

Biological potential of plants from the genus *Bauhinia*

Potencial biológico de las plantas del género *Bauhinia*

Pharmacist Iuri de França Bonilha, Pharmacist Camila Helena Ferreira Cuelho, Pharmacist Juliana Calil Brondani, Pharmacist Jocelene Filippin Cossetin, Forest Engineer Lucas Damo Marangoni, PhD Melânia Palermo Manfron

Phytochemical Research Laboratory, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazi

ABSTRACT

The genus *Bauhinia* (*Fabaceae*, *Leguminosae*) consists of approximately 300 species, which are commonly known as 'cow's paw' or 'cow's hoof', because of the shape of their leaves. They are widely distributed in most tropical countries, including Africa, Asia and South America. Their leaves and stem-bark have been used frequently in folk medicine as a remedy for different kinds of disease, particularly diabetes, infections, pain and inflammatory processes. *Bauhinia longifolia* flavonoids demonstrated the highest antiviral activity of all tested substances, being that quercetin had the highest antiviral activity amongst purified flavonoids. The aim of this article is to summarize concisely recent advances published in the last 5 years on the most recent works about the biological activities and phytochemical composition of plants of the genus *Bauhinia*. For this, was performed a search in some of the databases on the web as PubMed, Google Scholar and Medline, using the keywords *Bauhinia*, biological properties, diabetes, cancer.

Keywords: *Bauhinia* , biological properties, diabetes, cancer.

RESUMEN

El género *Bauhinia* (*Fabaceae*, *Leguminosae*) consta de aproximadamente 300 especies, que se conocen comúnmente como "pata de vaca" o "pezuña de

vaca, debido a la forma de sus hojas. Están ampliamente distribuidos en la mayoría de los países tropicales, incluyendo África, Asia y América del Sur. Sus hojas y el tallo de corteza se utilizan con frecuencia en la medicina popular como un remedio para diferentes tipos de enfermedades, en particular la diabetes, infecciones, dolor y en los procesos inflamatorios. Los flavonoides de *Bauhinia longifolia* demostraron la mayor actividad antiviral de todas las sustancias analizadas, y es la quercetina la de mayor actividad antiviral entre los flavonoides purificados. El objetivo de este artículo fue resumir los trabajos publicados en los últimos cinco años sobre la actividad biológica y la composición fitoquímica de las plantas del género *Bauhinia*. Para ello, se realizó una búsqueda en algunas de las bases de datos en la web como *PubMed*, *Google Scholar* y *Medline*, usando las palabras clave *Bauhinia*, actividad biológica, diabetes, cáncer.

Palabras clave: *Bauhinia*, actividad biológica, diabetes, cáncer.

INTRODUCTION

Medicinal plants and their derivatives consisted for a long time the basis of therapeutics and, currently, about 25 percent of the drugs used are of vegetable origin, while 50 % are of synthetic origin, but isolated principles related to medicinal plants.^{1,2} This is due in part to the wide variety of species (250 000–500 000) of plants existing in the world flora, many with important therapeutic properties.³ In recent years, there has been a large increase in the studies that prove what is known empirically, since folk medicine is rich in examples of plants used for various purposes, which replace often the prescription.⁴⁻⁸ It is believed that approximately 80% of the world population use plants like first therapeutic resource.^{9,10}

The genus *Bauhinia* (*Fabaceae*, *Leguminosae*) consists of approximately 300 species, which are commonly known as 'cow's paw' or 'cow's hoof', because of the shape of their leaves. They are widely distributed in most tropical countries, including Africa, Asia and South America. Their leaves and stem-bark have been used frequently in folk medicine as a remedy for different kinds of pathologies, particularly diabetes, infections, pain and inflammatory processes.^{7,11,12}

In recent years, interest in these plants has increased considerably throughout the world, especially in Brazil, since experimental studies have confirmed their reported therapeutic properties. The biological properties of different *Bauhinia* spp. phytopreparations and pure metabolites have been investigated in numerous experimental *in vivo* and *in vitro* models. Although some contradictory evidence has been reported, in general the results support the reported therapeutic properties, indicating that these are due mainly to the presence of flavonoids. Additional components include terpenes, steroids, aromatic acids, quinones, lactones and alkaloids, among others.^{11,13}

The aim of this article is to summarize the most recent works published in the last 5 years about the biological activities and phytochemical composition of plants of the genus *Bauhinia*. For this, was performed a search in some of the databases on the web as *PubMed*, *Google Scholar* and *Medline* using the keywords *Bauhinia*, biological properties, diabetes, cancer.

Bauhinia forficata

Bauhinia forficata Link is one of the most studied species in Brazil, because of its broad popular use as an aid in the treatment of diabetes and also for its wide geographic distribution.^{14,15} It is popularly known as 'cow paw' due to the characteristic bilobulated aspect of its leaves.¹⁶

The infusion has been used as a diuretic, a tonic, a cleanser, to combat elephantiasis, to control hypoglycemia and reduce glycosuria.¹⁷ In fact these two last properties, related to diabetes, have drawn the attention of researchers. In this sense, pharmacological studies were performed on the leaf extracts (aqueous and alcoholic) in order to verify the antidiabetic activity in different *in vivo* models.^{16,18-21}

B. forficata has been widely studied biologically. Its chemical composition has recently attracted attention, in particular due to the isolation of kaempferol-3,7-O-(alpha)-dirhamnoside (kaempferitrin), a flavonoid shown to occur only in the leaves of the plant and, consequently, one that can be used as a chemotaxonomical marker.²² The therapeutic potential of this plant was first confirmed in 1929, with the clinical studies of Juliani (1929) which showed the ability of *B. forficata* to lower blood sugar.²³ Later, the antidiabetic potential of the plant was confirmed in dogs, humans and rabbits.^{24,25}

Pepato *et al* demonstrated that a 1 month administration of an aqueous decoction of the fresh leaves of the plant to streptozotocin-induced diabetic rats, lowered the levels of serum and urinary glucose and urinary urea, compared with untreated diabetic rats.²⁰ A similar investigation revealed a significant blood-glucose lowering action in normal and diabetic rats.²⁶ Lino *et al.* showed that aqueous, ethanol and hexane extracts of *B. forficata*, evaluated in a model of alloxan-induced diabetes in rats, reduced glucose, triglycerides, total cholesterol and HDL-cholesterol levels, validating the clinical use of this plant for the treatment of diabetes mellitus type II.²⁷

Other study investigated the hypoglycemic activity of the dried extracts of *B. forficata* leaves *in vivo*, as well as the influence of the drying and granulation processes on this activity. The fluid extract was dried to generate oven-dried (ODE), spray-dried (SDE) and wet granulation (WGE) extracts. Each dried product was administered orally to male *Wistar* rats over 7 days old, for biomonitoring of the hypoglycemic activity profile. The effect of the extracts was studied in streptozotocin-induced diabetic rats. After 7 days of treatment, fasting glucose was determined, and the livers were removed, dried on tissue paper, weighed, and stored at -20 °C to estimate hepatic glycogen. The results showed that spray-drying or oven-drying processes applied to *B. forficata* extracts did not significantly alter its flavonoid profile or its hypoglycemic activity. Indeed, the dried extracts of *B. forficata* act differently from glibenclamide. Despite the lower active content in WGE, because of the higher concentration of adjuvants, the use of the granulation process improved the manufacturing properties of the ODE, making this material more appropriate for use in tablets or capsules.¹⁶

Recently, was evaluated the effects of *B. forficata* leaf tea on markers of oxidative damage and antioxidant levels in an experimental model of hyperglycemia in human erythrocytes *in vitro*. Human erythrocytes were incubated with high glucose concentrations or glucose and *B. forficata* tea for 24 h and 48 h. After incubation, lipid peroxidation and non-protein SH levels were analyzed. Moreover, quantification of polyphenols and flavonoids, iron chelating property, scavenging of DPPH, and prevention of lipid peroxidation in isolated lipids were also assessed. *B.*

forficata tea presented important antioxidant and chelating properties. Moreover, the tea was effective to increase non-protein SH levels and reduce lipid peroxidation induced by high glucose concentrations in human erythrocytes. The antioxidant effects of tea could be related to the presence of different phenolic and flavonoids components. Probably these components can be responsible to protect human erythrocytes exposed to high glucose concentrations against oxidative damage.²⁸

In plants, cysteine proteinases are widely distributed in various tissues, and are involved in physiological events such as germination, senescence and environmental stress responses. Andrade *et al.* described the purification and the functional characterization of a new cysteine proteinase from *B. forficata* leaves. By acetone precipitation and different chromatographic steps an endoproteinase named baupain detected in the *B. forficata* leaves was purified to homogeneity. While the N-terminal sequence similarity, molecular mass, circular dichroism spectra and intrinsic fluorescence profiles pointed to a close structural relationship to papain, its activity on fluorescence resonance energy transfer (FRET) and methylcoumaryl-amide (MCA) peptides indicates substrate specificity more related to the mammalian cathepsin L enzyme. The proteinase also shared with cathepsin L and papain the capacity of releasing bradykinin from human high molecular weight kininogen (HMWK). This work pointed out the kininogenase activity specificity profile of baupain that is distinct from other cysteine proteinases and similar to cathepsin L. This property may be relevant to regroup cluster papain-like cysteine proteinases.²⁹

In another study was ascertained the authenticity of two certified and two commercial *B. forficata* samples. Different flavonoids profiles were obtained, involving 39 compounds. Just kaempferol-3-O-(2-rhamnosyl) rutinoside was found in all analysed samples. Five compounds were common to the certified samples of *B. forficata* Link and *B. forficata* Link subsp. *pruinosa* (Vogel) Fortunato & Wunderlin, being kaempferol derivatives the most representative ones. The phenolic composition of *B. forficata* Link subsp. *pruinosa* (Vogel) Fortunato & Wunderlin is described herein for the first time, accounting for eight compounds, while 10 new compounds were identified in *B. forficata* Link. Commercial *B. forficata* Link showed higher contents of quercetin derivatives, in addition to the presence of myricetin derivatives and flavonoids-(galloyl)glycosides, for which the mass spectrometry fragmentation pattern is reported for the first time. *B. forficata* Link and the two commercial samples were able to inhibit α -glucosidase, with EC₅₀ values lower than that found for acarbose. Mild effects on cholinesterases were observed with the certified samples, while commercial ones were more effective. The same behavior was observed concerning the scavenging of DPPH, nitric oxide and superoxide radicals. The presence of high contents of quercetin derivatives in commercial samples seems to directly influence their biological properties.³⁰

Lectins are proteins or glycoproteins of non-immunogenic origin that specifically and reversibly bind to different types of carbohydrates or glycoproteins.³¹ Lectins exhibit many biological activities, including anti-insect,³² antifungal,³³ antiviral,³⁴ antibacterial,^{35,36} antiproliferative,³⁷ antiplatelet aggregating,³⁸ anti-inflammatory and analgesic activities.³⁹ A new lectin, BfL, was purified from *Bauhinia forficata* seeds by ammonium sulfate fractionation, DEAE-Sephadex ion exchange chromatography, Sepharose-4B and chitin affinity chromatographies and Superdex 75 size exclusion chromatography. The molecular homogeneity and purity of BfL were assessed by reversed-phase HPLC. BfL appeared as a single band of approximately 27,0 kDa on SDS-PAGE under non-reducing and reducing conditions, and its molecular weight was determined to be 27,850 Da by LC/ESI-MS. BfL is a glycoprotein with a carbohydrate content of 6.24 % determined by the phenol-sulfuric acid method. Fetuin, asialofetuin, thyroglobulin and azocasein

inhibited the hemagglutinating activity of BfL, whereas saccharides did not. BfL hemagglutinating activity was stable at 100 °C for 30 min, pH-dependent, with the highest activity at pH 6,0, and metal-independent. The primary structure of BfL shows similarity with other lectins from the genus *Bauhinia*. Deconvolution of the BfL circular dichroism (CD) spectrum indicated the presence of α -helix and β structures. BfL increases coagulation time, but this effect is not related to human plasma kallikrein or human factor Xa inhibition. BfL also inhibits ADP- and epinephrine-induced platelet aggregation in a dose-dependent manner and is the only currently described lectin from *Bauhinia* that exhibits anticoagulant and antiplatelet aggregating properties.⁴⁰

In a recent study, BfL inhibited the viability of the MCF7 breast cancer cell line but was ineffective on MDA-MB-231 and MCF 10A cells. It inhibits MCF7 adhesion on laminin, collagen I and fibronectin, decreases α 1, α 6 and β 1 integrin subunit expression, and increases α 5 subunit expression. BfL triggers necrosis and secondary necrosis, with caspase-9 inhibition. It also causes deoxyribonucleic acid (DNA) fragmentation, which leads to cell cycle arrest in the G2/M phase and a decrease in the expression of the regulatory proteins pRb and p21. BfL showed selective cytotoxic effect and adhesion inhibition on MCF7 breast cancer cells.⁴¹

The cytotoxic and antimutagenic potential of aqueous extracts of *B. forficata* on bone marrow cells of *Wistar* rats treated *in vivo* was evaluated. In this study was observed that the aqueous extracts of *B. forficata*, which are routinely used for the treatment of pain and diabetes, have considerable antioxidant activity, showed no cytotoxic activity, and may contribute to reducing the chromosomal damage induced by such chemotherapeutic agents as cyclophosphamide. Thus, the consumption of these plants can bring added benefits and protection to individuals undergoing treatment with cyclophosphamide or who use them for therapeutic purposes, improving their quality of life and health.⁴²

Some studies have proposed the use of natural compounds with antioxidant properties against involuntary movements induced by antipsychotics. Peroza *et al* examined the possible antioxidant activity of *B. forficata* on brain lipid peroxidation induced by different pro-oxidants. *B. forficata* prevented the formation of lipid peroxidation induced by both pro-oxidants tested. However, it was effective against lipid peroxidation induced by sodium nitroprusside ($IC_{50}=12,08 \mu\text{g/mL}$) and $Fe^{2+}/EDTA$ ($IC_{50}=41,19 \mu\text{g/mL}$). Moreover, the effects of *B. forficata* were analyzed on an animal model of orofacial dyskinesia induced by long-term treatment with haloperidol, where rats received haloperidol each 28 days (38 mg/kg) and/or *B. forficata* decoction daily (2,5 g/L) for 16 weeks. Vacuous chewing movements (VCMs), locomotor and exploratory activities were evaluated. Haloperidol treatment induced VCMs, and co-treatment with *B. forficata* partially prevented this effect. Haloperidol reduced the locomotor and exploratory activities of animals in the open field test, which was not modified by *B. forficata* treatment. The present data showed that *B. forficata* has antioxidant potential and partially protects against VCMs induced by haloperidol in rats. Taken together, these data suggest the protection by natural compounds against VCMs induced by haloperidol in rats.⁴³

Bauhinia longifolia

In a recent study conducted with the *B. longifolia* investigated the *in vitro* anti-Mayaro virus (MAYV) activity of the flavonoids quercetin and its derivatives. The arthropod-borne Mayaro virus (MAYV) causes 'Mayaro fever', a disease of medical significance, primarily affecting individuals in permanent contact with forested areas in tropical South America. Recently, MAYV has attracted attention due to its likely urbanization. Currently, there are no licensed drugs against most

mosquito-transmitted viruses. Flavonoids were purified by chromatographic fractionation from leaf extracts of *B. longifolia* and chemically identified as quercetin and quercetin glycosides using spectroscopic techniques. Cytotoxicity of purified flavonoids and of EtOAc- and n-BuOH-containing flavonoid mixtures was measured by the dye-uptake assay while their antiviral activity was evaluated by a virus yield inhibition assay. The following flavonoids were purified from *B. longifolia* leaves: non-glycosylated quercetin and its glycosides quaijaverin, quercitrin, isoquercitrin, and hyperin. EtOAc and n-BuOH fractions containing these flavonoids demonstrated the highest antiviral activity of all tested substances, while quercetin had the highest antiviral activity amongst purified flavonoids. Quercetin, EtOAc, or n-BuOH fractions inhibited MAYV production by more than 90 % at 25 µg/mL, displaying a stronger antiviral effect than the licensed antiviral ribavirin. A mixture of the isomers isoquercitrin and hyperin had a modest antiviral effect (IC₉₀= 104,9), while quaijaverin and quercitrin did not show significant antiviral activity. *B. longifolia* is a good source of flavonoids with anti-Mayaro virus activity. This is the first report of the activity of quercetin and its derivatives against an alphavirus.⁴⁴

Bauhinia vahlii

Bauhinia vahlii grows on undisturbed moist and sub tropical areas in different parts of India. As an important non timber forest product (NTFP) plant of forest in Uttrakhand state, the leaves of this plant are mostly used locally by grocery shops, petty hotels etc. as plates and packing material. The bark of this plant provides strong fiber which is put to multifarious uses. Tribal people of many part of India such as Madhya Pradesh, Uttrakhand used the different parts of plant extract for fever, diarrhea, dysentery, bone fracture, tonic and vermifuge.^{45,46} A recent study was carried out phytochemical characterization and antibacterial activity of *B. vahlii*. Phytochemical screening of the dried leaf extract showed the presence of proteins, carbohydrates, glycosides, triterpenoids, tannins and flavonoids in all the seven extracts, whereas alkaloid are present only in ethanol extract and amino acids present in all the extracts except the water. The antibacterial activity of this plant was carried out using agar disc diffusion method at different concentrations of crude extracts against nine bacterial strains pathogenic to human beings. Among the antibacterial assayed, ethyl acetate and acetone were found to be most active against most of the studied bacterial strains. Therefore, Minimum Inhibitory Concentration (MIC) of ethyl acetate and acetone extract was determined against the selected bacterial strains showing zones of inhibition ≥ 10 mm. The results indicate the potential of *B. vahlii* in treating bacterial infections. Thus, justifying their traditional uses in the treatment of urinary tract infection, diarrhea and food poisoning which are of infectious origin.⁴⁷

Bauhinia acuminata

Bauhinia acuminata (*Fabaceae*), an evergreen large shrub, grows in disturbed areas of Southeast Asia such as Indonesia, Malaysia or the Philippines. Several chemical compounds including phthalic acid, palmitic acid, three phthalic acid esters, gallic acid, ursolic acid were identified from the leaves of *B. acuminata*.⁴⁸ An study reported the antidiarrheal activity and the antimicrobial activity of the methanol leaf extract of this plant. The crude methanolic extract of *B. acuminata* showed a significance anti-diarrheal activity at dose of 200 mg/kg and 400 mg/kg-body weight as compared to the standard anti-diarrheal agent loperamide (dose: 1 mg/kg-body weight). At a dose of 200 mg/kg and 400mg/kg- body weight showed significant (P<0,01) reduction in animal model in magnesium sulphate induced enteropooling by 50,66 % to 66,66 % respectively. In castor oil induced diarrhea, it showed reduction in dose dependent manner. Anti-diarrheal activity was present in the methanolic extract (200 mg/kg and 400 mg/kg) which indicate that the crude

drug acted by causing decreased intestinal motility by 41,89 % to 58,33 % respectively. Any kind of antimicrobial activity was not shown by the methanolic extract of leaf of *B. acuminata*. This result suggests that *B. acuminata* leaves extract could be used for the treatment of diarrhea.⁴⁹

Bauhinia purpurea

Bauhinia purpurea, which has no medicinal uses in Malay traditional culture has been used in Indian, Pakistani and Sri Lankan folklore medicine to treat various ailments, including ulcers. Interestingly, the plant is also inserted in several formulation of ayurvedic medicine. Scientifically, the plant has shown evidence of anti-inflammatory activity.⁵⁰

The gastroprotective activity and mechanisms of protection of the methanol extract of *Bauhinia purpurea* leaves (MEBP) using ethanol-induced gastric ulcer model was determined in a recent study. Male Sprague Dawley rats (n=6) were administered orally with 10 % dimethyl sulfoxide (DMSO), 100 mg/kg ranitidine or MEBP (50, 250 and 500 mg/kg) daily for seven consecutive days prior to subjection to the ethanol-induced gastric ulcer assay. The mechanisms of gastroprotection were determined based on: antisecretory activity via pylorus ligation assay; the role of nitric oxide (NO) and sulfhydryl group via pre-treatment of MEBP-treated rats with the respective N-nitro-L-arginine methyl ester (L-NAME) or carbenoxolone (CBX) followed by the ethanol-induced assay; and antioxidant activity using superoxide anion radical scavenging assay and, oxygen radical absorbance capacity (ORAC) assay. Ranitidine (100 mg/kg) was used as the reference drug. MEBP exhibited a significant ($p < 0,05$) and dose-dependent gastroprotective activity against ethanol-induced gastric ulcer with ulcer formation ranging between 0 and 74 % (indicated by decrease in ulcer area from 21,3 to 5,5 mm²). The macroscopic observation was in line with the microscopic findings and further supported by the histological scores suggesting the antiulcer potential of MEBP. MEBP also significantly ($p < 0,05$) reduced volume gastric juice, as well as its free and total acidity while increasing its pH. Moreover, this activity was significantly ($p < 0,05$) modulated in the presence of sulfhydryl group, but not NO. The extract also exhibited significant ($p < 0,05$) antioxidant activity. MEBP exerts gastroprotective activity partly via its antisecretory and antioxidant activities, as well as by modulation of sulfhydryl group, but not NO action.⁵¹

Bauhinia malabarica

Bauhinia malabarica is a small or moderate sized deciduous tree. It is distributed throughout India, mainly on the sub-Himalayan tracts, Bengal, Assam and in south India. It is also found in peninsular India and in the western sub-Himalayan forests, deciduous and semi-evergreen forests, areas receiving 1000 to 3000 mm annual rainfall. The leaves of the plant are consumed in India, Indonesia and Thailand, among others. It is used in traditional medicine for wound healing, as a diuretic, to fight dysentery, to treat headache, fever and as an emmenagogue.⁵²

The antioxidant and free radical scavenging efficiencies of 70 % acetone and 50 % methanol extracts of *B. malabarica* pod and seed were examined. The extracts were screened for different antioxidant assays such as reducing power, DPPH, nitric oxide, hydroxyl radical scavenging, metal chelating, ABTS scavenging activity, lipid peroxidation preventive property and anti-haemolytic activities. On the basis of the results obtained, seeds of *B. malabarica* were found to be a potent source of natural antioxidants due to their marked antioxidant activity. Overall, the acetone was found to be the best solvent for the extraction of antioxidant compounds.

The results presented here imply that the consumption of such a legume food would not only improve the nutrient utilization but also provide the potential source of nutraceuticals for human health.⁵³

In other study, was reported the phytochemical, antimicrobial and antioxidant activities of *B. malabarica*. Leaf extracts and washed with tap water and for the removal of soil and dust particle then the extracts were dried in the oven at 40 °C to get thick paste. Then the collected samples were stored at 4 °C the obtained extract was divided into two parts. One part was kept at 8 °C for overnight and another one part was kept at room temperature (RT). After that various test are applied for phytochemical analysis like saponins (Foam Test), test for phenolic compounds many more. Antioxidant activity: DPPH stable free radical scavenging assay, free radical scavenging activity by ABTS method is used. Antimicrobial activity: The analyses of MIC against fungal species methods are used. In phytochemical analysis studies, the extracts prepared with hexane, chloroform, methanol, phosphate buffered saline-room temperature (PBS RT) and PBS 8 °C showing presence of many kinds of saponins, phenolic compounds, flavonoids, terpenoids, tannins, glycosides, carbohydrates and proteins. Phenolic compounds and carbohydrates were present in all extracts except hexane. Steroids and lipids were not found in any types of the leaf extracts. *Antioxidant activity*: five extracts, methanol, chloroform, hexane, PBS RT, PBS 8 °C are showing the free radical scavenging activity. In antimicrobial study against salmonella, *E. coli* and *P. aeruginosa* did not showed any clear zone of inhibition for any of the extracts except standard marker.⁵⁴ There are no reports in the literature on toxicological studies on mammals with *Bauhinia malabarica*.

CONCLUSION

A high number of plants belonging to the genus *Bauhinia* are prescribed in folk medicine to treat a variety of ailments, including diabetes and general pain, inflammation and infections. Scientific knowledge on the biological properties and active principles of these plants has progressed significantly in recent years. Although a number of chemical components described for the genus *Bauhinia* are also found in other species, the secondary metabolites produced by this genus, particularly the flavonoids, make these plants an important source of potential phytotherapeutic and medicinal agents. However, there are no reports of *in vivo* toxicity studies. Therefore, it is recommended to carry out toxicological studies on mammals in order to have a safe selection of isolated agents of these plants.

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Iuri de França Bonilha. Phytochemical Research Laboratory, Universidade Federal de Santa Maria. Avenida Roraima, 1000, Prédio 26, sala 1107, Camobi, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil. Phone: +55 55 8120 8030. E-mail: iurifbonilha@gmail.com