Journal of Medicinal Plants Research Vol. 6(24), pp.4142-4148, 28 June, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.691
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Antitumor and cytotoxic activity of *Kielmeyera coriacea* mart. Zucc. and *Pyrostegia venusta* (ker-gawl.) Miers extracts

Regildo M. G. Silva*, Débora T. M. Rodrigues, Fernanda S. Augustos, Fillipi Valadares, Pedro O. Neto, Lucinéia dos Santos and Luciana P. Silva

Universidade Estadual Paulista (UNESP), Departamento de Ciências Biológicas - Laboratório de Fitoterápicos, Faculdade de Ciências e Letras de Assis, Avenida Dom Antônio 2100, CEP: 19806-900, Assis, São Paulo, Brasil.

Accepted 22 May, 2012

This study aimed to investigate the antitumor and cytotoxicity activities of *Kielmeyera coriacea* and *Pyrostegia venusta* extracts. Therefore, the hydroalcoholic extracts of *P. venusta* flowers and *K. coriacea* leaves were prepared. The extracts were evaporated and the dry extracts were diluted at concentrations of 1.0, 0.1, 0.01 and 0.001 mg/ml for carrying out the bioassays. *Artemia salina* eggs were incubated in saline solution at 28°C for 24 h. The larvae were treated with different extracts concentrations and the mortality was evaluated after 24 and 48 h. Five discs of potato were placed in Petri dishes and 50 µl of inoculum of *Agrobacterium tumefaciens* were added to it at 28°C for 24 h incubation. So, 50 µl of the extracts in different concentrations were added. Positive and negative controls were made. The *P. venusta* and *K. coriacea* extracts did not show statistically significant acute toxicity. *K. coriacea* extract showed (mean% of tumor ± standard deviation) 15.30 ± 3.24, 6.34 ± 3.82, 7.57 ± 2.92 and 5.77 ± 2.85 and *P. venusta* showed 25.82 ± 5.15, 38.40 ± 8.28, 15.75 ± 4.44 and 13.38 ± 7.92, with their concentrations for the antitumor bioassay, and the positive control showed 25.80 ± 6.14. According to the obtained results it was established that the *K. coriacea* and *P. venusta* extracts showed antitumor activity but did not show significant cytotoxic activity in *A. salina* test.

Key words: *Kielmeyera coriacea*, *Pyrostegia venusta*, *Agrobacterium tumefaciens*, antitumor activity, cytotoxic activity.

INTRODUCTION

Popular reports and information from folk medicine in the region of Medium Paranapanema Valley- SP - Brazil, particularly in the city of Assis, reported the use of *Kielmeyera coriacea* and *Pyrostegia venusta* in the treatment of different tumor manifestations.

K. coriacea Mart. Zucc. (Clusiaceae) is a typical species from open areas of Cerrado (Lorenzi, 2002). This species is used in folk medicine for the treatment of schistosomiasis, leishmaniasis, malaria, fungal and bacterial infections (Alves et al., 2000). Studies have shown that organic fractions of this plant have cytotoxic activity (De Mesquita et al., 2009), anticancer and antiploriferative, suggesting the action of xanthones and derivatives found in extracts from stems and leaves (Gottlieb et al., 1969; Ferreira et al., 1972; Cortez et al., 1998). Pharmacological tests with natural xanthones showed different activities such as antiinflammatory, antileukemic, antitumor, antihepatotoxic, antiulcer, antiviral (herpes), fungal, stimulant of the myocardium and central nervous system, antihypertensive, antimicrobial, analgesic and immunosuppressive, which shows the potential of these special metabolites as medicinal agents (Bent and Ko, 2004; Diderot et al., 2006, Sela et al., 2009).

P. venusta (Ker-Gawl.) Miers (Bignoniaceae) flowers

*Corresponding author. E-mail: regildos@yahoo.com.br. Tel: +55 18 33025848. Fax: +55 18 33025802.

are used in folk medicine for treatment of white patches on the body (leukoderma, vitiligo) (Lorenzi, 1982) and its stem is used as tonic and antidiarrheal, and more recently as antitumor (Park et al., 2003, Ju et al., 2004). The ethanol extract of the roots of *P. venusta* has four substances that were identified based on the interpretation of spectral data, such as allantoin, the steroids β-sitosterol and 3β-O-β-D-glucopyranosylsitosterol and flavanone hesperidin. The compound β-sitosterol has been associated with antitumor activity (Park et al., 2003; Ju et al., 2004) and flavonoids have been recognized as responsible for allergenic, antiinflammatory, antiviral, antiproliferative and anti-cancer activity (Wattenberg, 1995).

With regard to tumors and cancer, these are among the leading causes of death in Brazil. They constitute the third most frequent cause of death (Ministério da Saúde, 2011). Thus, the research lines continue to expand for new anticancer and antitumor compounds, besides reviews in several tumor systems and tissue culture in order to select the most effective compounds (Hussar, 2000; Aslani et al., 2000).

The inhibition of *Agrobacterium tumefaciens*-induced tumors in potato disc tissue is an assay based on antimitotic activity and can detect a broad range of known and novel antitumor effects (McLaughlin and Rogers 1998). This assay uses a system based on neoplastic plant disease "Crown Gall", caused by *A. tumefaciens* (McLaughlin, 1991), and is also responsible for detecting the antitumor activity of chemotherapeutic drugs (Coker et al., 2003).

The bacterium *A. tumefaciens* transfers to the plant cells a portion of its DNA, single stranded, shielded by bacterial proteins, which integrates into the plant cell nucleus and there restore its double chain, triggering the production of hormones and other compounds (with which bacteria feeds) by the diseased tissue, causing growth of a tumor (Amara et al., 2008) with similar nucleic acids contents and histology to human and animal cancers (Agrios, 1997).

This study aimed to evaluate the antitumor potential of *P. venusta* and *K. coriacea* extracts by the inhibition of *A. tumefaciens*-induced tumors in potato disc bioassay. This study also evaluated the cytotoxicity by the Brine Shrimp (*Artemia salina* L.) bioassay.

MATERIALS AND METHODS

The leaves of *K. coriacea* and flowers of *P. venusta* were collected from specimens of remaining cerrado vegetation near the campus of UNESP / Assis (22°32'26"S and 50º22'31"N and 22°32'18"S; 50°22'47"N) and a copy of each species was taxonomically identified at the Herbarium of the Forestry Institute of São Paulo (voucher specimen: SPSF-40211 to *K. coriacea* and SPSF-40207 to *P. venusta*).

All collections were made in a sustainable manner and without causing environmental impact to the species collected.

Preparation of plant material in the laboratory, production and storage of extracts

Leaves of *K. coriacea* and flowers of *P. venusta* were selected and dried in air flow at a temperature of at most 40°C and then crushed and pulverized to prepare the hydroalcoholic crude extracts. The mechanical extraction of the powdered plant under stirring with a solution of 70% ethanol and distilled water happened for 24 h, repeating twice at 1:10 (w:v). After obtaining the extracts, they were taken to a rotary evaporator to remove all the alcohol, the resulting concentrated extract was taken to the drying chamber to obtain a dry extract.

Cytotoxicity assay of plant extracts by means of the Brine Shrimp assay

The cytotoxic effect of plants was tested by the method of Meyer et al. (1982) modified to suit the laboratory and consisted of dissolving the samples in each saline extract (34.2 g of sodium chloride, 1.425 g of magnesium sulfate, 4.75 g of sodium bicarbonate and 915 ml of distilled water) and divided into culture dishes, in which 10 *A. salina* larvae were placed in a final volume of 2.0 ml saline solution (pH 9.0). The cultures of *A. salina* were incubated at 28°C, being done the reading of surviving or dead larvae number after 24 and 48 h. We calculated the percent mortality for each of the concentrations tested and controls, which contained only where the larvae and saline solution (negative control) and the positive control, with the larvae and sodium hypochlorite to 1%.

Inhibition of *A. tumefaciens*-induced tumors in potato disc tissue

The liquid growth medium specific for *A. tumefaciens* used was the nutrient medium, containing 0.3% meat extract, 0.5% proteose peptone, 0.5% sucrose, 0.8% yeast extract, previously autoclaved for 15 min at 121°C for its sterilization.

The potatoes of the variety Monaliza were first washed in water and placed in sodium hypochlorite at 2% for 20 min and then put immediately in distilled water for 20 min. Cylinders were cut about 1.0 cm in diameter with the aid of a sterile surgical scalpel, always disposing the apex and base. Then, quickly, about 0.2 cm discs were cut. The discs were sterilized with sodium hypochlorite at 2% and washed in sterile distilled water for 20 min.

Five discs of potato were placed in each Petri dish with agar solid medium (1.5%). 5.0 µl of *A. tumefaciens* inoculum were added on each disc. These dishes were incubated for 24 h at 28°C and then 5.0 µl of each extract at concentrations of 1.0, 0.1, 0.01 and 0.001 mg/ml were added to the inoculated potato discs forming the experimental groups. Positive controls (inoculum of bacteria and solvent Tween80 and 0.03% water) and negative controls (enriched medium and solvent Tween80 and 0.03% water) were made and three dishes were placed to the experimental and control groups (n = 5 discs). The plates were sealed with PVC film and kept at 28°C for 30 days. On the tenth day, a counting of tumor occupied on each disk was performed by the percentage area.

Bacterial viability assay

The bacterial viability was determined by incubation of each extract (0.1 mg/ml) with 1×10^9 colony forming unit (CFU) of bacteria suspension, containing phosphate buffer solution (0.043% KH_2PO_4, 0.148% Na_2HPO_4 and 0.72% NaCl) in four *Eppendorf* tubes for test.

Table 1. Mean mortality of *Artemia salina* extract after incubation with *Kielmeyera coriacea.*

Concentration of *K. coriacea*	Mean mortality 24 h	Mean mortality 48 h	Sum of mortalities
100%	10.00	0.00	10.00
50%	10.00	0.00	10.00
25%	10.00	0.00	10.00
10%	10.00	0.00	10.00
1%	8.00	0.66	8.66
0.1%	5.66	1.66	7.32
0.01%	4.66	3.00	7.66
0.001%	4.33	3.00	7.33
0	3.66	3.00	6.66
PC	10.00	0.00	10.00

Legend: PC= Positive control (NaClO 1%).

Table 2. Mean mortality of *Artemia salina* extract after incubation with *Pyrostegia venusta.*

Concentration of *P. venusta*	Mean mortality 24 h	Mean mortality 48 h	Sum of mortalities
100%	6.66	2.66	9.32
50%	6.33	3.00	9.33
25%	6.00	3.00	9.00
10%	5.66	3.66	9.32
1%	5.66	3.66	9.32
0.1%	6.00	4.00	10.00
0.01%	4.33	4.33	8.66
0.001%	4.00	4.00	8.00
0	2.66	2.66	5.32
PC	10.00	0.00	10.00

Legend: PC= Positive control (NaClO 1%).

After 10, 20, 30 and 60 min after inoculation, 10 µl of this solution were removed and plated on nutrient medium and incubated for 24 h. The bacterial growth was evident in culture dishes. In addition this procedure, the bacterial growth was observed during experimentation by halo formation of colonies developed between the discs and the basis of Agar-agar.

Statistical treatment

The results were subjected to analysis of variance (ANOVA) and the averages compared by Tukey's test at α = 0.05 of probability or regression analysis (Sampietro et al., 2006).

RESULTS

Yield of plant extracts

The hydroalcoholic extracts showed the yield of 600 ml for both species and after drying the yield was 7.42 g for *K. coriacea* and 4.55 g of *P. venusta*. They were stored in amber glass container and diluted further in the concentrations required to achieve the bioassays.

Cytotoxicity test with *Artemia salina* (brine shrimp)

Acute toxicity evaluation of *K. coriacea* and *P. venusta* extracts was performed by the bioassay with *Artemia salina* using crude extract of these plants in different concentrations (100, 50, 25, 10, 1, 0.1, 0.01 and 0.001%). After 24 and 48 h of *A. saline* incubation with the extracts, the number of surviving and dead individuals was counted in three repetitions and then the average number of deaths was calculated (Tables 1 and 2).

The extracts of *K. coriacea* showed decreasing mortality in accordance with the successive dilutions, obtaining a high acute toxicity at all concentrations at 24 h of exposure to the extract, once the mortality of *A. salina* individuals exposed to incremental concentrations of 100% to 1% were greater than 80%, which is higher than the negative control. Within 48 h of exposure is noted that more individuals died, obtaining with the sum, over 70% of death in all the concentrations and this percentage is also greater than the negative control (Table 1).

The extracts of *P. venusta* also showed decreasing

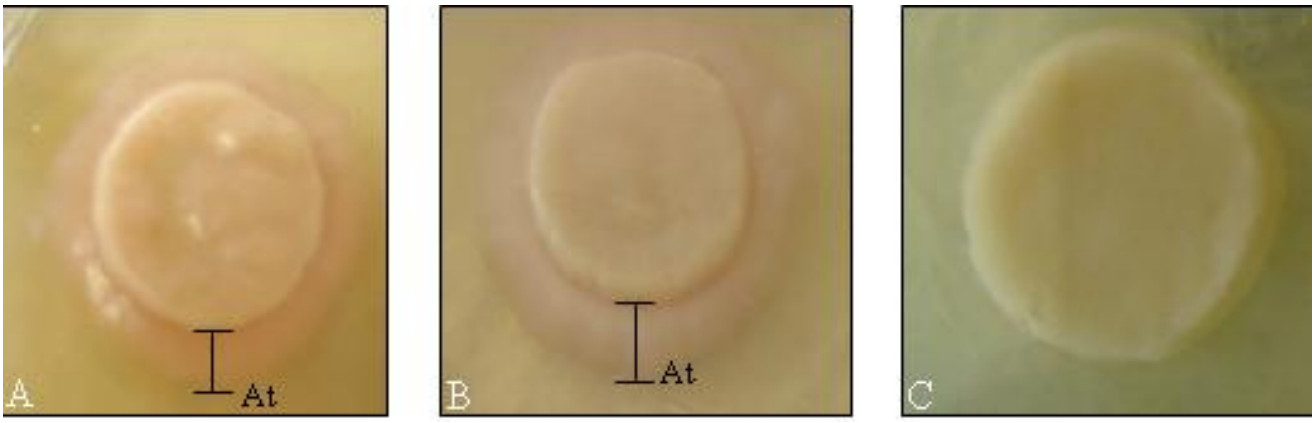

Figure 1. Potatoes discs infected by *Agrobacterium tumefaciens* (A - Positive control = untreated and B - Experimental group = treated with plant extract), At - halo of bacterial growth (A and B); Potato disc without infection by *Agrobacterium tumefaciens* (C).

Table 3. Average percentage ± standard deviation of *Agrobacterium tumefaciens*-induced tumors formation in potato disc tissue treated with different concentrations of *K. coriacea* and *P. venusta* hydroalcoholic extracts (experimental group) and untreated (positive control).

Extract concentration (mg/Ml)	Plant tested	
	K. coriacea	***P. venusta***
0.001	15.30±3.24[a]	25.82±5.15[a]
0.01	6.34±3.82[b]	38.40±8.28[b]
0.1	7.57±2.92[c]	15.74±4.44[c]
1.0	5.77±2.85[d]	13.38±7.94[d]
0	20.98±5.76[e]	25.80±6.14[a]

Same letters in column do not differ statistically (ANOVA), medium evaluated with Tukey's test (α=0.05).

mortality in accordance with the successive dilutions, obtaining a mild to moderate acute toxicity in all the concentrations at 24 h of exposure to the extract. The mortality of *A. salina* individuals exposed to all concentrations were 40% to 70%, being that greater than the negative control and well below than the positive control. Within 48 h of exposure is noted that more individuals died, obtaining with the sum more than 80% deaths in all the concentrations (Table 2).

The high mortality of *A. salina* individuals observed after 48 h incubation in two tested extracts may be the result of lack of oxygen and/or supply thereof, once the negative control of the two tests obtained deaths between 50 and 70%.

Considering the first 24 h of exposure, where the acute toxicity is measured by comparing the two tests, it is noted that the *P. venusta* extract is low toxicity since the mortality rate was decreased by 70%, based on the concentration of extract to 100%. Since the extract of *K. coriacea* showed a high acute toxicity in extracts of concentrations up to 1%, but showed low toxicity, even smaller than the *P. venusta*, at the lowest concentrations of 0.1, 0.01 and 0.001%.

Bacterial viability assay

After plating different bacteria inoculants with the extracts in different incubation times was observed bacterial growth in all culture dishes. This viability was also determined during the trial. In tests, the normal growth of bacteria was observed, since there was a halo formation of bacterial colony around the all experimental and positive control potato discs, which its size was constant, independent of the extracts concentrations (Figure 1)

Inhibition of *Agrobacterium tumefaciens*-induced tumors in potato disc tissue

The number of tumors per potatoes disc was quantified by determining the percentage of occupied area by the tumor in *pixel*^{2} on each evaluated disc in accordance with the experimental and control groups. For this purpose, the software *Image Processing and Analysis in Java* (*ImageJ* - freely available http://rsbweb.nih.gov/ij/) was used. Always considering the number of discs per dish (n=5) and the number of repetitions realized (n = 3).In Table 3, the average percentages are determined and

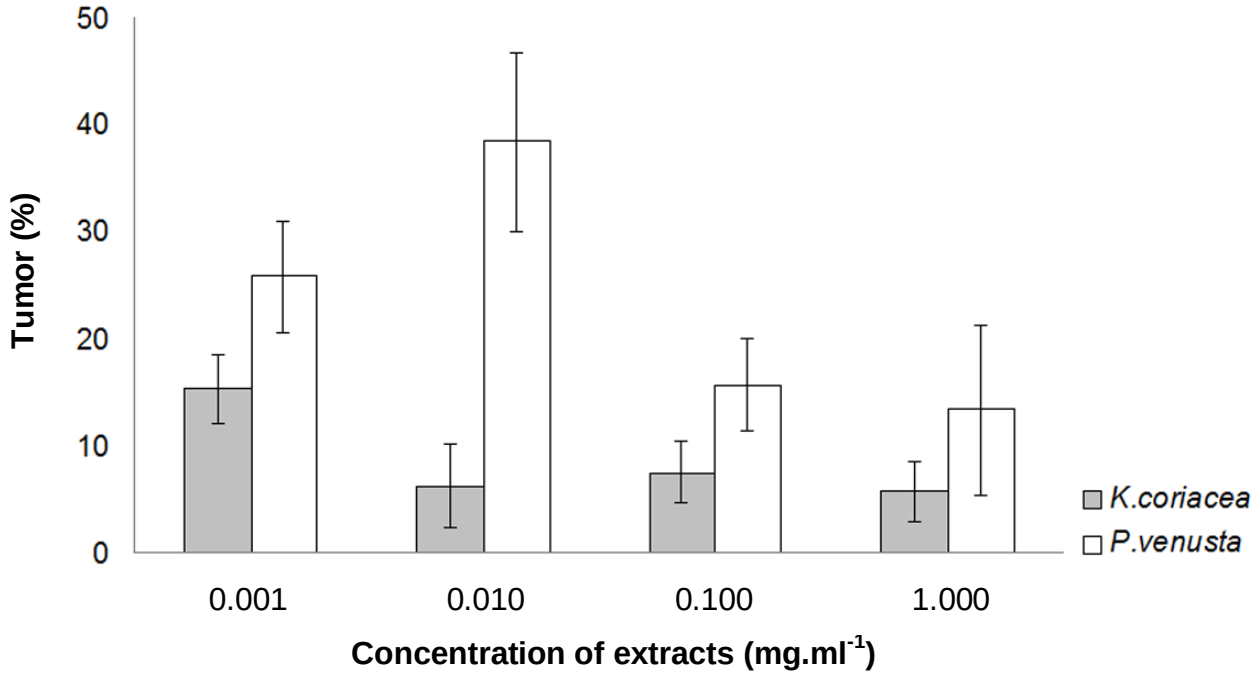

Figure 2. Average percentage of *Agrobacterium tumefaciens*-induced tumors in potato disc tissue and experimental groups treated with *K. coriacea* and *P. venusta* extracts.

standard deviations of *A. tumefaciens*-induced tumors formation in potato disc tissue treated with different concentrations of *K. coriacea* and *P. venusta* hydroalcoholic extracts (experimental group) and untreated (positive control).

For the treatment with *K. coriacea* it was possible to observe significant difference in the groups treated compared with the positive control and also between them. The difference was more pronounced in the group treated with the concentration of 1.0 mg/ml compared to positive control (Table 3).

In the experimental groups treated with *P. venusta* there was no significant difference between the group treated with 0.001 mg/ml and the positive control, but other treatments showed significant difference compared with the positive control and each other. Although the treatment with 0.01 mg/ml of *P. venusta* extract had differed statistically from the positive control, this difference was due to the greater number of tumors found in this experimental group, while the other experimental groups showed a significant reduction in the percentage of tumors found (Table 3).

About the inhibition of *A. tumefaciens*-induced tumors in potato disc tissue, the *K. coriacea* hydroalcoholic extract showed a lower percentage of tumors in all concentrations compared to the experimental group treated with *P. venusta*, therefore, presenting thus increased antitumor activity of extracts, according to the bioassay used in the experiment (Figure 2).

During the number of tumors evaluation in potato discs and the percentage determination of occupied area by the tumor in *pixel*^{2} was also possible to determine the antitumor action of the extracts used experimentally by observation of morphological characteristics and tumor prominences on the disks, which are noticeably smaller and less-developed in the treated groups compared to positive control, and also the absence of tumors and morphologic abnormalities in the negative control and in the extract treated groups, but without the addition of *A. tumefaciens* (Figure 3).

DISCUSSION

The experimental results of cytotoxicity for *K. coriacea* and *P. venusta* hydroalcoholic extracts in *A. salina* corroborate the work of McLaughlin et al. (1995), which demonstrated that the toxicity with *A. salina* shows good correlation with antitumor activity for substances with LC_{50}<1,0 mg/ml. On the other hand, a low toxicity can be considered an interesting feature for use plant extracts in natural (Ruiz et al., 2005; Lhullier et al., 2006; Silva et al., 2007; Nunes et al., 2008) and treatments with pharmacological purposes, such as cytotoxicity in human tumor cell lines for such products (Carballo et al., 2002).

The decrease of induced tumors in potato disc tissue treated with different concentrations of the *P. venusta* hydroalcoholic extract can be directly associated with the presence of an antitumor component in this extract, the β-sitosterol, as described by Raj and Katz (1984) and

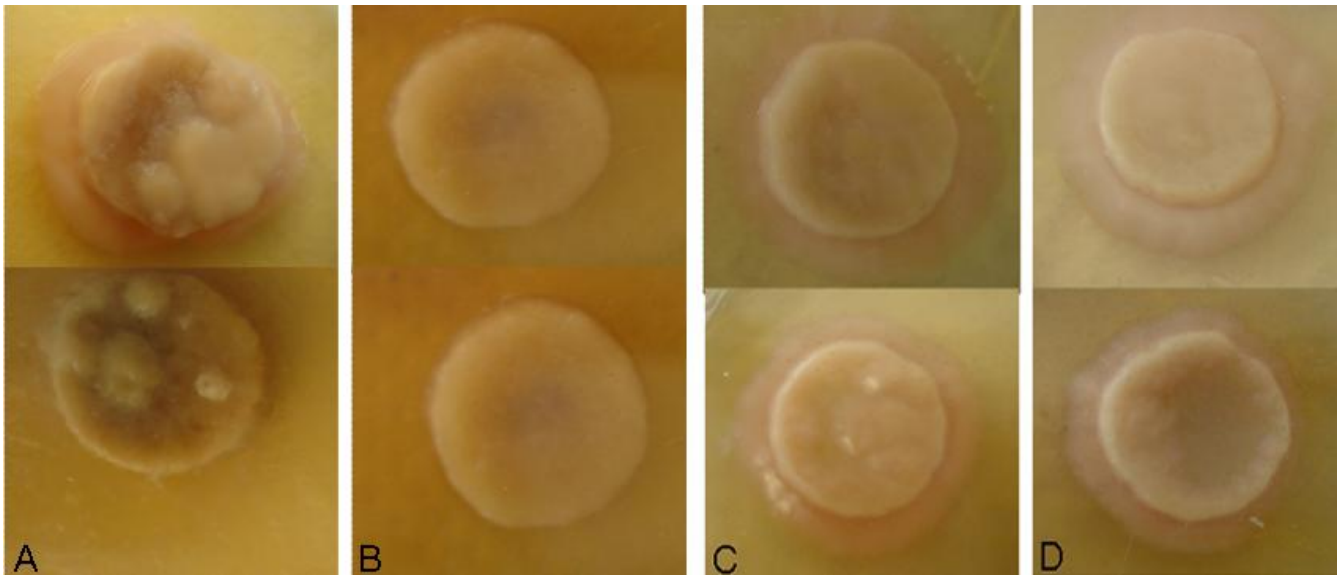

Figure 3. Morphological and development of *Agrobacterium tumefaciens*-induced tumors in potato disc tissue (A - positive control; B - negative control; C - Treatment with *Pyrostegia venusta* and D - *Kielmeyera coriacea*).

discovered in phytochemical analysis of *P. venusta* extracts performed by Dubey and Misra (1976).

The percentage of tumor decrease in the groups treated with *P. venusta* hydroalcoholic extract (0.1 and 1.0 mg/ml) were respectively 39 and 48% and when compared to positive control (PC). This reduction can also be associated with the presence of hesperidin in the *P. venusta* extract (Ferreira et al., 2000). Al-Majed et al. (2006) described the cytological and biochemical effects of this component, also present in extracts of valerian in somatic and germ cells of mammals.

The average percentage increase in tumor potato discs treated with the *P. venusta* extract concentration from 0.001 did not show statistically significant difference compared to the CP and the concentration of 0.01 showed an increase in the number of tumors per area of the examined discs. However, tumor flares, when evaluated morphologically, were lower than those of group CP, so the number of tumors was higher, but smaller in size than the positive control. This fact can also be seen in the work of Amara et al. (2008).

The treatment with *K. coriacea* in *A. tumefaciens*-induced tumors in potato disc tissue was possible to found a significant decrease at all concentrations (0.001, 0.01, 0.1 and 1.0 mg/ml) showing a decrease of 27, 70, 64 and 73% respectively. This decrease can be directly associated with phytochemical components such as xanthones, terpenes and biophenols that are important agents to the biological activities, including antitumor activity (Cortez et al., 1998; Cortez et al., 2002, Silva et al., 2009). And more recently, De Mesquita et al (2009) demonstrated significant cytotoxic action of *K. coriacea* realized *in vitro* studies using various cancer cell lines. Considering the experimental results obtained in this study it was concluded that *K. coriacea* and *P. venusta* hydroalcoholic extracts exhibit moderate cytotoxic activity and a significant antitumor activity according to the bioassays used in experiments.

ACKNOWLEDGEMENTS

The authors thank the laboratory technician José Gilberto Milani for collaboration during the performance of biological assays and the research foundation of the state of São Paulo (FAPESP) for financial support.

REFERENCES

Agrios GN (1997). Plant diseases caused by prokaryotes: bacteria and mollicutes. Plant Pathol., 4: 407–470.

Al-Majed AA, Al-Yahya AA, Al-Bekairi AM, Al-Shabanah OA, Qureshi S (2006). Studies on the cytological and biochemical effects of valerian in somatic and germ cells of Swiss albino mice. Food Chem. Toxicol., 44: 1830-1837.

Alves TMA, Silva AF, Brandão M, Grandi TSM, Smânia EF, Smânia A Jr, Zani CL (2000). Biological screening of Brazilian medicinal plants. Memórias do Instituto Oswaldo Cruz, 95: 367–373.

Amara AA, El-Masry MH, Bogdady HH (2008). Plant crude extracts could be the solution: extracts showing *in vivo* antitumorigenic activity. Pak. J. Pharm. Sci., 21(2): 159-171.

Aslani A, Smith RC, Allen BJ, Pavlakis N, Levi J (2000). A. The predictive value of body protein for chemoterapy-induced toxicity. Cancer, 88: 796-803.

Bent S, Ko R (2004). Commonly used herbal medicines in the United States: a review. Am. J. Med., 116: 478-485.

Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD (2002). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. BMC Biotechnol., 2: 1-5.

Coker PS, Radecke J, Guy C, Camper ND (2003). Potato disc tumor induction assay: a multiple mode of drug action assay. Phytomedicine, 10: 133–138.

Cortez DAG, Filho BAA, Nakamura CV, Filho BPD, Marston A, Hostettmann K (2002). Antibacterial activity of a biophenyl and xanthones from *Kielmeyera coriacea*. Pharm. Biol., 40: 485-489.
Cortez DAG, Ung MCM, Marston A, Wolfender JL, Hostettmann K (1998). Xanthones, triterpenes and a biphenyl from *Kielmeyera coriacea*. Phytochemistry, 47: 1367-1374.
De Mesquita ML, Paula JE, Pessoa C, Moraes MO, Costa-Lotufo LV, Grougnet R, Michel S, Tillequin F, Espindola LS (2009). Cytotoxic activity of Brazilian Cerrado plants used in tradicional medicine agaist câncer cell lines. J. Ethnopharmacol., 123: 439-335.
Diderot NT, Silvere N, Etienne T (2006). Xanthones as therapeutic agents: chemistry and pharmacology. Lead Mol. Nat. Prod., 2: 123-145.
Dubey RC, Misra K (1976). Chemical components of *Pyrostegia venusta* flowers. J. Indian Chem. Soc., 53: 378-381.
Ferreira GA, Gottlieb OR, Lins-Mesquita AA (1972). Xanthones from a Kielmeyera species. Phytochemistry, 11(4): 1512-1513.
Ferreira TD, Alvares PSM, Houghton PJ (2000). Constituintes químicos das raízes de *Pyrostegia venusta* e considerações sobre a sua importância medicinal. Química Nova, 23: 42-46.
Gottlieb OR, Lins AAM, Martins Da Silva E, Melo MT (1969). Xanthones of *Kielmeyera ferruginea*. Phytochemistry, 8(3): 665-666.
Hussar DA (2000). New drugs 2000, Part I. Nursing, 30: 55-62.
Ju YH, Clausen LM, Allred KF, Almada AL, Helferich WG (2004). Beta-sitosterol, beta-sitosterol glucoside and a mixture of beta-sitosterol and beta-sitosterol glucoside modulate the growth of estrogen-responsive breast cancer cells *in vitro* and in ovariectomized athymic mice. J. Nutr., 134: 1145-1151.
Lhullier C, Horta PA, Falkenberg M (2006). Avaliação de extratos de macroalgas bênticas do litoral catarinense utilizando o teste de letalidade para Artemia salina. Rev. Bras. Farmacogn., 16: 158-163.
Lorenzi H (2002). Árvores Brasileiras. Manual de Identificação e Cultivo de plantas Arbóreas Nativas do Brasil. Plantio direto e convencional, 1(4): 570.
Lorenzi H (1982). Plantas daninhas do Brasil; terrestres, aquáticas, parasitas, tóxicas e medicinais. p. 425.
Mclaughlin JL (1991). Grown gall tumors on potato discs and brine shrimp lethality: two single bioassays for plant screening and fractionation. Methods Plant Biochem., 6: 1–31.
Mclaughlin JL, Rogers LL (1998). The use of biological assays to evaluate botanicals. Drug Inf. J., 32: 513-524.
Mclaughlin JL, Colman-Saizarbitoria T, Anderson JE (1995). Tres bioensayos simples para quimicos de productos naturales. Revista de la Sociedad Venezolana Química, 18: 13-18.
Meyer BN, Ferrigini NR, Putnan JE, Jacobsen LB, Nichols DE, Mclaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Méd., 45: 31-34.
Ministério da Saúde (2011). Estimates of Cancer Incidence and Mortality in Brazil. Disponível em: <http://www.inca.org.br >. Accessed on: June 28, 2011.
Nunes XP, Mesquita RF, Silva DA, Lira DP, Costa VCO, Silva MVB, Xavier AL, Diniz MFFM, Agra MF (2008). Constituintes químicos, avaliação das atividades citotóxica e antioxidante de *Mimosa paraibana* Barneby (Mimosaceae). Rev. Bras. Farmacogn., 18: 718-723.
Park KY, Cho EJ, Rhee SH, Jung KO, Yi SJ, Jhun BH (2003). Kimchi and as active component, beta sitosterol, reduce oncogenic. H-Ras (v.12) induced DNA synthesis. J. Med. Food, 6: 151-156.
Raj AS, Katz M (1984). Corn oil and its minor constituents as inhibitors of DMBA-induced chromosomal breaks *in vivo*. Mutat. Res./Genet. Toxicol., 136(3): 247-253.
Ruiz ALTG, Magalhães EG, Magalhães AF, Faria AD, Amaral MCE, Serrano DR, Zanotti-Magalhães EM, Magalhães LA (2005). Avaliação da atividade tóxica em *Artemia salina* e Biomphalaria glabrata de extratos de quatro espécies do gênero Eleocharis (Cyperaceae). Rev. Bras. Farmacogn., 15: 98-102.
Sampietro DA, Vattuone MA, Isla MI (2006). Plant growth inhibitors isolated from sugarcane (*Saccharum officinarum*) straw. J. Plant Physiol., 163(8): 837-846.
Sela VR, Hattanda I, Albrecht CM, De Almeida CB, Obici S, Cortez DA, Audi EA (2009). Effect of xanthone from *Kielmeyera coriacea* stems on serotonergic neurons of the median raphe nucleus. Phytomedicine. online.
Silva FM, De Paula J, Espindola LS (2009). Evaluation of the antifungical potential of brazilian Cerrado medicinal plants. Mycoses, 52: 511-517.
Silva TMS, Nascimento RJB, Batista MM, Agra MF, Camara CA (2007). Brine shrimp bioassay of some species of *Solanum* from Northestern Brazil. Rev. Bras. Farmacogn., 17: 35-38.
Wattenberg L (1995). Chalcones, myo-inositol and other novel inhibitors of pulmonary carcinogenesis. J. Cell. Biochem., 22: 162-168.