Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
19(1A): 26-28, Jan./Mar. 2009
Received 5 January 2009; Accepted 1 March 2009

Artigo

Chemical constituents and lethality of *Paullinia weinmanniaefolia* (Sapindaceae) against *Artemia salina* Leach

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RESUMO: "Constituintes químicos e letalidade de *Paullinia weinmanniaefolia* (Sapindaceae) contra *Artemia salina* Leach". Os extratos obtidos de folhas e caule de *Paullinia weinmmaniaefolia* foram testados contra larvas de *Artemia salina* e mostraram significante letalidade. Os extratos das folhas em diclorometano e do caule em metanol e metanol-água (8:2) apresentaram atividade com valores de CL_{50} 366,87; 375,38 e 620,30 ppm, respectivamente. Sete triterpenos, dois esteróides e um ciclitol foram identificados através de técnicas espectrométricas como principais constituintes destes extratos.

Unitermos: *Paullinia weinmmaniaefolia*, Sapindaceae, triterpenos, *Artemia salina,* letalidade.

ABSTRACT: The extracts obtained from leaves and steam of *Paullinia weinmmaniaefolia* were tested against *Artemia salina* larvae and showed significant lethality. The extracts in dichloromethane of the leaves, in methanol and methanol-water (8:2) of the steam showed activity with LC_{50} values of 366.87, 375.38 and 620.30 ppm, respectively. Seven triterpenes, two steroids and one cyclitol were characterized by spectrometric methods with majoritary constituents of these extracts.

Keywords: *Paullinia weinmmaniaefolia*, Sapindaceae, triterpenes, *Artemia salina*, lethality.

INTRODUCTION

The Sapindaceae family, also know as the Soapberry family, has about 136 genera with 2.000 species (Ferruci, 2000). Sapindaceae includes many species of economically valuable tropical fruit, including the Lychee, the Guarana, the Longan, the Rabumtan, the Manoncilho and the Akee (Menzel et. al., 1998). The Sapindaceae species are trees, shrubs, herbs and lianas occurring in temperate to tropical regions throughout the world. The *Paullinia* genera comprise about 180 species all Neotropical lianas with the exception of *P. pinnata* occurring also in the African tropics (Henman, 1982). From the species *P. cupana* and *P. pachycarpa* were isolated alkaloids (Mattei et al., 1998), (Abourashed et al., 1999) and from *P. pinnata* were isolated flavones (Weckerle, 2003).

The *Artemia salina* larvae have been used as target organism to detect bioactive compounds in plant extracts and toxicity to this crustacean has good correlation with anti-tumor (McLaughlin, 1991; Lhullier et al., 2006; Shoeb et al., 2007; Silva et al., 2007; Subhan et al., 2008) and anti *Trypanosoma cruzi* (Zani, 1995).

P. weinmaniaefolia is a liana native to restinga region, known by the vernacular name of "cipó sangue" (Assunpção & Nascimento, 2000). There is no previous report in the literature concerning the isolation of the chemical constituents of *P. weinmmaniaefolia*. In this paper, we report the phytochemical study of leaves and steam of a specimen from *P. weinmanniaefolia*, involving isolation, structural characterization and evaluation of lethality against *A. salina* Leach, including of the crude extracts. The compounds were identified by spectroscopic methods, meanly spectral data obtained by ^{1}H and ^{13}C NMR spectra.

MATERIAL AND METHODS

Botanical materials

Leaves and steams of *P. weinmmaniaefolia* Mart. (Sapindaceae) were colleted in February 2003, in the city of São João da Barra, State of Rio de Janeiro, Brazil, in the Lagunar complex Grussaí-Iquipari, was identified by Jorge Assunção and a voucher specimen (No. 238) is deposited in UENF/CBB/LCA Herbarium in the Bioscience e Biotechnology Center, Campos dos Goytacazes, Brazil.

Extracts preparation and larvae evaluation

The dried and powdered leaves and steams of *P. weinmmaniaefolia* were extracted at room temperature

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ISSN 0102-695X

successively using hexane, dichloromethane, methanol and methanol-water (8:2). The solvents were removed under vacuum to yield the crude extracts. The crude extracts were tested against *A. salina* larvae as methodology proposed by McLaughlin and collaborators (McLaughlin et al., 1982). The lethal concentration 50% (LC_{50} value) and the standard error were calculated by Probit analysis (Finney, 1971).

Purification of constituents

The hexane and dichloromethane extracts were submitted to analytical TLC analysis and chromatographed on a silica gel column eluting with hexane/dichloromethane/methanol mixtures of increasing polarity, to produce the triterpenes (**1**-**8**) and the steroids (**9**-**10**). The crude methanol extract was acetylated with acetic anhydride in the presence of pyridine and purified on a silica gel column eluting with dichloromethane/ethyl acetate/ methanol mixtures of increasing polarity until isolation of peracetyl cyclitol (**11**).

Table 1. Results of brine shrimp lethality from extracts of *P. weinmmaniaefolia.*

Extracts	Material	LC_{50} (ppm)
Dichloromethane	Steam	> 1000*
Methanol	Steam	375.38 SE ± 0.08
Methanol-water (8:2)	Steam	620.30 SE ± 0.14
Dichloromethane	Leaf	365.87 SE ± 0.03
Methanol	Leaf	> 1000*
Methanol-water (8:2)	Leaf	> 1000*

* LC_{50} = Lethal Concentration 50% SE = Standard error.

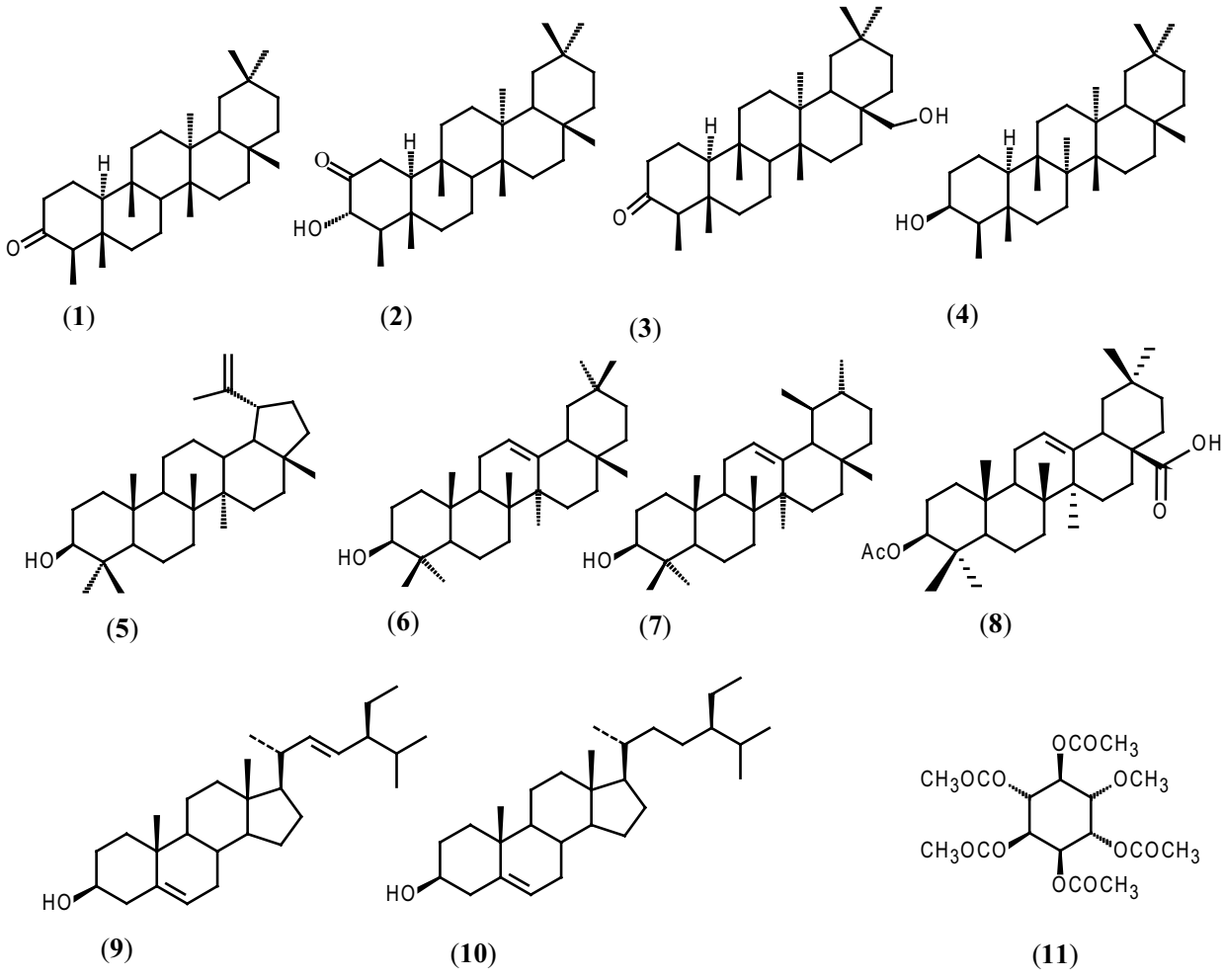

Figure 1. Constituents isolated from *P. weinmanniaefolia.*

RESULTS AND DISCUSSION

The results of brine shrimp lethality test from extracts of *P. weinmmaniaefolia* are described in Table 1. Among the extracts examined, the dichloromethane of leaves, methanol and methanol-water (8:2) of steam showed activity with LC_{50} values of 366, 375 and 620 ppm, respectively.

The triterpenes friedelin (**1**), lupeol (**5**), α-amirin (**6**) and β-amirin (**7**) were isolated from leaves and steam; friedelanane (**2**), canophyllol (3), *epi*-friedelinol (**4**), 3-*O*-acetylolean-12-en-28-oic (**8**) acid; the steroids stigmasterol (**9**) and β-sitosterol (**10**) and the peracetyl derivative of 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-L-*chiro*-inositol (**11**, derivative obtained by treatment with acetic anhydride in the presence of pyridine) were isolated from steam (Figure 1). The compounds were identified by spectroscopic methods, meanly spectral data obtained by ^{1}H and ^{13}C NMR (1D and 2D experiments) spectra involving comparison with values described in the literature for triterpenoids **1-8** (Ahmad & Atta-ur-Rahman, 1994) and steroids **9** and **10** (commonly isolated from plants).

ACKNOWLEDGMENTS

We thank Universidade Estadual do Norte Fluminense (UENF), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Coordenação e Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for fellowships and financial support and the biologist Jorge Assunção, LCA - Centro de Biociência e Biotecnologia - UENF, Campos dos Goytacazes, RJ, Brazil for the identification of this plant.

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