International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 3, 2011

Research Article

ANTIMICROBIAL ACTIVITY OF THE METHANOLIC EXTRACT AND COMPOUNDS FROM THE STEM BARK OF *GARCINIA LUCIDA* VESQUE (CLUSIACEAE)

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Received: 08 April 2011, Revised and Accepted: 09 May 2011

ABSTRACT

We investigated hereby the methanolic crude extract of the stem bark of *Garcinia lucida* Vesque. The genus *Garcinia* (Clusiaceae or *Guttiferae*) consists of about 200 species mainly encountered in lowland rain forests of the tropical world, particularly in Africa and Southeast Asia[1,2]. 21 *Garcinia* species are represented in Cameroon among which *Garcinia lucida* vesque[3]. Dichloromethane sub-fraction from the methanol crude extract of the stem bark of *Garcinia lucida* Vesque afforded eleven compounds namely putranjivic acid **1**, methyl putranjivate **3**, their intermediary lactone **2**, friedeline **4**, cycloartenol **5**, 1,2-dihydroxy- xanthone **6**, 1-hydroxy-2-methoxyxanthone **7**, betulinic acid **8**, oleanolic acid **9**, β-sitosterol **10** and stigmasterol **11**. Except **5**, all those compounds are reported for the first time from *Garcinia lucida*. The structures of isolated compounds were established on the basis of 1D/2D NMR experiments, mass spectrometric and by comparison of data with those of the literature.

The methanol crude extract and pure compounds from CH_2Cl_2 fraction were tested for their antimicrobial activity against ***Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus,*** **and** ***Candida albicans.*** The crude extract showed good inhibitory potential with a MIC value of **64 µg/mL** on *Candida albicans* while pure compounds were poorly active. Only compound **5** exhibited moderate and selected antimicrobial activity against *E. coli* and *P. aeruginosa*.

Keywords: *Garcinia lucida*, Antimicrobial, Clusiaceae, Crude extract, Putranjivic acid, Methyl putranjivate

INTRODUCTION

Garcinia lucida Vesque is well-known in south Cameroun as *ESSOK (Boulou)*. The seed, the fruit and the bark *are the* most commonly used parts in traditional medicine and food. It also represents an important economic potential for local population. The fresh bark and sometimes the seeds are used in fermentation of traditional alcohol from palm tree or raffia. The bark and the seeds dried or fresh, are widely used for medicinal purposes for the treatment of gastric and gynecological infections, diarrheas, and as antidote against poison as well as to cure snake bites. It is believed to possess some aphrodisiac properties and to hunt ghost[4,5]. *Garcinia lucida* is a small evergreen dioecious tree, reaching 25-30 cm of width and 12-15 m height. It grows in high -density in hilly moist forests. The geographical distribution is limited to parts of Cameroon (South), Equatorial Guinea and Gabon[6,7]. In our knowledge, this is the third phytochemical study of this species. The first one conducted by Nyemba[8] led to the isolation from the bark of three cycloartanes: 30-hydroxycycloartenol, 31-norcycloartenol and 24,25-epoxy-31-nor-cycloartenol (mixture of epimers). Fotié[9] investigations conducted to the isolation of trypanocidal and antileishmanial dihydro-chelerythrine derivatives in addition with five known products: stigmasterol, betulenic acid, *D*-glucopyranoside of sitosterol, sesamin, and *trans*-fagaramide were isolated as well. The crystalline structure of the *trans*-fagaramide was also described.

The purpose of this study is to identify and characterize the bioactive principles from stem bark of *Garcinia lucida*, and the evaluation of antimicrobial activities of crude extract and isolated compounds.

EXPERIMENTAL

General experimental procedure

Infrared spectra were recorded on a NICOLET 510 FT – IR spectrophotometer. Melting points were recorded with a LEICA GALEN III electronic microscope with the temperature probe TESTO 720. Alpha D were recorded on a Polarimeter Perkin Elmer, Model 341. ESI-HR mass spectra were recorded on a Bruker FTICR 4.7 T mass spectrometer. EI-MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance. The ^{1}H- and ^{13}C-NMR spectra were recorded at 400 MHz and 75 MHz respectively on Bruker Spectrospin Ultrashied NMR spectrometers. The $FeCl_3$ reagent was used for phenol groups detection. Chemical shifts were reported in δ (ppm) using TMS as internal standard, and coupling constants (J) were measured in Hz. A combination of vacuum liquid chromatography VLC, column chromatography CC, and preparative thin layer chromatography PTLC was used with different silica gel (20-45 µm, 35-70 µm, 70-230 µm, Merck) under pressure (200-250 mbar). TLC was performed on Merck precoated silica gel 60 F_{254} aluminum foil, and spots were detected using alcoholic sulfuric acid/vanillin spray reagent.

Plant material

Garcinia lucida stem bark was collected in September 2008 in the Dja reserve, South Province of Cameroon, The identification of the species was conducted by M. Victor NANA, botanist at the National Herbarium of Cameroon (Yaoundé), where voucher specimens are deposited under numbers: **N° 5768HNC** and **25666HNC.**

Extraction and isolation

The air-dried powdered stem bark (2.5 kg) of *G. lucida* was extracted by maceration in 30 L of methanol at room temperature for three days. The filtrate evaporated under reduced pressure yields an oily dark-brown crude extract (500 g). A part of the crude extract (200 g) was diluted with the mixture of water and acetone and the solution was partitioned successively with dichloromethane, ethyl acetate, n-butanol and water to yield after evaporation 15 g, 165 g and 13.8 g and 6.2 g fractions respectively.

The CH_2Cl_2 fraction (15 g) was subjected to column chromatography under reduced pressure over silica gel (35-70 µm) using a gradient system of cyclohexane, CH_2Cl_2 and ethyl acetate. More than 30 sub-fractions were collected and combined according to TLC analysis.

Putranjivic acid **1** (20 mg) and it lest polar lactone **2** (25 mg) were obtained in a CC with CH_2Cl_2 as eluent. methyl putranjivate **3** (27 mg) and friedeline **4** (32 mg), were obtained after CC with cyclohexane/ethyl acetate 93:3 (*V/V*) and 90:10 (*V/V*) systems respectively as eluents. The compound **5** (43 mg), obtained in cyclohexane/ CH_2Cl_2 10:90 (*V/V*) system, was the sole cycloartane obtained.

A yellow sub-fraction, obtained in CH_2Cl_2 fraction reacted positively with $FeCl_3$ reagent, suggesting the presence of phenolic compounds. A Preparative Thin Layer Chromatography using CH_2Cl_2 solvent gave compounds **6** (5 mg) and **7**(4 mg) as yellow powders. A Preparative Thin Layer Chromatography using CH_2Cl_2 solvent gave 1,2-dihydroxy- xanthone **6** (5 mg) and 1-hydroxy-2-methoxyxanthone **7**(4 mg) as yellow powders. The chemical structure of isolated compounds, established on the basis of NMR experiments, mass spectrometric and by comparison of data with those of the literature are presented in Fig. 1.

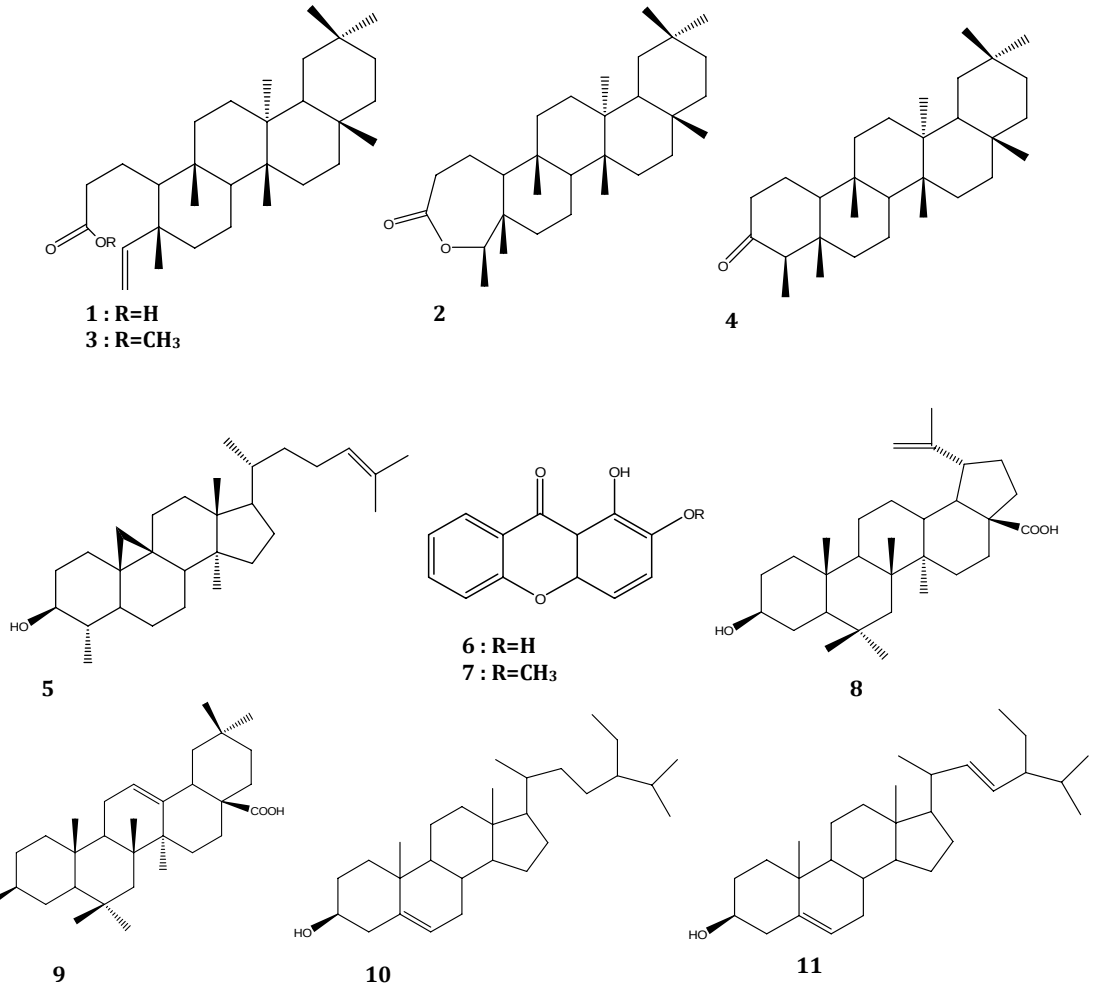

Fig. 1: Structures of compounds 1-11 isolated from CH_2Cl_2 extract of *Garcinia lucida* vesque

Biological activities

Microbial strains, culture media and chemicals

The microorganisms tested included *Staphylococcus aureus* ATCC25922 (Gram positive bacteria), three Gram-negative bacteria namely *Salmonella typhi* ATCC6539, *Pseudomonas aeruginosa* ATCC27853 *and Escherichia coli ATCC 10536* and *Candida albicans* ATCC 9002 (a yeast).

They were maintained on agar slant at 4°C and sub-cultured on a fresh appropriate agar plate 24 hours prior to any antimicrobial test. Nutrient Agar (NA) was used for bacterial culture. Sabouraud Glucose Agar was used for the activation of the fungi. The Mueller Hinton broth (MHB) was used to determine the minimal inhibition concentration (MIC) of all samples against the tested microorganisms. Nystatin (Maneesh Pharmaceutic PVT, China) for fungi and gentamycin (Jinling Pharmaceutic Group corp. India) for bacteria, were used as reference antibiotics (RA). Dimethylsulfoxide (DMSO; Sigma-Aldrich, South Africa) was used to dilute all tested samples.

Antimicrobial assays

The antimicrobial assays were conducted using rapid XTT colorimetry and viable count methods. The XTT colorimetric assay was performed according to Pettit and coll. (2005)[10] as modified by Kuete and coll. (2008)[11]. Briefly, the test sample was first of all dissolved in DMSO/MHB. The final concentration of DMSO was of lower than 1% and did not affect the microbial growth[12]. The solution obtained was then added to MHB, and serially diluted two fold (in a 96- wells microplate). 100 µl Of inoculum 1.5 10^6 CFU/mL prepared in MHB. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a plate shaker and incubated at 30°C for 48 hours. The assay was repeated in triplicate. Gentamicin, (bacteria) and nystatin (yeast) were used as positive control. Well containing MHB, 100 µl of inoculum and DMSO to a final concentration of 1% served as negative control. The MIC of samples was detected following addition (40 µl) of 0.2 mg/mL *p*-iodonitrotetrazolium chloride and incubated at 37°C for 30 minutes. Viable bacteria reduced the yellow dye to a pink color. MIC was the sample concentration that prevented this change and exhibited complete inhibition of bacterial growth.

RESULTS AND DISCUSSIONS

The partial chemical investigation of *Garcinia lucida*, and particularly the CH_2Cl_2 fraction led to the isolation of terpenoids and xanthones products. Because of the relative low quantity of xanthones obtained (less than 5 mg each), we were not able to proceed to their antimicrobial assays. The genus *Garcinia* is known to be rich in xanthones with several biological activities, such as antimicrobial and antibacterial[13,14], antifungal[15], antitumor-promotion[16], cytotoxic and anti-HIV-1[17], trypanocidal and antileishmanial[9].

Most triterpenes isolated such as putranjivic acid **1**, methyl putranjivate **3**, their intermediary lactone **2**, friedeline **4**, cycloartenol **5** are very rare and sometimes not present in the genus *Garcinia*. However, the compounds **1-4** and **6-7** were isolated for the first time from this species. The results of the antimicrobial assays summarized in Table **1** indicate that most of the studied compounds were not active. Only cycloartenol **5** exhibited moderate and selected antimicrobial activity with MIC of 512 µg/mL against *E. coli* and *P. aeruginosa*. Such values were still higher than those of reference drugs clearly indicating the weak inhibitory potential of compound **5**. The crude extract showed good inhibitory potential with a MIC value of 64 µg/mL on *Candida albicans*, indicating that the activity is not connected to our pure compounds. The present results point the possibility of investigating the polar fractions (butanol and H_2O), in order to explain the antimicrobial activity of the methanolic crude extract of stem bark *Garcinia lucida*.

Table 1: Minimal inhibitory concentration (µg/ml) of compounds and reference antibiotics on the studied microorganisms

Samples	*E. coli*	*P. aeruginosa*	*S. typhi*	*S. aureus*	*C. albicans*
Crude methanol extract	256	256	128	256	64
5	512	512	NA	NA	NA
RA*	**2**	**4**	**4**	**4**	**16**

RA*: gentamycin for bacteria and nystatin for *C. albicans;* **NA**: Not active up to 512 µg/mL

ACKNOWLEDGEMENT

This work is dedicated to the memory of the regretted Professor **François TILLEQUIN**[a] . It was supported by the Doctoral Mobility Fellowship Granted «Bourse Doctorale de Mobilité Universitaire BDMU» 2009 Paris-France. Joseph MOMO wishes to thank Dr. Raphael GROUGNET[a] for his comments and advice, and M. Roland NGUELE[2] for providing the plant material.

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