Issues in Biological Sciences and Pharmaceutical Research Vol. 2(9),pp. 090-105,October 2014
Available online at http://www.journalissues.org/IBSPR/
http://dx.doi.org/10.15739/ibspr.003

ISSN 2350-1588

Original Research Article

Free radical-scavenging activity and cytotoxicity from leaves of *Guettarda platypoda*

Accepted 8 October, 2014

Guilherme C. R. Rodrigues[1], Isla V. G. A. Bastos[1], Gibson G. Oliveira[1], José A. S. Pereira-Junior[1], Rômulo C. S. Peixoto Filho[1], Macia Cleane S. Almeida[2], Raimundo Braz-Filho[3], Teresinha G. da Silva[1], Telma L. G. Lemos[2], Sebastião J. de Melo[1]*

[1] Department of Antibiotics, Federal University of Pernambuco (UFPE), Brazil.
[2] Department of Organic and Inorganic Chemistry, Federal University of Ceará (UFC), Brazil.
[3] Laboratory of Chemical Sciences, State University of North Fluminense Darcy Ribeiro, Brazil.

*Corresponding Author
Email: melosebastiao@yahoo.com.br
Tel.: +558121268347

The specie *Guettarda platypoda* DC. a shrub present in the flora of the state of Pernambuco (Itamaracá) is used in folk medicine as an antipyretic. The aim of this study was to conduct a bioactivity-guided fractionation of the leaves of this species, thus evaluating the biological activity (Cytotoxic and antioxidant) of substance and/or fractions of different polarities. The raw methanol extract of the leaves of *G. platypoda* was subjected to fractionation using solvents of increasing polarity; cell viability was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay; antioxidant activity was determined based on the ability to sequester free radicals by the method of DPPH (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and the molecular characterization was performed by spectrometric methods. From this work it was possible to isolate a porphyrin (pheophytin a). During bioguided tests, it was evident that the crude methanolic extract (IC_{50} = 7.02 x 10^{-3} mg/mL), fractions of ethyl acetate (IC_{50} = 5.58 x 10^{-3} mg/mL) and methanol (IC_{50} = 8.10^{-3} mg/mL) had higher antioxidant activity than vitamin C. In conclusion, the substances present in the extract in ethyl acetate (F3) of *G. platypoda* and in the F2.2 subfraction, demonstrated cytotoxic and antioxidant activity against all cell lines tested, but the pheophytin a (P5), first identified to the genus *Guetarda*, showed no significant activity against the tested cell lines in this work.

Key words: *Guettarda platypoda*, phytochemical, pheophytina, cytotoxic activity, antioxidant.

INTRODUCTION

The *Guettarda* genera consist of 180 species widely distributed in tropical and neotropical regions. Throughout the Brazilian territory there are about 24 species. There are reports of the use of this genre in traditional medicine for the most diverse purposes, especially for the treatment of wounds and inflammations (Agra et al., 2007; Agra et al., 2008; Bertucci et al., 2008; Capasso et al., 1998).

Guettarda platypoda DC is a shrubby species widly distribution along the Brazilian coast, especially in the Northeast (Pereira and Barbosa, 2004; Zickel et al., 2007). It is commonly used as antipyretic, anti-inflammatory (Aquino et al., 1988a; Aquino et al., 1988b; Bhattacharyya and Almeida, 1985; Ferrari et al., 1986) and antiviral remedy against rhinovirus type 1B and the vesicular stomatitis vírus (Aquino et al., 1989).

An examination of the extract of the bark of the root of the plant verified the presence of saponines, alkaloids, steroids, tannins and triterpenes, as well as the lack of flavonoids. The quinovic acid, rotundic acid, β-sitosterol (Almeida, 2011), the 5α-carboxistrictosidina alkaloid, iridoid morroniside the secoiridoid sweroside (Ferrari et al., 1986), triterpene saponins, loganic acid, loganin

(Aquino et al., 1988a; Aquino et al., 1988b) and pentacyclic triterpenes (Soares et al., 1998) were isolated.

In the methanol extract of the leaves of *G. platypoda* was identified the presence of flavonoids, tannins (proanthocyanidins condensed leucoanthocyanidins) saponoside, triterpenes and steroids and the absence of alkaloids, coumarins, iridoids, as well as chlorogenic acid (cinnamic acid derivative), rutin, quercetin and kempferol (flavonoids) (Corrêa, 2007).

Given this variety of compounds and the few reports on the species in question, the present study aimed to conduct a guided fractionation and evaluate the cytotoxic and antioxidant activities of the crude methanol extract and subfractions of the leaves of the *G. platypoda* DC.

MATERIALS AND METHODS

Collection, Identification and Preparation of Extract

The plant material (leaves) was collected in the municipality of Itamaracá, Pernambuco, about 50 km from Recife, bounded on the north by the municipality of Goiana, on the south by Igarassu to the east by the Atlantic Ocean and to the west by Itapissuma (Zickel et al., 2007). A specimen of the species was identified and deposited in the Herbarium of the Agronomic Institute of Pernambuco (IPA) under number 86565.

After collection, the leaves were dried using an oven at 48 °C for 48 hours to eliminate moisture and stabilize the enzyme content. The material was powdered in electric mil and weighed (736.8 g). The extraction of the leaves of the fixed components was carried out by maceration for seven days using methanol PA (Merck) (20 g/L). The extracts were filtered and the residue re-estracted. The solutions obtained in this process were concentrated at reduced pressure in a rotary evaporator at 50 ± 5 °C until the solvent had been completely removed. The result was 173.3 g of the crude methanol extract (CME).

Fractionation bioassay-guided

The CME leaves of *G. platypoda* (8 g) was subjected to fractionation in a column using Traditional Liquid Chromatography (TLC) with silica gel 60 (0.063-0.2 mm, 70-230 mesh, Macherey-Nagel) as the stationary phase and with solvents PA (Merck) of increasing polarity. This procedure resulted in four fractions: *n*-hexane (F1), *n*-hexane/ethyl acetate (AcOE)t 1:1 (F2), AcOEt (F3) and methanol MeOH (F4) that were subsequently concentrated under reduced pressure in a rotary evaporator. The fractions were evaluated for their antioxidant and cytotoxic activities, determining their therapeutic potential by directing the fractionation (Houghton, 1996).

Then the F2 and F3 fractions were subjected to fractionation in TCL with silica gel to obtain subfractions F2.1, F2.2 and P5, respectively. The P5 subfraction led to the isolation of a substance (Pheophytin a) with a quantity and pure quality sufficient for analysis in Nuclear Magnetic Resonance (NMR) 1D ^{1}H (500 MHz) and ^{13}C (125 MHz).

Cytotoxicity aassay

Cell viability was measured by MTT reduction assay, which is based on the conversion of the salt 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a formazan product by the action of the enzyme succinyl dehydrogenase present in mitochondria of viable cells. The analysis used the CME leaves of *G. platypoda*, its fractions and pure compound isolated against the strains HEp-2 (human epidermoid Cancer Cells; HeLa derivative), HT-29 (colon carcinoma), MCF-7 (human breast adenocarcinoma cell line) and HL-60 (leukemia), each a concentration of 50g/mL. The cancer cell lines DMEM (Dulbecco's Modification of Eagle's Medium) were plated in 96-well microplates and incubated during 24 h at 37 °C. After this period, 25 µL of CME leaves of *G. platypoda* and its fractions (50 µg/mL) were added to each well and incubated for 72 h. Next, MTT dye (25 µL, 0.5 mg/mL) was added to the wells and the assay was incubated for 3 h more. After this period the medium was removed and dimethylsulfoxide (DMSO, 100 µL) was added to the wells for solubilization of the generated formazan salts. The optical density of the wells was measured at 540 nm and compared to that in the negative control (cells incubated only with medium). Two independent experiments were performed in duplicate. The samples were classified according to the inhibition percent of cell viability in the following categories: inactive (1-20%), weakly active (20-50%), moderately active (50-70%) or very active (70-100%) (Capasso et al.,1998).

Antioxidant activity test

The antioxidant activity was determined based on the ability of scavege free radicals, similar to that described by Hegazi et al. (2002), using the 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazino (DPPH). Methanolic solutions of the CME and the fractions obtained at concentrations of 0.001; 0.005; 0.01; 0.05; 0.1 and 1 mg/mL were prepared. An aliquot (1.0 mL) of each solution was mixed with another methanolic DPPH solution (1.0 mL) at a concentration of 60 µmol.L^{-1}. After 30 minutes the absorbance measurements in a UV-VIS spectrophotometer at 520 nm were carried out. The percentage of inhibition was obtained by comparing the absorption of the solution containing the sample, relative to a control DPPH solution without sample. The test was performed in triplicate, using as Trolox as the positive standards at concentrations of 0.001; 0.002; 0.004; 0.005; 0.1 and 1 mg/mL and vitamin C at 0.0025; 0.005; 0.0125; 0.025; 0.0375 and 0.050 mg/mL. The calculation of the antioxidant activity was made using

GraphPad Prism 4 for Windows program and the calculation of IC_{50} (concentration which inhibits 50% of free radicals) used the sigmoidal dose-response equation.

Molecular characterization

The molecular characterization was performed by spectrometric methods such as infrared spectrum (IR) and NMR (Bruker Daltonics)1D ^{1}H (500 MHz) and ^{13}C (125 MHz) and homonuclear 2D ^{1}H -^{1}H COSY (COrrelation SpectroscopY) and heteronuclear ^{13}C-^{1}H HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Correlation)

RESULTS AND DISCUSSIONS

Fractionation bioassay-guided

The chromatographic fractionation of 8 g of crude methanolic extract of the leaf of *G. platypoda* (EBMFG) was obtained through a column filled with silica gel powder with 70-230 mesh which was eluted with different solvents in order of increasing polarity. From this procedure four fractions were obtained: F1-*n*-hexane (0.13 g); F2-*n*-hexane/AcOEt [1:1] (0.55 g); F3-AcOEt (0.16 g); F4-methanol (4.5 g).

Then F3 fraction was subjected to TLC with silica gel, eluting with mixtures of hexane and AcOEt in increasing order of polarity. The P5 subfraction resulting from this process was possible to isolate a green amorphous solid, which after NMR (Tables 1 and 2) and infrared analysis and comparisons with similar procedures in the literature (Aquino et al., 1998a; Aquino et al., 1988b), it was possible to identify as pheophytin a (Figure 1), a porphyrin previously not reported for this genus.

Molecular characterization

The Infrared spectrum of pheophytin a (IR (KBr): 1698; 1738; 2926; 2847; 3434 cm^{-1}) showed bands attributed to conjugated carbonyl group (γ_{max} 1698 cm^{-1}), ester function (γ_{max} 1738 cm^{-1}), aliphatic and aromatic C-H (γ_{max} 2926, 2847 cm^{-1}) and N-H amines (γ_{max} 3434 cm^{-1}), along with characteristics of double bonds of alkenes and aromatic rings (Aquino et al., 1998a).

Comparative analysis of the {^{1}H}- and DEPT ^{13}C NMR spectra allowed us to identify signals (Tables 1 and 2) corresponding to nineteen non-hydrogenated carbons [all sp^2]: including three carbonyl groups at δ_C 189.87 (C-13^1), 169.83 (C-13^3) and 173.17 (C-17^3)]. Eleven methine carbons (including five sp^2) were identified. Fourteen methylene carbons [including one sp^2 at δ_C 122.96 (CH_2-3^2) and one sp^3 oxygenated at δ_C 61.67 (CH_2-3')] were identified. Eleven methyl carbons [including one methoxyl group at δ_C 53.04 (MeO-13^3 = $3H_3$-13^4). All of these were used to build the partial molecular formula $(C)_{16}(C{=}O)_3(CH)_{11}(CH_2)_{14}(CH_3)$ (Aquino et al., 1989).

The ^{1}H NMR spectra (1D and 2D ^{1}H-^{1}H-COSY) of pheophytin a showed singlet signals at δ_H 9.56, 9.35 and 8.56, attributed to characteristic hydrogen H-10, H-5 and H-20, respectively, of the presence of porphyrin moiety by corresponding to the carbon methinics bound to pyrrole rings. Signals were also observed corresponding to five methyl groups at δ_H 3.69 (s, Me-12^1), 3.40 (s, Me-2^1); 3.21 (s, Me-7^1), 1.82 (d, *J*=7.0 Hz, Me-18^1) and 1.69 (t, *J*=7.5 Hz, Me-8^2) appearing as substituents (four methyl and one ethyl-8 groups) on the pyrrole rings of the porphyrin unit (Table 1) (Aquino et al., 19988a).

Further data was found to confirm the presence of a porphyrin moiety: a triplet signal at δ_H 3.66 (q, *J*=7.5 Hz, 2H-8^1), methoxyl group at δ_H 3.90 (br s, 3H-13^4) and a vinyl substituent linked to C-3 by signals δ_H 7.97, [dd, 17.8 and 11.5 Hz, H-3^1 and 6.29 (d, *J*=17,8 Hz, H3^2a) and 6.14 (d, *J*-11.5 Hz, H3^2b) correlated in the HSQC with carbon signals at δ_C 129.28 (CH-3^1) and 122.96 (CH_2-3^2), along with additional signals to methines CH-13^2.[δ_H 6.28(s)/ δ_C 64.91], CH-17 [δ_H 4.25(m)/ δ_C 51.83] and CH-18 [δ_H 4.45(m)/δ_C 50.33] and methylenes CH_2-17^1 [δ_H 2.64(m),2.37(m)/δ_C 30.03] and CH_2-17^2 [δ_H 2.50(m),2.23(m)/δ_C 31.45], consistent with porphyrin ring (Zickel et al., 2007).

According Schwikkard et al. (1998), the pheophytin b in C-7^1 has an aldehyde group, differing from the compound found in this study due to the presence of a methyl signal at δ_H 3.21, reinforcing the suggestion that it is pheophytin a (Table 1).

The longe range heteronuclear coupling of the carbonyl C-17^3 (δ_C 173.17) with 2H-17^1 (δ_H 2.64 and 2.37, $^3J_{CH}$), 2H-17^2 (δ_H 2.50 and 2.23, $^2J_{CH}$) and 2H-1' (δ_H 4.57 and 4.44, $^3J_{CH}$) was used to confirm the esterification of the carboxyl group of the porphyrin moiety. All chemical shift assignments of the hydrogen and carbon atoms remaining of this moiety were also attributed on the basis of 1D and 2D NMR spectra (Table 1).

The attribution of the chemical shifts of the hydrogen and carbon atoms of the phytyl group (Table 2) was also based in the 1D and 2D NMR spectral data involving comparison with values described in literature (Capasso et al., 1998; Zickel et al., 2007).

Thus, ^{1}H and ^{13}C NMR spectral data, involving 1D and 2D [^{1}H-^{1}H-COSY and ^{1}H-^{13}C-COSY,HSQC, and HMBC correlations] and comparison with procedures reported in the literature were used to characterize and to allow the complete and unambiguous chemical shift assignments (Tables 1 and 2) of pheophytina.

Cytotoxic activity

Fouche et al, (2008) asserted that extracts tested at a maximum single dose of 100 µg/mL exhibit cytotoxic activity since they are able to inhibit the proliferation of

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) spectral data of porphyrin unit, in $CDCl_3$, inclusive results of HSQC and HMBC. Chemical shifts in δ (ppm).

	HSQC		HMBC	
	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$
C				
1	142.27	-		3H-21
2	132.06	-	3H-21	H-2; H-31
3	136.45	-	H-31	2H-32
4	136.74	-		H-31
6	155.85	-		3H-71
7	136.38	-	3H-7^1	H-5; 2H-8^1
8	145.49	-	2H-8^1	H-10; 3H-7^1; 3H-8^2
9	151.19	-		2H-8'
11	138.14	-	H-10	3H-12^1
12	129.30	-		H-10
13	129.19	-		H-13^2; 3H-12^1
14	149.85	-		H-13^2
15	105.46	-	H-13^2	
16	161.48	-		
19	172.45	-	H-18	3H-18^1
13^1	189.87	-	H-13^2	
13^3	169.83	-	H-13^2	MeO-13^4
17^3	173.17		2H - 17^2	2H-1'; 2H-17^1
CH				
5	97.75	9.35 (s)		
10	104.63	9.56 (s)		
17	51.83	4.25 (m)		3H-18^1
18	50.33	4.45	3H-18^1	
20	93.33	8.56 (s)		
3^1	129.28	7.97 dd,*J* 17.8, 11.5)		
13^2	64.91	6.28 (s)		
CH2				
3^2	122.96	6.29 (d, *J* = 17.8 Hz)		
		6.14 (d, *J* = 11.5 Hz)		
8^1	19.63	3.66 (q)	3H-82	
17^1	30.03	2.64 (m), 2.37 (m)		
17^2	31.45	2.50 (m), 2.23 (m)		
CH3				
2^1	12.28	3.40 (s)		
7^1	11.40	3.21 (s)		
8^2	17.59	1.69 (t, *J* = 7.5 Hz)	2H-81	
12^1	12.28	3.69 (s)		
13^4	53.04	3.90 (s)		
18^1	23.29	1.82 (d, *J* = 7.3 Hz)	H-18	

two or more cell lines by at least 75%. Based on this information, the F2.2 and F3 fractions exhibit cytotoxic activity against the HEp-2 and HT-29 (Table 3) strains. However, the pheophytin, showed no significant results.

The presence of terpenoids can justify the cytotoxic action of these two fractions when an MTT assay was studied. These secondary metabolites are well known for their antitumor activity. Between 1980 and 2000 they the main representative of the paclitaxel category, considered at that time to be the most promising antitumor agents (Simões et al., 2010).

Antioxidant activity

The CME leaves of *G. platypoda*, their primary pheophytin a and the fractions were analyzed. The results showed that both the CMB as F3 and F4 fractions exhibited antioxidant activity with 99% inhibition of free radicals in a concentration of 0.1 mg/mL. The IC_{50} was less than the standard used (vitamin C) has proven to have high

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) spectral data of **phytyl group**, in $CDCl_3$, inclusive results of HSQC and HMBC. Chemical shits in δ (ppm).

	HSQC		**HMBC**	
	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$
C				
3'	143.02	-	3H-20'/ 2H-4'/	2H-1'/ 2H-5'
CH				
2'	117.94	5.15 (t, *J* = 6.9 Hz)	2H-1''	3H-20'
7'	32.96	1.31 (m)	3H-19'	
11'	32.81	1.31 (m)	3H-18'	
15'	28.11	1.51 (hep, J = 6.6 Hz)	3H-16'/3H-17'	
CH2				
1'	61.67	4.52 (m), 4.44 (m)		
4'	39.99	1.89 (t, *J* = 7.7 Hz)		3H-20'
5'	25.19	1.61 (m)		
6'	36.83	1.02 (m)		3H-19'
8'	37.59	1.21 (m)		3H-19'
9'	24.61	1.12 (m)		
10'	37.53	1.23 (m)		3H-18'
12'	37.46	1.23 (m)		3H-18'
13'	24.98	1.27 (m)		
14'	39.55	1.12 (m)		
CH^3				
16'	22.91	0.86 (d, *J* = 6.5 Hz)	H-15'	
17'	22.81	0.86 (d, J = 6.5 Hz)	H-15'	
18'	19.91c	0.81 (d, *J* = 6.6 Hz)		
19'	19.63c	0.80 (d, *J* = 6.6 Hz)		
20'	17.59	1.59 (sl)		H-2'

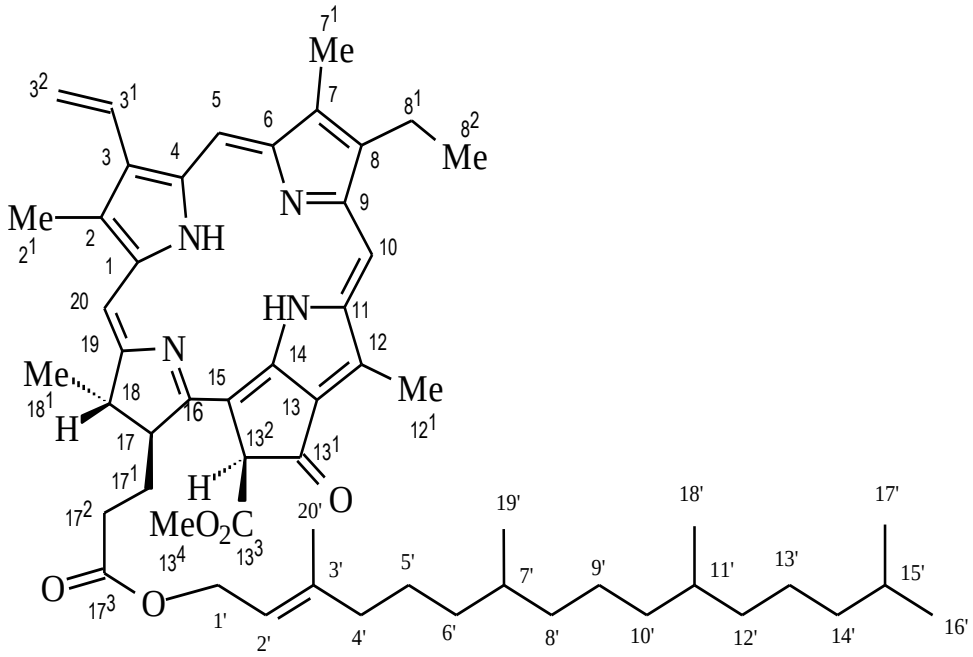

Figure 1: The structure of pheophytin

antioxidant activity (Mensor et al., 2001) (Table 4). This effect is probably due to the presence of flavonoids [14] in fraction F3 and flavonoids, tannins and reducing sugars in F4 and CME, secondary metabolites known to have antioxidant activity (Simões et al., 2010).

In their studies Endo et al. (1984, 1985) reported that the

Table 3. Evaluation of the cytotoxic activity of the crude methanol extract of the leaves of *G. platypoda* and its fractions.

Samples	HEp-2	HT-29	MCF-7	HL-60
Methanolic fraction (F4)	25.2	31.3	-	-
Ethyl acetate fraction (F3)	92.8	77.8	-	-
F2.1	80.5	38.4	-	-
F2.2	99.7	86.7	-	-
N-hexane fraction (F1)	40.3	35.5	-	-
CME	40.7	53.8	-	-
Doxorrubicine* (15 µg/mL)	0.7	0.4	-	0.02
Pheophytin a (P5)	-	-	25.49	26.79

Percentage of growth inhibition from crude methanol extract (CME) of the leaves of *G. platypoda*; (F1) *n*-hexane fraction, (F2.1 and F2.2) *n*-hexane/ ethyl acetate (1:1); (F3) ethyl acetate fraction, (F4) methanolic fraction; and * substances standart. (-) no active.

Table 4. Evaluation of antioxidant activity of the crude methanol extract of the leaves of *G. platypoda* and its fractions.

Amostras	IC_{50} (mg/mL)
CME	$7.02.10^{-3} \pm 2.8.10^{-5}$
F1	-
F2	-
F3	$5.58.10^{-3} \pm 7.0.10^{-4}$
F4	$8.10^{-3} \pm 1.1.10^{-5}$
Pheophytin a	$7.79.10^{-3} \pm 2.2.10^{-3}$
Trolox *	$2.6.10^{-3} \pm 2.3.10^{-4}$
Vitamin C *	$8.14.10^{-3} \pm 5.9.10^{-4}$

(CME) crude methanol extract of the leaves of *G. platypoda*; (F1) n-hexane fraction, (F2) n-hexane/ ethyl acetate (1:1); (F3) the ethyl acetate fraction, (F4) methanolic fraction; and * substances standart.

chlorophyll and the phaeophytins can move from pro-oxidants when subjected to light, catalyzing oxidation reactions and antioxidant, under the shelter of light. Thus, as expected, the pheophytin showed antioxidant activity, since the experiment was always protected from light.

Conclusions

In conclusion, the substances presents in fraction F3 (AcOEt) and F2.2 subfraction of *G. platypoda* demonstrated cytotoxic and antioxidant activity against all cell lines tested, but the pheophytin a, first identified to the genus *Guetarda*, showed no significant activity against the tested cell lines in this work.

ACKNOWLEDGMENTS

The authors acknowledge the support of Universidade Federal de Pernambuco (UFPE) and Universidade Federal do Ceará (UFC), CENAURENM, LBPN and the Foundation of Science and Technology of the State of Pernambuco (FACEPE) for financial aid.

REFERENCES

Agra MF, Freitas PF, Barbosa-Filho JM (2007). Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Rev. Bras. Farmacogn. 17 (1): 114-140.

Agra MF, Silva KN, Basílio IJLD, França PF, Barbosa-Filho JM (2008). Survey of medicinal plants used in the region Northeast of Brazil. Rev. Bras. Farmacogn. 18 (3): 472-508.

Almeida MCS (2011). Estudo fitoquímico e avaliação antioxidante de *Lippia sidoides.* Dissertação (Mestrado) – Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Centro de Ciências, Fortaleza. 121p.

Aquino R, Simone F, Pizza C, Cerri R, Mello JF (1988a). Quinovic acid glycosides from *Guettarda platypoda.* Phytochemistry 27 (9): 2927-2930.

Aquino R, Simone F, Pizza C, Mello JF (1989). Further quinovic acid glycosides from *Guettarda platypoda.* Phytochemistry 28 (1): 199-201.

Aquino R, Simone F, Senatore F, Pizza C (1988b). Iridoids and secoiridoids from *Guettarda platypoda.* Pharmacol. Res. Commun. 20 (5): 105-108.

Bertucci A, Haretche F, Olivaro C, Vázquez A (2008). Prospección química del bosque de galería del río Uruguay. Rev. Bras. Farmacogn. 18 (1): 21-25.

Bhattacharyya J, Almeida MZ (1985). Isolation of the constituents of the root-bark of *Guettarda platypoda.* J. Nat. Prod. 48 (1): 148-149.

Capasso A, Balderrama L, Silvila SC, Tommasi N, Sorretino L. (1998). Phytochemical and pharmacological studies of *Guettarda acreana*. Planta Med. 64 (4): 348-352.

Corrêa PG (2007) Defesas foliares em resposta à herbivoria em espécies lenhosas de restinga, Ipojuca-Pe. Dissertação (Mestrado), Centro de Ciências Biológicas, Universidade Federal Rural de Pernambuco, Recife. 53p.

Endo Y, Usuki R, Kaneda T (1984). Prooxidant activities of chlorophylls and their decomposition products on the photooxidation of methyl linoleate. J. Am. Oil Chem. Soc. 61 (4): 781-784.

Endo Y, Usuki R, Kaneda T. (1985). Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophyll. J. Am. Oil Chem. Soc. 62 (9): 1387-1390.

Ferrari F, Mesana I, Botta B, Mello JF (1986). Constituents of *Guettarda platypoda.* J. Nat. Prod. 49 (6): 1150-1151.

Fouche G, Cragg GM, Pillay P, Kolesnikova N, Maharaj VJ, Senabe J (2008). In vitro anticancer screening of South African plants. J. Ethnopharmacol. 119 (3): 455-461.

Hegazi AG, El Hady FKA (2002). Egyptian propolis: 3. antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. Z. Naturforsch. C., 57 (3-4): 395-402.

Houghton PJ (1996). Medicinal plants and the control of parasites: Compounds with anti-HIV activity from plant. T. Roy. Soc. Trop. Med. H. 90: 601 – 604.

Mensor LL, Menezes FS, Leitão GG, Reis AS, Dos Santos TC, Coube CS, Leitão SG (2001). Screnning of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15 (2): 127- 130.

Pereira MS, Barbosa MRV (2004). A família Rubiaceae na Reserva Biológica Guaribas, Paraíba, Brasil: subfamílias Antirheoideae, Cinchonoideae e Ixoroideae. Acta Bot. Bras. 18 (2): 305-318.

Schwikkard SL, Mulholland DA, Hutchings A. (1998). Phaeophytins from *Tapura fisheri*. Phytochemistry 49 (8): 2391-2394.

Simoes CMO, Schenkel EO, Gosmann G, Mello JCP, Mentz LA, Petrovick PR (2010). Farmacognosia da planta ao medicamento. (6rd ed.) UFSC: Florianópolis (SC), 1104p.

Soares FP, Ronconi CAV, Da Cunha EVL, Barbosa-Filho JM, Da Silva MS, Braz-Filho R (1998). Four known triterpenoids isolated from three Brazilian plants: ^{1}H and ^{13}C chemical shift assignments. Magn. Reson. Chem. 36 (8): 608–614.

Zickel CS, Almeida Jr EB, Medeiros DPW, Lima PB, Souza TMS, Lima AB (2007). Magnoliophyta species of restinga, state of Pernambuco, Brazil**.** Check List. 3: 224-241.