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Optimization of Extraction Conditions and Antioxidant Activity of *Solanum lycocarpum* Fruits

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Abstract: Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. The capacity of natural antioxidant from phytochemical has increase attention from researchers and public. However, the extraction process is affecting the activity and the bioavailability of bioactive compounds. The Solanum lycocarpum is a plant of the Brazilian "cerrado", popularly used as a hypoglycemic, hypocholesterolemic and control of metabolic diseases. Its effects are attributed to the presence of several glycoalkaloids (solamargine, solasonina) and solasodine. Therefore, the purpose of this communication was, investigate the optimization of extraction condition and evaluation of antioxidant activity from fruits of *Solanum lycocarpum*. The extracts were obtained using different solvent systems, i.e., water, 50% ethanol, ethanol absolute and ethyl ether (1:10 and 1:20) and different extraction processes: maceration with constant agitation at room temperature, maceration with constant agitation and heating at 30°C and ultrasound. The extracts were characterized by the amount of material extracted (1, 6 and 24 h) and the action of antioxidant activity by DPPH method. The results showed that the polar solvent (50% ethanol) and extractive process maceration with agitation to ambient temperature showed higher contents of extractable of fruits of *S. lycocarpum* (3.4 g %) and also showed higher antioxidant activity (88.57±2.41% de inhibition). This action whether the presence of glycoalkaloids (solamargine, solasonine and solasodine) in fruits *S. lycocarpum* which are polar compounds and may explain this increased antioxidant action of this extract.

Key words: Antioxidant activity, solanaceae, *Solanum lycocarpum*, glycoalkaloids, solasodine, cerrado

INTRODUCTION

The Solanaceae family is characteristically ethnobotanical, that is, extensively utilized by humans and comprises more than 2000 species many of which evolved in the Amazonian regions of South America and Cerrado. It is an important source of food, spice and medicine. Solanaceae are known for possessing a diverse range of alkaloids, steroids free or as glycosides, secondary metabolites structurally diverse and complex. These compounds and in general, are of therapeutic interest since they exhibit a wide range of activities such as cytotoxicity, anticancer, anti-inflammatory antiulcerogenic and molluscicide (Cruz, 1982).

Solanum lycocarpum A. St. Hil, is a species belonging to family Solanaceae and popularly known as "fruta-do-lobo", "lobeira" or "jurubebã", is a Brazilian plant, of the Cerrado, predominantly in the Southeast and Midwest of the Brazil. The fruit of lobeira is a food source for mammals, especially during the dry season when the availability of other fruits are scarce (Motta *et al*., 2002; Elias *et al*., 2003). The fruits are used in folk medicine as a calming, sedative, antiepileptic, antispasmodic (Cruz, 1982; Lorenzi, 1991) and anti-inflammatory (Vieira Jr. *et al*., 2003). However, the main popular use is for the treatment of diabetes and obesity, reducing cholesterol levels (Yoshikawa *et al*., 2007). Lobeira powder solution is also used by people to treat diabetes. Chemical investigations of *Solanum* species have revealed the presence of glycoalkaloids mainly solamargine, solasonine and solasodine (Motidome *et al*., 1970; Haragushi *et al*., 1978; Kerber *et al*., 1993; Yoshikawa *et al*., 2005) in the fruits of *S. lycocarpum*.

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Glycoalkaloids are a group of nitrogen-containing compounds that are naturally produced in various cultivated and ornamental plant species of the Solanaceae family. This large family of plants includes commonly consumed vegetables such as potatoes, tomatoes, eggplants and peppers. Glycoalkaloids may have evolved in selected plant species to protect against predators and pathogens such as bacteria, fungi, viruses, insects and animals. Glycoalkaloids also impart certain flavors in some plants (Sun *et al*., 2010; Milner *et al*., 2011).

So as to, when trying to extract the chemical components of a plant is necessary establishment of parameters to an optimal extraction. Several extraction methods are used to obtain extracts and the method used is normally dependant on what type of botanical material that is being used (Vinatoru, 2001). Depending on the nature of the extraction process, the temperature, pH, time, agitation, particle size of drug, polarity of extractor liquid could have an effect on the yield and selectivity. When you want to test the biological activity of medicinal plants is necessary to ensure that its chemical components have been extracted and preferably in larger quantities (Wang and Weller, 2006; Dai and Mumper, 2010).

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions (Hamid *et al*., 2010). Antioxidants are compounds which, when present in low (Beer *et al*., 2002). concentrations compared to oxidizable substrates, can quench free radicals and significantly delay or inhibit oxidation of the substrate and protect biological systems against potential harmful effects of free radicals (Arnao, 2000; Diplock *et al*., 1998). Natural antioxidants can be phenolic compounds (tocopherols, flavonoids, anthocyanins and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines) or carotenoids, as well as vitamins C and E and phospholipids (Hudson, 1990; Shahidi, 2002). Most of these antioxidant compounds are present in foods as endogenous constituents and are referred to as dietary antioxidants (Siddhuraju *et al*., 2002). The Food and Nutrition Board of the National Academy of Sciences (NRC, 1989) defined a dietary antioxidant as a substrate in foods that significantly decreases the adverse effects of free radicals such as Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) or both on normal physiological function in humans. Free radicals are molecules or molecular fragments containing one or more unpaired electrons. The presence of unpaired electrons confers a considerable degree of reactivity to free radicals (Valko *et al*., 2004). Free radicals are ubiquitous in the body and can be generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganisms (Hussain *et al*., 2003). Reactive oxygen species can be produced from endogenous sources such as mitochondria, cytochrome P450 metabolism, peroxisomes and inflammatory cell activation (Inoue *et al*., 2003). Trosznska *et al*. (2002) reported that imbalance between ROS/RNS and antioxidant defense systems may lead to chemical modification of biologically relevant macromolecules like DNA, proteins, carbohydrates or lipids. To avoid such modifications, antioxidants inhibit oxidation of these molecules and prevent initiation of oxidizing chain reactions (Klein and Kurilich, 2000; Velioglu *et al*., 1998). They scavenge free radicals by donation of an electron or hydrogen atom or by deactivation of prooxidant metal ions and singlet oxygen (Shahidi, 2002). Therefore, the purpose of this communication was to obtain the optimal extraction and antioxidant activity of *S. lycocarpum*.

MATERIALS AND METHODS

Samples and reagents: The ripe fruits of *Solanum lycocarpum* A. St. Hil, were collected from at Barretos (São Paulo State, Brazil) (S20°34'15.898"-W48°34'29.989"), in October and November, 2007. A voucher specimen (number SPFR 11.308) is deposited in the Herbarium, Department of Biology of the University of São Paulo-FFCLRP/USP.

Selection of extraction conditions: Ripe fruits of *S. lycocarpum* were dried at 40°C. Dried fruits of *S. lycocarpum* were subjected to different solvent systems, i.e., water, 50% ethanol, ethanol absolute and ethyl ether (1:10 and 1:20) and different extraction processes: maceration with constant agitation at room temperature, maceration with constant agitation and heating at 30°C and ultrasound. Extracts were filtered and concentrated under vacuum. We analyzed three times of extraction, with the aim of assessing the amount of material extracted and 1, 6 and 24 h.

1,1-diphenyl-2-picrylhydrazy(DPPH)radicalscavenging assay: The antioxidant activity of extracts, on the basis of the scavenging activity of the stable DPPH free radical (DPPH), was determined following a previously described method by Andrade *et al*. (2007). For these assays the

extracts were evaluated at concentrations at 1, 5, 10 and 15 μg mL^{-1}. The radical scavenging effect of each sample was calculated and compared with that rutin (0, 5 and 100 μg mL^{-1}). Appropriately diluted extracts of *S. lycocarpum* fruits and rutin (each 3.0 mL) were added to 0.1 mL of methanolic solution of DPPH 1 mM. A control sample was placed under same conditions. The resulting was placed, after 30 min, in a Hitachi (Tokyo, Japan) U-2000 UV-visible spectrophotometer for measurement of absorbance at 517 nm and the decrease in the absorbance was noted until the concentration of DPPH reached 50%. The remaining concentration of DPPH in the reaction medium was calculated from a standard calibration curve. All tests were conducted in triplicate.

RESULTS AND DISCUSSION

In recent decades, there has been an increasing worldwide interest in the use of medicinal plants and herbal medicines for the cure, prevention and treatment of various diseases. However, problems relating to the quality of the vegetable raw material such as extracts, does not appear to be fully resolved, leading to products with a variation of the effectiveness, safety and quality (Maria *et al*., 2006; Coradi *et al*., 2011).

The study of the extraction procedure is important because of changes in conditions that determine the quality and extraction yield of individual chemical constituents extractable (Hinneburg and Neubert, 2005).

The use of planning extraction of plant material plays a central role in the development and modernization of processes standardized medicinal plants preparations. This study evaluated of the best extractor liquid extraction and/or liquid binary mixtures of solvents at different concentrations of ethanol-water extraction to glycoalkaloids. The results indicated that all ratios tested could effectively extract glycoalkaloids but we were in search of the best ratio ethanol: water for much extraction of glycoalkaloids from fruits of *S. lycocarpum*. The results showed that 50% ethanol was the most effective proportion, significantly increasing the extraction of glycoalkaloids.

The optimized conditions for the extraction of glycoalkaloids, in terms of better liquid extracts for the species studied, was carried out using various processes already described in the literature (Vaananen *et al*., 2000; Alt *et al*., 2005; Cataldi *et al*., 2005).

Other authors have optimized extraction conditions for various medicinal plants and so could concentrate on extracts good amount of active (Luthria, 2006; Gribova *et al*., 2008; Uma *et al*., 2010; Pawar and Surana, 2010).

The optimization process involves the extraction under various conditions such as extraction temperature, comparison between different methods and liquid extracts (alcohol at various dilutions, addition of surfactants to the solutions mixed alcohol-water or water). As we have seen, there are a number of factors affecting extraction performance that interfere in solvent optimization. The selection of extractor liquid is often a neglected part of production of vegetables extracts because that importance not well understood and generally errors occur in your choice damaging the process of extraction of active principles. Therefore the optimizations of extraction conditions are important in the preparation of the extracts, mainly related to the choice of the extractor liquid (Vinatoru, 2001; Wang and Weller, 2006; Dai and Mumper, 2010). This article describes important parameters that should be considered when optimizing extraction systems for *S. lycocarpum*.

The results (Fig. 1) demonstrated that the extracts obtained by larger polarity solvents showed higher levels of the extractives compared to the solvent of low polarity (diethyl ether) , that showed the lowest percentage of extractives (0,06 g %). The polar solvent which extracted largest quantity of substances of fruits of *S. lycocarpum* (3,4 g %), was 50% ethanol, using the extraction process of maceration and agitation without heating.

When comparing the relationship between the amount of plant and solvent, it was observed that the lower this ratio, the higher extractive content are obtained, as can be seen in Fig. 1.

The same trend was observed for the other extractive techniques used maceration with stirring and heating and ultrasound but with less income and quarrying (Fig. 1). The heating during the extraction process does not seem to have affected significantly the extraction of active compounds, since the literature reports the presence of glycoalkaloids in fruits of *S. lycocarpum* and this class of substances is stable at temperatures used in extraction processes of this work.

DPPH scavenging activity: The antioxidant activity of extracts of fruits, based on free scavenging, was determined by the method described by Andrade *et al*. (2007). After determining the volumes required for 50% inhibition of DPPH free radical, can be seen that for the extract obtained by the technique of maceration with agitation and temperature, using a mixture of water and ethanol had a higher proportion of 1:1 DPPH inhibition (90%) and the lowest percentages of inhibition were presented by the ether extract (45%), as shown in Fig. 2a.

The results of the antioxidant activity of hydroalcoholic extract 1:1 obtained by the technique of

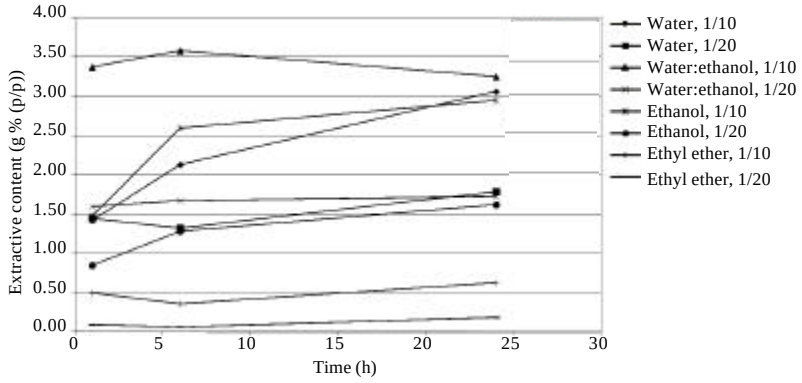

Fig. 1: Relation of the amount of extractives content compared with solvents and solvent:plant proportion using the technique of maceration with agitation and temperature

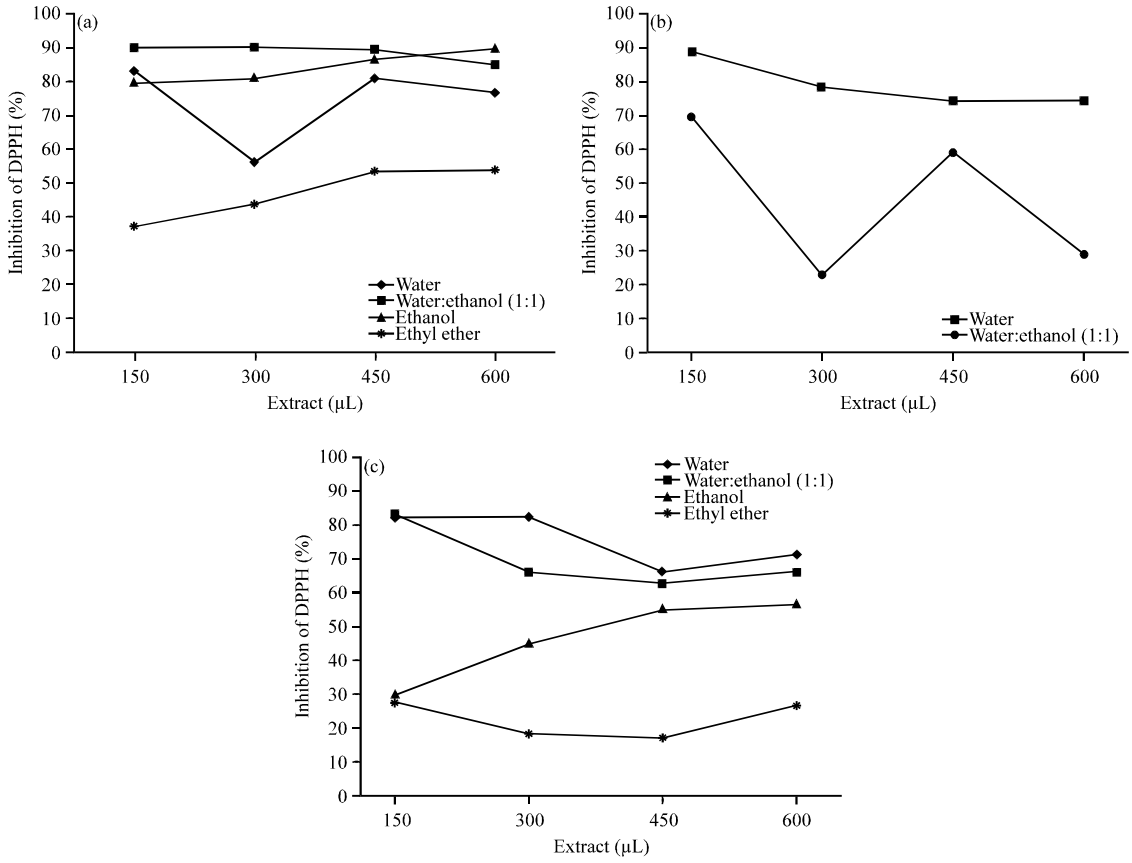

Fig. 2(a-c): Relation of percentage inhibition of DPPH radical and the volume of extract used in the analysis, (a) Technical maceration with agitation and room temperature, (b) Technical maceration with agitation and heating at 60°C and (c) Technical ultrasound

maceration with agitation and heated to 60°C are shown in Fig. 2b. Studies show that the oxidation process is accelerated when the temperature increase (Silva *et al*., 1999; Gribova *et al*., 2008), the results showed that using a temperature of 60°C for the production of crude extracts with 50% ethanol, these antioxidants are oxidized during

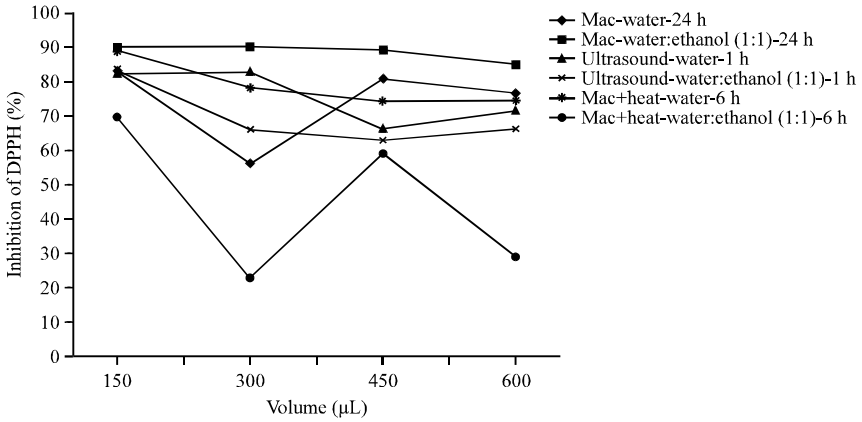

Fig. 3: Relation of percentage inhibition of DPPH radical and the volume of extracts obtained from different extraction techniques

the extraction process thereby reducing the antioxidant effect when compared with the technique of maceration without heating.

In Fig. 2c which represents the antioxidant activity of extract obtained by the ultrasound technique, one can observe that all results were below those obtained with the extracts obtained by maceration with stirring and without heating (Fig. 2a, b), showing that in this specific case, this technique has not proved suitable for obtaining extracts with antioxidant potential.

As can be seen in Fig. 3, the extract showed higher antioxidant activity was obtained by maceration with agitation at room temperature and ethanol-water mixture 1:1 (v/v) as extraction liquid and extraction time from 1 to 6 h and a relationship of equals' 1:10 plant solvent.

The fact of the presence of glycoalkaloids (solamargine, solasonine and solasodine) in fruits *S. lycocarpum* which are polar compounds, may explain this increased antioxidant action of this extract which is probably related to the extraction of these substances in larger amounts and may be responsible for this action.

CONCLUSION

The optimized condition for the extraction of glycoalkaloids *S. lycocarpum* was 50% ethanol by maceration. In this context, experiments like this can be extended to evaluate the extraction process other medicinal plants belonging to family Solanaceae. From the present study, can also conclude, that *S. lycocarpum* is an important source of natural antioxidants with good free radical scavenging and radical scavenging capacities. It may employed for treatment of various diseases and provide the development of products with antioxidant with possible applications in the pharmaceutical and food industries.

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