation of the triterpenoid ursolic acid **6**, and five known anthraquinones analogues: nordamnacanthal **1**, damnacanthal **2**, damnacanthol **3**, lucidin-ω-ethyl ether **4**, and anthraquinone **5**. The isolated compounds were characterized by NMR and mass-spectrometry and were evaluated for their antimicrobial properties. Compounds **1** and **4** displayed significant antimicrobial activities towards *Staphylococcus aureus.* The present study constitutes the first phytochemical examination of the leaves and roots of *Morinda geminata* DC.

Keywords: *Morinda geminata* DC; Anthraquinones; ursolic acid; Antimicrobial.

Introduction

Morinda geminata DC - known for its numerous therapeutic virtues is a plant of the traditional Senegalese pharmacopoeia. The leaves, bark and roots of this plant are commonly used in the Senegal and other African countries.

The ethnopharmacological studies previously carried out on the plant (leaves, bark and roots) proved its physiological and therapeutic importance [1-3]. *Morinda geminata* DC has been used in traditional medicine for the treatment of various diseases such as edema, fever, icteri, cough [4], malaria [5,6], yellow fever [7] and headaches [8]. It is also used for the treatment of wounds, as an antiseptic and hypertension. The previous phytochemical studies demonstrated the presence of quinones in the bark and roots [9,10]. Ethanolic, aqueous extracts of the root, bark and leaves of the plant have shown to possess antibacterial[11] and anti-inflammatory [3] activities.

This study presents for the first time the isolation and elucidation of the structure of five known anthraquinones and one triterpenoid from the aerial parts and roots of *Morinda geminata* DC. The isolated compounds were evaluated regarding their antibacterial activity on two reference strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213.

Experimental Section

General

The optical rotation was measured with an electronic Polarimeter Perkin Elmer 241. IR spectra were recorded on a spectrometer Nicolet Avatar 320 FT-IR. UV spectra were obtained by using a Philips PU 8720 UV/VIS spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer, operating at 400 MHz for ^{1}H and 125 MHz for ^{13}C. Coupling constants were expressed in Hz. High resolution mass spectra (HRESIMS) and ESIMS (positive-ion mode) were recorded using Micromass ESI-Q-TOF micro-instrument (Manchester, UK). Column chromatography (CC) was performed on silica gel (SiO_2) 60 (0.04-0.063 mm, Merck). Analytical and preparative TLC were performed on pre-coated kieselgel 60 F254 plates 250 µm (Merck) using cyclohexane (CyH)/EtOAc or petroleum ether (PE)/CH_2Cl_2 as eluents and detected by spraying with Dragendorff or H_2SO_4 (20%) followed by heating.

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Plant Material

Roots and leaves of *Morinda geminata* DC were collected in April 2015 from Mlomp department, region of Ziguinchor, Senegal. The plant was authenticated by Prof. E. Bassène, Pharmacognosy and botany Department, University Cheickh Anta Diop, Dakar, Senegal. A voucher specimen was deposited at the herbarium the Pharmacognosy and botany laboratory under number 2015/020.

Extraction and Isolation

The powdered roots (300 g) of *Morinda geminata* DC were successively macerated (24 h) and extracted with 1.5 L of CyH (2.15 g), 1.5 L of EtOAc (4.33 g), 1.5 L of EtOH (10.18 g), and 1.5 L of H_2O (27.52 g). One part of the crude roots extracts was chromatographed on silica gel (160 g, 0.04-0.063 mm, Merck). After eluting of the CyH extract (2.1 g) with a mixture of PE /CH_2Cl_2 (45/55 v/v 600 mL), 155 fractions were collected. Compounds **1** (69.8 mg) and **2** (137.7 mg) were isolated respectively in fractions 20-40 and 58-70. In the same manner, 275 fractions were obtained by elution of the EtOH extract (2 g) with a mixture of CyH/EtOAc (90/10 and 70/30 v/v 600 mL each). Fractions 43-48 and 140-161 gave compounds **3** (11.6 mg) and **4** (66.4 mg), respectively. Elution of crude EtOAc extract (2 g) with a mixture of CyH/EtOAc (7/3 v/v 600 mL) gave 456 fractions. Fractions 285-300 provided **5** (123.6 mg).

The powdered leaves (200 g) of *Morinda geminata* DC were successively macerated (24 h) and extracted with 1.5 L of CyH (8.28 g), 1.5 L of EtOAc (6.55 g), 1.5 L of EtOH (12.54 g), and 1.5 L of H_2O (25.05 g). One part of the crude leaves EtOAc extract (2 g) was subjected to a silica gel column chromatography with the mixture PE/EtOAc (8/2) to give 356 fractions. Fractions 82-125 yielded ursolic acid **6** (260.1 mg).

Antibacterial Assays

Isolated and characterized metabolites were tested on two reference strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213). 10 mg of product were solubilized with 2.5% DMSO in a volume of 4 mL at a concentration of 2500 µg/mL.

Preparation of the inoculum in a solid medium: several colonies of identical morphology were used in order to avoid selecting an atypical variant. These colonies were suspended in physiological water (saline of about 0.09% NaCl) with a sterile inoculation loop. The bacterial suspension was standardized using the 0.5 *McFarland* control.

Determination of the inhibition diameter (ID): in order to test the strains' susceptibility to the compounds, wells of approx. 6 mm in diameter were made in the agar using a sterile punch. Each well received 80 µL of the substance at a concentration of 5 mg/mL. After a diffusion period of 30 min at room temperature, the Petri dishes were incubated at 37 °C for 24 h.

Preparation of the inoculum in liquid medium: a bacterial inoculum was prepared from colonies of less than 24 h in Mueller Hinton broth (MHB). A colony isolated from the bacterial culture was extracted with a platinum loop, then homogenized in 10 mL of the broth and subsequently incubated for 3 to 5 h at 37 °C in order to obtain a pre-culture. A volume of 0.1 mL or 1 mL was taken respectively for *E.coli* and *Staphylococcus aureus* and was added to 10 mL of each sterile MHB.

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC): a series of eight hemolysis tubes numbered C1 through to C9 were each filled with 1 mL of the pure inoculum. Then, 1 mL of the plant product was added to the tubes according to the concentration range prepared. The plant product was distributed by feeding 1 mL of 2.5 mg/mL into tube C1, 1 mL of 1.25 mg/mL into tube C2 and so on until tube C8, which received 1 mL of the 19 µg/mL solution. 1 mL of sterile MHB was given into C9 tube as growth control. All tubes were incubated at 37 °C for a period of 24 h.

Results and discussion

This study describes the extraction, isolation and characterization of anthraquinones and triterpene from the roots and leaves of *Morinda geminata* DC and the examination of their antimicrobial activities. Column chromatography of extracts of the roots and leaves of *Morinda geminata* DC led to the isolation and elucidation of five anthraquinones and one triterpenoid.

The known compounds **1**-**6** were readily identified by their spectral data and by comparison with reported corresponding compounds in the literature [12-17] as nordamnacanthal **1**, damnacanthal **2**, damnacanthol **3**, lucidin-ω-ethyl ether **4**, anthraquinone **5** and ursolic acid **6** (Figure 1).

We conducted a bio-guided study on crude extracts from roots and leaves of *Morinda geminata* DC.

All tests for antimicrobial activity showed negative results, and as such a contradiction to the results described in the literature[11]. Hence, we decided to carry out the isolation of the molecules to improve the understanding of the bioactivity of this plant.

The elucidation of these particular molecules in the *Morinda geminata* DC plant confirms the previous performed ethnopharmacological studies and indicated the plant's interesting therapeutic aspect. The roots of *Morinda geminata* DC are a source of anthraquinones. Anthraquinones of Rubiaceae plants have been reported as molecules possessing in vitro biological activities with antimicrobial [18], antifungal [19], hypotensive, analgesic [20], antimalarial [18,21], anti-leukemic and mutagenic functions [22,23]. In addition, the anthraquinones [24-30] we have isolated from *Morinda geminata* DC have widespread bioactive potential.

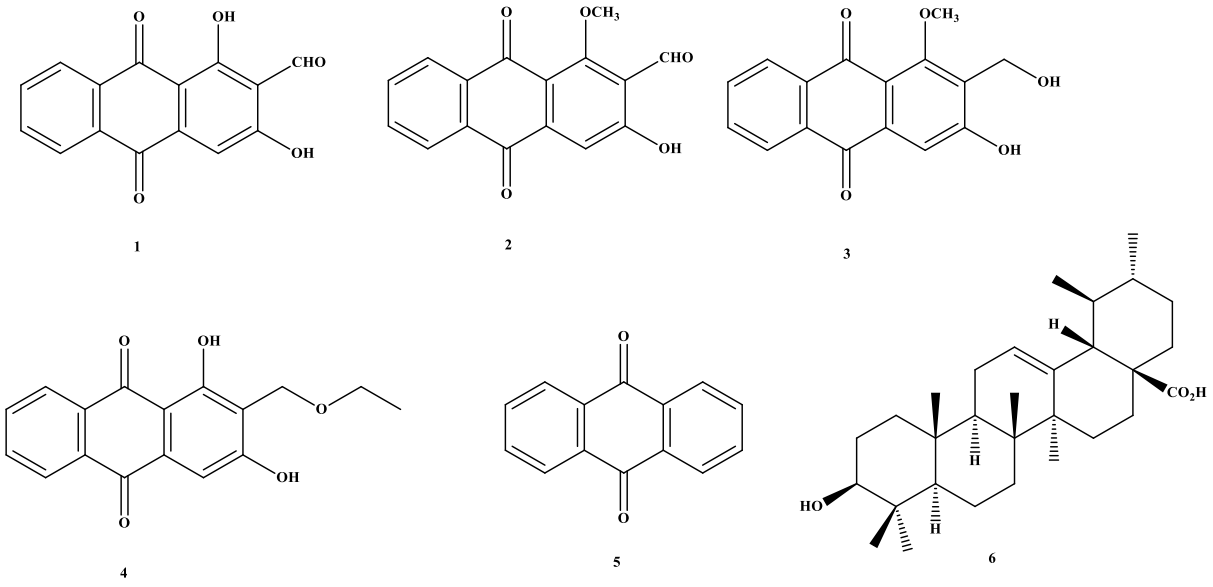

Figure 1: structure of the isolated molecules: nordamnacanthal **1**, damnacanthal **2**, damnacanthol **3**, lucidin- ω - ethyl ether **4**, anthraquinone **5** and ursolic acid **6**

Ursolic acid **6** –widely studied in the 21st century [31] exhibits a large spectrum [32-36] of pharmacological activities. An Iranian publication (2017) summarizes its known effects on the aging process [37].

The isolated compounds showed inhibition for *Staphylococcus aureus* (SA) and only anthraquinone **5** was sensitive (ID=10 and 13 mm) to the *Escherichia coli* (E.coli) strain. MIC values were assumed as the lowest concentration of product to inhibit organism growth after 24 h of incubation at 37 °C. The minimum bactericidal concentration (MBC) was determined by subculturing the tube with inhibition in an agar plate. Compounds **1** and **4** showed better inhibition at a concentration of 156 µg/mL against *Staphylococcus aureus*. Only compound **5** inhibited both strains with a MIC equal to MBC (625 µg/mL). The results are shown in Tables **1** and **2**.

Table 1: Inhibition diameter (ID) of isolated compounds

Compounds	**ID (mm)**	
	SA	E.coli
1	14	-
2	16	-
3	9	-
4	10	-
5	10	13
6	13	-

SA = *Staphylococcus aureus*, E. coli = *Escherichia coli*

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of isolated compounds

Compounds	**MIC (µg/mL)**		**MBC (µg/mL)**	
	Staphylococcus aureus	*Escherichia coli*	*Staphylococcus aureus*	*Escherichia coli*
1	156	-	312	-
2	312	-	312	-
3	ND	ND	ND	ND
4	156	-	312	-
5	625	625	625	625
6	1250	-	2500	-

- = *no activity*; ND = *not determined*

Conclusion

This paper reports for the first time, a phytochemical study of *Morinda geminata* DC. EtOH, EtOAc, and CyH extracts of the roots and the EtOAc extract of the leaves of *Morinda geminata* DC led to the isolation of one triterpenoid and five anthraquinones. Compounds **1** and **4** showed better inhibition at a concentration of 156 µg/mL against *Staphylococcus aureus*. Only compound **5** inhibited both strains with a MIC equal to MBC (625 µg/mL). Consequently, this study provides a molecular basis for comprehending the use of this plant in traditional Senegalese medicine.

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