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Sesquiterpene lactones of *Moquiniastrum polymorphum* subsp. *floccosum* have antineoplastic effects in Walker-256 tumor-bearing rats

Gracianny Gomes Martins [a], Francislaine Aparecida dos Reis Lívero [a], Aline Maria Stolf [a], Caroline Machado Kopruszinski [a], Cibele Campos Cardoso [a], Olair Carlos Beltrame [b], José Ederaldo Queiroz-Telles [c], Regiane Lauriano Batista Strapasson [d], Maria Élida Alves Stefanello [d], Ronald Oude-Elferink [e], Alexandra Acco [a,*]

[a] Department of Pharmacology, Federal University of Paraná, Curitiba, PR, Brazil
[b] Department of Veterinary Medicine, Federal University of Paraná, Curitiba, PR, Brazil
[c] Department of Medical Pathology, Federal University of Paraná, Curitiba, PR, Brazil
[d] Department of Chemistry, Federal University of Paraná, Curitiba, PR, Brazil
[e] Tytgat Institute for Liver and Intestinal Research, University of Amsterdam, Amsterdam, The Netherlands

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ABSTRACT

Background and aim: This study aimed to evaluate the *in vivo* antitumor actions and toxicity of the dichloromethane fraction (F1B) of *Moquiniastrum polymorphum* subsp. *floccosum* (formerly *Gochnatia polymorpha* ssp. *floccosa*), composed of sesquiterpene lactones, against Walker-256 carcinosarcoma in rats. *Methods:* Male Wistar rats received 100 mg kg^{-1} F1B per day orally for 16 days after subcutaneous inoculation of Walker-256 cells in the pelvic limb. The tumor progression was monitored, and after treatment, tumor weight, oxidative stress, plasma biochemistry, inflammatory parameters, gene expression and histology of tumor and/or liver were evaluated. The toxicity of F1B was analyzed through the relative weight of organs. Additionally, an LD_{50} test was performed in mice. *Results:* F1B treatment significantly reduced tumor volume and weight. There was no difference in oxidative stress in tumor tissue after treatment. F1B treatment modified hepatic glutathione and superoxide dismutase, and normalized plasma glucose, alkaline phosphatase, and amylase. F1B did not affect the activity of myeloperoxidase and *N*-acetylglucosaminidase or the nitric oxide levels in tumor tissue. However, F1B decreased the tumor necrosis factor (TNF)-α levels. Additionally, F1B increased apoptosis in the tumor, mediated by up-regulation of the *p53* and *Bax* gene expression. No clinical signs of toxicity or death were observed in the rats treated with F1B. The LD_{50} calculated for mice was 1209 mg kg^{-1}. *Conclusions:* F1B, which is rich in sesquiterpene lactones, showed antitumor activity against Walker-256 carcinosarcoma. This effect may be, at least in part, related to the induction of apoptosis and inhibition of TNF-α signaling.

1. Introduction

In recent decades, cancer has become a public health problem worldwide. Cancer, or malignant neoplasm, is characterized by uncontrolled proliferation and spread of abnormal cells, caused by alterations in oncogenes, tumor-suppressor genes, and microRNA genes [1]. The malignant characteristics of these cells results in dedifferentiation, increased cell invasion, and metastasis [2]. There are three main approaches to treating cancer: surgical excision, irradiation, and chemotherapy. The relative value of each of these approaches depends on the type and stage of the tumor. Importantly, chemotherapy may be used on its own or as an adjunct to other forms of therapy [3].

The compounds used in neoplastic chemotherapy exhibit marked differences in their structure and mechanism of action,

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Cat, catalase; DNCB, 2,4-dinitrochlorobenzene; EDTA, ethylenediaminetetraacetic acid; EtOAc, ethyl acetate; F1B, dichloromethane fraction of *M. polymorphum* subsp. *floccosum*; FOX2, ferrous oxidation of xylenol orange; GSH, reduced glutathione; GST, glutathione-*S*-transferase; HPLC, high performance liquid chromatography; HRP, streptavidin–horseradish peroxidase; HTAB, hexadecyltrimethylammonium bromide; LD_{50}, lethal dose 50%; LPO, lipid peroxidation; MAP kinase, mitogen activated protein kinase; MPO, myeloperoxidase; NAG, *N*-acetylglucosaminidase; NF-κB, nuclear factor κB; NO, nitric oxide; OPD, *o*-phenylenediamine; p53, pro-apoptotic proteins p53; ROS, reactive oxygen species; SEM, standard error of the mean; SLs, sesquiterpene lactones; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; VLC, vacuum liquid chromatography.

* Corresponding author at: Federal University of Paraná (UFPR), Biological Science Sector, Department of Pharmacology, Centro Politécnico, Cx. P. 19031, Curitiba 81531-981, PR, Brazil. Tel.: +55 (41) 3361 1742; fax: +55 (41) 3266 2042.
E-mail address: aleacco@ufpr.br (A. Acco).

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including alkylating agents, antimetabolites, natural products, hormones, and hormone antagonists, in addition to a variety of agents directed at specific molecular targets [4]. Despite the broad variety of antineoplastic drugs currently available, an intense search continues for novel treatment options at the molecular level, including agents that can act on specific signaling pathways, inhibit proliferation and angiogenesis, and promote cell death in tumors. However, despite the importance of discovering novel compounds that act on specific molecular targets, the ability of these drugs to be used in combination with other cytotoxic agents is essential, as this will produce a more beneficial response in patients [4–6].

Recently, there has been increasing focus on identifying natural products with antitumor activity. Among these, the guaianolide, a subtype of sesquiterpene lactones (SLs) have garnered interest, because they have displayed cytotoxic activity against several human tumor cell lines *in vitro* and antitumor activity *in vivo* [7]. SLs may inhibit cancer progression through the inhibition of inflammatory responses, prevention of metastasis, and induction of apoptosis [8]. SLs are abundant in *Moquiniastrum*, a genus of 21 species that is grown in South America [9]. Specifically, *Moquiniastrum polymorphum* (formerly *Gochnatia polymorpha*), also known as "Cambará," is a medium sized tree found in several Brazilian States, as well as Paraguay, Uruguay, and Argentina. Previous chemical studies have reported that this species contains SLs, dimeric guaianolides, diterpenes, triterpenes, flavonoids, coumarins and phenolic compounds [10–15]. The leaves and trunk of *M. polymorphum* have also been shown to have anti-inflammatory and antispasmodic activities [13,16]. In a recent study by Strapasson et al. [17], the *in vitro* cytotoxic activity of compounds isolated from the trunk bark of *G. polymorpha* ssp. *floccosa* was demonstrated in human cancer cell lines. The most active compounds, the dimeric lactones 10-desoxygochnatiolide A and gochnatiolide A, inhibited the growth of kidney, skin (melanoma), ovarian and brain (glioma) tumor cell lines at low concentrations (0.21–1.09 μg mL^{-1}). However, the antineoplastic effects of *G. polymorpha* were not evaluated *in vivo*.

The aim of this study is to evaluate the antineoplastic effect of a fraction obtained from the trunk bark of *M. polymorphum* subsp. *floccosum*, namely F1B, in rats bearing Walker-256 tumor, a model of solid cancer. To our knowledge, this is the first report of *in vivo* antitumor activity and toxicity evaluation of F1B obtained from this plant.

2. Materials and methods

2.1. Plant material

M. polymorphum subsp. *floccosum* (Cabrera) G. Sancho trunk bark (Asteraceae) was collected in June 2013, in Curitiba, Paraná State, Brazil, and identified by Dr. Armando C. Cervi. A voucher specimen (UPCB 30100) was deposited in the herbarium of the Federal University of Paraná (UFPR), Brazil. The plant nomenclature was checked in www.floradobrasil.jbrj.gov.br.

2.2. Extraction, isolation and chemical analyses

The extraction and isolation of compounds were carried out as previously reported [17]. Briefly, dried and powdered trunk bark (1.6 kg) was extracted at room temperature with hexane, followed by 95% ethanol (EtOH). The EtOH extract (65.2 g) was partitioned with dichloromethane, ethyl acetate (EtOAc), and 1-butanol. The dichloromethane fraction (F1, 19.2 g) underwent silica gel vacuum liquid chromatography (VLC), followed by elution with hexane, dichloromethane, EtOAc, and methanol. The fraction eluted with dichloromethane (F1B, 3.6 g) was used in biological assays. The major compounds of this fraction were isolated by successive chromatographic fractionation on silica gel, as previously described [15,17]. Compounds **1**–**6** were purified by semi-preparative HPLC and the pure compounds were used to identify the peaks in the HPLC fingerprint of F1B (Fig. 1; **1** retention time (R_t) 12.1 min; **2** R_t 18.5 min; **3** R_t 20.1 min; **4** R_t 22.3 min; **5** R_t 24.1 min; **6** R_t 28.6 min).

2.3. Animals

Male Wistar rats weighing 200–250 g were housed in a temperature controlled facility (22 ± 1 °C), with a constant 12-h light–dark cycles and free access to standard laboratory food (Nuvital®, Colombo, PR, Brazil) and tap water. All the experimental protocols followed the principles of laboratory animal care and were approved by the Ethical Committee for Animal Use (CEUA) of Biological Sciences Section of UFPR (authorization number 671).

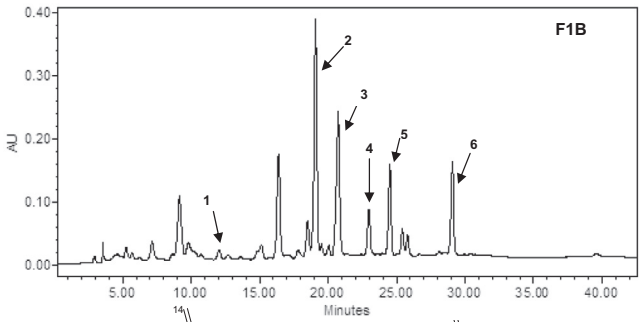
2.4. Tumor cells and experimental tumor inoculation

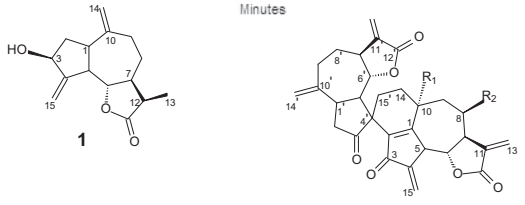
The maintenance of the Walker-256 cells was performed by weekly intraperitoneal (i.p.) inoculation passages of 10^7 cells/rat, followed by freezing cells at −80 °C [18]. After 5–7 days, the ascitic fluid was collected in a solution of ethylenediaminetetraacetic acid (EDTA) (0.5 M, pH 8.0, 1:1) and suspended in 1.0 mL of PBS (16.5 mM phosphate, 137 mM NaCl and 2.7 mM KCl).

For implantation in animals, tumor cells were injected subcutaneously (10^7 cells/animal) in the right pelvic limb after verifying viability by the Trypan blue exclusion method in a Neubauer chamber. The first tumor cells were kindly donated by Dr. Sandra Coccuzzo Sampaio of Butantã Institute (São Paulo, Brazil).

2.5. Experimental design

Rats were treated via oral gavage once a day, for sixteen consecutive days. Treatment began one day following subcutaneous inoc-





	2	3	4	5	6
R_1	α-OH	α-H	β-OH	α-OH	α-H
R_2	β-OH	β-OH	β-H	β-H	β-H

Fig. 1. Typical HPLC chromatogram of F1B (211 nm) and structures of the isolated compounds. (**1**) 11αH-13-dihydrozaluzanin C, (**2**) 8-hydroxigochnatiolide A, (**3**) 8-hydroxi-10-desoxigochnatiolide A, (**4**) gochnatiolide B, (**5**) gochnatiolide A, (**6**) 10-desoxygochnatiolide A.