Ethanolic extract of *Casearia sylvestris* Sw exhibits *in vitro* antioxidant and antimicrobial activities and *in vivo* hypolipidemic effect in rats


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**ABSTRACT:** The *Casearia sylvestris* Sw (Flacourtiaceae) is a shrub that occurs in forests of Southern Brazil; its leaves are widely used in folk medicine as a depurative, analgesic, anti-inflammatory and antiulcerogenic agent. The objective of this study was to perform the phytochemical description and to evaluate the pharmacological activities (antimicrobial, antifungal, antioxidant and toxicity) of the ethanolic extract (EE) of *C. sylvestris* Sw. In addition, we also evaluated the effect of the EE of *C. sylvestris* Sw on the glucose levels and lipid profile in blood serum of rats submitted to a model of streptozotocin-induced diabetes. Material and Methods: In *vitro* assay: the detection of chemical groups was done through chemical reactions with the development of color or precipitate and by chromatographic profile; the antioxidant activity was measured by the method of reduction of DPPH free radical (2,2-diphenyl-1-picrylhydrazyl); the Minimum Inhibitory Concentration was evaluated by the broth microdilution method, and the Minimum Bactericide Concentration and the Minimum Fungicide Concentration were performed in Petri dishes; the cytotoxic activity was measured by the *Artemia salina* test. In *vivo* assay: diabetic and non-diabetic rats were treated with EE of *C. sylvestris* Sw (300 mg/kg) for 45 days, and the glycaemia and lipid profile were analyzed. Results: The EE showed a Lethal Dose 50 of 724.76 µg.mL⁻¹ and important antioxidant, fungicide and fungistatic activities. The EE showed better antimicrobial activity regarding the microorganisms *Staphylococcus aureus*, *Escherichia coli* and *Salmonella setubal*. Conclusion: The EE of *C. sylvestris* Sw produces a significant decrease in triglycerides, total cholesterol and VLDL levels without any significant alteration in the glycaemia. The EE of *C. sylvestris* Sw presents antioxidant and antimicrobial activities and it exhibits a potent hypolipidemic effect.

**Keywords:** antioxidant activity, antimicrobial activity, diabetes, glycaemia, lipid profile.

**RESUMO:** Extrato etanólico de *Casearia sylvestris* Sw apresenta atividade antioxidante e antimicrobiana *in vitro* e efeito hipolipemiante em ratos. *Casearia sylvestris* Sw (Flacourtiaceae) é uma planta comumente encontrada em florestas do sul do Brasil; suas folhas são amplamente utilizadas na medicina popular como depurativa, analgésica, anti-inflamatória e anti ulcerogênica. O objetivo deste estudo foi apresentar uma descrição fitoquímica e da atividade farmacológica (antimicrobiana, antifúngica, antioxidant e toxicidade) do extrato etanólico (EE) da *C. Sylvestris* Sw. Adicionalmente, procurou-se avaliar o efeito do EE da *C. Sylvestris* Sw sobre os níveis séricos de glicose e perfil lipídico de ratos submetidos a um modelo de diabetes induzida por estreptozotocina. A detecção de grupos químicos foi realizada por reações químicas de coloração ou precipitação, e também por cromatografia; a atividade antioxidante foi mensurada pelo método de redução do DPPH (2,2-difenil-1-picril-hidrazil); a concentração mínima inibitória foi realizada pela técnica de micro-diluição, e concentração mínima bactericida e concentração mínima fungicida foram realizadas em placa de Petri; enquanto a atividade citotóxica foi conduzida pelo teste da *Artemia salina*. Nos ensaios *in vivo,*

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INTRODUCTION

Herbal medicine has been used throughout centuries similarly to the modern pharmaceuticals are used nowadays. The Brazilian territory covers a wide range of climates and soil types, providing a large biodiversity. Herbal medicines provide rational means for the treatment of many diseases (Vermani & Garg, 2002); between them, we call attention to diabetes. This disease is associated with impaired glucose tolerance and lipid metabolism. It is also linked to other common health problems, such as obesity, hypertension and atherosclerosis (Saltiel & Kahn, 2001; Modak et al., 2007). Although there are many approaches to reduce the ill effects of diabetes and its secondary complications, a lot of people prefer herbal formulations due to lesser side effects and low cost. Thus, screen for natural compounds or their derivatives with biological activity against diseases could provide a thorough knowledge of ethno botanical based on therapies and a rational exploration of new metabolites of therapeutic value.

Casearia sylvestris Sw (Flacourtiaceae) is a shrub that occurs in forests of Southern Brazil and in other countries throughout Latin America (Lorenzi, 2002; Ferreira et al., 2011) and it is popularly known as guaçatonga, chá-de-bugre or cafezinho-do-mato (Lorenzi & Matos, 2002). The leaves of this plant are widely used in folk medicine as a depurative, analgesic, anti-inflammatory, antiluercerogenic (Ruppelt et al., 1991; Esteves et al., 2005), antiviral (Simões et al., 1999), and antibacterial (Alves et al., 2000). It is also considered as an excellent functional food to heal dermal wounds and against several venoms of snakes (Cavalcante et al., 2007; Cintra-Francischinelli et al., 2008). In addition, previous study showed pronounced antioxidant of Casearia sylvestris Sw (Albano et al., 2013). The screening of medicinal plants presents an avenue for exploration of new metabolites of therapeutic value.

Casearia sylvestris Sw was reported as a plant, as demonstrated in phytochemical screening in this study. The antihyperlipidemic activity of C. sylvestris was reported in olive oil-loaded mice (Schoenfelder et al., 2008). Nevertheless, there are no studies of C. sylvestris action on lipid profile and glycaemia in animal models of diabetes. Therefore, our objective was described phytochemical screening and evaluated the pharmacological activities as antimicrobial, antifungal, antioxidant and toxicity of ethanolic extract of C. sylvestris Sw. In addition, we also evaluated the effect of the ethanolic extract of C. sylvestris Sw on glucose levels and lipid profile in blood serum of male rats submitted to a model of streptozotocin-induced diabetes.

MATERIALS AND METHODS

Plant material

The material was collected for analysis in Lajeado, Rio Grande do Sul, in February 2008. And, it was identified by Professor André Jasper PhD (PPGAD - UNIVATES) and voucher material, under number 2267, was filed in Herbarium of the Museum of Natural Sciences – Centro Universitário UNIVATES.

Preparation of the ethanolic extract (EE) of Casearia sylvestris

The plant was collect in the city of Lajeado, Rio Grande do Sul, Brazil, during the summer of 2008 and was identified by Professor Eduardo Ethur PhD (Department of Chemistry of Centro Universitário Unirates). The dried leaves were minced and extracted with 90% ethanol/water solution (ethanol:water 9:1), at room temperature, for 7 days. The solution was then totality evaporated and the extract was stored at –8 °C. The extracts were dissolved in a 3% Tween 80 solution to the desired concentration, just before use. The ethanolic extract of C. sylvestris was administered at concentration of 300 mg/kg (de Mattos et al., 2007).

Phytochemical Screening

It consisted of a set of qualitative analysis
performed with the choice of plant species in order to characterize the chemical composition of substances of plant origin. The detection of chemical groups is done through chemical reactions that show the development of color (phenolics compounds, tannins, coumarins, flavonoids), precipitate (alkaloids) or the presence of foam (saponins). This analysis is extremely important for an initial chemical characterization of the species, which has close relation with the pharmacological activity profile of the plant present. The methodology employed in this screening was adapted from Harborne (1998), Simões et al. (2004) and Brazilian Pharmacopoeia (1988).

**Pharmacological activities**

**Antibacterial and antifungal activities of the ethanolic extract (EE) of *Casearia sylvestris***

The analysis of the antimicrobial activity of ethanolic extract of *C. sylvestris* was performed using the microdilution method, using ATCC strains (American Type Culture Collection) of the following organisms - Gram Positive Bacteria: *Staphylococcus aureus* (ATCC 6538p), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633); Gram Negative: *Salmonella setubal* (ATCC 19796), *Escherichia coli* (ATCC 25792), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 27853); fungi (yeasts): *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 2601), *Cryptococcus neoformans* (ATCC 29952), *Candida glabrata* (ATCC 10231), *Candida dubliniensis* (CBS 7987). The minimum inhibitory concentration (MIC) was determined by the microdilution method, realized in triplicate. The microbial suspension was prepared on the day of analysis, obtained by adding the inoculum in 5.0 mL of sterile saline 0.8% to a standardized amount of microorganisms in solution. This standardization was carried out according to the McFarland nephelometric scale at 0.5, equivalent to approximately 1.5 x 10^8 UFC.mL^-1 (colony forming units per milliliter). For greater accuracy the amount of inoculum present, followed the recommendations of the NCCLS, whose reading of turbidity was performed in UV-VIS spectrophotometer at 625 nm wavelengths. We adapted the technique described by NCCLS (2003)[20], using a 96-microwell sterile. From solutions of the ethanolic extract of *C. sylvestris* at a concentration of 20.0 mg.mL^-1 in dimethyl sulfoxide (DMSO) were applied in microliter aliquots of 100.0 microwells in triplicate. Then there was a series of dilutions of these samples in casein soy broth for bacteria and Sabouraud broth for fungi, previously inoculated, resulting in the following test concentrations: 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg.mL^-1. The standard antibiotic was also applied in serial dilutions from a solution of chloramphenicol 0.2 mg.mL^-1, while the standard antifungal ketoconazole was used in a solution of 1 mg.mL^-1. During the test was also carried out control of the medium, inoculum and DMSO. The plates were incubated for 24 hours at 35 °C for bacteria and 48 hours at 25 °C for fungi. After this, there was microbial (bacteria and fungi) growth through the presence of turbidity in the wells. The well contains the lowest concentration where no more microbial growth was observed corresponded to the value of the minimum inhibitory concentration (MIC). For the visualization of microbial growth, were applied 10 mL of a solution of triphenyltetrazolium chloride (TTC) 5 mg.mL^-1, which results in red coloration in the wells where there was growth. The culture showed no growth plate was transferred to solid medium (nutrient agar and Sabouraud agar), to determine the minimum bactericidal concentration (MBC).

**Antioxidant activity of the ethanolic extract (EE) of *Casearia sylvestris***

The reduction potential of DPPH (2,2-diphenyl-1-picrylhydrazyl) from ethanolic extract of *C. sylvestris* was assessed spectrophotometrically as described by Elmastas et al. (2006). It was prepared a methanol solution of DPPH 0.051 mg.mL^-1, in 1.0 mL of this solution was added to 3.0 mL of methanol solution of ethanolic extract in concentrations of 0.10, 0.06, 0.04, 0.02, 0.01 and 0.005 mg.mL^-1. The mixtures were shaken and kept in the dark at room temperature for 30 minutes. The absorbance of the samples and controls were read in UV-VIS spectrophotometer (Perkin Elmer λ 25) at a wavelength of 517 nm (VIS), which corresponds to the maximum absorption of the free radical under study. The experiment was performed in triplicate, with the control the butylhydroxytoluene (BHT), synthetic antioxidant and quercetin (QUE), a natural antioxidant. The ability of the ethanolic extract of *C. sylvestris* to reduce the free radical DPPH was calculated according to the following equation: % Inhibition of DPPH = [(A0 - A1) / A0 × 100]. Where A0 is equal to the absorbance of the control and A1 is the absorbance of the samples. For the analysis of the results were used spreadsheet graphics to express the percentage of antioxidant activity.

**Cytotoxic activity of the ethanolic extract (EE) of *Casearia sylvestris***

For analysis of cytotoxicity activity (Figure 4) was used the microcrustacean *Artemia salina*, a methodology was adapted from Meyer et al. (1992). The samples were diluted to obtain concentrations of 10, 100 and 1000 μg.mL^-1, was applied along the positive control test tubes, in triplicate, leaving rest for 24 hours, drying the solvent. After this, it was applied 10 specimens of *A. salina* in each tube and 0.05 mL
of yeast. The tube was sealed and left to stand for 24 hours to evaluate the Lethal Dose 50 (LD50).

Experimental model
Animals
The experiments were carried out on 60 day-old male Wistar (180–190g) rats that was randomized according to the weight and housed in groups of five in home cages made of Plexiglass material (65 x 25 x 15 cm). The animals were maintained at 22 ± 2 °C with water and food ad libitum, under a 12:12h light/dark cycle. Experiments were conducted between 08h00 and 17h00. The protocol was approved by the on the Ethics and Research Committee of Federal University of Health Sciences of Porto Alegre (Number: 608/08), with procedures in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), the UK Animals Scientific Procedures Act 1986 and the European Community’s Council Directive of 24 november 1986 (86/609/EEC).

Chemicals and drugs
The following chemicals and drugs were used in this study: streptozotocin (STZ) and Tween 80 purchased from Sigma Chemical Co. (St. Louis, MO, USA), NPH insulin and regular insulin (Eli Lilly, São Paulo, SP, Brazil), ketamine (Parke-Davis, São Paulo, SP, Brazil) and xylazine (Bayer, São Paulo, SP, Brazil).

Experimental design
Before the beginning of experiment, the rats were habituated to the maintenance room by one week. The animals were firstly divided into two groups: non-diabetic (received citrate buffer, 1mL/Kg, i.p.) and diabetic groups [received an unique dose of streptozotocin (STZ), 65 mg/Kg, i.p.]. After, each group was divided into three groups, in total six groups: (CC): non-diabetic receiving drinking water by gavage (1 mL/kg); (CT): non-diabetic receiving 3% Tween 80 solution by gavage (extract of C. sylvestris Sw vehicle - 1 mL/kg); (CE): non-diabetic rats treated with ethanolic extract of C. sylvestris Sw by gavage (300 mg/kg); (DS): diabetic receiving drinking water by gavage (1 mL/kg) and saline s.c.; (DI): diabetic rats receiving drinking water by gavage (1 mL/kg) and injections insulin s.c. (2 UI/kg of regular insulin at 08:00 a.m., and 2-4 UI/kg of NPH insulin at 05:00 p.m.) dose of insulin was adjusted based on the severity of hyperglycemia, in order to maintain their blood glucose below 150 mg/dL (Kowluru, 2003); (DE): diabetic rats treated with ethanolic extract of C. sylvestris by gavage (300 mg/kg) and saline s.c.

Experimental induction of diabetes in rats
Rats were injected intraperitoneally with a single dose of 65 mg/kg STZ (Sigma Chemical Co., St Louis, MO, USA), freshly dissolved in citrate buffer (0.1 mol/L, pH 4.5) as previous described (Vats et al., 2004). Diabetes induction in rats was identified by polydipsia, polyuria and by measuring non-fasting serum glucose concentrations 48 h after injection of STZ. Rats with a serum glucose level above 300 mg/dL were selected for experimentation.

Pharmacological Treatment
The pharmacologic treatment started after the diabetes induction, i.e., 48 hours after STZ administration, and it was applied for 45 days consecutives: once a day, the animals received water (CC and DI), 3% Tween 80 solution (CT) and ethanolic extract of C. sylvestris Sw (CE and DE) by gavage; and twice a day the animals received saline (s.c., DS) and insulin (s.c., 08:00 a.m. and 05:00 p.m., DI).

Determination of blood glucose levels
Blood glucose levels were monitored using blood glucose test strips and a blood glucose meter (Accu-Check, Roche, Brazil). Non-fasting blood glucose was measured on day 1 (basal), 15, 30 and 45 from blood obtained from the tail vein in all groups.

Determination of serum lipids
Triglycerides (TG), total cholesterol (TC), LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) were determined on day 45 using commercial enzymatic kits (Diagnostic Labtest, MG, Brazil). VLDL cholesterol (VLDL-C) was calculated by the Friedewald equation (Friedewald et al., 1972) after measurement of total serum cholesterol, triglycerides and HDL cholesterol as previously reported (Wood et al., 2006). After fasting for 12h, animals were submitted to anesthesia with xylazine (0.5mg/kg) and ketamine (10mg/kg) and blood samples collected by cardiac puncture, centrifuged at 3000 rpm for 10 min and serum samples were obtained.

Statistical analysis
Data were expressed as mean + Standard Error of the Mean (S.E.M.) or Standard Deviation (S.D.) as indicated. For the antioxidant activity, it was used one-way ANOVA test, followed by Student-Newman-Keuls test when necessary. The cytotoxic activity was calculated from linear regression obtained by Origin Graph version 5.0 program, which allows the calculation of LD50. For confirm the diabetes condition was applied Student t test for independent samples. Repeated measures two-way ANOVA with independent factors diabetes and treatment was performed followed by multiple
comparisons test (Bonferroni test) when indicated for glucose measures, and two-way ANOVA followed by multiple comparisons (Student-Newman-Keuls test - SNK) for lipid profile. Differences were considered to be statistically significant if $P<0.05$.

RESULTS

Income

After removing the solvent in a rotary evaporator, we obtained dark brown extract with a yield of approximately 3.7%.

Phytochemical Screening

Through the phytochemical screening tests detected the following constituent groups: phenolic compounds, flavonoids, alkaloids, saponins, coumarins and tannins.

Pharmacological activities

Antibacterial and antifungal activities of ethanolic extract (EE) of *C. sylvestris*

The EE of *C. sylvestris* showed activity against the *Staphylococcus aureus*, *Escherichia coli* and *Salmonella setubal* (Table 1). In addition, the EE of *C. sylvestris* presented also a fungicide activity and be lethal to *Saccharomyces cerevisiae* and *Cryptococcus neoformans*, and fungistatic activity characterized by inhibition the growth of *Candida albicans*, *Candida glabrata* and *Candida dubliniensis* (Table 2). For the standard MBC, was found $3.12 \times 10^{-1}$ mg.mL$^{-1}$. For the standard MFC was found $1.25 \times 10^{-1}$ mg.mL$^{-1}$.

Antioxidant activity of ethanolic extract (EE) of *C. sylvestris*

We observed an important antioxidant activity of this extract, where the EE at the lowest dose tested showed a significant antioxidant activity. Importantly, BHT concentrations 0.005 and 0.01 mg.mL$^{-1}$, like QUE 0.005 mg.mL$^{-1}$ had lower potential to reduce the DPPH radical than in all of the EE concentrations (ANOVA, SNK $P<0.05$).

Cytotoxicity activity of ethanolic extract (EE) of *C. sylvestris*

This test it was possible to assess the cytotoxic activity and calculate the LD50 value provided by Figure 4. The behavior of the extract was different in each test performed. According to Meyer et al. (1982), the larger the LD50 value, the lower the degree of toxicity to extract evaluated, as such, the lower the LD50 value found, the greater the toxicity. Then, test the value found for the LD50 was $724.76 \mu g.mL^{-1}$, and values of LD50 less than 1000 $\mu g.mL^{-1}$ are consider toxic samples, however our EE presented moderate toxicity.

Biochemical analysis

Effect of ethanolic extract (EE) of *C. sylvestris* on blood glucose

Initially, 48h after the induction of diabetes with STZ i.p., and before the first dose of ethanolic extract or its vehicle, we observed difference between the level of glucose in relation to diabetics (DS, DI and DE) and non-diabetics groups (CC, CT and CE; one-way ANOVA/SNK, $P<0.001$, data not shown) in the first time (day 1).

We found a significant difference in the mean of glycaemia level between diabetic and non-diabetic rats, when diabetic rats presented higher level of glycaemia ($P<0.001$). We found a significant difference in the mean of glycaemia level among treatment, the animals that received vehicle (tween 80) and insulin presented lower level of glycaemia in comparison to other groups (two-way ANOVA, $P<0.001$). The *C. sylvestris* treatment for 45 days had no effect on glycaemia in normal or streptozotocin-induced diabetic rats (repeated measures two-way

### TABLE 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanolic extract (EE) of *C. sylvestris*. *Initial concentration of 20.0 mg.mL$^{-1}$. $^*$Standard antibiotic chloramphenicol 0.2 mg.mL$^{-1}$. NA: Not active.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>EE*(mg.mL$^{-1}$)</th>
<th>MIC</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>2.50</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>2.50</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1.25</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Salmonella setubal</td>
<td>1.25</td>
<td>2.50</td>
<td>2.50</td>
</tr>
</tbody>
</table>

### TABLE 2. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ethanolic extract (EE) of *C. sylvestris*. *Initial concentration of 20.0 mg.mL$^{-1}$. $^*$Standard antifungal, ketoconazole of 1.0 mg.mL$^{-1}$. NA: Not active.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>EE*(mg.mL$^{-1}$)</th>
<th>MIC</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Candida dubliniensis</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>5.0</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Saccharomyces cerevisae</td>
<td>5.0</td>
<td>5.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>
Values presented in mg/dL and data were expressed as mean ± S.D. (n = 6). *different from non-diabetic rats (repeated measure two-way ANOVA, P < 0.05). ** different from other groups (repeated measure two-way ANOVA, P < 0.05). *** different from other times (repeated measure two-way ANOVA, P < 0.05). (CC): non-diabetic receiving drinking water by gavage; (CT): non-diabetic receiving 3% Tween 80 solution by gavage; (CE): non-diabetic rats treated with EE of C. sylvestris Sw by gavage; (DS): diabetic receiving drinking water by gavage and saline s.c.; (DI): diabetic rats receiving drinking water by gavage and injections insulin s.c.;

FIGURE 3. Antioxidant activity.

Data expressed as mean ± S.E.M. of the percentage of antioxidant activity. *Groups with similar activities (ANOVA, P > 0.05). # Significant difference compared to other groups (ANOVA/SNK, P < 0.05). * different from other groups (ANOVA/SNK, P < 0.05). ** different from other groups that are not flagged as equal to it (ANOVA/SNK, P < 0.05). QUE: quercetin, BHT: butylhydroxytoluene, EE: Ethanolic Extract.

FIGURE 4. Cytotoxicity of ethanolic extract of C. sylvestris. (R = 0.98712).
(DE): diabetic rats treated with EE of *C. sylvestris* by gavage and saline s.c.

**Effect of ethanolic extract (EE) of *C. sylvestris* on lipid profile**

We found significant difference on lipid profile between diabetic and non-diabetic rats, where diabetic rats presented higher levels of triglycerides and VLDL, associated to lower levels of total cholesterol, LDL and HDL (two-way ANOVA, \( P < 0.01 \), Table 3).

We observed effect of the treatment by two-way ANOVA (\( P < 0.05 \)). It was observed which animals that received ethanolic extract of *C. sylvestris* presented lower triglycerides and VLDL levels in comparison to other groups (two-way ANOVA/SNK, \( P < 0.05 \)). The total cholesterol level of the animals that received ethanolic extract of *C. sylvestris* presented lower levels in comparison to animals that received water and vehicle (tween 80) by gavage (two-way ANOVA/SNK, \( P < 0.05 \)). While, the animals that received insulin presented even total cholesterol lower level in relation to animals that received water, and animals that received vehicle (tween 80) by gavage presented higher levels than other groups (two-way ANOVA/SNK, \( P < 0.05 \), Table 3).

In addition, we observed interaction between diabetes and treatment in relation to level of triglycerides, total cholesterol, LDL and VLDL (two-way ANOVA, \( P < 0.05 \)).

**DISCUSSION**

In this study, the EE extract showed an important antioxidant activity, checked by the test of DPPH. In addition, it showed an antioxidant activity higher than the standards BHT and quercetin in lower concentrations, corroborating a recent study using hydroalcoholic extract of *C. sylvestris* (Albano et al., 2013). In the last years, it has been described the role of free radicals and pro-oxidant molecules in aging, cancer, cardiovascular diseases, and brain dysfunction (Molyneux, 2004). Thus, it is need to study new compounds to protect the body against free radical, and based on our results, we suggest that the potential antioxidant activity observed in our study can be related to the chemical composition of *C. sylvestris*. It is known that coumarins, flavonoids, lignans and several diterpenes, especially the clerodane (de Carvalho et al., 1998; Tininis et al., 2006) are present, in its composition. The results of the phytochemical screening confirmed the presence of phenolic compounds, alkaloids, flavonoids, saponins, tannins and coumarins. And, phenols...
TABLE 3. Effect of ethanolic extract of Casearia sylvestris on serum lipid profile of rats treated for 45 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
<th>VLDL cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>144.33±10.54</td>
<td>36.00±3.58</td>
<td>31.67±4.87</td>
<td>8.33±0.82</td>
<td>41.17±4.17</td>
</tr>
<tr>
<td>CT</td>
<td>163.83±13.82</td>
<td>45.17±6.62</td>
<td>84.34±12.70</td>
<td>10.83±2.14</td>
<td>53.33±11.18</td>
</tr>
<tr>
<td>CE</td>
<td>130.40±9.53</td>
<td>46.40±3.51</td>
<td>37.31±5.20</td>
<td>5.80±0.84</td>
<td>28.60±3.29</td>
</tr>
<tr>
<td>DC</td>
<td>114.04±8.06</td>
<td>26.40±5.59</td>
<td>82.40±7.99</td>
<td>49.80±1.92</td>
<td>250.00±9.62</td>
</tr>
<tr>
<td>DI</td>
<td>58.25±15.73</td>
<td>31.00±5.88</td>
<td>28.12±11.79</td>
<td>13.25±1.98</td>
<td>66.25±8.97</td>
</tr>
<tr>
<td>DE</td>
<td>49.33±6.35</td>
<td>44.67±4.03</td>
<td>20.34±5.57</td>
<td>8.33±2.25</td>
<td>40.33±10.71</td>
</tr>
</tbody>
</table>

Values expressed in mg/dL and data were expressed as mean ± S.D. (n = 4-8). *different from other groups (two-way ANOVA/SNK, P<0.05); a different from water group (two-way ANOVA/SNK, P<0.05); b different from insulin group (two-way ANOVA/SNK, P<0.05). (CC): non-diabetic receiving drinking water by gavage; (CT): non-diabetic receiving 3% Tween 80 solution by gavage; (CE): non-diabetic rats treated with EE of C. sylvestris Sw by gavage; (DC): diabetic receiving drinking water by gavage and saline s.c.; (DI): diabetic rats receiving drinking water by gavage and injections insulin s.c.; (DE): diabetic rats treated with EE of C. sylvestris by gavage and saline s.c.

compounds, tannins, flavonoids and coumarin, are generally described with the antioxidant activity (Martín-Sánchez et al., 2014). Deserving emphasis on phenolic compounds and flavonoids that determine the antioxidant activity of the extract of C. sylvestris (Menezes et al., 2004), acting as free radical scavenger. In addition, the EE showed an antimicrobial activity against the microorganisms Staphylococcus aureus, Escherichia coli and Salmonella Setubal. Likewise, the antimicrobial activities can be linked to the presence of saponins, which presents bactericidal and fungicidal activities (Chung et al., 2006).

Additionally, in opposing to popular medicine, the chronic treatment of EE (45 days) did not promote any benefits in the glycaemia serum level of rats. However, it is important to highlight that our model of diabetes induction (STZ) worked very well, the diabetic rats presented high levels of glycaemia. It is important to bring to light that the diabetes model used is involved to the inhibition of biosynthesis and secretion of insulin, culminating in the β cells death; and we can not discard that the folk used of C. sylvestris may be related to at least in part the production, releasing or action of endogenous insulin. Interestingly, the vehicle (tween 80) presented lower levels of glycaemia in comparison to other treatments (two-way ANOVA); this effect observed showed a limitation of our study, since, we did not have diabetic rats treated with the vehicle (tween 80). In the present study, we did not evaluate a possible synergic effect between insulin and EE of C. sylvestris Sw, that it can be considered a limitation of the present protocol.

In relation to diabetic condition, we observed that diabetic rats presented lower levels of total cholesterol and LDL in relation to non-diabetic rats, and these levels were decreased in high proportion after insulin treatment revealed by two-way ANOVA corroborating previous study (Pinheiro et al., 2011). These rats also presented higher serum levels of triglycerides and VLDL, and this condition was reverted by insulin treatment. In addition, the same animals showed lower level of HDL that did not suffer any influence of insulin treatment. Interestingly, we found reduced levels of total serum cholesterol in diabetic rats in comparison to non-diabetic rats. This finding can be interpreted as high absorption and low synthesis of cholesterol. Streptozotocin-induced diabetes in rats hypertrophies intestinal mucosal function, enhancing fat and cholesterol absorption and reducing cholesterol synthesis (Young et al., 1988). It is also known that fasting down regulates cholesterol synthesis and reduction of dietary cholesterol has been observed to effectively reduce serum cholesterol in type 1 diabetic subjects (Kaufmann et al., 1975).

Important results were found in relation to experimental model, we observed that the EE of C. sylvestris treatment presented a significant improvement in lipidic profile values in rats (decrease total cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides levels), this corroborates previous study that demonstrated the antihyperlipidemic activity of C. sylvestris (Schoenfelder et al., 2008). We highlight the hypolipidemic activity by ethanolic extract of C. sylvestris including in a not specific model to develop lipid profile alterations. Moreover, it was observed that the HDL levels were increased for both vehicle tween 80 and ethanolic extract of C. sylvestris treatments, highlighting the vehicle action. However, we call attention that tween 80 also increased the total cholesterol and LDL levels (Table 1) while the ethanolic extract of C. sylvestris decreased them, highlighting the action of extract of C. sylvestris. This difference observed by treatment brings to light the protective effect of ethanolic extract of C. sylvestris against cardiovascular diseases.

Previous study showed that flavonoids are...
able to improve the lipid profile in normal rats (Ito et al., 2008); and the C. sylvestris extract is a flavonoid-rich plant, as demonstrated in phytochemical screening in this study. The activity of HMG-CoA reductase, the key enzyme of cholesterol biosynthesis, is inhibited by flavonoids and it probably also exerts their influence on steroid metabolism at other pivotal points (Havsteen, 2002; Sung et al., 2004; Min & Kim, 2007). In diet-induced obesity, tea flavonoids increased fecal excretion of cholesterol and bile acids and reduced cholesterol absorption from intestine (Ikeda et al., 1992). These studies indicate that flavonoids modulate lipid metabolism not only in diabetic or obese animals, but also in normal animals. Therefore, we suggest that the flavonoids present in the extract may account at least in part of the hypolipidemic effect observed in our study.

Many studies showed the inhibition of phospholipase A2 by C. sylvestris (Raslan et al., 2002; Cavalcante et al., 2007; da Silva et al., 2008) and inhibition of cytosolic phospholipase A2 has been reported by suppresses the production of cholesterol ester (Li et al., 2008). It may be also another mechanism by which C. sylvestris improves the lipid profile.

In contrast, we observed that the diabetic rats showed higher triglycerides levels, it can be characterizing a hyperlipidemic state. There are several reports in the literature trying to explain the possible mechanisms underlying the hyperlipidemia presented by insulin-deficient diabetes (Reaven & Reaven, 1974; Chen et al., 1979; Rauramma et al., 1980; Tavangar et al., 1992; Gleeson et al., 1999; Horii et al., 2004). Insulin deficiency stimulates lipolysis in adipose tissues, increasing the delivery of free fatty acids (FFA) from adipose tissues to liver and consequently, the production of triglycerides (TG) in liver. Insulin deficiency also reduces plasma lipoprotein lipase (LPL) activity, a key enzyme in plasma lipid metabolism. It hydrolyzes triglycerides (TG) from chylomicrons and VLDL, controls fatty acid uptake into tissues, and releases components for HDL formation (Merkel et al., 2002; Rizos et al., 2005; Loeffler et al., 2007) demonstrated that lipoprotein-associated phospholipase A2 (Lp-PLA2) activity was higher in patients with the metabolic syndrome than in those without it. In addition, increased levels of Lp-PLA2 mass concentration was noted in diabetic patients requiring insulin, suggesting that Lp-PLA2 may raise significantly when the metabolic syndrome is present and β cell insufficiency coexists with high insulin resistance (Noto et al., 2006). 3-Hidroxi-3-metilglutaril-CoA (HMG-CoA) reductase inhibitors (statins) and fenofibrates can reduce Lp-PLA2 concentrations in plasma, being orally active. Also, specific Lp-PLA2 inhibitors have been developed and tested in clinical trials to evaluate the potential of Lp-PLA2 as a therapeutic target (McConnell & Hoefner, 2006).

CONCLUSION
In summary, first, we did not find any improvement in the glycaemia serum level after 45 days of treatment with EE of C. sylvestris in a rat model of type 1 diabetes. Second, we suggest that the use of C. sylvestris in folk medicine has a high association with the data generated in this study, i.e., the extract has shown significant hypolipidemic effect in rats. We can suggest a possible effect cardio-protective associated to lipid profile enhancement. The exact mechanism(s) underlying the reduction of the cholesterol and triglycerides by the EE of C. sylvestris was not investigated in the current study. However, further studies would be required to determine the exact mechanism(s) of lipids lowering effects and site of action of the extract.

Competing interests
The authors report no conflicts of interest.

REFERENCES


