

# Pharmacological screening and acute toxicity of bark roots of *Guettarda platypoda*

**Evelyn M. L. Pina,<sup>1,2</sup> Fernando W. C. Araújo,<sup>1</sup> Ivone A. Souza,<sup>1</sup> Isla V. G. A. Bastos,<sup>1</sup> Teresinha G. Silva,<sup>1</sup> Silene C. Nascimento,<sup>1</sup> Gardenia C. G. Militão,<sup>1</sup> Luiz A. L. Soares,<sup>2</sup> Haroudo S. Xavier,<sup>2</sup> Sebastião J. Melo\*<sup>1</sup>**

<sup>1</sup>Departamento de Antibióticos, Universidade Federal de Pernambuco, Brazil,

<sup>2</sup>Departamento de Farmácia, Universidade Federal de Pernambuco, Brazil.

**Abstract:** Due to its folk use, scientific reports and phytochemical screening, the purpose of this work was to study the phytochemical and the biological properties of the methanol extract and to evaluate the anti-inflammatory activity as well as determine the acute toxicity, antitumor and cytotoxic activity of the root barks of *Guettarda platypoda* DC., Rubiaceae. In this analysis the presence of flavonoids and terpenoids were identified. These data and the ones in the literature indicated it as a potential antioxidant and motivated the cytotoxic analysis related with three tumoral cell strains as well as to evaluate its antitumoral activity (sarcoma 180 and Ehrlich carcinoma) in female mice. Due to the presence of esterooids and the previous study of the ethanolic extract, its anti-inflammatory activity and toxicity were also evaluated. Absence or low toxicity in 2000 mg/kg doses was verified and the attention to their phytochemical and pharmacological properties is constantly increasing.

## Article

Received 19 Mar 2012  
Accepted 6 Jul 2012  
Available online 9 Oct 2012

### Keywords:

acute toxicity  
anti-inflammatory activity  
antitumor activity  
cytotoxicity  
*Guettarda platypoda*

ISSN 0102-695X  
<http://dx.doi.org/10.1590/S0102-695X2012005000118>

## Introduction

The chemical and pharmacological potential of higher plants due to the identification of their secondary metabolites has long been known and the attention to their phytochemical and pharmacological properties is constantly increasing. Stems, flowers, leaves, roots, tubercle, seeds and fruits are targets of interdisciplinary studies in order to broaden the knowledge of their properties.

It is reported that from nearly 400000 plant species existing on our planet (IGEduc, 2010), Brazil has about 55000 of these in its territory. Thus, Brazil has one of the highest biodiversity in the world, featuring a variety of biomes that reflects the richness of flora and fauna. For this reason, it is one of the most important regions among the so-called mega-diverse and, many species found in Brazilian biomass are unique (endemic) (Portal Brasil, 2011). Due to this, it is easy to deduce the potential of bioactive products from the plants, and a large number of plant species in Brazil has still not been studied from the chemical and/or pharmacological point of view (Cunha et al., 2003; David et al., 2006).

Among the angiosperms, the most representative is the Rubiaceae family, being the fourth largest in Dicotyledons, comprising about 13000 species in 650 genera and 44 tribes (Delprete, 2004). Its species are found

in tropical and subtropical regions, and can also reach the temperate and cold regions of Europe and northern Canada (Pessoa, 2009). In the Americas, the family Rubiaceae is represented by 217 genera and 5000 species and in Brazil, about 120 genera and 2000 species, representing one of the leading families of our flora. This family is present in almost all natural formations: forest, *Cerrado*, *Caatinga*, shoal, rainforest, forest and mountain altitude, trays, dunes and open fields (Delprete, 2004; Lima et al., 2010; Pereira, 2002). According to Barbosa et al. (2011), the Rubiaceae family also stands out among the most diverse in the Brazilian Northeast, with 66 genera and some 310 species.

One of the representatives of this family is the genus *Guettarda*, composed of about 180 species known widely distributed in the tropical and neotropical zone, with the occurrence of 24 species distributed throughout the Brazilian territory (Mól, 2010). They are used in folk medicine for various purposes, mainly in the treatment of wounds and inflammation (Agra et al., 2007, 2008; Bertucci et al., 2008; Capasso et al., 1998).

*Guettarda platypoda* DC., Rubiaceae is found in the coastal plain of northeast of Brazil and is known as “angelica” or “angelica-do-mato” (Andersson, 1992; Pereira & Barbosa, 2004). *G. platypoda* is a shrub and its root is used in folk medicine as a febrifuge (Aquino et al., 1988a,b; Bhattacharyya & Almeida, 1985; Ferrari

et al., 1986), as well as during the puerperal period (Bhattacharyya & Almeida, 1985; Matos, 1997) and to correct menstrual irregularities (Bahia, 1979).

The objective of this work was to perform the phytochemical analysis and to evaluate the anti-inflammatory activity as well as to determine the acute toxicity, antitumor and cytotoxic activity. As the population of the northeast of Brazil uses this plant for medicinal purposes, and very few data were available, it was decided to evaluate its pharmacological potential, toxicity and safety levels of employment.

## Materials and Methods

### Plant material

The barks of *Guettarda platypoda* DC., Rubiaceae, were collected in August 2009 in the Island Itamaracá (Pernambuco, Brazil). The plant was identified by Rita de Cássia Araújo Pereira and a voucher specimen was deposited at Dárdano de Andrade Lima Herbarium, Agronomic Institute of Pernambuco (IPA), under the number 86565.

### Preparation of methanolic extract

After *G. platypoda* roots were collected the barks were immediately removed from them and left to dry at room temperature ( $25\pm 2$  °C) for seven days. They were weighed and triturated in an electric mill to obtain a fine granulated powder. The preparation of the methanolic extract was obtained by an infusion of 700 g of bark roots in 2000 mL of methanol, followed by 72 h maceration with sporadic agitation. The extract was filtered and the plant residue was re-extracted two more times. The filtered was concentrated in a rotary evaporator at 50 °C, to obtain the Crude Dry Extract (CDE).

### Phytochemical analysis

Using silica gel plates (Alugram® SIL G/UV254, Ref: 818133), 10 µL aliquots were subjected to thin layer chromatography (TLC) to analyze the presence of the main secondary metabolites: flavonoids, cinnamic derivatives, phenylpropanoid glycoside (Neu, 1956; Wagner & Blatt, 1996), condensed proanthocyanidins, leuco-anthocyanins (Roberts et al., 1957), monoterpenes, sesquiterpenes, diterpenes (Wagner & Blatt, 1996), triterpenes, steroids (Harbone, 1998; Sharma & Dawra, 1991), cardiac glycosides, alkaloids (Wagner & Blatt, 1996) and reducing sugars (Russel, 1982), employing the respective standards and specific revelators. The saponin presence was tested by afrogenicity, according to the methodology by Costa (2001).

### Animals

Adult female mice (*Mus musculus*) weighing 25-35 g were obtained from the Antibiotics Department of the Federal University of Pernambuco. The animals were kept in polypropilene cages with water and ration (Labina Purina Brazil) *ad libitum*, under controlled lightness, light and dark cycles of 12 h and of temperature ( $23\pm 2$  °C).

The experimental procedure was performed according to the ethical principles of CEEA (Ethics Committee of Animal Experiments) of Federal University of Pernambuco under the number 23076.013788/2009-82.

### Acute toxicity evaluation

The mice were submitted to acute toxicity essay according the OECD 423 protocol (2001). The CDE was given orally. After 8 h of fasting, the animals received a 2000 mg/kg and 3000 mg/kg doses and were observed during the first 24 h and daily for fourteen days period. At the end of fourteen days, they were sacrificed in a CO<sub>2</sub> chamber and taken to autopsy for macroscopic observation of the organs (liver, kidney and spleen).

### Anti-inflammatory activity evaluation

The carrageenan-induced peritonitis experiment was conducted in female mice (n=6) as describe by Uchôa et al. (2009). One hour before the experiment, the animals received the *G. platypoda* CDE orally in doses of 30, 50, 100, 150 and 200 mg/kg. The standard group received the vehicle (10% propylene glycol in saline, orally). Mice were injected intraperitoneally with 0.25 mL of carrageenan (1% in saline solution). After 4 h, the animals were sacrificed in CO<sub>2</sub> chamber and immediately submitted to surgery to have their chest opened, which was flushed with 3% of saline solution containing 3 µM of EDTA. The collected exudates were centrifuged and re-suspended in saline solution and the count of polymorph nuclear leucocytes (PMNL) was performed in cell automatic counter (Micros 60) (Vital et al., 2009).

### Antitumor evaluation (*Sarcoma 180* and *Ehrlich carcinoma*)

The antitumor experiment was performed according to Sugiura et al. (1955) method. After removing the tumor mass from the donor animal, it was placed in physiologic solution associate with an antibiotic, separating necroses from the other parts. After this, the fragments (about 3 mm) were induced by subcutaneous way in the receptor animals. The

animals were then divided in three groups (six animals per group). During the experiment (seven days), the animals were weighed and the control group received 0.5 mL of physiological solution orally, the positive group was treated with methotrexate (2.5 mg/kg oral way) and to the test group *G. platypoda* CDE in doses of 100 and 200 mg/kg was administered. The experimental chemotherapy was started 48 h after the transplant. At the end of the treatment (8<sup>th</sup> day) all the animals were weighed and sacrificed, and the organs (liver, kidneys, heart, lung and spleen) were removed and weighed, as well as the tumors were also excised, dissected and weighed. The tumor inhibition was calculated as: Inhibition ratio (%) was calculated as: inhibition ratio (%) =  $[(A-B)/A] \times 100$ , where A is the average tumor weight of the negative control, and B is that of the treated group.

#### *Cytotoxicity evaluation*

The cytotoxic potential of Crude Dry Extract was evaluated by the MTT assay (Mossman, 1983), compared with three human tumor cell lines: HT29 (human colon carcinoma), MCF-7 (breast human carcinoma) and NCI H-292 (lung human cancer) obtained from Rio de Janeiro's Cell Bank Brazil. All cell lines were maintained in DMEN medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin, at 37 °C with 5% CO<sub>2</sub>. The cells were plated in 96-well plates at  $1 \times 10^5$  cells/mL. The *G. platypoda* CDE was diluted in DMSO 0.1% and tested in quadruplicate in 50 µg/mL concentration. After 72 h of incubation, 25 µL of MTT (5 mg/mL) was added. Three hours later the MTT formazan product was dissolved in 100 mL DMSO and absorbance was measured at 595 nm. The experiments were carried out in duplicate and they were analyzed according to their averages and standard deviations employing the Graph Pad Prism program.

#### *Statistical analysis*

All results were expressed as mean values ± SD for each experimental group. Statistical analysis between groups was performed by one-way analysis of variance (ANOVA), followed by Tukey's test. For a confidence interval of 95%, only *p* values less than 0.05 (*p* < 0.05), as determined by BioEstat (version 5.0), were considered to be indicative of statistical significance.

## **Results**

#### *Phytochemical analysis*

The phytochemical analysis enables the

identification of the secondary metabolites, which may be related to biological activities. The phytochemical profile from the methanol extract of the root bark of *Guettarda platypoda* DC., Rubiaceae, detected the presence of flavonoids, cinnamic derivatives, phenylpropanoid glycoside, monoterpenes, sesquiterpenes, diterpenes, triterpenes, steroids, saponins and reducing sugars. The other metabolites (condensed proanthocyanidins, leuco-anthocyanins, cardiac glycosides and alkaloids) were not detected in the tests. A strong violet coloration was observed in the chromatographic test for triterpenes/steroids identification, which may indicate the presence of triterpene acids (oleanolic acid or ursolic acid). The presence of steroid beta-sitosterol was found in the extract, afrogenicity was found in the test, and the presence of saponins was indicated by the existence of an intense foam which lasted for more than 24 h.

#### *Acute toxicity evaluation*

Some signs and unusual symptoms during the first 60 min of the acute toxicity protocol were observed (Table 1): as exciting behavioral (agitation, touch response, tachycardia and tremor), inhibitory (sleepiness) and others characteristics (piloerection and tail quivering).

However, in this study, no death was observed fourteen days after acute treatment with high doses (2000 and 3000 mg/kg) of *G. platypoda* CDE, so it was not possible to calculate the LD<sub>50</sub>, considered here as greater than 3000 mg/kg. The treated animals did not show any significant alteration in water or food consumption, or in body weight (Table 2) during the experimental period. In addition, macroscopic examination revealed that the organs taken presented aspect of color, size and texture, showing no difference from the control group, with the remaining aspects preserved.

#### *Anti-inflammatory activity evaluation*

The results obtained in the carrageenan-induced peritonitis test are shown in Table 3. The data revealed that the doses tested with the Groups 2, 3, 4 and 5 (50, 100, 150 and 200 mg/kg, respectively) caused a significant reduction in the migration of polymorphonuclear leukocytes in comparison to the control group, presenting a promising anti-inflammatory activity. But, between these groups no statistically significant difference was observed.

#### *Antitumor activity evaluation*

The effects of *G. platypoda* CDE in mice

transplanted with the tumor sarcoma 180 and Ehrlich carcinoma are shown in Figure 1 and 2, respectively. Compared with the mean tumor weight control group treated with saline solution, the groups treated with 100 mg/kg (74,26%) and 200 mg/kg (84,77%) doses, had the mean weight of sarcoma 180 tumors reduced (Figure 1). The positive control group, metotrexato (2.5 mg/kg, oral way) had 89% tumor inhibition. In the animals transplanted with Ehrlich carcinoma, of the treatment with *G. platypoda* CDE presented a slight increase of the average weight of the tumor, however this decrease was not statistically significant. The positive control group (metotrexate) showed 92% inhibition in the growth of Ehrlich carcinoma.

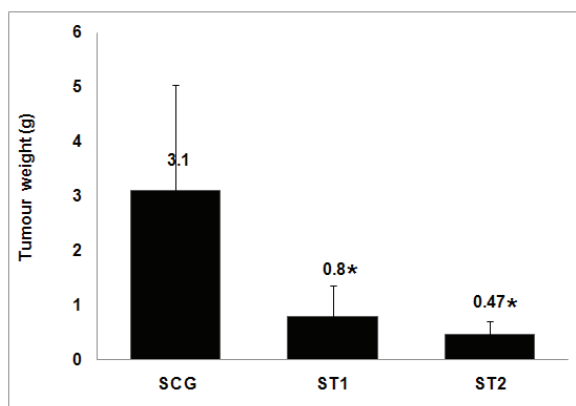
**Table 1.** Clinical signs of acute toxicity observed in adult female mice (*Mus musculus*), treated with different doses CDE of *Guettarda platypoda* by oral doses.

Parameters	Groups		
	2,000 mg/kg	3,000 mg/kg	Group control
Stimulants			
Agitation	+++	+++	++
Convulsions	—	—	—
Vocal fremitus	+	—	—
Irritacion	—	—	—
Stereotyped movements	—	++	—
Touch Response	++	++	—
Salivation	—	—	—
Tachycardia	++	+++	—
Tremors	++	++	—
Inhibitory			
Abdominal writhing	—	+	—
Stretch	—	—	—
Ptosis	—	—	—
Sleepiness	++	+++	+
Hindquarter	—	+	—
Other			
Cyanosis	—	—	—
Defecation	++	++	+
Diarrhea	—	—	—
Tearing	—	—	—
Urination	+	+	—
Piloerection	+	+	—
N° of deaths	0	0	0

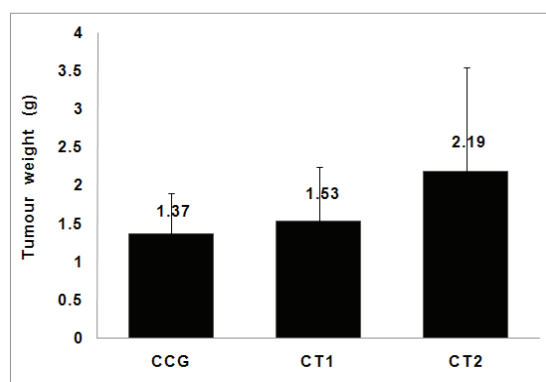
—: no effect; +: light effect; ++: moderate effect; +++: major effect.

During the experiments it was observed that all the animals presented no significant weight changes. Mice with Sarcoma 180: 90% of the ST1 or ST2 experimental groups presented hyperemic, friable, fibrous-looking lungs, one of the ST1 group presented

kidneys with a cystic appearance. Mice with Ehrlich carcinoma; it was macroscopically verified that two animals of CT1 group presented lung hemorrhage. With these exceptions, the mice of both strains, and independent of treatment, showed no macroscopic changes in their organs, with no fibrous consistency, thrombus, lesions and atrophy on the edge of the organs, preserving aspects in relation to structure, texture and shape. The tumors were well-defined and had a solid consistency (Figure 3).



**Figure 1.** Antitumor activity of *Guettarda platypoda* CDE in *Mus musculus* female mice after implantation of Sarcoma 180. Saline (0.5 mL) was orally administered in the Control Group (SCG) and experimental treated groups (ST1=100 mg/kg; ST2=200 mg/kg) for seven days. \* $p < 0.05$ .



**Figure 2.** Antitumor activity of *Guettarda platypoda* CDE in *Mus musculus* mice after implantation of Ehrlich carcinoma. Saline (0.5 mL) was orally administered in the Control Group (SCG) and experimental treated groups (CT1=100 mg/kg; CT2=200 mg/kg) for seven days. \* $p < 0.05$ .

#### Cytotoxicity evaluation

According to the results, CDE did not present cytotoxicity for any of the strains tested. The intensity scale used to evaluate the cytotoxic potential of the samples tested was: samples with no activity (10-20% inhibition), with few activity (cell growth inhibition ranging from 20

**Table 2.** Weight gain and physiological habits (consumption of water/diet) in adult female mice (*Mus musculus*), treated with different doses of *G. platypoda* CDE in acute toxicity by oral doses.

Groups	Body weight		Consumption of water (mL)	Consumption of diet (g)
	Initial (g)	Final (g)		
Control	22.6±0.85	27.7±1.11	31.07±6.59	11.28±4.69
2000 mg/kg	24.6±0.52	30.6±1.39	30.71±9.83	12.77±2.21
3000 mg/kg	23.9±1.41	29.7±1.38	31.78±8.64	11.66±7.96

to 50%) with moderate activity (cell growth inhibition ranging from 50 to 70%) and with large activity (cell growth inhibition ranging from 70 to 100%). The average cell growth inhibition (IC%) of cell treated with 50 µg/mL CDE is shown below (Table 4).

**Table 3.** Effect of *Guettarda platypoda* CDE, in different doses, on cell migration into peritoneal cavity on carrageenan-induced peritonitis in mice. Data are the mean±SD of at least six animals.

Treated	Dose (mg/kg)	N° of PMNL/mL (x10 <sup>6</sup> )	Inhibition (%)
Group 1	30	9.48±2.0	6.7
Group 2	50	3.35±0.7 *	67.0
Group 3	100	4.72±1.4 *	53.5
Group 4	150	3.40±0.7 *	66.5
Group 5	200	6.54±1.7 *	35.6
Indomethacin	10	4.90±0.5 *	51.8
Control	-	10.16±3.1	-

\* $p < 0.01$ . Significance was determined with ANOVA one-way followed by Tukey's Test when compared with control group.

## Discussion

The phytochemical analysis indicated the presence of significant secondary metabolites in the *Guettarda platypoda* DC., Rubiaceae, CDE. Some authors have reported the presence of several triterpene saponins, such as quinovic acid derivatives (Aquino et al., 1988a, 1989; Almeida, 1982; Bhattacharyya & Almeida, 1985; Ferrari et al., 1986, Melo et al., 2008). In this study alkaloids were not identified but

their presence was described in the ethanol extract by Almeida (1982) and detected the presence of 5 $\alpha$ -carboxistictosidina was detected by Ferrari et al. (1986) and Melo et al. (2008).

Results indicated that *G. platypoda* CDE has low oral acute toxicity. According to the criteria for acute toxicity by the GHS (Globally Harmonized Classification System) the fact that no serious injury or death occurred in any animal tested at the highest recommended dose (2000 mg/kg), indicate that the compounds were fit in class 5 (very low or no toxicity) (GHS, 2005). The absence of atypical behavior is also another indication of lack of toxicity of the compounds (Vital et al., 2009).

In order to evaluate the effect of *G. platypoda* CDE on cell migration to the peritoneal cavity, we tested its effects on the induction of peritonitis by carrageenan. The statistical analysis revealed also that *G. platypoda* CDE evaluated, exhibited an inhibitory profile similar to indomethacin in the doses 50, 100, 150 and 200 mg/kg. The potential anti-inflammatory activity by CDE may be due to synergistic action of quinovic acid glycosides already reported in the literature (Bhattacharyya & Almeida, 1985; Aquino et al., 1988a, 1989, 1991; Ferrari et al., 1986) and the presence of beta-sitosterol (Bhattacharyya & Almeida, 1985), flavonoids and the triterpnes identified in this study, since anti-inflammatory action of the metabolites mentioned above have been already described.

The action of  $\beta$ -sitosterol is similar to the hydrocortisone (Gupta, 1980) and the action of flavonoids was explained in part by its inhibitory effect on arachidonic acid metabolism (Silva et al., 2002).

**Figure 3.** Illustration of antitumor evaluation sarcomatous tumors. A. Control group; B. Group treated with CDE *Guettarda platypoda* dose 100 mg/kg (ST1); C. Group treated with CDE *G. platypoda* dose 200 mg/kg (ST2).

**Table 4.** Percent inhibition of cell growth (IC%) of the three tumor cell lines tested in a single dose (50 µg/mL) of *Guettarda platypoda* CDE.

Tumor lineage	Mean (IC%) CDE (50 µg/mL)	Standard Deviation (SD) CDE	Mean (IC%) Doxorrubicina (15 µg/mL)
HT29	15.2	0.6	0.4
MCF-7	0	0	N.d.
NCI-H-292	1.7	0.5	0.2

N.d: no determined.

Some flavonoids can block the biosynthetic processes of eicosanoids, which lead to an anti-inflammatory action, because it causes inhibition of COX-2 and nitric oxide synthase (Machado et al., 2008). It is also reported that triterpenes have anti-inflammatory activity, due to inhibition of nitric oxide and prostaglandin E2 synthesis (Moraes et al., 2012).

However, other disorders can lead to a decrease in PMNL migration. This probably happened with doses above 150 mg/kg, observing an activity decrease anti-inflammatory, probably because exudate presented hemolysate due to the abundant presence of saponins in the roots of *G. platypoda* as described in this paper and by several other authors (Aquino et al., 1988a, 1989; Almeida, 1982; Bhattacharyya & Almeida, 1985; Ferrari et al., 1986; Melo et al., 2008).

In the antitumor activity evaluation, the *G. platypoda* CDE is quite promising for the treatment of Sarcoma 180, but did not provide satisfactory results against the Ehrlich carcinoma. Therefore, it is important to emphasize the positive results of this study, which may improve our understanding in the use of this plant as an alternative anti-cancer therapy. For Sarcoma 180, as this strain is the most difficult to treat, according to Almeida et al. (2005), who emphasizes that sarcomatose tumors are more difficult to treat because they affect of bone and muscle regions. The identification of active principles and their mechanisms of action still need to be studied in order to enhance the promising effects in treatment of this strain. Probably flavonoids are responsible for the antitumor action due to its antioxidant capacity, and cell growth inhibitory activity in several tumor cell lines and induce apoptosis (Machado et al., 2008).

It was verified in this study that of *G. platypoda* CDE did not demonstrate cytotoxic activity. Analysis of cytotoxicity by MTT method has been used in the screening program of the National Cancer Institute of the USA (NCI), which tests more than 10000 samples each year (Skehan et al., 1990). It is a rapid, sensitive and inexpensive test. It was first described by Mosman (1983) as having the potential to analyze the viability and metabolic state of the cell. This is a colorimetric analysis based on conversion of 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-2-H-tetrazolium bromide salt (MTT) into blue formazan due

to mitochondrial enzymes present only in metabolically active cells. The study carried out employing cytotoxic the MTT method allows to define the cytotoxicity easily, but not the action mechanism (Berridge et al., 1996).

## Conclusions

The methanolic extract of *Guettarda platypoda* DC., Rubiaceae has low toxicity and promising anti-inflammatory activity at doses of 50, 100 and 150 mg/kg and antitumor activity to Sarcoma 180 at doses of 100 mg/kg and 200 mg/kg. It's necessary to continue the studies with fractions to determine which fraction may will be more active. The cytotoxic activity did not indicate satisfactory results. These tests don't necessarily discard the potential of CDE against this activity and it is necessary additional tests using other cell strains and experimental models.

## Acknowledgment

To CNPq for its financial support.

## References

- Agra MF, França PF, Barbosa-Filho JM 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Rev Bras Farmacogn* 17: 114-140.
- Agra MF, Silva KN, Basilio IJLD, França PF, Barbosa-Filho JM 2008. Survey of medicinal plants used in the region Northeast of Brazil. *Rev Bras Farmacogn* 18: 472-508.
- Almeida MZ 1982. *Estudo fitoquímico e triagem farmacológica da casca da raiz da Guettarda platypoda DC.* João Pessoa, p.133. Dissertação de Mestrado, Programa de Pós-graduação em Produtos Naturais, Universidade Federal da Paraíba.
- Almeida VL, Leitão A, Reina LCB, Montanari CA, Donnici CL, Lopes MTP 2005. Câncer e agentes antineoplásicos ciclo-celular específicos e ciclo-celular não específicos que interagem com o DNA: uma introdução. *Quim Nova* 28: 118-129.
- Andersson L 1992. A provisional checklist of neotropical Rubiaceae. *Scripta Botan Belg* 1: 1-199.
- Aquino R, Feo V, Simone F, Pizza C, Cirino G 1991. Plant

- Metabolites. New Compounds and Anti-Inflammatory Activity of *Uncaria tomentosa*. *J Nat Prod* 54: 453-459.
- Aquino R, Simone F, Pizza C, Cerri R, Mello JF 1988a. Quinovic acid glycosides from *Guettarda platypoda*. *Phytochemistry* 27: 2927-2930.
- Aquino R, Simone F, Pizza C, Mello JF 1989. Further quinovic acid glycosides from *Guettarda platypoda*. *Phytochemistry* 28: 199-201.
- Aquino R, Simone F, Senatore F, Pizza C 1988b. Iridoids and secoiridoids from *Guettarda platypoda*. *Pharmacol Res Commun* 20: 105-108.
- Bahia 1979. Governo do Estado. Seplanteq - Subsecretaria de Ciência e Tecnologia. *Inventário de plantas medicinais do Estado da Bahia*. Salvador.
- Barbosa MRV, Souza EB, Jardim JG 2011. *Rubiaceae*. In: Checklist das Plantas do Nordeste (Versão 1.5). <http://www.cnip.org.br/bdnpn/checklistNE.pdf>, accessed Jan 2011.
- Berridge MV, Tan AS, McCoy KD, Wang R 1996. The Biochemical and Cellular Basis of Cell Proliferation Assays that Use Tetrazolium Salts. *Biochem J* 4: 14-19.
- Bertucci A, Haretche F, Olivaro C, Vázquez A 2008. Prospección química del bosque de galería del río Uruguay. *Rev Bras Farmacogn* 18: 21-25.
- Bhattacharyya J, Almeida MZ 1985. Isolation of the constituents of the root-bark of *Guettarda platypoda*. *J Nat Prod* 48: 148-149.
- Capasso A, Balderrama L, Silvilá SC, Tommasi N, Sorretino L 1998. Phytochemical and pharmacological studies of *Guettarda acreana*. *Planta Med* 64: 348-352.
- Costa AF 2001. *Farmacognosia*. 3<sup>rd</sup> Ed., vol.3, Lisboa: Calouste Gulbenkian.
- Cunha AP, Silva AP, Roque OR 2003. *Plantas e produtos vegetais em fitoterapia*. Lisboa: Calouste Gulbenkian, p. 21-61.
- David JPL, David JM, Soares MBP, Costa JFO, Santos RR 2006. *Introdução*. In Lucchese AM (ed.). *Instituto do Milênio do Semi-árido. Plantas da Caatinga: perfil botânico, fitoquímica e atividade biológica*. vol.4. Recife: Associação de Plantas do Nordeste, p. 15-17.
- Delprete PG 2004. Rubiaceae (Coffea or Quinine family). In Smith N, Mori SA, Henderson A, Stevenson DWm., Head SV (ed.) *Flowering plants of the neotropics*. Princeton: Princeton University Press, p. 328-333.
- Ferrari F, Mesana I, Botta B, Mello JF 1986 Constituents of *Guettarda platypoda*. *J Nat Prod* 49: 1150-1151.
- GHS 2005. A Guide to The Globally Harmonized System of Classification and Labeling of Chemicals (GHS). <http://www.osha.gov/dsg/hazcom/ghsguideoct05.pdf>, accessed Feb 2011.
- Gupta MB, Nath R, Srivastava N, Shanker K, Kishor K, Bhargava KP 1980. Anti-inflammatory and antipyretic activities of  $\beta$ -sitosterol. *Planta Med* 39: 157-163.
- Harbone JB 1998. *Phytochemical Methods*. 3<sup>rd</sup> ed., London: Chapman & Hall.
- IGEduc 2010. Biodiversidade. <http://www.igeduc.com.br/artigos/acontece/biodiversidade.html>, accessed Feb 2011.
- Lima LF, Lima PB, Almeida-Jr EB, Zickel CS 2010. Morfologia de frutos, sementes e plântulas de *Guettarda platypoda* DC. (Rubiaceae). *Biota Neotrop*. 10: 155-160.
- Machado H, Nagem TJ, Peters VM, Fonseca CS, Oliveira TT 2008. Flavonóides e seu potencial terapêutico. *Bol Centro Biol Reprod* 27: 33-39.
- Matos FJA 1997. *O formulário fitoterápico do professor Dias da Rocha*. Fortaleza: UFC.
- Melo SJ, Braz-Filho R, Lemos TLG, Santos HS, Fonseca AM, Xavier HS 2008. Derivados terpenóico e indólico glicosilados das raízes de *Guettarda platypoda* D.C. (Rubiaceae). 31<sup>a</sup> Reunião Anual da Sociedade Brasileira de Química. Águas de Lindóia, São Paulo.
- Mól DFF 2010. *Rubiaceae em um remanescente de floresta atlântica no Rio Grande do Norte, Brasil*. Natal, 69 p. Dissertação de Mestrado, Programa de Pós-graduação em Ciências Biológicas, Universidade Federal do Rio Grande do Norte.
- Moraes WF, Galdino PM, Nascimento MVM, Vanderlinde FA, Bara MTF, Costa EA, Paula JR 2012. Triterpenes involved in the anti-inflammatory effect of ethanolic extract of *Pterodon emarginatus* Vogel stem bark. *J Nat Med* 66: 202-207.
- Mossman T 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65: 55-63.
- Neu R 1956. A New reagent for differentiating and determining flavones of paper chromatograms. *Naturwissenschaften* 43: 82.
- OECD (Organization for economicco-operation and development) 2001. *Guideline for Testing of Chemicals: Acute Oral Toxicity-Acute Toxic Class Method*. Guideline: 423. [http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD\\_GL423.pdf](http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL423.pdf), accessed Jan 2012.
- Pereira MS 2002. *A família Rubiaceae na reserva biológica Guaribas, Paraíba, Brasil*. Recife, 117 f. Dissertação de Mestrado, Programa de Pós-graduação em Biologia Vegetal, Universidade Federal de Pernambuco.
- Pereira MS, Barbosa MRV 2004. A família Rubiaceae na Reserva Biológica Guaribas, Paraíba, Brasil. Subfamílias Antirheoideae, Cinchonoideae e Ixoroideae. *Acta Bot Bras* 18: 305-18.
- Pessoa MCR 2009. *Diversidade e riqueza da família Rubiaceae Juss. no Cariri paraibano*. Recife, 83 f. Dissertação de Mestrado, Programa de Pós-graduação em Biologia Vegetal em Biologia Vegetal, Universidade Federal de Pernambuco.
- Portal Brasil 2010. *Por dentro do Brasil-Meio Ambiente*. 34p. <http://www.brasil.gov.br/cop/materiais-para-download/por-dentro-do-brasil-2013-meio-ambiente->

- 2013-setembro-2010, accessed Jan 2011.
- Roberts EAH, Cartwright RA, Oldschool M 1957. Phenolic substances of manufactured tea. I. Fractionation and paper chromatography of water-soluble substances. *J Sci Food Agr* 8: 72-80.
- Russel JE 1982. Contribution a l'estude botanique et chimiotaxonomique du genre *Verbascum* en Languedoc-Cevennes. França. Thèse de Doctorat, Université de Montpellier I.
- Sharma OP, Dawra RK 1991. Thin-layer chromatography separations of lantadens, the pentacyclic triterpenoids from (*Lantana camara*) plant. *J. Chromatogr* 587: 351-354.
- Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bodesch H, Kenney S, Boyd MR 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer I* 82: 1107-1112.
- Silva FRMB, Szpoganicz B, Pizzolatti MG, Willrich MAV, Sousa E 2002. Acute effect of *Bauhinia forficata* on serum glucose levels in normal and alloxan-induced diabetic rats. *J Ethnopharmacol* 83: 33-37.
- Sugiura K, Stock CC, Sugiura MM 1955. The effect of phosphoramides on the growth of a variety of mouse and rats tumors. *Cancer Res* 2: 38-50.
- Uchôa FT, Silva TG, Lima MC, Galdino SL, Pitta Ida R, Dalla Costa T 2009. Preclinical pharmacokinetic and pharmacodynamic evaluation of thiazolidinone PG15: an anti-inflammatory candidate. *J Pharm Pharmacol* 61: 339-345.
- Vital FAC, Melo SJ, Silva TG, Carneiro S, Araújo JM, Mendonça-Jr FJB 2009. Avaliação da toxicidade aguda e das atividades citotóxica, antimicrobiana e antiinflamatória de 7-aryl-2,3-diidrotiazolo[3,2- $\alpha$ ] pirimidin-5-ona-6-carbonitrila. *Lat Am J Pharm* 28: 507-512.
- Wagner H, Blatt S 1996. *Plant drugs analysis. A thin layer chromatography atlas*. 2<sup>nd</sup> ed., Munich: Springer.

**\*Correspondence**

Sebastião J. Melo  
Departamento de Antibióticos, Universidade Federal de Pernambuco  
Av. Artur Sá s/n, Cidade Universitária, 50670-901 Recife-PE, Brasil  
sebastiao.melo@ufpe.br  
Tel/Fax: +55 81 2126 8347