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Research Article

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Isolation, Characterization and Cytotoxic Activity of Isolated Compounds from Seed of *Carapa angustifolia* (Meliaceae)

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ABSTRACT

Phytochemical investigation on Carapa angustifolia, an endemic Cameroonian Meliaceae species, led to the isolation and characterization of four andirobin-type limonoids, known as methyl angolensate (1), andirobin (2), 7-deacetoxy-7-oxogedunin (3) and 7-deacetoxy-7-hydroxygedunin (4) together with six known compounds. The structures of these compounds were identified by NMR and MS spectroscopic data and by comparison of these data with those reported in literature. The compounds 1-10 are reported for the first time from C. angustifolia. The cytotoxic activity of some compounds has been evaluated and the chemotaxonomic significance of isolated compounds has also discussed.

Keywords: *Carapa angustifolia*; Andirobin-type limonoids; Cytotoxic activities; Chemotaxonomy

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INTRODUCTION

Carapa angustifolia [1], one of the thirteen species of the genus *Carapa* growing in Cameroon, is an evergreen tree which could reach a height of 12-35 m with 0.2 m of diameter and growing mainly in tropical areas of Africa [1]. Previous phytochemical investigation of the Meliaceae family including *Carapa* species revealed the presence of a wide range of secondary metabolites, including terpenes [2] and limonoids [3], some of which showed interesting biological properties, such as antimicrobial, anti-inflammatory [3]. In our continuing search for bioactive secondary metabolites from Cameroonian *Carapa* species, bioassay-guided study of the active constituents of seed of *Carapa angustifolia* was carried out as well as their chemotaxonomic relevance. This study also involved the first phytochemical investigation on *Carapa angustifolia.*

EXPERIMENTAL SECTION

General Considerations

IR spectra were recorded on a Shimadgu 8900 FT-IR spectrophotometer in KBr disks. Mass spectra were obtained with a JEOL JMS-600H mass spectrometer. The NMR spectra in $CDCl_3$, DMSO-d_6, and pyridine-d_5 were obtained

using Bruker Av-300, Av-500 and Avance-500 Cryo-Probe instruments. Chemical shifts are given in □ (ppm) using tetramethylsilane (TMS) as internal standard. Silica gel (70-230 mesh; Merck) and pre-coated silica gel plates (Merck 60F254; 20 × 20; 0.25 mm) were used for chromatography. TLC plates were visualized by spraying with a solution of 10% H_2SO_4 or under ultraviolet light of wavelength 254 and 366 nm.

Plant Collection

Fresh seeds of *Carapa angustifolia were* collected from mount Kala, Yaounde, Centre region, Cameroon in December 2018. Voucher specimens were authenticated by plant taxonomist (M. Ngansop) and maintained at the National Herbarium of Cameroon.

Extraction and Isolation

The air-dried, ground seeds of *Carapa angustifolia (734 g) were* extracted by maceration with 10 L of a solvent mixture of CH_2Cl_2/MeOH (1:1) at room temperature for 48 hours. After filtration, the organic solution was concentrated in vacuo and this entire process was repeated three times to yield a viscous crude extract (97 g). The crude extract was solubilized in the mixture of MeOH/H_2O (9:1) and partitioned sequentially with hexane (3 × 100 ml), CH_2Cl_2 (3 × 100 ml), EtOAc (3 × 100 ml) and *n*-BuOH (2 × 100 mL) to afford the semi-crude fractions.

The CH_2Cl_2 fraction (27 g) was subjected to column chromatography eluting with solvent gradient of Hex/EtOAc and monitoring by means of TLC. A total of 297 fractions of 100 mL each were collected.

Fractions 1-82 were combined on the basis of TLC to give sub-fraction F1 (4 g) which was rechromatographed over silica gel using hexane increase polarity with EtOAc to afford compounds **5** (5.3 mg), **6** (3.7 mg), **7** (4.8 mg) and **9** (5.5 mg). Fractions 83-105, eluent in the mixture of Hex/EtOAc was also combined to give sub-fraction F2 (2 g). Submitted to repeated column chromatography, this sub-fraction gave limonoids which consist of compound **1** (3.5 mg), **2** (2.7 mg), **3** (3.0 mg) and **4** (3.3 mg). Fractions 106-151 were also combined to as sub-fraction F3 (0.97g), which was subjected to further column chromatography to afford 8 (7.1 mg). Fractions 109-145 (0.98g) obtained from the elution using mixture of Hex/EtOAc (7:3) were subjected to a further column chromatography over silica gel with the mixture of Hex/EtOAc (4:1) as eluent, yielding to compound **10** (3.9 mg).

Cytotoxic Activity

Compounds **1** to **6** were evaluated for their cytotoxic activities against human hepatoma cell HepG2 using the MTT method [4] with doxdorubicin as positive control.

RESULTS AND DISCUSSION

Phytochemical investigation of the crude extract of the seeds of *Carapa angustifolia led to the isolation of ten compounds whose structures were* elucidated via comparison of their spectroscopic data (MS and NMR) with those reported in the literature. These compounds were identified as methyl angolensate (**1**), andirobin (**2**), 7-deacetoxy-7-oxogedunin (**3**), 7-deacetoxy-7-hydroxygedunin (**4**), betulinic acid (**5**), taraxerol (**6**), *β*–sitosterol (**7**), *β*-sitosterol-3-O-*β*-D-glucopyranoside (**8**), scopoletin (**9**) and sorbitol (**10**) [5-11]. All these compounds were isolated from *Carapa angustifolia* for the first time (Figure 1).

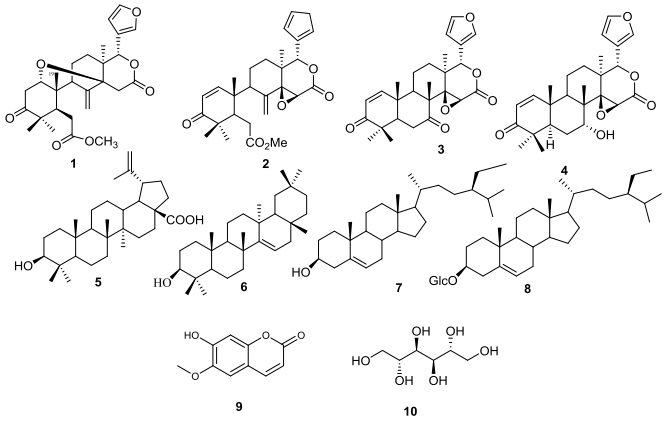

Figure 1. Structure of compounds **1-10.**

Concerning cytotoxic activities (Table 1), compound **5** exhibited more significant cytotoxicity against *HepG2* cell lines with IC_{50} value of 0.79 µg/mL while compound **1** showed moderate activity with IC_{50} value of 17.04 µg/mL. Compounds **2, 3, 4** and **6** were inactive, that is, showed no cytotoxicity on *HepG2* cell (IC50>20 μg/mL).

Table 1. Cytotoxic activity of isolated compounds from *Carapa angustifolia*

Compound	Cytotoxicity IC50 values on HepG2 (µg/mL)
1	17.04 ± 0.61
2	23.47 ± 0.48
3	22.20 ± 0.89
4	> 200
5	0.79 ± 0.20
6	39.91 ± 5.42
Doxorubicin	3.57 ± 1.03

This is the first report of compounds **1**-**4** from *Carapa angustifolia* species which were characterized and classified as andirobin-type limonoids. These group of limonoids provide new information for chemotaxonomy study on this species amongst the genus *Carapa. Indeed, these compounds were isolated from the seeds of Carapa guianensis which confirmed* their abundance from genus *Carapa* [12,13]. Compounds **1**, **2** and **3** were also isolated from *Soymida febrifuga* [14] and *Swietenia macrophylla* [15] whereas compound **4** was isolated from *Khaya grandifoliola* [16]. All these species as cited belong to Meliaceae family. Due to less phytochemical research on genus *Carapa*, therefore, the isolation of compounds **1**-**4** from *Carapa angustifolia* could be of great chemotaxonomic significance and can serve as valuable chemotaxonomic markers for *C. angustifolia* and other species from Meliaceae family, specifically genus *Carapa.*

The other representative components belonging to various class of secondary metabolites, are widely distributed in the plants of the species from Meliaceae family, especially in *Trichilia monadelpha* and *Trichilia rubescens* [17,18].

CONCLUSION

In conclusion, the phytochemical study of the seeds of *Carapa angustifolia* reported in this work is in conformity with the chemical profile of *Carapa guianensis* thereby suggesting a close chemotaxonomic relationship between these two species. Nevertheless, information on chemical compounds from *Carapa angustifolia* are still limited and it is necessary to conduct more investigation in order to confirm the chemotaxonomic relevance.

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