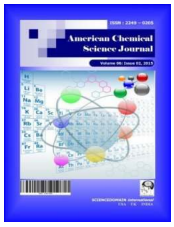

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Phytochemical Study of *Sacoglottis gabonensis* (Baill.) Urb. Isolation of Bioactive Compounds from the Stem Bark

Guy Raymond Feuya Tchouya[1*], Giresse Delphang Nguema Obiang[1], Jean-bernard Bongui[1] and Jacques Lebibi[1]

[1]Department of Chemistry, Faculty of Science, Scientific and Technical University of Masuku, Box. 223(Potos), Franceville, Gabon.

Authors' contributions

This work was carried out in collaboration between all authors. Author GRFT designed the study, wrote the protocol, managed the literature search, did the spectral interpretation and wrote the first draft of the manuscript. Author GDNO did the chromatographic separation of the stem bark extract. Author JBB was involved in the literature search of data on the plant and the critical revision of the manuscript for important intellectual content. Author JL supervised the work and reviewed all drafts of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The main objective of the study was to isolate biomolecules from *Sacoglottis gabonensis*, and also to identify its antimicrobial active principles.
Study Design: Chromatographic separation of biomolecules from the stem bark extract of *S. gabonensis* and spectral analysis of the isolated compounds.
Place and Duration of the Study: Department of Chemistry, Faculty of Science, Scientific and Technical University of Masuku, Box. 223(Potos), Franceville, Gabon, and International Center for Chemical and Biological Sciences, H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan, between January 2014 and March 2015.
Methodology: The stem bark extract of *S. gabonensis* was fractionated by means of silica gel

**Corresponding author: E-mail: gfeuya@yahoo.fr;*

column chromatography, and the isolated biomolecules characterized through extensive spectroscopic analyses.
Results: Two bioactive substances, involved in the antimicrobial property of the plant were isolated, and their structures assigned as known compounds, bergenin and gallic acid.
Conclusion: The compound gallic acid was isolated from *S. gabonensis* by using Column chromatography for the first time. Bergenin and gallic acid can be considered as antimicrobial active principles of *S. gabonensis*.

Keywords: Sacoglottis gabonensis; column chromatography; phytochemistry; bergenin; gallic acid.

1. INTRODUCTION

The search for new drugs from nature is generally based on biological and ecological rationales, since natural sources such as plants, have provided many effective drugs including morphine, quinine, artemisinin, paclitaxel (taxol) and others [1-5]. *Sacoglottis gabonensis* (Baill.) Urb. (Humiriaceae), is a large evergreen tree with an immense, heavy-branched crown that can grow up to 40 metres or more tall. The bole can be free of branches for up to 20 metres, but more often branches from low down. It is often crooked, knotty and deeply fluted; up to 180 cm in diameter with buttresses up to 2.5 metres high. The tree is exploited from the wild for its timber, which is used locally and also exported. The bark is used in the Gabonese folk medicine in the treatment of venereal diseases [6].

In our previous work on plants used in traditional medicine in the treatment of HIV/AIDS opportunistic diseases in Gabon [6], we screened the leaves and stem bark crude extracts of *S. gabonensis*, for their antimicrobial activities and their chemical composition. The tested extracts showed interesting antimicrobial activities *in vitro*, and high potentially active biomolecules content, including alkaloids, polyphenols and sugars [6].

The present study includes the fractionation of the methanolic extract of the stem bark along with the identification of active principles through analysis of spectroscopic data.

2. MATERIALS AND METHODS

2.1 Plant Material

The stem bark of *S. gabonensis* was collected in May 2012 in the Mondah forest near Libreville, in the Estuaire Region (Gabon) and was identified by Mr Thomas NZABI, a botanist of the National Herbarium of Gabon. A specimen is kept in the Scientific and Technical University of Masuku, Franceville, Gabon (N°Sg 127/UM) [6].

2.2 Extraction

The air-dried and powdered stem bark (4 kg) was extracted with 8 l of MeOH (1 week, repeated three times) at room temperature. The extract obtained was concentrated to dryness under vacuum at low temperature to give 150 g of crude extract (dark brownish colour) that was thereafter submitted to chromatographic separation on a silica gel column.

2.3 Fractionation, Purification and Identification of Compounds

40 g of the methanol extract obtained was chromatographed in a 70-230 mesh silica gel column (1 kg) with stepwise gradient elution by *n*-hexane/EtOAc mixtures (80:20; 70:30; 60:40; 50:50; 30:70; 0:100). Eighty column fractions, each containing 300 ml, were collected and combined according to their TLC profiles on pre-coated silica gel 60 F_{254} plates, developed with *n*-hexane/EtOAc and CH_2Cl_2/MeOH mixtures to give 4 groups of fractions: $\mathbf{F_1}$ (1-15); $\mathbf{F_2}$ (16-30); $\mathbf{F_3}$ (31-65) and $\mathbf{F_4}$ (66-80). Fraction $\mathbf{F_1}$ (10 g) contained a complex oily mixture. Fraction $\mathbf{F_2}$ (100 mg) was again subjected to column chromatography (CC) over silica gel, eluted with *n*-hexane/EtOAc mixtures starting from 100% *n*-hexane to 50% of the mixture. This resulted in the isolation of three compounds indexed $\mathbf{F_{2\text{-}1}}$ (1.4 mg), $\mathbf{F_{2\text{-}2}}$ (15 mg) and $\mathbf{F_{2\text{-}3}}$ (2 mg). Fraction $\mathbf{F_3}$ was also subjected to CC using the same quality of silica and eluted with a mixture of EtOAc and *n*-hexane (1:9 - 100% EtOAc) to give: $\mathbf{F_{2\text{-}2}}$ (30 mg), $\mathbf{F_{3\text{-}1}}$ (5 mg) and a mixture of 2 polar compounds with spots very close on TLC plates, that was recrystallized in a *n*-hexane/EtOAc mixture to afford $\mathbf{F_{3\text{-}2}}$ (3 mg). Fraction $\mathbf{F_4}$ appeared as a trail on the TLC plate and was not submitted to any chromatographic separation.

Compounds **F_{2-2}** and **F_{3-1}**, after spectroscopic analysis and comparison of their spectroscopic and physical data with those reported in the literature were identified respectively to bergenin **(1)** and gallic acid **(2)**. **F_{2-1}** and **F_{2-1}**, obtained in little quantities were not submitted to NMR spectroscopic analysis. The structure determination of **F_{3-2}** is still going on.

From the chemotaxonomic point of view, it is of interest to note that, although the occurrence of gallic acid in *Caesalpinia* genus (Leguminoseae) [7] and in many plant tissues [8], this is the first time its isolation is being reported from *S. gabonensis*.

3. RESULTS AND DISCUSSION

The phytochemical investigation of the stem bark of *S. gabonensis* (Humiriaceae) led to the isolation of two known phenolic compounds: bergenin **(1)** and gallic acid **(2**), together with three other unknown compounds.

Bergenin **1**, $C_{14}H_{16}O_8$ (0.0042% from the starting material), a beige powder crystallizing in *n*-hexane/EtOAc (v/v 9:1), mp 238-240°C, was identified on the basis of its NMR spectra and compared with reported data in the literature [9]. The bergenin structure is shown in Fig. 1. NMR spectra were recorded on a Brucker-500 spectrometer. ^{1}H NMR (500 MHz, CD_3OD-d4): δ 7.09 (s, 1H); 4.97 (d, 10, 1H); 3.68 (m, 1H); 3.45 (dd, 9.5; 8.5, 1H); 3.83 (t, 8.75; 8.5, 1H); 4.06 (t, 9.5; 10.0, 1H); 3.91 (s, 3H); 3.68 (m, 1H); 4.04 (dd, 10.7; 2.5, 1H). ^{13}C NMR (125 MHz, CD_3OD-d4): δ 165.9 (C); 150.9 (C); 140.6 (C); 148.0 (C); 115.9 (C); 118.0 (C); 109.4 (CH); 73.7 (CH); 81.7 (CH); 70.7 (CH); 74.2 (CH); 79.8 (CH); 59.8 (OCH_3); 61.1 (CH_2).

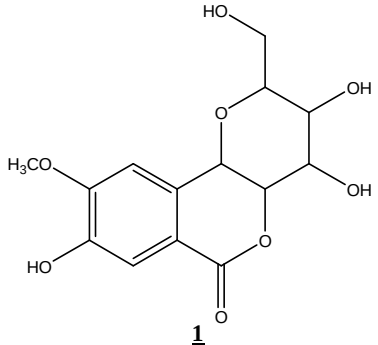
Gallic acid **2**, $C_7H_6O_5$ (0.00047% from the starting material), white powder crystallizing in *n*-hexane-EtOAc (v/v 8:2), mp 256-258°C, was also identified on the basis of its NMR spectra and compared with reported data in the literature [7]. The gallic acid structure is shown in Fig. 1. NMR spectra were recorded on a Brucker-400 spectrometer. ^{1}H NMR (400 MHz, Acetone-d6): δ 7.14 (s, 2H). ^{13}C NMR (100 MHz, Acetone-d6): δ 167.6 (C); 145.9 (C); 138.6 (C); 122.1 (C); 110.1 (CH).

The isolated compounds are well known in the scientific literature to have been subjected for many pharmacological studies.

Bergenin has also been isolated from many plants species such as *Bergenial crassifolia*, *Corylopsis spicata*, *Caesalpinia digyna*, *Mallotus japonicas* and *Thomandersia hensii* etc [10-12]. Bergenin has been shown to exhibit antihepatotoxic, antiulcerogenic, anti-HIV, antiarrhythmic, neuroprotective, anti-inflammatory, antiulcer, antifungal, immunomodulatory activities and burn wound healing effects [12-15].

A work done by Silva et al. [11,16] revealed important antimicrobial activities of bergenin tested against *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans, C. guilliermondii*, *Aspergillus flavus*, *A. nidulans*, *C. tropicalis*, *A. niger*, *Shigella sonnei, Serratia marcenses* and *Klebsiella pneumoniae* ATCC and Clinical isolated strains. Results showed that bergenin strongly inhibited the growth of the yeasts *Candida albicans*, *C. tropicalis* and *C. guilliermondii*, and the bacteria *P. aeruginosa in vitro*.

In the same way, gallic acid is a bioactive phytochemical that commonly occurs in a wide



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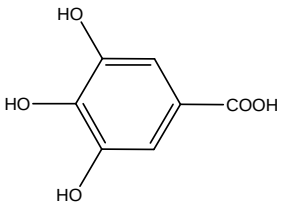

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Fig. 1. Chemical structures of isolated and identified compounds: bergenin 1, gallic acid 2

range of land plants [17]. This compound has been shown to exhibit many biological activities, especially antimicrobial activities against many microorganisms such as *Salmonella typhimurium* [18], *Escherichia coli*, *Staphylococcus aureus* [7] and *Campylobacter jejuni* [18,19-21].

In our previous study, we showed that *S. gabonensis* possessed antimicrobial activities, inhibiting the growth of many microorganisms, such as *Pseudomonas aeruginosa*, *Shigella flexnerii*, *Staphylococcus aureus* and *Streptococcus B* [6]. In the literature, it is indicated that isolated biomolecules showed interesting bioactivities when tested against several microbial strains. The antimicrobial property of the plant can be attributed to the content of its stem bark, namely – bergenin and gallic acid. The compounds have also been reported as good antioxidants, and strong antimicrobials [11-16,18-21].

4. CONCLUSION

The phytochemical study of the stem bark of *S. gabonensis*, allowed us to isolate two bioactive compounds, identified as bergenin and gallic acid. The known antimicrobial activities of the crude extracts and isolated biomolecules of this plant, partially validate the use of *S. gabonensis* in the Gabonese traditional medicine in the treatment of venereal diseases. However, more pharmacological studies, including the determination of the toxicity of the crude extract and isolated biomolecules of *S. gabonensis* are necessary.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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