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ANTITUMOR ACTIVITY of EXTRACTS of Caulerpa taxifolia in the SARCOMA 180 TUMOR TREATMENT

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ABSTRACT

Cancer is a disease characterized by the multiplication and spread out of control in the form of anomalous cells. A normal cell becomes cancerous cells as a result of one or more mutations of DNA. Marine algae are a rich source of bioactive compounds, some of which are precursors of pharmacological tools and potentially useful substances for the development of new drugs. The present study aims to evaluate the antitumor activity of Caulerpa taxifolia front of Sarcoma 180 in albino mice (Mus musculus). The transplanted animals with sarcoma 180 (n = 24), were divided into four groups (n = 6) treated with two extracts: the MeOH extract (T1) and from hydroalcoholic C. taxifolia (T2) at a dose of 50 mg/kg ip. The control group (S1) received saline for the same route of administration. The default group (S2) received the reference drug to the tumor lineage. The data revealed that the S3 and S4 group responded to treatment with reduction of 63.6 and 42, 0 % respectively, as compared to EN/PA, when compared with the control group. The activities of extracts were higher than those of the reference drug for the Sarcoma 180.

Keywords: phytotherapy, bioassay, bioactivity.

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Introduction

Cancer cells manifest to varying degrees, with four features that distinguish them from normal cells: uncontrolled proliferation, differentiation and loss of function, invasion and metastasis of power [1]. The growth of the malignant tumor is the result of the imbalance between proliferation capacity of your cells and the body's reaction to stop him [1-3].

Cancer is characterized by the uncontrolled multiplication and dissemination of abnormal forms of the body's own cells [4]. i.e. the existence of cells that undergo changes in the control mechanisms that regulate the ability of differentiation and cellular proliferation, tissue penetration, the compression of blood vessels and migrate to other places in the body where these uncontrolled cells can maintain their ability to proliferation and growth [2,5].

The term tumor is used to describe a volume increase of organ or tissue; already the word cancer is intended to describe an invasive neoplasm. Neoplasm and tumor can refer to benign and malignant growths, while the term always cancer refers to malignant growth. The benign neoplasms, commonly, do not invade surrounding tissue or spread to other organs, are able to be treated and is unlikely to lead the patient to death. On the other hand, the evil, when not treated, invade and spread throughout the body through the Mets, with a high chance of getting the patient to death [2.3]. Currently, in the United States of America, one in every four deaths by diseases is due to cancer [6]. This being considered the second leading cause of death in the world, behind only of deaths due to cardiovascular problems [5].

The malignant neoplasms are among the leading causes of mortality, corresponding to the second most common cause of death from natural causes. According to the data of records of cancer in Brazil, the most frequent are tumors of the prostate, lung, stomach, colon, rectum and oesophagus in the male population. Women breast cancer predominates, followed by cancers of the cervix, colon and rectum, lung and stomach [3,7,8].

In Brazil, the mortality from neoplasms has been growing considerably over the last few decades. In 2008, according to the information of mortality, according to the Group of causes (ICD10), neoplasms (tumors) represented the second most common cause of death in the population (except the "Other causes set"), which represents more than 14.6% of total deaths in the country [3].

Marine algae are a rich source of bioactive compounds potentially useful for the development of new drugs [9]. *Caulerpa taxifolia* are rich in caulerpine, which presents a broad spectrum of biological activities, such as the antioxidant and antitumor activity [10,11]. In the face of the present study aims to evaluate the antitumor activity of seaweed *C. taxifolia* in Swiss albino mice (*Mus musculus*) patients with sarcoma 180.

Materials and methods

Obtaining the extracts

Samples of seaweeds *C. taxifolia* has been collected at the beach of Barra do Sirinhaém, municipality of Sirinhaém, State of Pernambuco, Brazil. Were stored in polyethylene bags being conducted at the laboratory of cellular communication-UFPE. The extraction procedures used in this study were designed according to methodology of [12, 13].

Samples of biological material after cleaning procedures and accommodation were pounded into mechanical mill, later being the material made available to the extraction procedures. Then, a sample of 500 g of this material has been added to a volume of one litre of ethanolic solution to 50%, getting in maceration at room temperature for a period of one week. After this step, the material was filtered on filters and route paper evaporated in At low pressure model 4000 rpm the Laborota Heidolph ®.

In addition, a 500 g sample of the biological material, properly processed and crushed in similar conditions in relation to previous extract was added to a volume of one litre of methanol for subsequent maceration at room temperature for a period of one week. After this step, the material has been filtered with paper filters, and evaporated in rotaevaporator model 4000 rpm the Laborota Heidolph ®.

Experimental animals

The animals used in this activity were albino Swiss mice (*Mus musculus*), with approximately 60 days of born weighing between 25 and 30, kept under controlled conditions of illumination (cycle of 12 hours dark/light) and temperature (22 ± 2° C), in polypropylene cages getting specific feed and water ad libitum.

Antitumor activity

Tumor cells lineage ascites sarcoma 180, with eight days after implantation, were sucked from the peritoneal cavity of mice and implanted subcutaneously (0.2 mL-25 x 106 cells/mL), in subaxilar region of experimental animals (n = 6). The 24 hours after implantation, the extract was solubilized in tween 80 (Sigma-Aldrich ®) (5%) and administered by intraperitoneal route for seven days, in doses of 50 and 100 mg/kg. Cyclophosphamide (10 mg/kg) was used as positive control. The negative control group was administered a solution of Tween 80 (MONTENEGRO, 2008), still used a fifth group (sound control), the animals (n = 6) remained under the same experimental conditions of treated and control groups, but have not been transplanted with the tumor cells (sarcoma 180) on the ninth day all the animals were euthanized and the tumors excised, weighed and fixed in formaldehyde (10 %). Tumor inhibition was calculated according to the formula:

TWI (%) = (C-T) x 100/C Where:

TWI = percentage of inhibition of the tumor weight

C = average of the weights of the tumors in the control group T = average of the weights of the tumors of the treated group.

The tests were carried out according to the methodology described by [15].

Biochemical and hematological parameters evaluation

On the ninth day of the experiment, after fasting for 6 hours, the animals were anesthetized with sodium thiopental (Thiopentax®, crystal clear) and blood samples were collected by the orbital Plexus with a heparinized needle.

For the analysis of biochemical and Hematological parameters (glucose, urea, creatinine, bilirubin, AST and ALT) blood was subjected to centrifugation for 10 minutes at 3500 rpm for obtaining plasma. These parameters were determined using specific kits for the automatic biochemical Analyzer Cobas Mira Plus ® (Roche Diagnostic System).

Analyze histological

The animals were euthanized and the organs and tumors removed, heavy and fixed in 10 % formaldehyde for 24 hours, washed with distilled water and processed in increasing concentrations of alcohol (70 %, 80 %, 90 % and 100 %), soaked in paraffin and stained to the technique Hematocilin-eosin (HE) for subsequent microscopic analysis [16].

Microscopic analyses were conducted binocular microscope model AC-L1000b Kinon ® brand, and photographs of histological sections held with digital camera in Samsung ® brand model PL120 14.2 MP 5 x Optical Zoom.

Statistical analysis

For the three experiments were carried out tests in triplicate. The results obtained in the experiments were analyzed employing – if the test analysis of variance (ANOVA) one way, followed by the *Tukey* test where the values are expressed as mean ± standard error of media (e.p.m), and the results considered significant when $p < 0.05$. [17].

Results

On the relationship between the tumor weight and the weight of the animal (EN/PA) of animals with Sarcoma 180 groups showed that the treated group 1 (S3) showed an average of 0.0376 (table 1) and when compared with the Control Group's average value (S1), which was the Group S3 0.1032 presented a 63.6 % lower value than the control group. Compared treated group 2 (S4), which featured an average of 0.0599, and the control group, the S4 group presented an average 42.0 % smaller in proportion to PT/PA. The average value of the default group, which was when 0.0700 related to the control group, presented 32.2 % average less than this.

Table 1. Averages and standard deviations of the animals ' weights ratio (PA) (*Mus musculus*) of Sarcoma 180 (EN) and PT/PA relations of control groups (S1) (control with saline 0.9 via ip), standard (S2) 10 mg/kg/Cyclophosphamide ip, treated with meoh extract of c. taxifolia at a dose of 50 mg/kg/ip (S3), hydroalcoholic extract of *C. taxifolia* at a dose of 50 mg/kg/ip (S4).

Grupos	P A	PT	PT/PA
S1 – Control	32,0 ± 0, 2	3,305 ± 0,002	0,1032 ± 0,05
S2 – Padrão	35,0 ± 0, 03	2, 452± 0,002	0,0700 ± 0,01
S3- Treated 1	34,72 ± 0,3	1,305± 0,002	0,0376**± 0,001
S4 – Treated 2	30,3± 0, 4	1,814* ±0,002	0,0599** ± 0,002

Significant difference relative to the control (ANOVA followed by *Tukey* test **).

Table 2. The averages and standard deviations of reason between the biochemical and Hematological data obtained by colorimetric method described in Frankel and Reitman (1957) (*Mus musculus*) in groups with Sarcoma 180 of control groups (S1) (control with saline solution 0.9 via ip), standard (S2) 10 mg/kg/Cyclophosphamide ip, treated with meoh extract of c. taxifolia at a dose of 50 mg/kg/ip (S3) hydroalcoholic extract of *C. taxifolia* at a dose of 50 mg/kg/ip (S4).

Grups	Glucose	Urea	Creatinine	Bilirrubin	TGO	TGP
S1 – Control	106,3 ± 4,6	45,9 ± 2,3	0,58 ± 0,02	1,3 ± 0,2	84,6 ± 4,4	50,6 ± 6, 7
S2 – Padrão	103, ± 2,4	49,0 ± 1,3	0,61 ± 0,06	1,2 ± 0,3	77,3 ± 5,8	51,9 ± 4,6
S3 – Treated 1	99,6±5,2	42,5 ± 3,5	0, 79± 0,04	1,2 ± 0,3	73,6 ± 3,6	49,3 ± 10,5
S4 Treated 2	102,1± 3,2	48,4±4,2	0,68 ±0,08	1,3±0,4	82,6 ± 3,8	47,4 ± 4,3

The reference value for mice: glucose = 100; Urea = 48.0; creatinine = 0.5 IU; Bilirubin = 0.9; TGO = 80 IU; TGP = 50 IU (DANTAS et al., 2006).

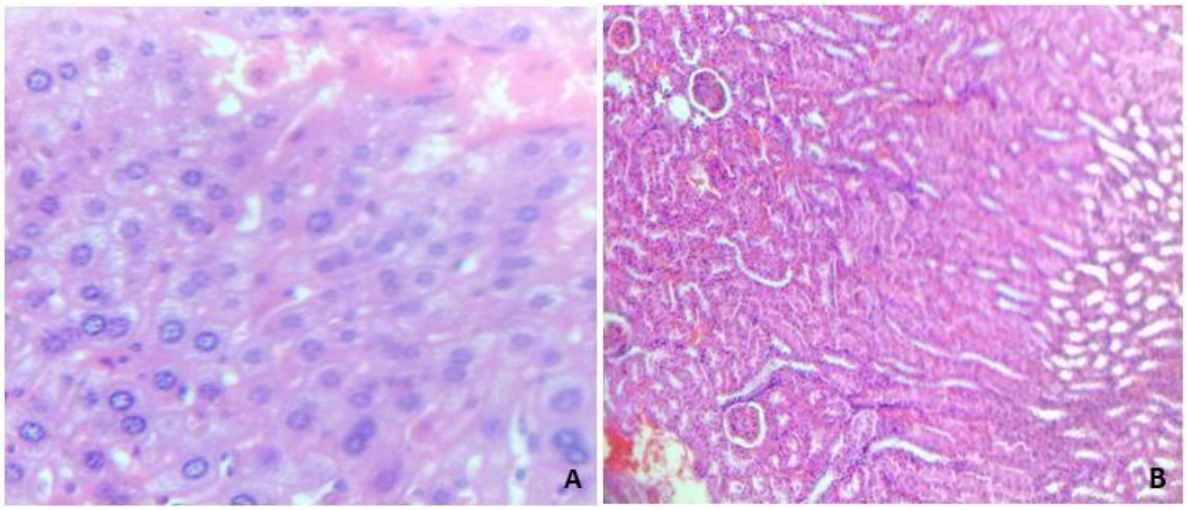

Figure 1. Hepatic Tissue (A) and (B) renal Tissue stained in HE observed in optical microscope 10 x.

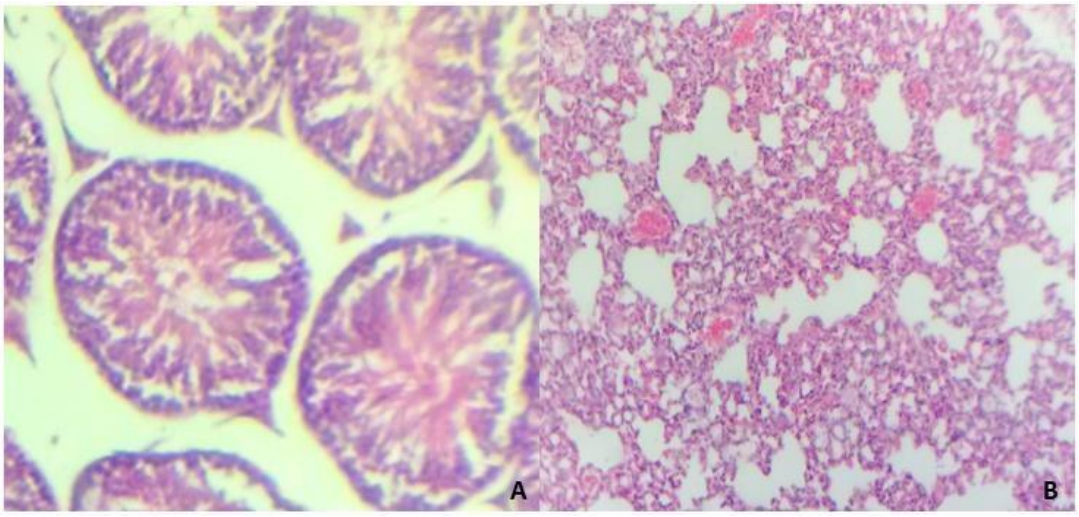

Figure 2. Histological Cut of the testis and lung (B) stained in HE observed in optical microscope 10 x.

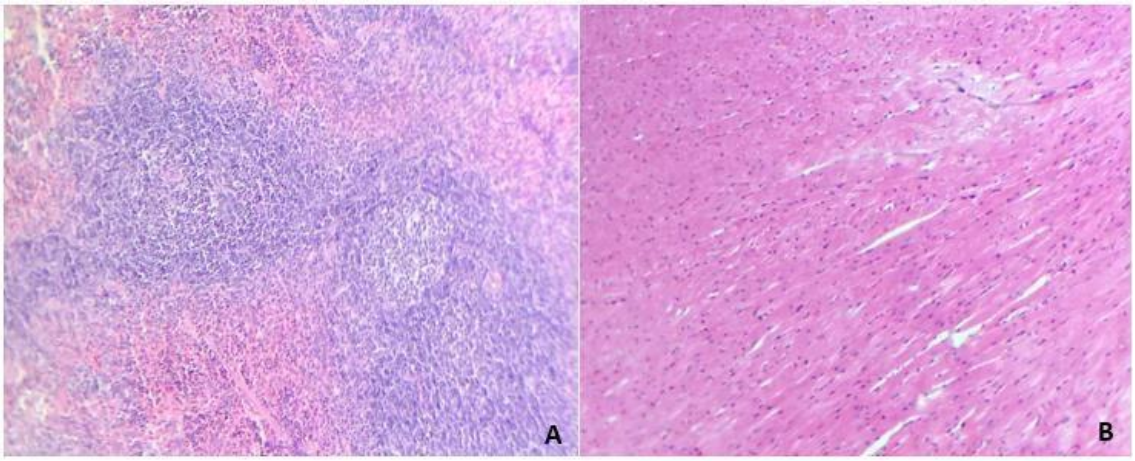

Figure 3. Histological Cut of splenic tissue (A) and (B) heart stained in HE observed in optical microscope 10 x.

The serum values of glucose, urea, bilirubin, SGOT and SGPT to all groups studied were within the reference levels for mice, as can be seen in table 2; the creatinine levels were higher than those of reference were. Both the MeOH extract as the hidroalcoholic extract showed significant reduction in tumor mass, when compared to control groups, with results superior to that achieved with the recommended dose of the drug for sarcoma. Several works involving preclinical in vivo studies of natural products, using biochemical parameters to evaluate possible signs of toxicity [18, 19, 20, 21, 22, 23].

Pharmacological/toxicological studies these after exposure to drugs, are analyzed the parameters to assess possible changes in liver function as through the levels of TGP and TGO, kidney function as urea and creatinine.

Analysis of hepatic and renal tissues present preserved morphology (Figure 1). The hepatocytes and liver sinusoids with preserved architecture, however was observed the presence of bleeding points in the fabric. The renal system does not seem to be affected by the extract (Group T1), not being observed changes in cortical and Medullary regions of these individuals, structures like the contorted twitch of the proximal and distal tubules clusters showed normal anatomy.

The microscopic Anatomy of the gonads of the treated animals was viewed Sertoli cell and spermatocytes, as well as interstitial tissue and Leydig cells without morphological change (Figure 2 - A). The lungs and the pulmonary artery branches within the standard of normality. It was viewed that the structure of the respiratory epithelium (Figure 2 - B).

Spleen presenting cells of the white pulp red pulp, trabecula, corona, germinal Center and arterioles of preserved morphology (Figure 3 - A), heart tissue showing interleaved disk, mononucleated with core compatible with normality (Figure 3 - B).

Discussion

According to the results, we can suggest that the extract used in Group T1 presented in front of the Sarcoma 180 antitumor activity with 95 % higher than the activity presented by reference drug to the tumor lineage. When compared to the activity of the drug administered to the default group (S2) and extract administered in Group T2, this presented an activity 31 % higher than the reference drug on tumor lineage.

The values of glucose, urea, bilirubin, SGOT and SGPT to all groups studied were within the reference levels for laboratory animals, as can be seen in table 2, creatinine levels already showed higher values of reference. The biochemical parameters such: glucose, urea, creatinine and bilirubin of the treated group showed similar values to the control groups and default, which became one of the reference values for mice.

TGO levels already and TGP suffered a significant reduction in the group treated with hidroalcoholic extract, which showed a significant reduction in tumor mass, when compared to control groups, having better results than achieved with the recommended doses of the drugs. Several works involving preclinical in vivo studies of natural products, using biochemical parameters to evaluate possible signs of toxicity [18, 19, 20, 21, 22, 23]. Pharmacological/toxicological studies these after exposure to drugs, are analyzed the parameters to assess possible changes in liver function as through the levels of TGP and TGO, kidney function as urea and creatinine.

In analysis of antitumor activity, in this study, it was a better performance of the MeOH extract of *C. taxifolia*, front line of Sarcoma 180, that reduction of 63.6 % in PT/PA relationship, while the hidroalcoolic 42.0 % reduction extract, and this may be the activities of secondary metabolites present in the extract, being the MeOH extract what drag as many secondary metabolites contained in the plant. The MeOH extract crude indicated the presence of alkaloids, terpenoids, steroids and saponins

[24]. Alkaloids that have anticancer activity belong to a new class of bisindolic's derivatives, which may suggest that the anticancer activity of *C. taxifolia* relates the alkaloids present in the extract [25]. An indolic alkaloid derived from tryptophan, off isolated from species of the genus Caulerpa considered toxic. Morphological changes were not observed in animals treated with studied extracts, which can characterize the secondary metabolites contained in the extracts at studied, have little or no side effects [26,27].

References

1. Goodman LS, Gilman AG. As bases farmacológicas da terapêutica. edited by Guanabara, Rio de Janeiro, RJ: McGrawHill, 2012,pp.85-123.
2. INCA – Instituto Nacional de Cancer, Rio de Janeiro, 2012, 2ª edição revista e atualizada. Disponível em:<http://bvsms.saude.gov.br/bvs/publicacoes/inca/ABC_ do cancer_2ed.pdf.>. Acesso em: 10 de maio de 2014.
3. INCA. Fisiopatologia do câncer. Disponível em: <http://www.inca.gov.br/enfermagem/docs/cap2.pdf.> .Acesso em: 16 de junho de 2014.
4. Silva AER, Fiori ATM, Matsumoto C, Cruz A, Silva NMO, Bernardes ACA, Carvalho RP, Mazzola PG. Levantamento bibliográfico de antineoplásicos padronizados no Hospital da Mulher – Prof. Dr. José Aristodemo Pinotti (CAISM) UNICAMP In: Congresso dos Profissionais das Universidades de São Paulo, São Paulo: USP, Unicamp e Unesp, 2011.
5. Oliveira JS, Costa-Lotufo LV, Bezerra DP, Alencar NM, Marinho-Filho JD, Figueiredo IS, Moraes MO, Pessoa C, Alves AP, Ramos MV. In vivo growth inhibition of Sarcoma 180 by latex proteins from Calotropis procera. Naunyn Schmiedeberg's Archives of Pharmacology. 2010; 382(2):139-49.
6. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics. CA Cancer J. Clin.2007; 57(1):39-43.
7. Guerra MR, Gallo CVM, Mendonça G. Risco de câncer no Brasil: tendências e estudos epidemiológicos mais recentes. Revista Brasileira de Cancerologia.2005; 51(3):227-34.
8. Santos 2011
9. Natali ALC. Ação inotropica positiva do extrato metanolico de Bryopsis pennata (CHLOROPHYTA CAULERPALES). Dissertação (Metrado em Biociencia) Universidade de São Paulo. 2007.
10. Hay ME, Fenical W. Marine plant-herbivore interactions. The ecology of Chemical defense. Ecol. Syst, 1988; 19(1):111-45.
11. Solè-Cava AM, Kelecom A. Dialogo quimico dos mares, Ciência Hoje. 1988; 8(46):18-29.
12. Matos FJA, Oliveira F. Lippia sidoides Cham. Rev. Bras. Farmacognosia. 1998;79(1):84-87.
13. Di Stasi LC: Plantas medicinais: arte e ciência. Edited by Unesp. São Paulo, 1996,p. 129-56.
14. Geran RI, Greenberg NH, Mcdonald MM, Schumacher AM, Abbot BJ. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemoth.1972; 33(1):1–103.
15. Geran RI, Greenberg NH, Mcdonald MM, Schumacher AM, Abbot BJ. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemoth.1972; 33(1):1–103.
16. Robbins SL, Angell M, Kumar V. Patologia básica, Editora da Universidade de São Paulo.1984 Nov; 16(1):577-625.
17. Morettin LG. Estatística Básica: Probabilidade e Inferência. São Paulo:Pearson Prentice Hall.2010; 1(1): pp1-376.
18. Bezerra D P, Castro FO, Alves AP, Pessoa AC, Moraes MO, Silveira ER, Lima, MA, Elmiro FJ, Alencar NM, Mesquita RO, Lima MW, Costa-Lotufo LV. In vitro and in vivo antitumor effect of 5-FU combined with piplartine and piperine. Journal of Applied Toxicology. 2008; 28(2):156-63.
19. Bezerra DP, Marinho-Filho, FD, Alves AP, Pessoa C, Moraes MO, Pessoa OD, Torres MC, Torres MC, Silveira ER, VIANA FA, Costa-Lotufo LV. Antitumor activity of the essential oil from the leaves of Croton regelianus and its component ascaridole. Chemistry & Biodiverity.2009; 6(8):1224-31.
20. Gonzaga ML, Bezerra DP, Alves AP. In vivo growth of Sarcoma 180 by analpha-(14)-glucan(16)- glucan-protein complex polyccharide obtainned from Agaricus blazei Murrill. J.Nat.Med. 2009; 63(1):32-40.
21. Lins KO, Bezerra DP, Alves AP, Alencar NM, Lima MW, Torres VM, Pessoa C, Moraes, MO, Costa-Lotufo LV. Antitumor properties of a sulfated polysaccharide from the red seaweed Champia feldmannii (Diaz-Pifferer). Jornal of Applied Toxicology.2009;29(1):20-6.
22. Magalhães HI, Ferreira PM, Moura ES, Torroes MR, Alves AP, Pesso OD, Costa-Lotufo LV, Moraes, MO, Pessoa C. In vitro and in vivo

antiproliferative activity of Calotropis procera stem extracts. Anais da Academia Brasileira de Ciências. 2010; 82(2):407-16.
23. Oliveira-Júnior RJ. Análises citogenéticas e expressão da telomerase em Sarcoma 180. Dissertação (Mestrado em Genética e Bioquímica) – Universidade Federal de Uberlândia, MG. 2008.
24. Moura CA, Silva EV, Cavalcanti KPS, Anjos FBR, Jimenez GC, Oliveira SF, Souza IA. Perfil Fitoquímico da Macroalgas Marinha *Caulerpa taxifolia* (VAHL) C.AGARDH, 1817 (caulerpaceae) Congresso Brasileiro de Oceanografia, Rio de Janeiro, Nov. 2012.
25. Robbins SL, Cotran RS. Patologia: Bases – Patologicas das doenças, ed.Elsevier,Rio de Janeiro, 2000, 7:1592.
26. Aguilar-Santos G, Chem J, SOC C. Chemical studies on three species of marine algal genus Caulerpa. In. Froedenthal, H.D. (ed.) Drugs from the sea. Marine Technology Society.1970; 84(2): 173-76.
27. Maiti BC, Thamson RH, Mahendran M. The structure of caulerpina, a pigment from Caulerpa algae. J. Chem. 1978 May; 34(5):126-27.