ARTÍCULO DE REVISIÓN

Chemical constituents, biological activity and therapeutic uses, of *Scutia buxifolia Reissek.*

Componentes químicos, actividad y usos terapéuticos, de *Scutia buxifolia Reissek*.

Pharmacist Juliana Calil Brondani,[I] PhD. Melânia Palermo Manfron[II]

[I] Phytochemical Research Laboratory, *Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.*
[II] Industrial Pharmacy Department, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.

ABSTRACT

Scutia buxifolia has been used in the treatment of a number of diseases, which includes bacterial and fungal infections, hypertension, Alzheimer's Disease and cancer. S. buxifolia contains biologically active compounds such as flavonoids, steroids, tanins, lipids, terpens and alkaloids. A range of biological activities has been found from plant extract and fractions, including antioxidant, acetylcholinesterase inhibitor, antiviral and antibiotic. Some studies about the potential toxicity were performed; however the results are not conclusive, suggesting that a single administration of the extract is safe, whereas prolonged use has deleterious effects for the body. The revised databases were *SciELO, PubMed, ScienceDirect and Portal da Capes* considering studies between 1964 and 2014 and by searching for terms like *Scutia buxifolia, Rhamnaceae family, Scutia buxifolia Constituents, Scutia buxifoli*a use and OECD.

Keywords: biological activities, Scutia buxifolia, chemical constituents, toxicity.

RESUMEN

Scutia buxifolia se utiliza en el tratamiento de una serie de enfermedades que incluye infecciones bacterianas y fúngicas, hipertensión, enfermedad de *Alzheimer* y

cáncer. *S. buxifolia* contiene compuestos biológicamente activos tales como flavonoides, taninos, esteroides, lípidos, terpenos y alcaloides. A partir del extracto y las fracciones de la planta surgen una gama de actividades biológicas, que incluyen antioxidante, inhibidor de la acetilcolinesterasa, antiviral y antibiótico. Se realizaron algunos estudios sobre el potencial tóxico, sin embargo los resultados no son concluyentes, lo que sugiere que una sola administración del extracto es segura, mientras que el uso prolongado tiene efectos perjudiciales para el organismo. Las bases de datos revisadas fueron *SciELO*, *PubMed*, *ScienceDirect* y Portal de Capes, teniendo en cuenta los estudios entre 1964 y 2014 y mediante la búsqueda de términos como *Scutia buxifolia*, *Rhamnaceae family*, *Scutia buxifolia* constituents, *Scutia buxifolia* uses and OECD.

Palabras clave: actividad biológica, *Scutia buxifolia*, compuestos químicos, toxicidad.

INTRODUCTION

The use of medicinal plants in therapy is an ancient practice, historically built on the wisdom of common sense, linking culture and health. Since early times, men seek in nature the solution to your woes, whether spiritual or physical.[1]

The emergence of "natural" concept contributed, in recent decades, to a significant increase in the use of medicinal plants in the world. For many people this concept means "no chemicals", being natural products seen as healthy products without any risk to health. Misconception, since many plants contain substances that can exert toxic effects on the organism.[2]

BOTANY

Scutia buxifolia is a plant that belongs to Rhammnaceae family, being popularly known as coronilha or espinho de touro. It is a native tree from South America with a dispersion area that comprises Rio Grande do Sul State in Brazil and countries like Argentina and Uruguay.[3] In Rio Grande do Sul, occurs mainly in araucarias forests, riverine forests, mountains of southeastern and southern coast. Occasionally, also can be found in the central depression of the State[4]. The bushes can reach 6 meters height. The leaves are opposite and alternate, entire or with few teeth, measuring 1.5 to 4 cm long by 1 to 2 cm wide. Flowers from October to January and fruits in the month of March. The inflorescences are in axillary fascicles with small and green flowers.[3]

CHEMICAL CONSTITUENTS

Flavonoids: several flavonoids have been isolated from *Scutia buxifolia*. Boligon *et al*[5] dosed the flavonoids content in the plant branches. The concentration of these compounds was higher in the butanol fraction (140,71±2,14 mg rutin/g dried plant), followed by the ethyl acetate fraction (137,28±0,39 mg rutin/g dried plant), dichloromethane fraction (93,21 ±1,23 mg rutin/g dried plant) and the crude extract with ethanol 70 % (83,47±0,93 rutin/g dried plant). A similar result was obtained by the same group in 2012, in which the content of flavonoids was also

lower in the crude extract (100,37±0,56 mg g^{-1} quercetin), however higher in the ethyl acetate fraction (145,72±0,27 mg g^{-1} quercetin), followed by the utanolic fraction (138,92±0,83 mg g^{-1} quercetin).[6]

In another work,[7] the ethyl acetate fraction obtained from the leaves of *S.buxifolia* was subjected to column chromatography on silica gel 60 using CH_2Cl_2(700 mL) as mobile phase. The result was the identification of four flavonol compounds (quercetin, quercetin–3–0–rhamnoside/quercitrin, quercetin–3–0-β–D–glucopyranosid/isoquercitrin and rutin) based on H NMR, C NMR spectra and by comparison with the literature.

Many flavonoids are antioxidants.[8] Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation,[9] the process by which unsaturated fatty acids are converted to free radicals via the abstraction of hydrogen.The oxidation of low–density lipoproteins (LDL) can result in the formation of atherosclerotic plaques, leading to cardiovascular disease.[10] Also, the vulnerability of brain lipid membranes to lipid peroxidation can cause neurodegenerative disease, such as Alzheimer's and Parkinson's Disease.[11]

Rutin is also considered able to neutralize hydroxyl radicals and superoxide.[12,13] Rodrigues *et al*[14] demonstrated the effects of rutin raising HDL cholesterol, reducing the risk for atherosclerosis and cardiovascular diseases. The authors related this action to the superoxide radical destruction, due to elevation in SOD (superoxide dismutase enzyme) activity.

Steroids: e sterified steroids, as well as terpenes, reduce the synthesis of ergosterol, a component offungal cell membrane, leading to defective cell wall formation and leakage of cell contents.[15] So, these could be responsible for the antimicrobial actions of *S.buxifolia.*[16]

From the *S. buxifolia* stem bark and leaves, Boligon *et al*[17] examined the dichlorometanic fraction by liquid chromatography high efficiency in order to identify and quantify β-sitosterol and stigmasterol steroids. The stem bark fraction showed the highest amount of steroids (40,4 % β–sitosterol+stigmasterol) when compared to the same fraction of leaves (20 % β–sitosterol+stigmasterol), whereas the β–sitosterol was the main steroid found in both fractions.

β–sitosterol is chemically very similar to cholesterol, differing only by the presence of an ethyl group at carbon 24 of the side chain. [18] It is recognized as beneficial in the treatment of immune disorders, inflammatory diseases, breast and colon câncer.[19,20]

In another study from the same research group,[21] the leaves's and stem bark's dichlorometanic fraction of *S.buxifolia* was analyzed by GC–MS, identifying the presence of three esterified steroids in the stem bark (cholesta–3,5–dien–7–one; lanost–8–em–24–al and stigmastan–3,5–diene) and an esterified steroid in the leaves (stigmastan–3,5–diene).

Tanins: u sing the method described by Morrison *et AL*,[22] the presence of tannins in the stem bark was quantified from the crude extract, butanol, ethyl acetate and dichloromethane fractions. The study results showed that the greater amount of tannins was found in the ethyl acetate fraction (176,70±0,24 mg g^{-1} fraction), followed by butanolic fraction (174,83 ±0,65 mg g^{-1} fraction), dichloromethane fraction (79,61±0,51 mg g^{-1} fraction) and crude extract (66,67±0,17 mg g^{-1} extract).[6]

Tannins are recognized for their antioxidant potential as free radical scavengers, chelating of transition metals, inhibitors of prooxidative enzymes and lipid peroxidation.[23] In the work developed by Zhang,[24] condensed tannins extracted from the stem bark and fine root showed a good DPPH radical scavenging activity and ferric reducing power, confirming that this compounds can be considered as new sources of natural antioxidants for food and nutraceutical products.

Lipids: a large number of lipophilic components has been identified and quantified in the leaves and stem bark of *Scutia buxifolia*. In the work of Boligon *et al*,[21] fatty acids represented the major class of nonpolar compounds in the dichloromethane fraction of leaves (L) and stem bark (S), including tetradecanoic acid (3,23 % L and 0,22 % S), hexadecanoic acid/palmitic acid (3,58 % L and 0,30 % S), octadeca–9,12–dienoic acid/linoleic acid (3,48 % L and 2,57 % S) and octadec–9–enoic acid/oleic acid (0,90 % L and 1,14 % S).

Terpens: s pathulenol, timol and β–cubene were isolated in *S.buxifolia*. From the leaves's dichloromethane fraction, they found 4,28 % of spathulenol and 3,15 % of timol. For the stem bark only the β–cubene was identified, representing 3,08 %.[21]

Terpenes are also known due to its antimicrobial action, since it increases the permeability of the bacterial and mammalian cells integrating themselves into the lipid layer of cell membrane and thereby changing the selective permeability.[25] Also, they can reduce the synthesis of ergosterol, causing deformation in the fungal cell membrane.[15]

Alkaloids: Scutia buxifolia has been tested positive for cyclopeptidic alkaloids. The first identified member of this group was called scutianine A.[26] Latter, the same research group isolated a second compound, scutianine B.[27] Scutianines C and D were identified simultaneously.[28,29] Followed by the isolation of scutianine E,[29] F[30] and G.[31] After, several others cyclopeptidic alkaloids were isolated and identified.[32-35]

Marangon *et al*[36] demonstrated the difference in the chemical constitution of *S.buxifolia* stem bark from different regions of Rio Grande do Sul. The ethereal alkaline fractions, originating from Quaraí, Santana do Livramento and São Sepé were subjected to chromatographic analysis by HPLC with four patterns of neutral cyclopeptides, scutianines X, Y, W and Z. The authors concluded that the location influences the plant metabolism and, hence, on its chemical composition, since the samples from Quaraí and São Sepe presented many scutianine Y. On the other hand, the sample from Santana do Livramento presented many scutianines X and Y.

Plants of the Rhamnaceae family are known by the cyclopeptidic alkaloids with antimicrobial properties[37] and immunostimulatory actions. [38]

Essential oil: Boligon *et al*[39] identified qualitatively and quantitatively twenty-five components (98,38 %) from the essential oil of *S.buxifolia* leaves. Of these, 73,69 % are sesquiterpenes and 18,74 % are monoterpenes. The main constituents isolated were spathulenol (27,09 %), β–cubene (5,36 %), germacrene D (9,81 %), carvacrol (7,01 %), globulol (5.36 %), α–copaene (4,17 %), γ–eudesmol (3,59 %), thymol (3,27 %), 1,8–cineol (3,08 %), p–cymene (2,56 %), α–eudesmol (2,34 %), β–elemene (2,04 %), butylated hydroxytoluene (2,00 %) along with eugenol acetate, n–hexanol, α–pinene, α–humulene, eugenol, humulene epoxide and phytol as minor constituents. According to the authors, the main components identified in the essential oil may be one of the responsible for the antimicrobial activity, since spathulenol and carvacol have been reported to present antimicrobial action against bacterial infections.[40,41]

BIOLOGICAL ACTIVITY

Scutia buxifolia is used for different purposes in traditional medicine around the world. Because of this, researchers have tested it for different types of biological activities on crude extracts and fractions. The results of some studies are listed below and include positive and negative results.

Antimicrobial activity: s everal studies have demonstrated the antibacterial and antifungal action of *Scutia buxifolia.*[39,42]

Dichloromethane, ethyl acetate and n-butanol fractions from the branches of *S.buxifolia* were tested for their antimicrobial potential against*Candida albicans*, *Candida glabrata*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacilus subtilis* and *Prototeca zopfii*. The results showed that, for the microorganism *S. cerevisiae*, the butanol fraction had lower MIC (62,5 mg mL^{-1}), while ethyl acetate fraction obtained MIC 250 mg mL^{-1}.
About the two species of *Candida*, all fractions tested showed MIC greater tan 2 000 mg mL^{-1}. The Gram negative organisms, *E. coli* and *P. aeruginosa* , were resistant to all fractions. *S. aureus* was sensitive to ethyl acetate (MIC 250 mg mL^{-1}) and butanolic (MIC 500 mg mL^{-1}) fractions. The authors considered acceptable the greater resistance by Gram negative microorganisms, since they exhibit structural features that hinder antibiotics penetration.[43]

Maldaner *et al*[44] likened this antimicrobial activity with the structure of cyclopeptidic alkaloids and neutral cyclopeptide found in *S. buxifolia*. According to the authors, the presence of β–phenylserine unit and the N, N–dimethyl (or N–methyl) make the alkaloids more active against microorganisms. Furthermore, consider the stereochemistry of the amino acids that form alkaloids other intervening factor in the aforementioned activity.

Natural inhibitor of acetylcholinesterase (AChE): AChE is an enzyme responsible for regulating the levels of the neurotransmitter acetylcholine. The increase of the activity in the body is related to the onset of Alzheimer's Disease, since the resulting cholinergic deficiency causes progressive cognitive and neuropsychiatric manifestations until an eventual incapacitation.[45,46]

Acetylcholinesterase inhibitors cause an increase in the capacity of acetylcholine to stimulate the brain muscarinic and nicotinic receptors. Therefore, in clinical practice, are the symptomatic treatment of choice for Alzheimer's Disease.[47,48]

Maldaner *et al*[49] studied the inhibition ability of acetylcholinesterase by crude extract, the acidic and alkaline ethereal fractions and isolated compounds (cyclopeptidic alkaloids scutianine B, C, D and neutral cyclopeptide scutianene C) of *Scutia buxifolia*. The crude extract and the ethereal alkaline fraction did not inhibit AChE enzyme, however the ethereal acidic fraction showed some points of inhibition. On the other hand, the isolated metabolites seems to be promising candidates of AChE inhibitors. For scutianine B, C and scutianene C was possible to observe an inhibition of the enzyme (up to 0,39 mg/µL, 6,25 mg/µL and 6,25 mg µL, respectively). Scutianina D didn't show inhibitory activity.

Antiviral activity: the crude extract with ethanol 70 %, dichloromethane, ethyl acetate and n-butanol fractions from the leaves and stem bark of *S. buxifolia* were tested for their antiviral activity by MTT assay. The n-butanol and ethyl acetate fractions of the stem bark plus the ethyl acetate fraction of the leaves exhibited potent antiviral activity with SI values of 25,78, 15,97 and 14,13, respectively.

Subfractions were also evaluated for anti–HSV activity. The subfractions where phenolic acids and flavonoids (rutin) were quantified showed antiviral activity.[50]

Antioxidant activity: v ery good antioxidant activities were found for *S.buxifolia*. In the study developed by Boligon *et al*,[51] ethyl acetate and n-butanol fractions obtained from the stem bark exhibited, at a concentration of 250 g/mL, an DPPH inhibition of 97,45 % and 96,29 %, respectively. The antioxidant activity, even at low concentrations (7,81 mg/mL), still existed with an inhibition of 89,60 % by butanolic and 90,23 % by ethyl acetate fractions. On the other hand, the dichloromethane fraction showed a much lower activity, having 34,14 % of DPPH inhibition at the concentration of 7,81 mg/mL. This fact is justified by the authors based on the chemical composition of the different fractions, because some compounds quickly react against DPPH, while others have a slower mechanism. Excellent antioxidant activity can be observed in n-butanol and ethyl acetate fractions obtained from the leaves. Also, Fe (II)=induced TBARS production in brain preparations was significantly decreased by the *S.buxifolia* stem bark. The ethyl acetate fraction had the highest activity, butanolic fraction and crude extract showed intermediated activities and, dichloromethane fraction, had the lowest activity.

Other biological activities of *Scutia buxifolia* is being studied as cardiotonic, hypotensive and diuretic properties.[3,52] Also, its capacity to protect DNA against damage.[53]

TOXICITY

The toxic potential of *S.buxifolia* is not fully elucidated. De Freitas *et al*[54] tested the hepatotoxic effect of the stem bark aqueous crude extract of *Scutia buxifolia* (SBSB) in rats treated sub–chronically at concentrations of 100, 200 and 400 mg SBSB/kg body weight. For this, they considered markers of liver damage such as the aminotransferase AST and ALT (dosed on day 15º and day 30º of the experiment), the quantification of non-protein sulfhydryl groups (NPSH), histopathological studies and hepatic redox balance was determined by thiobarbituric acid reactive substances (TBARS).

Statistical analyzes showed that subchronic treatment with 100 mg of SBSB/kg body weight did not alter the serum aminotransferase levels during the experimental protocol. Animals treated with 200 mg of SBSB/kg body weight did not have any change in ALT values, however, the AST dosage increased 3,7 times on day 30º (519,5±6,51 U/L) in comparison to the 15º day (130,0±6,28 U/L). The highest tested dose, 400 mg SBSB/kg body weight, did not alter the parameters evaluated. Furthermore, the level of MDA in hepatic tissue, determined by TBARS technique, showed no change among the control group and the groups treated with SBSB. The same was found for the NPSH test.

The morphological analysis of hepatocytes showed no alterations induced by sub–chronic treatment with *Scutia buxifolia*. The authors conclude that, apparently, the use of *Scutia buxifolia* stem bark is safe and does not bring risks to hepatocytes. Also, the AST dosage cannot be directly related to the toxicity of *S.buxifolia*, since aminotransferase activity are influenced by haemolysis, muscular stress and xenobiotics.

De Freitas *et al*[55] tested the acute oral toxicity of the aqueous extract of *Scutia buxifolia*'s stem bark (SBSB). Four groups of six rats each received a single dose of SBSB intragastrically at concentrations of 0 mg/kg (water), 100 mg/kg, 200 mg/kg and 400 mg SBSB/kg body weight. The animals were observed for eventual signs of

toxicity or death during 14 days. Alterations in food and water intake, mortality and/or changes in behavioral pattern were analyzed. The study did not reveal any statistically significant difference in the water and food consumption, as well as no changes were observed in general animal behaviour and motor capacity.

Grecco *et al*,[56] testing the acute oral toxicity of SBSB aqueous crude extract, didn't find any signs of toxicity and / or death. In this study, no significant changes were detected in serum urea, creatinine, GOT and GPT. Also, any behavioral and histological changes were observed. Similary, the study developed by Da Silva *et al*[57] evaluating the toxic effects of acute and subchronic exposure of stem bark ethyl acetate fraction from *Scutia buxifolia*in mice showed that, when a single dose (2 000 mg/Kg) is administered, no sign of toxicity were observed, being considered category 5 of OECD classification. But the administration, at different doses, for 28 days showed biochemical, hematological and histological alterations, indicating that this plant does not present high safety when used for prolonged periods.

The serum ASAT activity was increased (~73%) in male mice treated with 400 mg/Kg of the plant extract. Glucose levels were reduced in male mice that received 200 and 400 mg/Kg in 34 and 32 %, respectively. No modifications were observed in female mice. In relation to the hematological parameters, male mice, treated with 400 mg/Kg of plant extract, presented a decrease in the HGB and HCT of 21 and 22 %, respectively. Also, the RDW increased by approximately 13 %. At a dose of 200 mg/Kg, the MCV decreased approximately 7 %. In female mice, alterations in the HGB, HCT, RDW and MCV values were not observed.

The hepatic histological analysis showed that, male mice treated with a dose of 200 mg/Kg of plant extract, presented moderate inflammatory cells infiltration and cytoplasmic vacuolization. Treatment with 400 mg/Kg of plant extract promoted intense inflammatory cells in the tissue. For renal histological analysis of male mice, the group treated with 200 mg/Kg of *S. buxifolia* extract presented normal aspects with slight renal morphological changes in some areas, with tubular dilation and glomerular alteration. The treatment with 400 mg/Kg caused tissue disintegration in the proximal and distal tubules and glomerular alteration in the kidney of male mice.All histological results for female mice were similar to the control group, indicating a greater biological susceptibility of male mice when compared to females.

CONCLUSIONS

Considering the antimicrobial activity, it is not only one single compound that is responsible for this effect. The activity may be due to the presence of cyclopeptidic alkaloids and neutral cyclopeptide found in *S. buxifolia* extracts. Also, esterified steroids (β–sitosterol and stigmasterol), terpens (spathulenol, timol and β–cubene) and the essential oil (spathulenol and carvacol) could be responsible for this effect.

Cyclopeptidic alkaloids scutianine B, C and scutianene C, seems to be promising candidates to inhibit acetylcholinesterase. The antiviral and antioxidant claimed activities could be explained by the presence of phenolic acids, tanins and flavonoids, like quercetin and rutin.

Due to the traditional use of *Scutia buxifolia*, its chemical composition, biological activities and the not fully elucidated toxic potential, seems to be worth the effort of exploring this plant further.

REFERENCES

1. Alvim NAT, Ferreira MA, Cabral IE, Almeida Filho AJ. O uso de plantas medicinais como recurso terapêutico: das influências da formação profissional às implicações éticas e legais de sua aplicabilidade como extensão da prática de cuidar realizada pela enfermeira. Rev Latino-Am Enfermagem. 2006;14(3):316-323.

2. Mengue SS, Mentz LA, Schenkel EP. Uso de plantas medicinais na gravidez. Rev Bras Farmacogn. 2001;11(1):21-35.

3. Wasicky R, Wasicky M, Joachimovits R. Erstuntersuchungen na Coronilha-*Scutia buxifolia* Reissek. Planta Med. 1964; 12:13-25.

4. Sobral M, Jarenkow JA, Brack P, Irgang B, Larocca J, Rodrigues RS. Flora Arbórea e Arborescente do Rio Grande do Sul, Brasil. São Carlos: Novo Ambiente; 2006.

5. Boligon AA, Magoga BR, Feltrin AC, Janovik V, Athayde ML. Potencial antioxidante *in vitro*, conteúdo de fenóis e flavonóides nos ramos de *Scutia buxifolia* Reissek. Saúde. 2009;35(1):34-38.

6. Boligon AA, De Brum TF, Frolhich JK, Froeder ALF, Athayde ML. HPLC/DAD Profile and determination of total phenolics, flavonoids, tannins and alkaloids contents of *Scutia buxifolia* Reissek Stem Bark. Research Journal of Phytochemistry. 2012;6(3):84-91.

7. Boligon AA, Feltrin AC, Machado MM, Janovik V, Athayde ML. HPLC Analysis and phytoconstituents isolated from ethyl acetate fraction of *Scutia buxifolia* Reiss. Leaves. Latin American Journal of Pharmacy. 2009;28(1):121-124.

8. Bohm H, Boeing H, Hempel J, Raab B, Kroke A. Flavonols, flavones and anthocyanins as native antioxidants of food and their possible role in the prevention of chronic diseases. Zeitschrift fur Ernahrungswissenschaft. 1998;37:147-163.

9. Sakanashi Y, Oyama K, Matsui H, Oyama TB, Oyama TM, Nishimura Y *et al*. Possible use of quercetin, an antioxidant, for protection of cells suffering from overload of intracellular Ca2+: a model experiment. Life Sci. 2008;83(5-6):164-9.

10. Abdalla DSP, Sena KCM. Lipid peroxidation biomarkers in atherosclerosis. Rev Nutr. 2008;21(6):749-756.

11. Balazs L, Leon M. Evidence of an oxidative challenge in the Alzheimer's brain. Neurochem Res. 1994;19(9):1131-7.

12. Metodiewa D, Kochman A, Karolczak S. Evidence for antiradical and antioxidant properties of four biologically active N,N-diethylaminoethyl ethers of flavanone oximes: a comparison with natural polyphenolic flavonoid (rutin) action. Biochem Mol Biol Int. 1997;41(5):1067-75.

13. Azevedo MI, Pereira AF, Nogueira RB, Rolim FE, Brito GAC, Wong DVT *et al*. The antioxidant effects of the flavonoids rutin and quercetin inhibit oxaliplatin-induced chronic painful peripheral neuropathy. *Molecular Pain.* 2013;9:53-67.

14. Rodrigues HG, Diniz YS, Faine LA, Almeida JA, Fernandes AAH, Novelli ELB. Suplementação nutricional com antioxidantes naturais: efeito da rutina na concentração de colesterol-HDL. Rev Nutr. 2003;16(3):315-320.

15. Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. J Med Microbiol. 2009;58(11):1454-1462.

16. Boligon AA, Janovik V, Frohlich JK, Froeder ALF, Alves SH, Athayde ML. Antimicrobial and cytotoxic activities of leaves, twigs and stem bark of *Scutia buxifolia* Reissek. Nat Prod Res. 2012;26:939-944.

17. Boligon AA, De Brum TF, Frohlich JK, Zadra M, Froeder ALF, Gindri AL *et al*. Quantificação de β-sitosterol e estigmasterol nas cascas do tronco e folhas de *Scutia buxifolia* Reissek. SEPE–XV Simpósio de ensino, pesquisa e extensão. Santa Maria: SEPE; 2011.

18. Pegel KH. The importance of sitosterol and sitosterolin in human and animal nutrition. S Afr J Sci. 1997;93: 263-268.

19. Bouic PJ, Etsebeth S, Liebenberg RW, Albrecht CF, Pegel K, Van Jaarsveld PP. Beta-sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as na immunomodulatory vitamin combination. Int J Immunopharmac. 1996;18:693-700.

20. Awad AB, Gan Y, Fink CS. Phytosterols as anticancer dietary components: evidence and mechanism of action. Nutr Cancer. 2000;36:74-78.

21. Boligon AA, Schwanz TG, Froeder ALF, Athayde ML. GC-MS Analysis of dichloromethane fractions of *Scutia buxifolia* Reissek leaves and stem bark. Research Journal of Phytochemistry. 2014;8(1):16-20.

22. Morrison IM, Asiedu EA, Stuchbury T, Powell AA. Determination of lignin and tannin contents of cow pea seed coats. Annal Botany. 1995;76:287-290.

23. Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L *et al*. Condensed and hydrolysable tannins as antioxidants influencing the health. Mini Rev Med Chem. 2008;8(5):436-47.

24. Zhang SJ, Lin YM, Zhou HC, Wei SD, Lin GHL, Ye GF. Antioxidant tannins from stem bark and fine root of *Casuarina equisetifolia*. Molecules. 2010;15:5658-5670.

25. Villani FA, Chiarra SS, Piccinini F. Effect of terpenes on the cell permeability to tetracycline. Arzneimittelforscung. 1972;22:1364-1367.

26. Tschesche R, Welters R, Fehlhaber HW. Scutianinm ein cyclisches Peptid-Alkaloid aus *Scutia buxifolia* Reiss. Chem. Ber. 1967;100:323.

27. Tschesche R, Ammermann E, Fehlhaber HW. Alkaloide aus rhamnaceen, X: Scutianin-B, ein weiteres Peptidalkaloid aus *Scutia buxifolia* Reiss. Tetrahedron Letters. 1971;12(46):4405-4408.

28. Sierra MG, Mascaretti OA, Merkuza VM, Tosti EL, Ruveda EA. Peptide alkaloids of *Scutia buxifolia*. Phytochemistry. 1974;13:2865-2869.

29. Tschesche R, Ammermann R. Alkaloide aus Rhamnaceen, XXIII. Scutianin-C, -D und -E, drei weitere Cyclopeptidalkaloide aus *Scutia buxifolia* Reiss. Chem. Ber. 1974;107:2274.

30. Tschesche R, Hillebrand D, Wilhelm H, Ammermann E, Eckhardt G. Hysodricanin-a, mauritin-h, scutianin-f und aralionin-c, vier weitere cyclopeptidalkaloide aus ziziph us, *Scutia* und araliorhamnus. Phytochemistry. 1977;16:1025.

31. Tschesche R, Hillebrand D. Scutianin-g, ein weiteres cyclopeptidalkaloid aus *Scutia buxifolia*. Phytochemistry. 1977;16:1817.

32. Menezes AS, Mostardeiro MA, Zanatta N, Morel AF. Scutianine-J, a cyclopeptidic alkaloid isolated from *Scutia buxifolia*. Phytochemistry. 1995;38(3):783-786.

33. Morel AF, Bravo RFB, Reis FAM, Ruveda EA. Peptide alkaloids of *Scutia buxifolia*. Phytochemistry. 1979;18:473-477.

34. Maldaner G, Marangon P, Ilha V, Caro MSB, Burrow RA, Dalcol II *et al*. Cyclopeptide alkaloids from *Scutia buxifolia* Reiss. Phytochemistry... 2011;72:804-809.

35. Morel AF, Machado ECS, Moreira JJ, Menezes AS, Mostardeiro MA, Zanatta N *et al*. Cyclopeptide alkaloids of *Scutia buxifolia*. Phytochemistry. 1998;47(1):125-129.

36. Marangon P, Morel AF, Maldaner G, Silva UF, Dalcol II. Comparação de metabólitos secundários de *Scutia buxifolia* por CLAE. XXII Simpósio de plantas medicinais do Brasil. Bento Gonçalves: SBQ; 2012.

37. Emile A, Waikedre J, Herrenknecht C, Fourneau C, Gantier JC, Hnawia E *et al*. Bioassay-guided isolation of antifungal alkaloids from *Melochia odorata*. Phytother Res. 2007;21:398-400.

38. Lin HY, Chem CH, You BJ, Liu KCS, Lee SS. Cyclopeptide alkaloids from *Paliurus ramossisimus*. J Nat Prod. 2000;63:1338-1343.

39. Boligon AA, Schwanz TG, De Brum TF, Frohlich JK, Nunes L, Mario DN *et al*. Chemical composition, antioxidant and antimicrobial activities of the essential oil of *Scutia buxifolia* Reissek leaves. Pharmaceut. Anal. Acta. 2012;3:10.

40. Chinou IB, Bougatsos C, Perdetzouglou D. Chemical composition and antimicrobial activities of *Helichrysum amorginum* cultivated in Greece. Journal of Essential Oil Research. 2004;16:243-245.

41. Burt S. Essential oil: their antibacterial properties and potential applications in foods – A Review. Int. J. Food Microbiol. 2004;94:223-253.

42. Boligon AA, De Brum TF, Zadra M, Piana M, Alves CFS, Fausto VP *et al*. Antimicrobial activity of *Scutia buxifolia* against the honeybee pathogen *Paenibacillus larvae*. Journal of Invertebrate Pathology. 2013;112:105-107.

43. Boligon AA, Boligon AA, Lorentz LH, Mahlke JD, Machado MM, Orige SC *et al*. Estudo da atividade antimicrobiana de extratos e frações obtidos a partir dos ramos de *Scutia buxifolia* Reissek. Horticultura Brasileira. 2008;26(2):13-17.

44. Maldaner G, Ilha V, Giacomelli SR, Stuker CZ, Morel AF, Dalcol II *et al*. Estudo da relação estrutura atividade antimicrobiana de alcalóides ciclopeptídicos e ciclopeptídeo neutro de *Condala buxifolia* e *Scutia buxifolia* pertencentes à familia Rhamnaceae. 30ª Reuniao Anual da Sociedade Brasileira de Química. Águas de Lindóia: SBQ; 2007.

45. Zhao Q, Tang XC. Effects of huperzine A on an acetylcholinesterase isoforms *in vitro*: comparison with tacrine, donepezil, rivastigmine and physostigmine. Eur J Pharmacol. 2002;455(2-3):101-107.

46. Selkoe DJ. Alzheimer's Disease: genes, proteins, and therapy. Physiol Ver. 2001;81(2):741-66.

47. Sereniki A, Vital MABF. A Doença de Alzheimer: aspectos fisiopatológicos e farmacológicos. Ver. Rev Psiquiatr RS. 2008;30(1 Supl):1-17.

48. Bottina CMC, Carvalho IAM, Alvarez AMMA, Avila R, Zukauskas PR, Bustamante SEZ *et al*. Reabilitação cognitiva em pacientes com Doença de Alzheimer. Arq Neuropsiquiatr. 2002;60(1):70-79.

49. Maldaner G, Marangon P, Ilha V, Dalcol II, Morel AF, Adolpho L *et al*. Inibidores naturais da acetilcolinesterase isolados de *Scutia buxifolia*. 32ª Reuniao Anual da Sociedade Brasileira de Química. Fortaleza: SBQ; 2009. 50. Boligon AA, Kubiça TF, Mario DN, De Brum TF, Piana M, Weiblen R *et al*. Antimicrobial and antiviral activity-guided fractionation from *Scutia buxifolia* Reissek extracts. Acta. Physiol. Plant. 2013;35:2229-2239.

51. Boligon AA, Pereira RP, Feltrin AC, Machado MM, Janovik V, Rocha JBT *et al*. Antioxidant activites of flavonol derivatives from the leaves and stem bark of *Scutia buxifolia* Reiss. Bioresource technology. 2009;100:6592-6598.

52. Da Silva RCVAF, Souza SCP, Boligon AA, Athayde ML, Santos ARS, Marques MCA *et al*. Endothelium-dependent and independent vasorelaxation induced by na n-butanolic fraction of bark of *Scutia buxifolia* Reiss (Rhamanaceae). J. Ethnopharmacol. 2012;141:997-1004.

53. Boligon AA, Sagrillo MR, Machado LF, Filho OS, Machado MM, Cruz IBM *et al*. Protective effects of extracts and flavonoids isolated from *Scutia buxifolia* Reissek against chromosome damage in human lymphocytes exposed to hydrogen peroxide. Molecules. 2012;17:5757-5769.

54. De Freitas RB, Rovani BT, Boligon AA, De Brum TF, Piana M, Jesus RS *et al*. Hepatotoxicity evaluation of aqueous extract from *Scutia buxifolia*. Molecules. 2013;18(7):7570-7583.

55. De Freitas RB, Araldi ICC, Boligon AA, De Brum TF, Rovani BT, Piana M *et al*. Phytochemical analysis and toxicity investigation of stem bark of *Scutia buxifolia* Reissek. Natural Product Research. 2013;27(18):1620-1624.

56. Grecco GA, Santos KT, Santanna LS, Moreira CM. Avaliação da toxicidade oral aguda do extrato aquoso de *Scutia buxifolia* Reiss em ratos. Anais do Salão Internacional de Ensino, Pesquisa e Extensão (SIEPE-Universidade Federal do Pampa) 2011;3(2).

57. Da Silva ARH, Moreira LR, Brum ES, De Freitas ML, Boligon AA, Athayde ML *et al*. Biochemical and hematological effects of acute and sub-acute administration to ethyl acetate fraction from the stem bark *Scutia buxifolia* Reissek in mice. Journal of Ethnopharmacology. 2014;153(3):908-916.

Recibido: 14 de junio de 2014.
Aprobado: 22 de diciembre de 2014.

Juliana Calil Brondani. Address: Avenida Roraima, 1000, Prédio 26, sala 1107, Camobi, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil. Phone: +55 55 99199080 E-mail: juliana.brondani@gmail.com