Determination of Content of Phenolic Compounds and Flavonoids in Leaves Extracts of Plectranthus sp. (“Boldos”), Potential Antioxidant and Antibacterial Action

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ABSTRACT

Plectranthus barbatus (PB) and P. ornatus (PO) in folk medicine was reported using these species as anti-microbial to the liver and heart. The aim of this study was to evaluate the levels of phenolic compounds, anti-oxidant and anti-bacterial properties of extracts of dried leaves of P. barbatus and P. ornatus. The assay of phenolic compounds was done using Folin-Ciocalteau and flavonoids by vanillic acid. Antioxidant activity was measured using the DPPH method and the antimicrobial activity performed by the technique of disc plate. The best effective method of preservation of content of phenolic compounds is by drying using hot air circulation oven. Extracts of P. barbatus and P. ornatus were classified as good antioxidant properties. At the concentrations tested no inhibition of microbial growth for Staphylococcus aureus was observed. The species possess antioxidant properties and are capable of inhibiting the oxidation forming products and also have potential effectiveness against S. aureus.

Key words: Plectranthus barbatus, Plectranthus ornatus, Brazilian “boldos”, drying methods, biocompounds, antimicrobial activity.

INTRODUCTION

Medicinal plants and herbal medicines were investigated in recent years. This is mainly due to the increase in research seeking new bioactive compounds. These new drugs should be at a reduced cost to the patient and viable to be produced and marketed by the pharmaceutical industry.

The World Health Organization reports approximately 75% of the world’s population already made use of some sort of plants in order to relieve unpleasant symptoms, such as pain or fever. It was estimated that 25% of prescription drugs are derived from plants worldwide (WHO, 2011).

Natural products are used as a form of poultices, infusions, teas, potions and others pharmaceutical forms since ancient times. Searches systematized these plant species are essential to the validation of popular knowledge. Natural products must have the use, efficacy, safety and therapeutic potential validated, thus, ensuring viability and credibility in the field of medical science.

Species of this study are used in folk medicine for the treatment of gastric diseases in inhibiting gastric acid secretion and heart problems (Dubey et al., 1981; Dellar et al. 1996; Lukhoba et al. 2006). These species are popularly known as “Boldos”, included in the genus Plectranthus which has 300 species distributed in tropical Africa, Asia and Australia (Lukhoba, 2006).

The main chemical compounds fro main abietanos, labdane and neoclerodanos were isolated from Plectranthus barbatus (“boldo-peludo”, “hair boldo”) and m Plectranthus sp. are essential oils, phenolic compounds and diterpenoids (major component or chemical marker) attributed to the potential medicinal (Abdel-Mogib et al., 2002; Kerntopf et al., 2002; Sousa et al., 2004). Diterpenoids Plectranthus ornatus (“boldo-miúdo”, “small
It is important to clarify that in most studies on medicinal plants, the pharmacological action of a given species can be attributed to a set of active substances and not only a chemical constituent or marker. This study sought to relate phenolic compounds present in *P. barbatus* and *P. ornatus* with pharmacological, microbiological and antioxidant activities.

In *P. barbatus* (PB) was isolated only a flavonoid (genkwanina), a phenylpropanoid and three phenolic compounds (caffeic acid and colexantona coleosídeo B) (Yao et al., 2002; Ahmed et al., 1991; Liu et al., 2007). In *P. ornatus* (PO) there are no reports in the literature concerning the identification and isolation of phenolic compounds present in *P. ornatus* (Ahmed et al., 1991; Rijo et al., 2002, 2005, 2007; Oliveira et al., 2005; Mendes et al., 2006; Liu et al., 2007; Alasbahi et al., 2010).
compounds. Flavonoids present in these species are phenolic compounds that act in response to lipid peroxidation and consequently, have antioxidant activity at the cellular level.

Anti-oxidant drugs can be used as auxiliaries in the treatment of various diseases. The use of natural products, including raw vegetables, such as drug therapy has important perspective on the development of new herbal medicines in the treatment of diseases associated with oxidative stress. The consumption of vegetables rich in antioxidants may reduce the incidence of some diseases (Barreiros et al., 2006; Gomes et al., 2008; Oliveira et al., 2007).

The analysis of antioxidant potential by methodology uses 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) based on the quantitative consumption the DPPH radical by antioxidants. This method is validated for ease of implementation; the accuracy in spectrophotometric quantification in vitro scavenging activity and have wide application in plant extracts (Braga et al., 2008; Gomes et al., 2008; Rufino et al., 2007; Sousa et al., 2007).

The antioxidant activity of the species P. barbatus and P. ornatus is mainly related to reducing properties and chemical structure of the phenolic compounds that play an important role in neutralizing or sequestering "reactive oxygen species" (ROS) and "reactive nitrogen species" (ERNS) acting both as the initiation step in the propagation of the oxidative process (Bandeira, 2011). Chemically, the aromatic ring of the phenolic compounds (terpenoids, tannins and flavonoids) with one or more hydroxyl substituents, including functional groups allows for the removal and stabilization of free radicals, reduced oxygen and acts on lipid oxidation reactions as well as, chelation of metals (Oliveira, 2011).

Anti-microbial use in infection control is the most widely used prophylactic measure; however, rates of bacterial resistance to antimicrobials reference are increasing. Staphylococcus aureus is an example of organism widely studied for its pathogenicity, being responsible for several types of infections (Haida et al., 2007) as dermatitis and acne, to more serious infections such as osteomyelitis, endocarditis, pneumonia, food poisoning, meningitis, arthritis bacterial and yet, scalded skin syndrome, bullous impetigo and toxic shock syndrome (Silva et al., 2007; Santos et al., 2007). The high prevalence of infections justifies the search of resources that enable the development of new antimicrobials from plant species (Haida et al., 2007).

For validation of herbal medicine, the processing of the plant drug is essential to ensure its therapeutic potential. The use of medicinal plants in fresh or natural form ensures that all bioactive compounds be present. However, when the purpose is to store and sell products of natural origin, it is necessary to use the process of drying, depending on the water content, means facilitating the proliferation of microorganisms and enzymatic reactions that can cause deterioration or even the loss of active principle (Martins et al., 2003). Low concentrations of chemical constituents can also occur through inadequate processing such as drying and storage.

Therefore, the objectives of this study were to evaluate the levels of phenolic compounds, anti-oxidant and anti-bacterial properties of extracts of dried leaves of PB and PO obtained by the methods of natural and artificial drying. The most effective method of drying was assessed in leaves of PO and PB in relation to the preservation of bioactive compounds.

MATERIALS AND METHODS

Plant material

Approximately 3.0 kg of fully expanded leaves were collected from the middle third of adult plants of P. barbatus and P. ornatus, in May, 2010, the medicinal garden of the Universidade Federal de OuroPreto (UFOP), located and attached to the Institute of Biological Sciences (coordinates 20°23’47.36”S; 43°30’35,51” W), in OuroPreto, Brazil. The exsiccate of P. barbatus and P. ornatus were made and identified by taxonomist, V. R. Scalon. Deposit was made in the Herbarium of Professor José Badini (UFOP) under numbers OUPR12671 OUPR7043, respectively. Material witness was also deposited in the Health Centre/UFOP.

Post-harvest and drying method

Immediately after harvesting was done segregation of leaves of P. barbatus (PO) and P. ornatus (PO) were subjected to natural and artificial drying processes. In artificial drying, conventional oven (CO), hot air circulation oven (HAC) and microwave (MW) were used, while natural drying was by thin layer (TL). The dried leaves of PO and PB were designated after this process as vegetable drug.

Extracts

The extracts of dried leaves of P. barbatus (EPB) and P. ornatus (EPO) were made by vegetable drug from the treatments CO, HAC, TL, and MW with 5 g fresh leaves (FL) of PB and PO made into crude plant extract. All ten treatments (CO, HAC, TL, MW and FF) of PB and PO were extracted by maceration in Ethanol PA using successive extractions. The final extracts were filtered and evaporated obtaining dry extracts of PO and PB. The standard time of extraction lasted for two days. The yield of dried extract was calculated according to the initial fresh weight.

Determination of phenolic compounds

The determination of total phenolic compounds was
Table 1. Comparison of mean levels of fresh matter, dry matter, water loss, phenolic compounds and flavonoids content of fresh and dried leaves of *P. barbatus* and *P. ornatus* in relation to natural drying methods (TL) and artificial (HAC, CO and MW).

<table>
<thead>
<tr>
<th>Specie</th>
<th>Treatment</th>
<th>Fresh matter (g)</th>
<th>Dry matter (g)</th>
<th>Water loss (%)</th>
<th>Plenolic compounds (mg/ml)</th>
<th>Flavonoids content (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td><em>P. ornatus</em></td>
<td>FL</td>
<td>5.0800&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>0.2440&lt;sup&gt;DE&lt;/sup&gt;</td>
<td>0.2310&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HAC</td>
<td>5.1075&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>0.2553&lt;sup&gt;D&lt;/sup&gt;</td>
<td>95.12&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.2342&lt;sup&gt;E&lt;/sup&gt;</td>
<td>0.2034&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>5.1134&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>0.2492&lt;sup&gt;D&lt;/sup&gt;</td>
<td>95.12&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.1125&lt;sup&gt;C&lt;/sup&gt;</td>
<td>0.1860&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MW</td>
<td>5.0711&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>0.5105&lt;sup&gt;C&lt;/sup&gt;</td>
<td>89.93&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.1420&lt;sup&gt;FG&lt;/sup&gt;</td>
<td>0.0750&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TL</td>
<td>5.1300&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>0.4300&lt;sup&gt;C&lt;/sup&gt;</td>
<td>91.62&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.2152&lt;sup&gt;F&lt;/sup&gt;</td>
<td>0.0330&lt;sup&gt;E&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The means followed by the same letter not differ statistically by Tukey test at 5% of significance level. FL=fresh leaves; HAC=Hot air circulation oven; CO=Conventional oven; MW=Microwave; TL=Thin layer; CV=Coefficient of variation.

adapted from Murthy et al. (2002). Dried extracts (0.2 mg) of the treatments CO, HAC, TL, MW and FF were added to 1.0 ml of Folin-Ciocalteu (1:10) and 0.8 ml of sodium carbonate (7.5%) and were then homogenized by vortexing and incubated at temperature of 30°C for 30 min. The absorbance was measured at 765 nm in spectrophotometer (FEMTO). All treatments were made in four replicates. The standard curve was plotted as reference tannic acid PA (0.01 to 0.1 mg/ml) under the conditions earlier described. The results were expressed in tannic acid (mg/ml).

**Determination of flavonoids**

When doses of flavonoids 0.2 mg of extracts of treatments (CO, HAC, TL and FL) were dissolved in methanol and solution of vanillin (0.5%) and HCl solution (4%) homogenized in vortex and stored away from light and heat at 23°C for 20 min according to the methodology adapted by Jayaprakasha et al. (2001) and further proceeded to the reading of the treatments in spectrophotometer at 500 nm (FEMTO). The standard curve was constructed using rutin as a reference under the same conditions described. The results were expressed in rutin (g/ml).

**Antioxidant activity**

In the evaluation of the antioxidant activity (AAT) of PO and PB the wavelength of greatest absorbance of DPPH was determined by spectral scan and performed in the range 500 to 570 nm in spectrophotometer (FEMTO) with 60 μM solution for the construction of analytical curve of absorbance versus concentration of DPPH as described by Rufino et al. (2007). The concentration values (30 to 100 μM) were chosen respecting the Beer Lambert law. In determining the AAT of PO and PB, solution of DPPH (800 ppm) was prepared from which aliquots were taken in 3.9 ml and 0.1 ml of extract treatments PB and PO added (sample solution) and a homogenization performed at 546 nm readings in a time interval of 1 to 30 min by measuring the DPPH consumption versus time. The absorbance of the solution of DPPH (reference solution) and methanol PA (White) were then measured. The antioxidant activity of PB and PO were calculated according to the equation of the line. The results were expressed as DPPH concentration (mM) remaining in the reaction medium.

**Antibacterial activity**

The antibacterial test was performed on Mueller-Hinton agar inoculated with *S. aureus* ATCC strain...
(25923), starting from the direct colony suspension (0.5 McFarland scale). Filter paper discs were impregnated with 10 μl extracts PB and PO (50 mg/ml). The positive control was 1 mg of Oxacillin (UNSIDISC) and negative control ethanol PA. After incubation, the plate was incubated at 35°C for 18 h and the inhibition halo around the disc measured (NCCLS, 2005). Tests were made with six replications.

**Statistical analysis**

Data were subjected to analysis of variance (ANOVA), regression (ANAREG) and mean tests (Tukey) at 5% significance level and adapted to the experimental model using the program SAEG for Windows (UFV, SAEG, 2010).

**RESULTS AND DISCUSSION**

Table 1 shows the results of evaluations of fresh matter (FM), dry matter (DM) and water content (WT). General mean fresh weight (FW), dry matter (DM) and water tenor (WT) in leaves of PB and PO were 5.14 ± 0.14 g; 0.51 ± 0.19 g and 90.12 ± 8.22% respectively.

The highest medium of WT was in PO. In PB, the drying method more effective was in HAC (WT=88.87%) and PO were CO (95.12%) and EFV (95.0%) where the values of WT were higher. In PB and PO the lower values were in MW in PO and PB. In PB and PO, the lower values were in MW (PO=89.94% and PB=85.05%). The higher yield of dry matter (PB) was in TL (20.96%), whereas in PO it was CO (41.34%). The lower yields of the extracts were dried in FF (PB=3.32% and PO=4.82%) (Table 1).

The values of MS and WT showed that the loss of water was considerably high in both species. The high content of water loss for desiccation was in PO (Table 1) where was found 23.85%, more water reserved in the cellular structures, such as vacuoles and parenchyma cells. This result is justified in function of the leaf structure of PO with smaller leaves, fleshy, slightly velvety and smaller number of trichomas in leaf surface, which facilitates water loss through transpiration.

In storage of the vegetable drug during processing and marketing, the values of partial loss of water was found around 60 to 70% (Farm, 2010); in order to maintain the preservation of the active principle.

The drying method most appropriate was in HAC because it causes less damage to plant material and maintains the sensory characteristics (color, odor and flavor), thereby providing less risk of loss of active principle, or bioactive substances that may be involved in therapeutic potential as flavonoids and phenolic compounds.

For the calibration equations of phenolic compounds ($\bar{y}=0.0042x+0.0801; \ r^2=0.9911$) and flavonoids ($\bar{y}=0.0083x+0.0209; \ r^2=0.9927$) were calculated tenors of flavonoids and phenolic compounds in PB and PO (Table 1).

In the efficacy analysis, the drying processes (vegetable drug) as compared to fresh plant in the preservation of active compounds were detected as average concentration of phenolic compound (0.84±0.79 mg/ml) and FF (2.4430 and 0.2440 mg/ml in PB and PO, respectively) statistically superior followed by HAC (1.4683 and 0.2342 mg/ml in PB and PO, respectively) (Table 1). The lowest concentrations of phenolic compounds were in MW (0.21 mg/ml) and CO (1.41 mg/ml) in PB and MW (0.14 mg/ml) and CO (0.11 mg/ml) PO, respectively (Table 1).

At the levels of phenolic compounds, the concentration of flavonoids was higher in PB (0.19±0.17 μg/μl). The concentrations of flavonoids were higher in treatments FF and HAC, with values in PB of 0.61 and 0.23 µg/μl and PO of 0.23 and 0.20 µg/µl, respectively (Table 1).

The lowest concentrations of flavonoids were in MW (0.0470 and 0.0750 µg/µl in PB and PO, respectively) and TL (0.1455 and 0.0330 µg/µl in PB and PO, respectively) and result consistent with the assay of phenolic compounds (Table 1). The extract FF had higher content of phenolic compounds and flavonoids, both in PB and PO and expected result considering the natural plant, preserved with all bioactive compounds used. The extraction process by maceration was effective in preserving flavonoids and phenolic compounds in all treatments.

The flavonoids were more susceptible to drying methods and process viewed in function of the considerable difference found in relation with FF and HAC where there was loss of activity of approximately 62.08% (PB) to 11.95% (PO). Comparing the drying methods (artificial and natural), HAC had better results confirming that this drying process showed lower structural loss in the leaves of the species, favoring the stabilization and preservation of ergastic substances.

In processing plant it was noted that natural drying (TL) interferes in the concentrations of flavonoids and phenolic compounds. Thus, it was observed that drying, although mild or fast, is still a factor that influences the quantity and quality of these compounds.

The method of artificial drying at MW provided reduction in the concentration of flavonoid and phenolic compounds. This result may be related to the type of drying being faster causing abrupt water loss and overheating responsible for major damage to the leaf structures of PB and PO and consequent alteration in the levels of compounds.

The preservation of the highest content of flavonoids is aimed at ensuring the medicinal property of the species under study. Phenolic compounds, including flavonoids, are usually described in the literature for its antioxidant properties, which act in protective action against oxidative reactions, in the prevention of degenerative processes such as cancer, atherosclerosis, diabetes, arthritis, malaria, AIDS and cardiovascular diseases.
The result of spectral scanning was in 546 nm for analyses of antioxidant activity (AAT) by DPPH method in PB and PO. The analytical curve adjusted was $\bar{y}=0.0072x+0.0189$ ($r^2=0.9991$). The time spent front the beginning of the reaction to stabilize them was 10 min in PB and 2 min at PO. The absorbance value of the DPPH reacted after half the time of onset of the reaction to the stabilization was $53.16 \pm 0.08 \mu M$ in PB (10 min) and $58.25 \pm 0.16 \mu M$ in PO (2 min). These values relate, therefore, the concentration of DPPH remaining in the reaction medium.

Analyzing the results of AAT in function of the levels of flavonoids and phenolic compounds in PB and PO inferred that, PO showed antioxidant property immediately (2 min) and PB was slower in neutralizing free radicals, that is, the higher the consumption of DPPH in a sample, the more the effective concentration (EC50) or inhibitory concentration (IC50) is reduced. IC50 is defined as the amount of antioxidant in the sample test required to consume the production of spectral scanning was in 546 nm for analyses of antioxidant activity (AAT) by DPPH method in the levels of phenolic compounds in PB. The probable mechanism of action of phenolic compounds to clarify the effectiveness in antimicrobial therapy is related by acting on bacterial cytoplasmic membrane; altering the structure and function by interfering with active transport and also coagulating the cell content (Almeida et al., 2007).

Conclusion

The drying processes natural and artificial, though mild, are a factor that influences the levels of phenolic compounds and especially the flavonoids in the species under study. The drying is more effective at preservation of phenolic compounds using hot air circulation oven at the temperature of 40°C. The species possess antioxidant activity, because at low concentration, they are capable of inhibiting the stable oxidation forming products and have the potential effectiveness against S. aureus.

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REFERENCES


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