Journal of Applied Pharmaceutical Science Vol. 2 (9), pp. 061-066, September, 2012
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2012.2913
ISSN 2231-3354

Antibacterial, allelopathic and antioxidant activity of extracts and compounds from *Rourea induta* Planch. (Connaraceae)

Milena Kalegari*, Marilis Dallarmi Miguel, Andressa Frizzo Philippsen, Josiane de Fátima Gaspari Dias, Sandra Maria Warumby Zanin, Cristina Peitz de Lima, Obdulio Gomes Miguel
Department of Pharmacy, Federal University of Paraná, Curitiba, Paraná, Brazil

ARTICLE INFO

Article history:
Received on: 08/08/2012
Revised on: 24/08/2012
Accepted on:05/08/2012
Available online: 28/09/2012

***Key words**: Rourea induta*. Connaraceae. *Staphylococcus aureus*. *Staphylococcus epidermidis*. *Lactuca sativa*. Antioxidant.

ABSTRACT

This study evaluated some biological activities of the leaves from *Rourea induta* Planch., Connaraceae. Fractions of the ethanolic extract from leaves of this species were obtained by liquid/liquid partition and their antibacterial, allelophatic and antioxidant activities were analyzed. By the agar diffusion method the ethyl acetate, chloroform fractions and hyperin showed antimicrobial activity against *Staphylococcus epidermidis* with an inhibition halo of 15.0, 12.3 and 9.3 mm respectively, and *Staphylococcus aureus* with 7.6 mm for the fractions. For antioxidant activity all samples have demonstrated a significant potential, especially the chloroform, ethyl acetate fractions and the hyperin which IC50 were, 5.33 ±0.19; 3.21 ± 0.00 and 3.89 ± 0,02 respectively. This result is close to the standards vitamin C and rutin. In the allelopathic activity the hexane fraction at 0.8 mg and 0.4 mg, inhibited 33.24% and 20.54% the hipocotyl's growth of *Lactuca sativa* seeds, and the compound tetracosane inhibited 18.05% the hipocotyl's growth at 0.4 mg. The obtained results stimulate the continuity of this study.

INTRODUCTION

Connaraceae is an angiosperm family that the species are distributed around tropical areas of the world and they are also spread on several ecosystems, mainly in the Amazon forest. Shrubby species are represented by genera *Rourea* and *Connarus* (Lorenzi and Souza, 2005; Lenza et al., 2008). The species *Rourea induta* is abundant in "cerrado campo sujo" and "cerrado sentido estrito" (cerrado *sensu stricto*) areas and is distributed around the following Brazilian states: Maranhão, Bahia, Ceará, Pernambuco, Minas Gerais, Distrito Federal and São Paulo (Forero, 1976; Fonseca and Proença, 2002). It comprises small trees and bushes that blossom from May to September, fruiting from September to December (Fonseca and Proença, 2002). Some of these family species bear properties which allow their use as popular medicine: *Agelaea emetica* leaves are used to induce vomit; *Agelaea lamarckii* is an agent against *Neisseria gonorrhoeae*, and *Agelaea villosa* leaves are used in the treatment of dysentery. Studies involving species of Connaraceae family showed important biological activities. The chloroform extract and two isolated substances from *Rourea minor*, rourinoside and rouremin, demonstrated antimalarial activity (He et al., 2006). The aqueous extract from *Byrsocarpus coccineus* presented positive response in diminishing edemas and it can be used as an anti-inflammatory medication (Akindele and Adeyemi, 2007a). The extract has also shown antipyretic activity (Akindele and Adeyemi, 2007b), hepatoprotective and *in vivo* antioxidants effects (Akindele et al., 2010). The flavonoid tricin, isolated from the species *Agelaea pentagyna*, displays important anti-histaminic activity

* *Corresponding Author*
Departamento de Farmácia, Laboratório de Fitoquímica
Universidade Federal do Paraná. Av. Prefeito Lothário Meissner, 632, Jardim Botânico, CEP: 80210-170, Curitiba-Brasil
Tel.: 55 41 3360-4066

(Kuwabara et al., 2003), and the hexane extract from stems of *Rourea doniana* species showed a larvicidal promising activity against *Aedes aegypti* (Oliveira et al., 2010).

Under such perspective, this study evaluated the antibacterial, allelopathic and antioxidant activities of ethanol extract fractions obtained from the *R. induta* leaves, as an initial screening of this species properties.

MATERIAL AND METHODS

Plant material

The leaves from *R. induta* Planch., Connaraceae were collected in Rondonópolis city, Mato Grosso State, in November 2007, and they were dried in the shadow. After identification by Gert Hatschbach, botanist of Curitiba Botanical Museum, the voucher was registered under number 261574.

The dried plant material (2 kg of leaves) was submitted to ethanol extraction with the use of Soxhlet equipment followed by filtering, concentrating to 300 mL and liquid-liquid partitioning. From that, hexane, ethyl acetate and chloroform fractions were obteined. After filtering a residue was obtained, which was dissolved with the hexane fraction, and fractioned with hexane in silica column (silica 60 Merck 0.063-0.200 mm) in the Soxhlet equipment for a solid-liquid partitioning, hexane 1 and hexane 2 fractions were obtained as a result.

The compounds hyperin, a flavonoid isolated by precipitation from ethyl acetate fraction and tetracosane, a hydrocarbon isolated by silica column chromatography from hexane 1 fraction, were identified before(Kalegari et al., 2011). These compounds were tested in the biological activities.

Antibacterial activity

For this test was used the agar diffusion method (Romeiro, 2001). Strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella thyphimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228) were tested after being replicated in tryptic soy broth (TSB) and incubated at 35^{o}C for 24 hours. Each bacteria culture was diluted in sterile saline (5 mL) and adjusted 0.5 MacFarland scale's turbidity.

The tested fractions, hexane 1, hexane 2, chloroform, ethyl acetate, hyperin and tetracosane were impregnated in the sterile discs in two concentrations, 1000 µg and 500 µg, from a 50 mg mL^{-1} initial solution. Negative control discs were also prepared with the solvent that was used. Chloramphenicol 30 µg was used as positive control. The inoculated plates with disperse disks were incubated at 35°C for 24 hours and inhibition halos were measured, the final result corresponded to the halos average found in the test's triplicate (Romeiro, 2001).

Allelopathic activity

This activity was tested as described by Malheiros and Peres (2001) and Dias (2005). The tested samples were diluted with methanol to obtain 0.8; 0.4; 0.2 e 0.1 mg in 2 mL, they were soaked in filter paper Whatman n.6 and taken to the incubator at 40°C for 24 hours to evaporate the solvent. After 24 hours, each filter paper with the sample was placed in Gerbox boxes together with a filter paper free from sample. Three milliliters of distilled water and 20 seeds of *Lactuca sativa* were added to the samples following the pattern of 4 consecutive repetitions of 5 seeds per part (Dias, 2005). Boxes with distilled water and seeds, and boxes with solvent (methanol or chloroform), distilled water and seeds, were submitted to the same experiment conditions, and they were used as control. Aluminum foil protected the boxes from light exposure during the whole experiment, and the boxes were placed in a Mangelsdorf (Biomatic) germinator with temperature ranging from 17 to 20°C. Seven daily readings were conducted during the germination test. Daily, the germinated seeds were extracted from the boxes and in the end of the test, the germination speed rate (GSR) was calculated for each repetition of each treatment. The averages were submitted to Scott-Knott test for average comparison. Growth readings were taken on the material that was kept in the germinator for seven days and only opened in the reading day. Radicle's and hypocotyl's length sizes were measured, with a graph paper, in each seed to compare with controls measures. Results were submitted to Scott-Knott test for average comparison (p<0.05).

Phosphomolybdenum complexometry method

This method was prepared based on Prieto, Pineda and Aguilar (1999). A methanol solution at 200 µg mL^{-1} concentration was prepared for each fraction. Three hundred micro liters from each sample were added to a 3 mL reagent solution of the phosphomolybdenum complex. Tubes were shut and kept in a double boiler at 95ºC for 90 minutes. After cooling, the reading at 695 nm was run. Three hundred microliters of methanol with 3 mL of the phosphomolybdenum complex was used as the blank reagent. The antioxidant capacity of the samples were compared to the standards rutine (200 µg mL^{-1}) and vitamin C (200 µg mL^{-1}), which the antioxidant activity was considered 100% (Prieto, Pineda and Aguilar, 1999; Balestrin et al., 2008).

Radical DPPH (2,2- diphenyl -1- picrylhydrazyl) reduction

In this test five methanol solutions were prepared from the fractions, the concentration ranged from 2.0 to 12.5 µg mL^{-1}, of which 2.5 mL were added to 1 mL of DPPH methanol solution at 0.03 mmol mL^{-1} concentration. For the hexane fractions, five solutions that concentration ranged from 100 to 300 µg mL^{-1} were used. A blank reagent with 2.5 mL of the sample solution and 1 mL of methanol was prepared for each sample. In parallel, a control containing 2.5 mL of methanol and 1 mL of DPPH solution was carried on. After 30 minutes, readings were conducted in a spectrophotometer at 518 nm. The standards used were rutine and vitamin C (Mensor et al., 2001). The extract's ability to reduce the radical was calculated as follows:

$$IC\% = 100 - \{(\underline{A\ sample - A\ blank\ reagent}) \times 100\}$$
$$A\ control$$

The percentage of DPPH inhibition was calculated for each sample and a straight line, obtained from linear regression analysis, was used to calculate the IC_{50}. Each test was run three times and ANOVA and Tukey test ($p<0.05$) were used to analyze the results.

RESULTS AND DISCUSSION

The crude ethanolic extract was fractioned with different solvents. A partition of hexane fraction was performed and four fractions were obtained, and for these samples were analyzed the antibacterial, allelophatic and antioxidant activities, which have shown interesting results presented below.

Antibacterial test results (Table 1) indicate that the ethyl acetate and chloroform fractions have an effect against gram positive microorganisms *Staphylococus epidermidis* and *Staphylococus aureus.* To *S. epidermidis* the inhibition was proportionally to tested concentrations of ethyl acetate fraction (1000 and 500 µg), lower concentration showed a decrease in inhibition halo of 5 mm when compared to the higher one, the same was observed for the compound hyperin, in the lower concentration the halo was smaller than the higher concentration. For chloroform fraction the inhibition occurred just at 1000 µg. *S. aureus* inhibition was obtained when 1000 µg of both fractions were used. Consequently *S. epidermidis* strain shows higher sensibility to the samples. No growth inhibition was observed to gram negative bacteria; also, hexane 1, hexane 2 fractions and the compound tetracosane did not cause inhibition to any of the tested microorganisms.

The natural products can be an alternative to the bacterial resistance to usual antibacterial therapy, once they are important source of new drugs (Silva et al., 2010). The *Staphylococus aureus* can cause endocarditis, gastroenteritis, pneumonia and skin infections, and the *Staphylococcus epidermides* is a skin normal habitant, that can cause endocarditis and it can be an opportunist pathogen of urinary tract (Medline, 2009). So this extract can be investigated as an alternative against these pathogens to be use for the treatment of these bacterial infections. This extract has an antibacterial potential like the species *Rourea santaloides*, which showed a promissory activity against *S. aureus* and *S. epidermides*, an inhibition of 3 and 9 mm respectively (Parekh and Chanda, 2008), and other species of the same family, *Cnestis ferruginea*, *Byrsocarpus coccineus* and *Manotes longiflora,* that showed activity against *S. aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (Boakye-Yiadom and Konning, 1975).

Based on Scott-Knot's statistic test, none of the samples showed influence over the *Lactuca sativa*'s seeds germination in the allelophatic test. The samples do not differ statistically being equal to the solvent and water controls ($p<0.05$). The germination test results are shown in Table 2.

In the growth test (Table 3), it was observed that hexane fraction 2 had influence on the growth of the hypocotyl of *Lactuca sativa*'s seeds at 0.8 mg and 0.4 mg concentrations, an inhibition of 33.24% and 20.54% respectively occurred. The compound tetracosane showed an inhibition of 18.05% in hypocotyl growth. According to the statistics analysis the other sample results were equal to the control, therefore they did not demonstrate influence on *Lactuca sativa's* radicle or hypocotyl growth.

The alellochemicals presence, secondary metabolites that are involved in direct and indirect plant defense, is an important ecological mechanism; they can control the insects herbivores and pathogens (Moraes et al., 2008). They can cause significant influences in agricultural management, and they can be used to control weeds (Andrade et al., 2003), they can be use, alternatively, as potential herbicides to standardize the local vegetation, some of compounds constitute chemical weapons (Jasicka-Misiak, Wieczorek and Kafarski 2005). Tetracosane is a hydrocarbon, and the use of hydrocarbons as pesticides is already known (Siddiqui et al., 2004), so this compound is a potential herbicide and deserves greater attention in the study of this kind of activity. As the species of Connaraceae family do not present alellophatic studies registered in the literature, and many secondary metabolites, including tetracosane, and a synergism between them may show allelophatic activity, it is important more detailed studies about the phytochemical composition of *R. induta* hexane fraction 2 to find the compounds responsible for this activity.

The antioxidant activity evaluated by phosphomolybdenum complexometry method (Table 4) demonstrated that all the tested fractions showed antioxidant potential. Considering rutine and vitamin C activities as 100%, the ethyl acetate and chloroform fractions achieved 70% of vitamin C's activity and were higher than rutine's activity. The flavonoid hyperin showed an expressive activity, higher than rutine and almost 50% of vitamin C's activity. Hexane 2 fraction showed an antioxidant activity next to rutine (106.98%), on the other hand, hexane 1 and tetracosane showed low antioxidant activity, less than 10% of vitamin C's activity, considered the less active sample.

Regarding the radicle DPPH reduction method (Table 5), it was possible to verify that the chloroform fraction has as much antioxidant activity as the standards (vitamin C and rutine). The acetate fraction presents the lowest IC_{50} level, being statistically different from the other fractions and standards. It can be considered the most active sample. Hyperin has an activity equal to the ethyl acetate and chloroform fractions, being more active than the standards. The hexane fractions are different themselves and from the other samples, showing relatively high IC_{50}. This result indicates that the hexane fractions are less active than the analyzed standards, because a larger amount of the sample is necessary to reduce 50% of the initial DPPH concentration.

According to the results, the most important activity of this species is the antioxidant. This is an important characteristic of the extract because antioxidants can disable reactive oxygen species, which can cause tissue injury at inflammatory process; they can delay or prevent the oxidation of substrates (Coutinho, Muzitano and Costa, 2009; Andrade et al., 2010). The species

Table 1:- Average of inhibition halos in millimeters

Samples	Inhibition halos average (mm)					
	Concentrations (µg)	*S.epidermidis*	*S.aureus*	*E. coli*	*S. typhimurium*	*P. aeruginosa*
Hexane 1	1000	-	-	-	-	-
Fraction	500	-	-	-	-	-
Hexano 2	1000	-	-	-	-	-
Fraction	500	-	-	-	-	-
Chloroform	1000	12.30	7.60	-	-	-
Fraction	500	-	-	-	-	-
Ethyl Acetate Fraction	1000	15.00	7.60	-	-	-
	500	10.00	-	-	-	-
Hyperin	1000	9.3	-	-	-	-
	500	7.0	-	-	-	-
Tetracosane	1000	-	-	-	-	-
	500	-	-	-	-	-
Chloramphenicol	30	36.80	26.50	28.20	27.00	15.00

- no activity

Table. 2: *Lactuca sativa's* seed germination speed rate Scott-Knott test.

Sample	Concentration				Water Control	Solvent Control
	0.8 mg	0.4 mg	0.2 mg	0.1 mg		
Hexane 1 fraction	5.00 a1	4.83 a1	5.00 a1	5.00 a1	4.50 a1	5.00 a1
Hexane 2 fraction	4.00 a1	4.25 a1	4.12 a1	4.87 a1	4.50 a1	4.50 a1
Ethyl acetate fraction	3.87 a1	3.49 a1	3.87 a1	3.25 a1	3.50 a1	3.14 a1
Chloroform fraction	4.37 a1	3.81 a1	4.00 a1	4.00 a1	3.50 a1	3.14 a1
Hyperin	4.33 a1	4.83 a1	4.58 a1	4.75 a1	4.50 a1	4.50 a1
Tetracosane	4.63 a1	4.87 a1	4.87 a1	5.00 a1	4.50 a1	5.00 a1

a1 = statistic classification. Samples that are followed by the same statistic group in the line don't differ statistically.

Table . 3: Scott-Knott test for radical and hypocotyl growth test.

Treatment	Repetition	Radicle (average mm)						Hypocotyl (average mm)					
		H1	H2	AE	CL	HYP	n-TETR	H1	H2	AE	CL	HYP	n-TETR
0.8 mg	1	33.6a1	23.6a1	21.2 a1	25.4 a1	27.2 a1	31.8 a1	31.8 a1	19.7a1	21.8a1	25.0 a1	29.8 a1	32.2 a2
	2	36.0a1	15.6a1	24.8 a1	25.8 a1	25.6 a1	36.0 a1	32.0 a1	15.4a1	26.4a1	23.0 a1	24.2 a1	36.0 a2
	3	35.8a1	32.4a1	26.2 a1	25.6 a1	26.4 a1	28.8 a1	32.4 a1	23.0a1	24.4 a1	27.0 a1	28.4 a1	29.8 a2
	4	40.0a1	31.4a1	28.4 a1	25.0 a1	24.4 a1	32.0 a1	34.4 a1	21.2a1	27.4 a1	26.0 a1	28.4 a1	28.8 a2
0.4 mg	1	32.20a1	31.2a1	27.0 a1	22.2 a1	27.8 a1	34.0 a1	37.0 a1	24.6a1	24.0 a1	24.6 a1	30.6 a1	26.4 a1
	2	32.6a1	27.4 a1	24.6 a1	28.6 a1	24.4 a1	35.8 a1	35.4 a1	22.2a1	23.2 a1	29.4 a1	23.2 a1	27.4 a1
	3	26.4 a1	25.0 a1	28.0 a1	31.6 a1	26.6 a1	30.2 a1	30.4 a1	22.6 a1	25.6 a1	27.6 a1	26.8 a1	25.6 a1
	4	29.4 a1	31.0 a1	21.2 a1	26.2 a1	31.2 a1	30.2 a1	28.8 a1	25.0a1	22.5 a1	27.2 a1	30.2 a1	27.8 a1
0.2 mg	1	39.4a1	29.6 a1	28.4 a1	31.0 a1	31.4 a1	32.8 a1	35.4 a1	32.2a2	26.4 a1	28.2 a1	25.0 a1	32.0 a2
	2	36.00a1	31.2 a1	26.2 a1	23.0 a1	28.2 a1	29.2 a1	32.0 a1	30.4 a2	22.2 a1	23.6 a1	24.2 a1	24.6 a1
	3	32.8a1	27.0 a1	31.0 a1	23.2 a1	33.0 a1	33.2 a1	32.0 a1	31.2a2	22.6 a1	22.4 a1	28.2 a1	25,. a2
	4	28.0a1	27.8 a1	27.2 a1	23.0 a1	31.6 a1	33.8 a1	20.6 a1	29.0a2	23.4 a1	26.0 a1	25.0 a1	30.8 a2
0.1 mg	1	28.8 a1	27.0 a1	25.6 a1	27.4 a1	29.0 a1	30.8 a1	34.8 a1	30.4 a2	26.4 a1	26.4 a1	26.4 a1	30.2 a2
	2	34.0a1	29.8 a1	22.6 a1	27.4 a1	32.6 a1	30.2 a1	35.6 a1	28.4 a2	25.2 a1	24.6 a1	26.0 a1	28.2 a1
	3	36.6 a1	26.6 a1	25.0 a1	28.2 a1	29.4 a1	29.2 a1	37.4 a1	26.6 a2	26.2 a1	25.8 a1	24.0 a1	23.5 a1
	4	28.7 a1	23.6 a1	26.6 a1	28.6 a1	30.0 a1	38.2 a1	34.0 a1	26.4 a2	26.2 a1	23.2 a1	29.8 a1	26.8 a1
Water control	1	34.2 a1	31.8 a1	15.2 a1	15.2 a1	31.8 a1	34.2 a1	32.4 a1	31.2 a2	20.7 a1	20.7 a1	31.2 a1	32.4 a2
	2	38.0 a1	32.4 a1	27.2 a1	26.6 a1	32.4 a1	38.0 a1	35.4 a1	32.2 a2	28.2 a1	28.2 a1	32.2 a1	35.4 a2
	3	32.8 a1	28.6 a1	25.0 a1	25.2 a1	28.6 a1	32.8 a1	30.0 a1	28.8 a2	24.8 a1	24.8 a1	28.8 a1	30.0 a2
	4	32.8 a1	18.0 a1	24.2 a1	24.2 a1	18.0 a1	32.8 a1	33.0 a1	26.6 a2	26.5 a1	26.5 a1	21.6 a1	33.0 a2
Solvent control	1	30.0 a1	24.2 a1	26.6 a1	26.6 a1	24.2 a1	30.0 a1	30.0 a1	29.0 a2	24.4 a1	24.4 a1	23.2 a1	30.0 a2
	2	28.4 a1	28.8 a1	30.0 a1	30.0 a1	28.8 a1	28.4 a1	29.4 a1	28.0 a2	27.0 a1	27.0 a1	28.0 a1	29.4 a2
	3	32.0 a1	29.2 a1	27.0 a1	27.0 a1	29.2 a1	32.0 a1	29.6 a1	32.2 a2	23.4 a1	23.4 a1	32.2 a1	29.6 a2
	4	31.4 a1	32.0 a1	18.6 a1	18.6 a1	32.0 a1	31.4 a1	32.0 a1	32.2 a2	21.2 a1	21.2 a1	32.2 a1	32.0 a2

H1 – hexane fraction 1; H2 – hexane fraction 2; AE – ethyl acetate fraction; CL – Chloroform fraction, HYP – hyperin, n-TETR – tetracosane.
*averages followed by the same letter in the same column don't differ statistically.

Table. 4: Antioxidant activity by the reduction of the phosphomolybdenum complex.

Sample	Antioxidant activity in relation to rutine (%)	Antioxidant activity in relation to vitamin C (%)
Hexane 1 fraction	32.03	6.58
Hexane 2 fraction	106.98	32.45
Chloroform fraction	249.39	77.96
Ethyl acetate fraction	196.68	61.49
Hyperin	127.80	42.34
Tetracosane	23.67	7.18

Table. 5: Antioxidant activity by radical DPPH reduction.

Sample	IC_{50} (µg) ± SD
Vitamin C	5.80 ± 0.08 a3[a]
Rutine	5.91 ± 0.04 a3[a]
Hexane 1 fraction	199.86 ± 1.54 a5[a]
Hexane 2 fraction	113.43 ± 0.35 a4[a]
Ethyl acetate fraction	3.21 ± 0.00 a1[a]
Chloroform fraction	5.33 ± 0.19 a2[a] a3[a]
Hyperin	3.89 ± 0,02 a1[a] a2[a]

[a] tukey test, samples that are followed by the same number don't differ statistically.

Byrsocarpus coccineus, also showed a significant antioxidant activity by reducing the activity of catalase, SOD, peroxidase and GSH *in vivo*. This activity can be explained by the presence of natural antioxidants in *B. coccineus*, flavonoids and alkaloids, but studies are ongoing to isolate and identify the active compounds (Akindele et al., 2010). The *R. induta* species, that has been reported to contain flavonoids (Kalegari et al., 2011), probably has the antioxidant activity related to this phenolic compounds, like hyperin, but needs further studies about its potential and studies to find and elucidated if hyperin is the bioactive compound.

CONCLUSION

There is no data in the literature about the *R. induta* biological activities, therefore the obtained results stimulate the continuity of this study, especially for ethyl acetate, chloroform fractions and hyperin regarding their antimicrobial and antioxidant potential, and the tetracosane allelopathic activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

To Gert Hatschbach, botanist of Curitiba Botanical Museum, for identifying the species, and to Camila de Albuquerque Mercali for the text grammatical revision.

REFERENCES

Akindele, A.J.; Adeyemi, O.O. Antiinflamatory activity of the extract of *Byrsocarpus coccineus*. Fitoterapia. 2007a; 78:25-28.

Akindele, A.J.; Adeyemi, O.O. Antipyretic activity of *Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae). Int. J. Pharmacol. 2007b; 3: 357-361.

Akindele, A.J.; Ezenwanebe, K.O.; Anunobi, C.C.; Adeyemi, O.O. Hepatoprotective and in vivo antioxidant effects of *Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae). J. Ethnopharmacol. 2010; 129: 46-52,.

Andrade, C. A.; Miguel, M.D.; Miguel, O.G.; Ferronato, M.L.; Peitz, C.; Cunico, M.; Dias, J.F.G.; Balestrin, L.; Kerber, V.A. Efeitos alelopáticos das flores da *Acacia podalyriaefolia* A. Cunn. Visão Acadêm. 2003; 4: 93-98.

Andrade, C.A.; Carvalho, J.L.S.; Cunico, M.M.; Lordello, A.L.L.; Higaskino, C.E.K.; Almeida, S.C.C.; Dias, J.F.G.; Kerber, V.A.; Miguel, M.D.; Miguel, O.G. Antioxidant and antibacterial activity of extracts, fractions and isolated substances from the flowers of *Acacia podalyriifolia* A. Cunn. ex G. Don. *Braz. J. Pharm. Sci.* 2010; 46: 715-721.

Balestrin, L.; Dias, J.F.G.; Miguel, O.G.; Dall'Stella, D.S.G.; Miguel, M.D. Contribuição ao estudo fitoquímico de *Dorstenia multiformis* Miquel (Moraceae) com abordagem em atividade antioxidante. Rev. Bras. Farmacog. 2008; 18: 230-235.

Boakye-Yiadom, K.; Konning, G. H. Incidence of antibacterial activity in the Connaraceae. Planta Med. 1975; 28: 397-400.

Coutinho, M. A. S.; Muzitano, M. F.; Costa, S. S. Flavonóides: potenciais agentes terapêuticos para o processo inflamatório. Rev. Virt. Quím. 2009; 1: 241-256.

Dias, J.F.G.; Cirio, G.M.; Miguel, M.D.; Miguel, O.G. Contribuição ao estudo alelopático de *Maytenus ilicifolia* Mart. Ex Reiss., Celastraceae. Rev. Bras. Farmacog. 2005; 15: 220-223.

Fonseca, L.C.M.; Proença, C.E.B. Connaraceae. In: Cavalcanti, T.B.; Ramos, A.E. *Flora do Distrito Federal*. 2.ed. Embrapa Recursos Genéticos e Biotecnologia, Brasília (2002) 41-47.

Forero, H. A revision of american species of *Rourea* Subg. *Rourea* (Connaraceae). Memoirs of The New York Botanical Garden. 1976; 26: 1-119.

He, Z.D.; Ma, C-Y.; Tan, G.T.; Sydara, K.; Tamez, P.; Southavong, B.; Bouamanivong, S.; Soejarto, D.D.; Pezzuto, J.M.; Fong, H.H.S.; Zhang, H.J. Rourinoside and Rouremin, antimalarial constituents from *Rourea minor*. Phytochemistry. 2006; 67:1378-1384.

Jasicka-Misiak, i.; wieczorek, p.p.; kafarski, p. Crotonic acid as a bioactive factor in carrot seeds (*Daucus carota* L.). Phytochemistry. 2005; 66: 1485-1491.

Kalegari, M. Miguel, M.D.; Dias, J.F.G.; Lordello, A.L.L.; Miyazaki, C.M.S., Zanin, S.M.W.; Verdam, M.C.S.; Miguel, O.G. Phytochemical constituents and preliminary toxicity evaluation of leaves from *Rourea induta* Planch.. (Connaraceae). Braz. J. Pharm. Sci. 2011; 47: 635-642.

Kuwabara, H.; Mouri, K.; Otsuka, H.; Kasai, R.; Yamasaki, K. Tricin from a Malagasy Connaraceous plant with potent antihistaminic activity. J. Nat.Prod. 2003; 66: 1273-1275.

Lenza, E.; Ferreira, J.N.; Consolaro, H.; Aquino, F.G. Biologia reprodutiva de *Rourea induta* Planch.. (Connaraceae), uma espécie heterostílica de cerrado do Brasil Central. Rev. Bras. Bot. 2008; 31: 389-398.

Lorenzi, H.; Souza, V.C. Botânica sistemática*:* guia ilustrado para identificação das famílias de angiospermas da flora brasileira, baseado em APG II.: Instituto Plantarum, São Paulo (2005) 640.

Maguire, J.D. Speed o germination - aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 1962; 2: 176-177.

Malheiros, A.; Peres, M.T.L. Alelopatia: interações químicas entre as espécies. In: Yunes, R.A.; Calixto, J.B. Plantas medicinais sob a ótica da química medicinal moderna. Argos Universitária, Chapecó/SC (2001) 504-521.

Medline Plus. *Staphylococcal Infections*. Available at: <http://www.nlm.nih.gov/medlineplus/staphylococcalinfections.html> Accessed on: 01 ago. 2009.

Mensor, L.L.; Menezer, F.S.; Leitão, G.G.; Reis, A.S.; Santos, T.C.; Coube, C.S.; Leitão, S.G. Screening of brazilian plant extracts for antioxidant activity by the use of DPPH free radical method**.** Phytoter. Res. 2001; 15: 127-130.

Moraes, M.C.B.; Birkett, M.A.; Gordon-Weeks, R.; Samart, L.E.; Martin, J.; Pye, B.J.; Bromilow, R.; Pickett, J.A. cis-Jasmone induces accumulation of defence compounds in wheat, *Triticum aestivum. Phytochemistry*. 2008; 69: 9-17.

Oliveira, P.V.; Ferreira, J.C.; Moura, F.S.; Lima, G.S.; Oliveira, F.M.; Oliveira, P.E.S.; Conserva, L.M.; Giulietti, A.M.; Lemos, R.P.L. Larvicidal activity of 94 extracts from ten plant species of northeastern of Brazil against *Aedes aegypti* L. (Diptera: Culicidae). Parasitol. Res. 2010; 107: 403-407.

Parekh, J.; Chanda, S. V. Antibacterial activity of aqueous and alcoholic extracts of 34 indian medicinal plants against some *Staphylococcus* species. Turk. J. Biol. 2008; 32: .63-71.

Prieto, P.; Pineda M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: specific application to the determination of vitamin E. Anal. Biochem. 1999; 269: 337-341.

Romeiro, R.S. Métodos em bacteriologia de plantas. Editora UFV, Viçosa (2001) 297.

Siddiqui, B.S.; Rasheed, M.; Ilyas, F.; Gulzar, T.; Tariq, R.M.; Naqvi, S.N.H. Analysis of Insecticidal *Azadirachta indica* A. Juss. Fractions. Z. Naturforsch. 2004; 59c: 104-112.

Silva, V.A.; Freitas, AF.R.; Pereira, M.S.V.; Siqueira Junior, J.P.; Higino, J.S. Avaliação *in vitro* da atividade antimicrobiana do extrato da *Lippia sidoides* Cham.sobre isolados biológicos de *Staphylococcus aureus*. Rev. Bras. Plantas Med. 2010; 12: 452-455.

How to cite this article:

Milena Kalegari, Marilis Dallarmi Miguel, Andressa Frizzo Philippsen, Josiane de Fátima Gaspari Dias, Sandra Maria Warumby Zanin, Cristina Peitz de Lima, Obdulio Gomes Miguel**.** Antibacterial, allelopathic and antioxidant activity of extracts and compounds from *Rourea induta* Planch. (Connaraceae). J App Pharm Sci. 2012; 2(9): 061-066.