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The influence of seasonality on the content of goyazensolide and on anti-inflammatory and anti-hyperuricemic effects of the ethanolic extract of *Lychnophora passerina* (Brazilian arnica)

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ABSTRACT

Ethnopharmacological relevance: *Lychnophora passerina* (Mart ex DC) Gardn (Asteraceae), popularly known as Brazilian arnica, is used in Brazilian folk medicine to treat pain, rheumatism, bruises, inflammatory diseases and insect bites.

Aim of the study: Investigate the influence of the seasons on the anti-inflammatory and anti-hyperuricemic activities of ethanolic extract of *L. passerina* and the ratio of the goyazensolide content, main chemical constituent of the ethanolic extract, with these activities.

Materials and methods: Ethanolic extracts of aerial parts of *L. passerina* were obtained from seasons: summer (ES), autumn (EA), winter (EW) and spring (EP). The sesquiterpene lactone goyazensolide, major metabolite, was quantified in ES, EA, EW and EP by a developed and validated HPLC-DAD method. The *in vivo* anti-hyperuricemic and anti-inflammatory effects of the ethanolic extracts from *L. passerina* and goyazensolide were assayed on experimental model of oxonate-induced hyperuricemia in mice, liver xanthine oxidase (XOD) inhibition and on carrageenan-induced paw edema in mice.

Results: HPLC method using aqueous solution of acetic acid 0.01% (v/v) and acetonitrile with acetic acid 0.01% (v/v) as a mobile phase in a gradient system, with coumarin as an internal standard and DAD detection at 270 nm was developed. The validation parameters showed linearity in a range within 10.0–150.0 μg/ml, with intraday and interday precisions a range of 0.61–3.82. The accuracy values of intraday and interday analysis within 87.58–100.95%. EA showed the highest goyazensolide content. From the third to the sixth hour after injection of carrageenan, treatments with all extracts at the dose of 125 mg/kg were able to reduce edema. Goyazensolide (10 mg/kg) showed significant reduction of paw swelling from the second hour assay. This sesquiterpene lactone was more active than extracts and presented similar effect to indomethacin. Treatments with ES, EA and EP (125 mg/kg) and goyazensolide (10 mg/kg) reduced serum urate levels compared to hyperuricemic control group and were able to inhibit liver XOD activity. One of the mechanisms by which ES, EA, EP and goyazensolide exercise their anti-hyperuricemic effect is by the inhibition of liver XOD activity. Goyazensolide was identified as the main compound present in ES, EA, EW and EP and it is shown to be one of the chemical constituents responsible for the anti-inflammatory and anti-hyperuricemic effects of the ethanolic extracts.

Conclusion: The anti-inflammatory and anti-hyperuricemic activities of the ethanolic extracts from *L. passerina* were not proportionally influenced by the variation of goyazensolide content throughout the seasons. The involvement of goyazensolide on *in vivo* anti-inflammatory and anti-hyperuricemic activities of *L. passerina* extracts was confirmed, as well as the possibility of participation of other constituents on these effects. This study demonstrated that the aerial parts of *L. passerina* may be collected in any season for use as anti-inflammatory agent. For use in hyperuricemia, the best seasons for the collection are summer, autumn and spring. The ethanolic extract of *L. passerina* and goyazensolide can be considered promising agents in the therapeutic of inflammation, hyperuricemia and gout.

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1. Introduction

The *Lychnophora* genus (Asteraceae) is known in folk medicine as Brazilian arnica (Cerqueira et al., 1987). Like other species of the genus, *Lychnophora passerina* (Mart ex DC.) Gardn is used in traditional medicine in the form of alcoholic preparations to treat inflammation, pain, bruises, rheumatism and insect bites (Saúde et al., 1998; Chaves et al., 2015). Despite the traditional use of *L. passerina* as anti-inflammatory agent was supported by scientific results (Guzzo et al., 2008; Capelari-Oliveira et al., 2011).

There are few studies on the quantification of chemical markers in *Lychnophora* species. In addition, the most of the studies about influences on the concentration of metabolites are still directed to a limited number of commercially available species. The content of secondary metabolites may vary over time due to seasonal and daily variations and may change by spatial conditions (Gobbo-Neto and Lopes, 2007). Furthermore, the existence of other factors such as intra- and interspecies variations and biological and environmental factors may affect the occurrence of plant metabolites (Harborne, 1993). These variations may directly influence the therapeutic value of herbal preparations (Calixto, 2000) and reinforce new standardization and quality studies.

Phytochemical analysis of *L. passerina* revealed a variety of metabolites such as flavonoids, triterpenes and sesquiterpene lactones of which goyazensolide have been often reported as chemical marker of species (Borella et al., 1998; Chicaro et al., 2004). The anti-inflammatory activity of goyazensolide has been demonstrated in previous studies (Kanashiro et al., 2006; Rüngeler et al., 1999).

Gouty arthritis is a metabolic disease belongs to the group of rheumatism, in which there is excessive production or decreased excretion of uric acid, resulting in the deposition of mono sodium urate (MSU) in joints and soft tissues (Hou et al., 2007; Popovich et al., 2006). In recent decades, the prevalence of hyperuricemia and gout increased significantly due increasing life expectancy, use of drugs and organ transplants. The hyperuricemia is known as the main risk factor in the development of gout and its association with the development of cardiovascular disease, diabetes and nephrolithiasis was reported (Dalbeth and So, 2010). Previous studies have demonstrated the anti-hyperuricemic effect of the ethanolic extract from *Lychnophora trichocarpha* (De Souza et al., 2012).

This study aimed evaluate the anti-hyperuricemic activity of ethanolic extracts of *L. passerina* and goyazensolide; identify and quantify the main chemical constituent of the ethanolic extract and evaluate the seasonal influence on anti-inflammatory and anti-hyperuricemic activities of the ethanolic extract and on content of this major compound.

2. Material and methods

2.1. Chemicals and reagents

Ethanol, chloroform, hexane, ethyl acetate, methanol, dimethylsulphoxide (DMSO), Tween80 were of analytical grade. HPLC grade acetonitrile (ACN), methanol and acetic acid were obtained from J.T. Baker (EUA). Xanthine (≥99%), potassium oxonate (97%), uric acid (≥99%), indomethacin (≥99%), allopurinol (98%), coumarin (≥99%) and carrageenan were obtained from Sigma-Aldrich (USA). Ketamine and xylasine were obtained from Sespro Indústria e Comércio Ltda (Brazil). Uric acid assay kit was purchased from Bioclin (Brazil). Pure water (Milli-Q, Millipore) was employed in all the experiments.

2.2. Plant material

Aerial parts of *Lychnophora passerina* (Mart ex DC.) Gardn were collected in Serra do Cipó, Minas Gerais, Brazil. The plant was identified by Prof. Aristônio Magalhães Teles of the Federal University of Goiás (UFG). A voucher specimen was deposited in the Herbarium of the Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto – UFOP, under the number OUPR 28840. Aerial parts were collected from the same population, at three months intervals of the same year (in March/10, June/10, September/10 and December/10 of 2013) and always in the afternoon. As soon as possible, the plant material was dried under forced ventilation at 37 °C, for a week, and stored in a dry place.

2.3. Preparation of ethanolic extracts

Dried aerial parts (200.0 g) of *L. passerina* collected of each season were ground and extracted with ethanol (4.0 L) by percolation, at room temperature, for seven days. The solvent was removed under reduced pressure for obtain the dried ethanolic extracts of the seasons: summer (ES, 16.7 g), autumn (EA, 15.6 g), winter (EW, 19.3 g) and spring (EP, 14.1 g).

2.4. Isolation and identification of goyazensolide

Dried aerial parts from *L. passerina* (579.0 g) were exhaustively extracted with chloroform by percolation. The solvent was eliminated resulting in the dried crude chloroformic extract (44.0 g). The extract was submitted to filtration column chromatography on silica gel (63–200 μm, Merck), eluted with hexane, ethyl acetate and methanol to yield the hexanic (FH, 2.0 g), hexanic/ethyl acetate (1:1) (FHAc, 18.39) and methanolic (FM, 13.48 g) fractions, respectively. FHAc yielded a white solid (P1, 1.3 g).

2.5. Analytical HPLC method

The following mobile phase gradient was employed for HPLC-DAD analysis, at a flow rate of 1.0 ml/min: solvent A=aqueous acetic acid, 0.01% (v/v); solvent B=acetonitrile with acetic acid, 0.01% (v/v); elution profile=0–10 min, 10–25% B (linear gradient, 10–30 min, 25–30% B (linear gradient), 30–40 min, 30–60% B (linear gradient), 40–45 min, 60–80% B (linear gradient), 45–50 min, 80–10% B (linear gradient), 50–55 min, 10% B (isocratic); the UV-DAD detect was set to record spectra between 210 and 600 nm, UV chromatograms were recorded at 270 nm. A LC-RP-18 column (5 μm, 4.6/150 mm; Waters) was utilized and its temperature was maintained at 30 °C. Coumarin was chosen as the internal standard (I.S.) at fixed concentration of 10.0 μg/ml.

2.6. Method validation

An analytical methodology of goyazensolide quantification in ethanolic extracts of *L. passerina* was validated in accordance with current Brazilian and international legislations (Brasil, 2012), using the same parameters employed for the previously described HPLC-DAD method. Solutions of goyazensolide and coumarin (IS) were added to ethanolic extract solution and injected under method conditions for the retention times and peak purity evaluation. For this last, the purity angle and purity threshold were obtained from IS and goyazensolide peaks. According to Waters software, there is no co-elution or interference of other substance when peak purity angle obtained is lower than its purity threshold. The limits of detection (LOD) and quantitation (LOQ) were estimated as the minimum concentration of goyazensolide able to produce signal-to-noise ratios (S/N) of 3 and 10, respectively. To assess the linearity of the method, goyazensolide solutions (1.0 mg/ml, triplicate) was added in extract solution (1.0 mg/ml) at concentrations of zero, 10.0, 25.0, 50.0, 75.0, 100.0, 150.0 μg/ml. Analytical curves were obtained by ratio of goyazensolide and IS peaks areas at 270 nm. The precision and accuracy were performed for injection of triplicate of low (10.0 μg/ml), medium (75.0 μg/ml) and high (150.0 μg/ml) concentrations of goyazensolide

by injections in one day and three consecutive days. The precision were expressed as residual standard deviation (RSD %) and accurate was measured as percent deviation from nominal concentration. For the stability test, the samples were immediately analyzed after preparation and after 24 h.

2.7. Determination of goyazensolide content in ethanol extracts from L. passerina

Methanolic solutions at 4.0 mg/ml of ethanolic extracts obtained from each seasons added 10.0 µl of IS (1.0 µg/ml) were injected under validated conditions after the construction of an analytical curve.

2.8. Animals

The experiments were conducted on male Swiss mice (25–30 g; six-seven weeks) supplied by Animal House of Universidade Federal de Ouro Preto (UFOP). Animals were divided into experimental groups (n=6), housed in plastic cages and maintained on light/dark period of 12 h. They received feed and water *ad libitum*. Experiment protocol was approved by the Ethical Committee of Animal Experimentation of UFOP, Brazil (no. 2012/68). The protocols were prepared in accordance to the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication, revised in 1985).

2.9. Carrageenan-induced paw edema assay

The assay was performed using the methodology described by Winter et al. (1962) with modifications. Ethanolic extracts (ES, EA, EW and EP), goyazensolide and indomethacin were solubilized in DMSO: Tween 80: water (1:1:8). Animals were divided into 16 experimental groups (n=6) and fasted 2 h before administration of tests solutions. The animals of the control condition group received only the injection of a saline solution into the sub-plantar tissue of the right hind paw. The animal groups were treated, by gavage, with ES, EA, EW, EP (62.5, 125.0, 250.0 mg/kg body weight each), goyazensolide (10 mg/kg body weight) and indomethacin (10 mg/kg, positive control group). Mice of the untreated group received only vehicle by gavage. One hour after administration of the various agents, edema was induced by injection of carrageenan (20 µl, 0.1%, w/v) into the sub-plantar tissue of the right hind paw. Only needle introduction was performed into the left paw, corresponding damage induced by mechanical perforation. Measurements of paw thickness were performed by a digital caliper rule (Starred) at immediately and every hour for 6 h after carrageenan application. The results were expressed as percentage change compared to the initial thickness (Δ). The untreated group was considered as maximum of inflammation and all others treatments were compared to this group.

2.10. Anti-hyperuricemic effect in oxonate-induced hyperuricemic mice and inhibition of liver xanthine oxidoreductase (XOD) activity

2.10.1. Study design and tests solution administration

ES, EA, EW, EP, goyazensolide and allopurinol were solubilized in DMSO: Tween 80: water (1:1:8). Potassium oxonate (250 mg/kg) was prepared in suspension with 0.9% saline immediately before its use. Animals were divided into 16 experimental groups (n=6) and fasted 2 h before administration of tests solutions. Mice of normal and hyperuricemic groups received only vehicle by gavage. In positive control group, animals were treated with allopurinol (10 mg/kg body weight, gavage). The animal groups that remain were treated, by gavage, with ES, EA, EW and EP (62.5, 125 and 250 mg/kg body weight) and goyazensolide (10 mg/kg body weight). The treatment was carried out for three days in a row.

2.10.2. Animal model hyperuricemia in mice

In order to evaluate the anti-hyperuricemic activity of ES, EA, EW, EP and goyazensolide has been used an animal model of hyperuricemia induction with potassium oxonate previously described with modifications (Hall et al., 1990; De Souza et al., 2012). Potassium oxonate is an uricase inhibitor, enzyme present only in animals, responsible for metabolizing uric acid into allantoin (Miguel and Mediavilla, 2011). Potassium oxonate (250 mg/kg) was administrated intraperitoneally to each animal, except those of normal control group, in the first and third days of the experiment. Extracts, goyazensolide and allopurinol solutions were given by gavage, 1 h after the potassium oxonate administration, once a day, for 3 consecutive days. At the third day, mice were anesthetized with ketamine and xylasine (100 and 20 mg/kg, respectively), 1 h after the final drug administration. It performed abdominal opening of animals for blood collection from abdominal aorta. The blood was kept at room temperature and centrifuged at 2500×g for 10 min. Serum was stored at −20 °C for later quantification of uric acid.

2.10.3. Uric acid assay

Serum concentrations of uric acid in blood samples of animals were determined by the enzymatic-colorimetric method, using a diagnostic kit (Bioclin, Brazil) according to the manufacturer's instructions.

2.10.4. Liver sample preparation

The liver extraction was performed immediately after blood collection and euthanasia. Livers were washed in 0.9% cold saline solution and quickly stored at −80 °C. Enzyme extraction has been performed as described previously (Haidari et al., 2008). Livers were macerated in 5.0 ml of 80 mM sodium phosphate buffer (pH 7.4) and centrifuged at 3000×g for 10 min at 4 °C. Lipid layer was removed and the residual supernatant removed was centrifuged at 10,000g again for 60 min at the same temperature. The supernatant from this final step was used for enzymatic assays.

2.10.5. Liver XOD activity assay

XOD activity was assayed spectrophotometrically by monitoring the uric acid formed by the action of xanthine oxidase, as described previously (Hall et al., 1990; De Souza et al., 2012) with modifications. Liver homogenate (100 µl) was added 5.4 ml of a mixture containing 50 mM phosphate buffer (pH 7.4) and potassium oxonate (0.2 mg/ml). Samples were preincubated for 15 min at 35 °C. The reaction was initiated by addition of 1.2 ml of the 250 µM xanthine. The reaction was stopped after 0 and 30 min by adding 0.5 ml of 0.6 M HCl. The resulting solution was centrifuged at 3000×g for 5 min, the supernatant was collected and its absorbance was measured at 295 nm using a Varian 50Bio UV/VIS spectrophotometer. The protein content of liver homogenate was performed according to the spectrophotometric method described of Bradford (1976). The inhibition of XOD was expressed in nanomoles (nM) of uric acid formed per minute per milligram protein.

2.11. Statistical analysis

Results of *in vivo* assays were analyzed using GraphPad Prism 5.0 soft-ware (Inc. San Diego, CA, U.S.A.) and presented as mean values S.E.M. (n=6) for each experimental group. Data were submitted to analysis of variance (One-way ANOVA) followed by Bonferroni test. P values ≤0.05 were considered statistically significant.

3. Results

3.1. Goyazensolide identification

The chromatographic signals identification of the goyazensolide was confirmed by co-injection of sample previously isolated and characterized by our group (Fig. 1). Goyazensolide was identified by

comparison of melting point (mp 168.7–169.5 °C) and IV, ^{1}H and ^{13}C NMR data with literature data of goyazensolide (Vichnewsky et al., 1976; Bohlmann et al., 1981; Herz and Goedken, 1982; Perry et al., 2001).

3.2. Method development and validation

The HPLC-DAD method validation ensured reliability of results for goyazensolide quantification in *L. passerina* extracts. The retention times in the HPLC method developed were 19.92 min for goyazensolide and 12.79 min for the IS (Fig. 2). The peak purity angles (goyazensolide: 0.230 and IS: 0.209) were lower than their respective purity thresholds (goyazensolide: 0.448 and IS: 0.382) showing the absence of interfering co-elution between the analytes and other constituents of extract. The method was considered linear between concentrations of 10.0 and 150.0 µg/ml for the three calibration curves. The mean regression equation was y=0.03314x+1.250 (r=0.9955). The LOD and LOQ for goyazensolide were 1.13 µg/ml and 3.75 µg/ml, respectively. The precision at low, medium and high concentrations of goyazensolide showed RSD values of 3.82%, 0.61% and 2.80% to intraday and 1.83%, 1.51% and 2.15%, to interday analysis. Under same concentrations, the variations obtained for accuracy were 87.58, 100.95, 100.15%, and 93.06, 97.47, 100.08%, respectively. The RSD and variations obtained from analyses were lower that preconized (Brasil, 2012). Therefore, all these data demonstrated that HPLC-DAD method was precise and accurate. Standards and extract evaluated immediately analyzed and also 24 h after their acquisition showed no significant differences in plotting peak areas.

3.3. Determination of goyazensolide content in ethanolic extracts (ES, EA, EW and EP)

Goyazensolide content determination showed significant variations in ethanolic extracts of *L. passerina* and the highest mean concentrations were detected in autumn (EA, 4.73%) and they were decreasing until the summer (ES, 2.51%) (Fig. 3). Analysis of ES, EA, EW and EP HPLC chromatograms showed no variation in the chemical composition of the extracts of the summer to winter, there was only variation in the content of the chemical constituents. EP had fewer components compared to other extracts.

3.4. Effects of ES, EA, EW, EP and goyazensolide on carrageenan-induced paw edema in mice

The carrageenan injection caused a significant increase in paw edema observed in animals that received only vehicle (untreated group). This group was considered as the maximum inflammation, and it was used to compare to the others groups aiming to evaluate anti-inflammatory activity. From the third to the sixth hour after injection of carrageenan, treatments with all extracts at the dose of 125 mg/kg were able to significantly reduce edema. The paw thickness was reduced in mice treated with EA at the dose of 250 mg/kg from the first to the fifth hour experiment. Mice treated with goyazensolide at the dose of 10 mg/kg showed significant reduction of paw swelling from of the second hour assay. This compound was statistically more active than extracts and similar to indomethacin (positive control). No significant differences were identified between the anti-inflammatory effects of ES, EA, EW and EP at the dose of 125 mg/kg (Table 1).

3.5. Effects of ES, EA, EW, EP and goyazensolide on serum urate levels in hyperuricemic mice

In this work it was evaluated the first time the anti-hyperuricemic activity of the ethanol extract from *L. passerina* and of the goyazensolide. Animals of the hyperuricemic control group were treated with uricase inhibitor potassium oxonate showed increase serum urate levels compared to normal control group (Fig. 4). Positive control group (allopurinol, 10 mg/kg) had serum urate levels reduced lower normal values. Treatment with ES and EA at the doses of 125 and 250 mg/kg reduced serum urate levels compared to hyperuricemic control group. Treatment with EP showed anti-hyperuricemic effect only at the dose of 125 mg/kg. EW not was active in any of the evaluated doses. Goyazensolide (10 mg/kg) induced a significantly reduction of serum urate levels and its statistically similar to allopurinol.

3.6. Effects of ES, EA, EP and goyazensolide on XOD activity in mice liver

The evaluation of liver xanthine oxidase (XOD) inhibition activity was performed only for the effective extracts and at doses that presented anti-hyperuricemic activity. ES and EA at the doses of 125 and 250 mg/kg and goyazensolide (10 mg/kg) were significantly able to inhibit liver XOD activity, when compared to hyperuricemic control group (Table 2). EP at the dose of 125 mg/kg was able to inhibit liver XOD activity. Allopurinol caused a significant reduction (68.8%) of XOD.

4. Discussion

This work evaluated the anti-hyperuricemic activity and the influence of the seasonality on the anti-inflammatory and anti-hyperuricemic effects of the ethanolic extracts from *L. passerina* and on content of goyazensolide in this extract. Sesquiterpene lactones are reported to be isolated and/or identified in 90% of the species of subtribe *Lychnophorinae* (Keles et al., 2010). The high occurrence of these metabolites may justify the different research on these substances.

A novel standard addition method was developed and it was suitable for to quantify goyazensolide in presence of other constituents of the *L. passerina* ethanolic extracts. The HPLC-DAD method showed acceptable separation and relatively a short analysis time for goyazensolide compared to the data available in the literature (Gouvea et al., 2012) and contributed in reducing solvent consumption in routine work. Moreover, the method validation covered a wide concentration range and the value of LOQ was small and consistent for analysis of

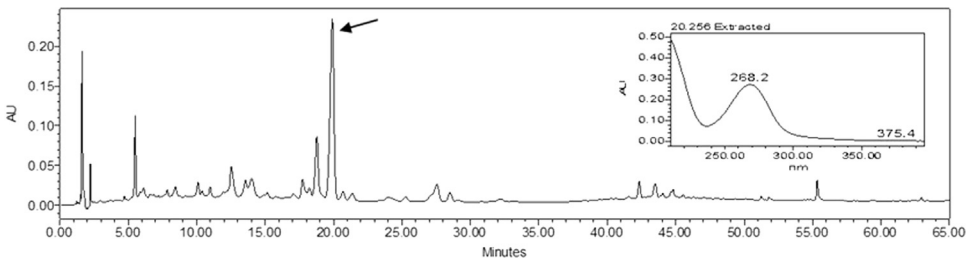

Fig. 1. Goyazensolide peak on chromatogram obtained during analysis of the ethanolic extract from *Lychnophora passerina* (2.0 mg/ml). Mobile phase: aqueous acetic acid 0.01% (v/v) and acetonitrile with acetic acid 0.01% (v/v). Flow rate of 1.0 ml/min. UV-DAD detection at 270 nm.

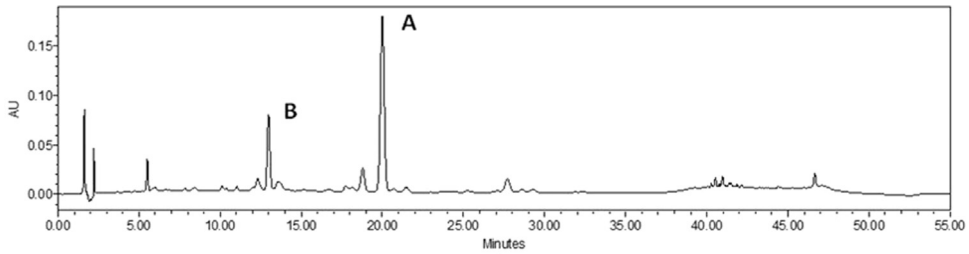

Fig. 2. Peaks on chromatogram obtained during analysis of the ethanol extract from *Lychnophora passerina* (1.0 mg/ml). **A**: Goyazensolide peak (50.0 μg/ml); **B**: Internal standard peak (10.0 μg/ml). Mobile phase: aqueous acetic acid 0.01% (v/v) and acetonitrile with acetic acid 0.01% (v/v). Flow rate of 1.0 ml/min. UV-DAD detection at 270 nm.

goyazensolide variability in species, thereby ensuring reliability and credibility of the results.

Goyazensolide was identified as the main compound present in ES, EA, EW and EP maintaining its majority throughout the study time. Previous studies indicate goyazensolide as one of the most present sesquiterpene lactones in species of subtribe *Lychnophorinae* (Chicaro, 2004) and genus *Eremanthus* (Gouvea et al., 2012). In addition, goyazensolide showed to be one of the chemical constituents responsible for the anti-inflammatory and anti-hyperuricemic effects of the ethanolic extracts. However, it was shown that other components of the extract also contribute to these activities. Previous phytochemical investigations of the aerial parts of *L. passerina*, collected in same region of the species used in this study, resulted in the isolation of the taraxasterol, α-and β-amyrin, lupeol, lupeol acetate, α-humulen, lychnophoric acid, stigmasterol, eremantin, goyazensolide, 15-desoxygoyazensolide, kaempferol, apigenin, luteolin, quercetin and tiliroside (Bohlmann et al., 1981; Perry et al., 2001; Chicaro et al., 2004). Some of this compounds, such as α and β - amyrins, lupeol, α-humulen and flavonoids had their anti-inflammatory activity demonstrated (Akihisa et al., 1996; Rajic et al., 2000; Rodrigues et al., 2013). Besides, the inhibitory activity of sesquiterpene lactones in transcription factor of inflammatory cytokines (NF-κB) was confirmed and goyazensolide is one of the most potent of the evaluated substances (Sakamoto et al., 2003).

The carrageenan-induced paw edema method is an experimental model of acute inflammation involving different phases. In the first phase (1.5 h), carrageenan stimulates the release of pro-inflammatory cytokines that induce the release of serotonin and histamine. In middle phase (1.5–2.5 h) occurs the release of kinins. Vasoactive amines are responsible for the initial phase (first 3 h), followed by activation of prostaglandins, lysosomal enzymes and proteases in the later phase (4–6 h) (Akkol et al., 2008). In the present study, the ethanolic extracts from *L. passerina* were able to reduce edema significantly of the third to sixth hour of the administration of carrageenan. No anti-inflammatory activity differences were found between ethanolic extracts of the plant collected in different seasons. Goyazensolide acted at different phases of inflammation and showed statistically more anti-inflammatory activity than extracts and similar to indomethacin.

The control of hyperuricemia and the treatment of inflammation are the therapeutic approaches against gout. Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used to treat acute inflammation in gout, however, may develop various side problems such as gastrointestinal toxicity, renal toxicity or gastrointestinal bleeding (Cronstein and Terkeltaub, 2006). Probenecid and benzbromarone increase uric acid elimination and are called uricosuric agents, useful in underexcreters patients that have been associated with adverse effects including renal and gastrointestinal toxicity (Miguel and Mediavilla, 2011). Allopurinol is the drug of choice for the control of hyperuricemia that acts inhibiting the xanthine oxidase activity, the enzyme that converts xanthine and hypoxanthine into uric acid, but it has been associated with adverse effects, such as fever, skin rashes, allergic reactions, hepatitis and nephropathy (Umamaheswari et al., 2009). ES, EA and goyazensolide were able to reduced serum urate levels by liver XOD activity inhibit and showed anti-inflammatory effect. Lychnopholide, eremantholide C, lupeol, apigenin and luteolin isolated of the ethanolic extract from *Lychnophora trichocarpha* were the compounds responsible by anti-hyperuricemic activity of this species (De Souza et al., 2012). In another study, β-amyrin and stigmasterol

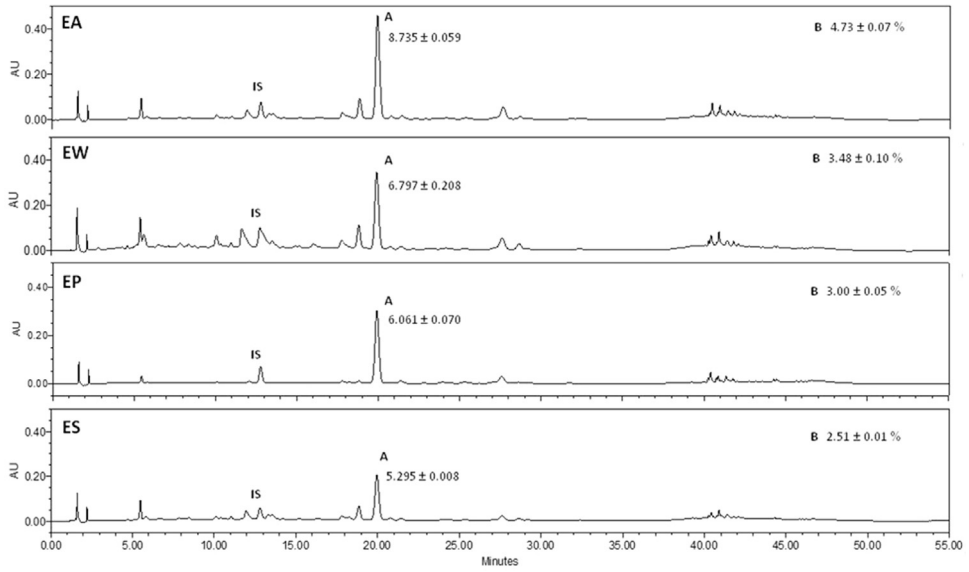

Fig. 3. Chromatograms obtained during goyazensolide-content quantification in ethanolic extracts of *Lychnophora passerina* (4.0 mg/ml). **A**: Mean and standard deviation values of goyazensolide peak area/IS peak area. **B**: Mean of goyazensolide content (% w/w). **EA**: autumn ethanolic extract, **EW**: winter ethanolic extract; **EP**: spring ethanolic extract; **ES**: summer ethanolic extract; **IS**: internal standard.

Table 1
Effects of treatment with ethanolic extracts from *Lychnophora passerina* obtained of aerial parts collected in summer (ES), autumn (EA), winter (EW) and spring (EP) and goyazensolide on carrageenan-induced paw edema.

Groups	Dose	Edema variation (%)					
	(mg/kg)	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**
Control condition (without carrageenan)	–	1.56 ± 1.45	1.14 ± 1.32	1.33 ± 1.21	0.94 ± 1.41	0.44 ± 0.55	0.12 ± 0.31
Untreated control	–	9.18 ± 2.00	13.40 ± 4.67	23.14 ± 6.46	22.00 ± 3.55	21.45 ± 3.91	16.64 ± 6.68
Indomethacin	10	1.80 ± 3.88***	6.96 ± 3.33***	7.31 ± 4.41***	2.91 ± 4.30***	5.33 ± 6.24***	5.03 ± 4.94***
ES	62.5	2.49 ± 2.37***	11.49 ± 5.54	15.37 ± 2.40	22.53 ± 6.82	16.31 ± 6.60	11.59 ± 5.44
EA	62.5	7.14 ± 4.32	10.82 ± 5.27	14.13 ± 0.95	15.86 ± 2.09	13.15 ± 2.34	10.67 ± 6.64
EW	62.5	12.12 ± 4.23	16.17 ± 4.92	19.37 ± 6.22	21.62 ± 9.40	15.64 ± 3.99	15.74 ± 1.33
EP	62.5	9.34 ± 3.08	13.83 ± 6.46	25.48 ± 3.93	17.89 ± 3.19	24.88 ± 7.48	13.5 ± 4.29
ES	125	6.56 ± 6.67	7.74 ± 4.77	6.41 ± 4.61***	6.87 ± 5.52***	4.06 ± 4.37***	2.47 ± 2.55***
EA	125	8.40 ± 6.10	8.55 ± 6.24	11.40 ± 6.81**	8.60 ± 5.43***	9.39 ± 5.23***	7.33 ± 3.96***
EW	125	1.56 ± 2.33	2.40 ± 3.22***	3.82 ± 4.07***	3.16 ± 3.85***	1.17 ± 1.99***	1.70 ± 2.42***
EP	125	7.94 ± 5.19	9.33 ± 2.42	11.26 ± 3.96**	11.99 ± 3.05**	3.87 ± 4.04***	8.06 ± 2.56**
ES 250	250	6.71 ± 3.87	8.24 ± 4.94	14.30 ± 3.51	19.5 ± 2.51	16.02 ± 3.17	14.39 ± 3.70
EA 250	250	0.54 ± 1.20***	1.98 ± 3.19***	10.48 ± 4.62**	14.36 ± 1.45	9.73 ± 5.18*	11.59 ± 6.11
EW 250	250	10.18 ± 5.00	7.66 ± 4.16	13.55 ± 6.21	17.70 ± 6.70	15.95 ± 4.26	13.71 ± 6.89
EP 250	250	13.06 ± 2.82	8.74 ± 4.41	23.65 ± 7.38	20.44 ± 5.81	19.25 ± 7.67	17.79 ± 6.60
Goyazensolide	10	4.34 ± 5.82	2.05 ± 2.88***	1.03 ± 2.03***	0.70 ± 1.86***	2.15 ± 4.00***	2.87 ± 4.97***

Results represent the mean ± S.E.M. variation of the percentage of full length between the dorsal and ventral faces of the paws before and 1, 2, 3, 4, 5 and 6 h after carrageenan. Values represent mean ± S.E.M., (n=6).
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$ compared to untreated control group (ANOVA followed by Bonferroni's test).

showed anti-hyperuricemic activity (Ferraz–Filha et al., 2016). Thereby, can be conclude that besides goyazensolide, lupeol, β-amyrin, stigmasterol, apigenin and luteolin can be responsible to anti-hyperuricemic activity of the ethanolic extract from *L. passerina*. These results demonstrate that ES, EA and goyazensolide are candidate for treatment of the gout.

Secondary metabolism of plants can vary considerably depending on several other factors as climatic, biotic, environmental and genetic. All these factors, as well as the collection conditions, stabilization and storage can influence the quality and therapeutic value of the species (Calixto, 2000). The plant collection period is considered an important factor that influences the diversity and content of secondary metabolites, as in some species both the quantity and the nature of the active constituents could not be constant throughout the year (Gobbo-Neto and Lopes, 2007). This study became relevant to be the first to assess the seasonal variations of the *L. passerina* ethanolic extracts.

The anti-inflammatory activity of *L. passerina* extracts was not affected by seasonal variation and nor by goyazensolide content enabling the use of the species at any time of year which characterizes an advantage of the species considering its acquisition. The source and quality of raw material play a central role in the production of herbal medicines with therapeutic property reproducible (Calixto, 2000).

Table 2
Effects of ethanolic extracts from *Lychnophora passerina* obtained of aerial parts collected in summer (ES), autumn (EA) and spring (EP) and goyazensolide on *in vivo* liver xanthine oxidase (XOD) activity in mice.

Treatment	Dose (mg/kg)	XOD activity (U/mg protein)	Inhibition (%)
Hyperuricemic control	–	15.82 ± 1.35	–
Allopurinol	10	4.82 ± 3.04***	68.76
ES	125	7.67 ± 1.93***	50.38
ES	250	6.75 ± 4.50***	56.32
EA	125	4.73 ± 1.44***	69.36
EA	250	6.00 ± 4.59***	61.16
EP	125	5.99 ± 3.05***	61.18
EP	250	10.07 ± 1.62	–
Goyazensolide	10	5.72 ± 2.23***	63.83

Data represent mean ± S.E.M. of 6 animals. One-way ANOVA followed by Bonferroni's test was used for statistical significance.
*** $P < 0.001$ compared to hyperuricemic control.

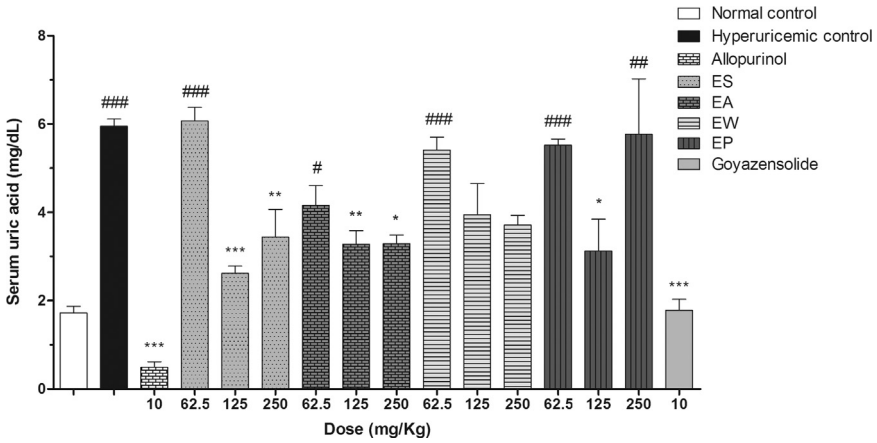

Fig. 4. - Anti-hyperuricemic effects of ethanolic extracts from *Lychnophora passerina* obtained of aerial parts collected in summer (ES), autumn (EA), winter (EW) and spring (EP) and goyazensolide in mice pretreated with potassium oxonate. Assay was performed as described in Section 2. Values represent mean ± S.E.M., (n=6) $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared to hyperuricemic control group; $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ compared to normal control group (ANOVA followed by Bonferroni's test).

Indeed, this work provides basis for further investigations about domestication and biotechnological production.

5. Conclusion

This study showed the higher goyazensolide content in aerial parts of *L. passerina* can be obtained from autumn collection. It was evident the contribution of goyazensolide on the pharmacological effects of the ethanolic extracts, although further investigations will be required. Other active constituents or adjuvant substances present in extracts can synergistically act which could justifying their therapeutic potential and thereby reinforces the need to quantification of other targets.

This study demonstrated that for use in inflammatory processes, the aerial parts of *L. passerina* may be collected in any season. For use in hyperuricemia, the best seasons for collection are summer and autumn. The search for drugs or herbal medicines that may act as both anti-hyperuricemic and anti-inflammatory agents are interesting alternatives in the treatment of gouty arthritis (Ahmad et al., 2008). Thus, according the results of this study, the ethanolic extract from *L. passerina* and goyazensolide can be considered promising agents in the therapeutic of inflammation, hyperuricemia and gout.

Conflict of interest

The authors declare no conflict of interests.

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