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TECHNOLOGICAL APPROACHES TO AQUEOUS EXTRACTION OF PHENOLIC COMPOUNDS FROM *PLECTRANTHUS ORNATUS*: ANTIOXIDANT POTENTIAL AND TOXICOLOGICAL ASSESSMENT[1]

ABORDAGENS TECNOLÓGICAS PARA EXTRAÇÃO AQUOSA DE COMPOSTOS FENÓLICOS DE PLECTRANTHUS ORNATUS: POTENCIAL ANTIOXIDANTE E AVALIAÇÃO TOXICOLÓGICA

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ABSTRACT

Plectranthus ornatus Codd is a species of the genus *Plectranthus*, popularly known as "Boldo". In traditional medicine, its leaves are commonly prepared as tea, either by infusion or decoction. The species of the genus *Plectranthus* are rich in secondary metabolites; however, studies on the phenolic composition and biological activities of *P. ornatus* are scarce. This study aimed to produce aqueous extracts from leaves of *P. ornatus* through different methods (decoction, infusion, reflux, and freeze-dried) and evaluate their antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) method, the content of total phenolic compounds by Folin Ciocalteu, and in vivo toxicity using *Artemia salina*. Antioxidant activity was expressed as IC_{50}, with the best result obtained for the lyophilized extract (6.85 ± 0.60 mg/mL). The same was found for total phenolic content (23.21 ± 0.60 mg gallic acid equivalents/g). All processes showed a strong positive correlation ($r>0.9$; $p<0.05$) between total phenolic content and antioxidant activity. The lyophilized aqueous extract was non-toxic to *Artemia salina* at all tested concentrations. These findings highlight the importance of extraction technology in optimizing the bioactive potential of *P. ornatus*, reinforcing its potential as a safe and effective natural antioxidant source.

Keywords: natural products; extraction technology; phenolic acids; redox capacity; oxidative stress.

RESUMO

Plectranthus ornatus Codd é uma espécie do gênero Plectranthus, popularmente conhecida como "Boldo". Na medicina tradicional, essa planta é utilizada na forma de chá, por infusão ou decocção. As espécies do gênero Plectranthus são ricas em metabólitos secundários; no entanto, os estudos sobre a composição fenólica e as atividades biológicas de P. ornatus ainda são escassos. O objetivo deste estudo foi produzir extratos aquosos das folhas de P. ornatus por meio de métodos diferentes (decocção, infusão, refluxo e liofilização) e avaliar sua atividade antioxidante pelo método do 2,2-difenil-1-picril-hidrazil (DPPH•), o conteúdo de compostos fenólicos totais pelo método de Folin Ciocalteu e a toxicidade in vivo utilizando Artemia salina. A atividade

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antioxidante foi expressa em IC_{50}, e o melhor resultado foi obtido para o extrato liofilizado (6,85 ± 0,60 mg/mL), o qual também foi observado para o teor de fenólicos totais (23,21 ± 0,60 mg equivalentes de ácido gálico/g). Todos os processos apresentaram uma forte correlação positiva ($r > 0,9$; $p < 0,05$) entre o conteúdo fenólico total e a atividade antioxidante. O extrato aquoso liofilizado não foi tóxico para Artemia salina em nenhuma das concentrações testadas. Esses achados destacam a importância da tecnologia de extração na otimização do potencial bioativo de P. ornatus, reforçando seu potencial como uma fonte antioxidante natural segura e eficaz.

Palavras-chave: *produtos naturais; tecnologias extrativas; ácidos fenólicos; capacidade redox; estresse oxidativo.*

INTRODUCTION

The genus *Plectranthus*, belonging to the family Lamiaceae, comprises approximately 300 species distributed worldwide, with a greater prominence in regions of Africa, India, and some Eastern countries (Ávila *et al*., 2017). *Plectranthus ornatus* Codd. has the synonym *Coleus comosus* (Hochst. ex Gürke) and is documented as originating from Africa and some Eastern countries. It was brought to Brazil by the Portuguese and is popularly known as "Boldo-chinês," "Boldo-miúdo," "Boldo-gambá," or "Boldinho" (Nascimento *et al*., 2017). The plant grows in tropical and subtropical regions and is used in both ethnobotany and as an ornamental species (Mota *et al*., 2014).

Figure 1 - Images of herbarium specimens of *P. ornatus* Codd. (A-B) and the *in natura* plant (C).

Source: Images A-B provided by F. Gonzatti (Herbarium of the Universidade de Caxias do Sul). Image C from the author's collection.

P. ornatus Codd. is a perennial herb characterized by aromatic, succulent leaves that are petiolate, pubescent, and prominently veined, with a rounded apex and a cuneate base. The bright green, ovate leaves are covered with fine hairs, giving them a soft, velvety texture (Figure 1). It has a terminal inflorescence resembling a raceme, with flowers arranged in whorls and ranging in color from light blue to violet. It typically forms dense garden beds and can reach a height of up to 50 cm

(Cordeiro, 2021). These characteristics contribute to its ornamental use in landscaping, as it is a plant with natural beauty (Mota *et al*., 2014).

In ethnobotany, its leaves are used in infusions to treat digestive disorders and dyspepsia (Mota *et al*., 2014). Cerqueira *et al*. (2020) report the popular use of *P. ornatus* leaves as a decoction to treat gastrointestinal problems, and Oliveira Melo *et al*. (2022) confirm the use of teas prepared from the leaves to relieve diarrhea, abdominal and intestinal pain, as well as hangover symptoms (Furlanetto, Novakowski & Correa, 2012). Furthermore, research conducted in a fishing community in Porto Alegre, Rio Grande do Sul, confirms the popular use of the species to treat digestive and hepatic problems (Baptista *et al*., 2013).

The *Plectranthus* genus can be used to obtain essential oils (Ávila *et al*., 2017). It is rich in compounds such as diterpenes (Andrade *et al*., 2020) and phenolic compounds (Andrade *et al*., 2020; Ávila *et al*., 2017). Regarding *P. ornatus*, the literature describes diterpenes in its composition (Andrade *et al*., 2020; Ávila *et al*., 2017; Mesquita *et al*., 2020), but little is known about its phenolic compounds content. Andrade *et al.* (2020) reported polyphenols with antioxidant activity in methanolic extracts of *P. ornatus*, and Medrado *et al*. (2017) isolated rosmarinic acid, caffeic acid, and derivatives of cinnamic acid from the plant infusion.

Diterpenes are known for their toxic effects in humans. According to Thawabteh *et al.* (2021), some diterpenes act as cardiotoxins and neurotoxins. Since these compounds are poorly soluble in water, they are minimally extracted into aqueous solutions. However, toxicity testing remains essential to ensure the safe medicinal use of plants containing diterpenes.

Phenolic compounds are particularly important in medicine, as they are widely recognized for their antioxidant properties (Yahfoufi *et al*., 2018). These compounds are composed of molecules with benzene rings linked to hydroxyl groups, which allow them to donate electrons and stabilize other unstable molecules, such as free radicals. The antioxidant capacity is influenced by the number of hydroxyl groups present in the molecule (Rodrigues *et al*., 2021). In humans, free radicals arise as natural by-products of metabolism, but their excess leads to oxidative stress.

Oxidative stress is associated with several diseases, including neurodegenerative disorders, cancer, and diabetes, as well as it is linked to aging. It is a consequence of reactive oxygen species (ROS) interacting with lipids, proteins, deoxyribonucleic acid (DNA), and other biological molecules, causing cellular damage (Andrade *et al*., 2020). Given that phenolic compounds can naturally counteract oxidative stress and protect cellular membranes and biomolecules (Matias *et al*., 2019), their role in human health is particularly significant.

Given the limited literature on phenolic compounds in aqueous extracts of this species, despite its recognized traditional use, this study aims to: (1) analyze whether tea-like aqueous extracts contain phenolic compounds with antioxidant activity, and (2) produce a concentrated extract for lyophilization to evaluate the potential toxicity of this extract in an *in vivo* model.

METHODOLOGY

PLANT MATERIAL

This study was conducted using leaves of *P. ornatus* Codd. The plant was cultivated in an urban area of Nova Prata - RS, Brazil (Latitude: 28º 47’ 02” S and Longitude: 51º 36’ 36” W), and was harvested in the morning during the month of February 2022. The specimen was identified at the Herbarium of the University of Caxias do Sul, where it was cataloged and assigned to an identification code: HUCS: 48639 (SISGEN n° A5824F3). The leaves were dried in a forced-air circulation oven at 40 ºC and ground using a knife mill (Willye TE-650).

PREPARATION OF EXTRACTS

The extracts were prepared from the dried and ground leaves using infusion, decoction, and aqueous reflux methods. For the infusion and decoction methods, a concentration of 10% (w/v) was used: the infusion was carried out in water at 100 °C for 10 minutes, and the decoction at 80 - 90 °C for 10 minutes after boiling. In the reflux method, a concentration of 5% (w/v) was adopted, supported by previous results from our laboratory (Michelon *et al.,* 2012; Chies *et al.,* 2013; Chilanti *et al.,* 2025) demonstrating that this concentration is adequate for extracting phenolic compounds. The extract obtained through the reflux system was lyophilized in a bench lyophilizer (model L101, LIOTOP, Brazil), and subsequently, 1% (w/v) solutions were prepared for the *Artemia salina* toxicity assay.

ANTIOXIDANT CAPACITY

Antioxidant activity was assessed by measuring the scavenging capacity of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), following the methodology adapted from Yamaguchi *et al.* (1998). For each 100 µL of the extracts, 500 µL of DPPH$^{\bullet}$ reagent and 400 µL of freshly prepared Tris-HCl buffer (100 mM, pH 7.0) were added. The samples were mixed and incubated in the dark for 20 minutes. Afterwards, absorbance was measured at 517 nm using a spectrophotometer (IL-226-NM, Kasuaki). Results were expressed as IC_{50} (mg/mL), which is defined as the concentration required to scavenge 50% of the DPPH$^{\bullet}$ radical.

TOTAL PHENOLIC CONTENT

The phenolic content of the extracts was determined using the colorimetric Folin-Ciocalteu method with modifications (Singleton, 1965). For each 100 µL of the extracts, 500 µL of

1 N Folin-Ciocalteu reagent and 400 µL of 7.5% sodium carbonate (Na_2CO_3) were added. Gallic acid was used for the standard curve in concentrations ranging from 0.025 to 0.75 mg/mL. The samples were mixed and incubated in the dark for 30 minutes. Absorbance was measured at 765 nm using a spectrophotometer (IL-226-NM, Kasuaki). The results were expressed as mg of gallic acid equivalents (GAE) per gram of plant material.

TOXICITY ASSESSMENT

To assess the *in vivo* toxicity of *P. ornatus* extract, the *Artemia salina* bioassay was conducted based on the method described by Meyer *et al.* (1982), with some modifications. The lyophilized aqueous extract (the one with the highest phenolic content) was used for this assay. *A. salina* nauplii were obtained from Artêmia Salina do RN, Brazil. *A. salina* eggs were incubated in a rectangular aquarium (20 × 15 × 10 cm) containing 2 L of saline solution at a concentration of 0.1 g/L, under controlled pH (7.0 - 9.0), artificial light, and ambient temperature (27 - 30 °C). After 48 hours, approximately 10 to 15 nauplii were transferred to 36 well plates containing 0.8 mL of saline solution. Then, it was added 0.2 mL of the *P. ornatus* Codd. extract at different concentrations (0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 mg/mL). The negative control consisted of a saline solution, and the positive control used various concentrations of potassium dichromate. The plates were maintained for 24 hours under the same environmental conditions as the incubation system. Analysis was performed using a colony counter (CP 608, Phoenix). Nauplii that were immobile after light stimulation were considered dead.

STATISTICAL ANALYSIS

The results were presented as mean ± standard deviation from experiments conducted in triplicate. For comparative data analysis, Pearson correlation, Student t-test, or analysis of variance (ANOVA) followed by Tukey's post hoc test ($p < 0.05$) was used. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS®), version 22.0.

RESULTS AND DISCUSSION

The use of medicinal plants in the form of tea is one of the most widespread home remedies for treating ailments, with knowledge often passed down from generation to generation (Furlanetto, Novakowski & Correa, 2012). However, little is known about *P. ornatus* Codd., and its use as a tea lacks strong scientific support.

Aqueous extracts prepared by decoction and infusion were designed to mimic traditional homemade tea preparations, while the reflux method was used as a comparison due to its higher efficiency in extracting phenolic compounds, making it suitable for toxicity evaluation. Accordingly, this extract was lyophilized to further concentrate the bioactive compounds and used in the *A. salina* assay, as described in the methodology section. All extracts were tested for phenolic compound content and antioxidant activity using the Folin-Ciocalteu assay and DPPH• radical scavenging method, respectively, as outlined in the methodology.

The antioxidant activity and total polyphenol content (TPC) of the *P. ornatus* Codd. infusion and decoction extracts are presented in Table 1. The extract with the highest antioxidant activity (expressed as IC_{50} in mg/mL) was the decoction extract (45.48 ± 0.67 mg/mL), followed by the infusion extract (47.89 ± 0.89 mg/mL). The highest TPC (expressed as mg of gallic acid equivalents per gram of plant) was also observed in the decoction extract (4.72 ± 0.1 mg/g).

Table 1 - Evaluation of Antioxidant Capacity (DPPH•) and Total Polyphenol Content (TPC) of *Plectranthus ornatus* extracts.

Extract Type	Concentration (% w/v)	DPPH (IC_{50} mg/mL)	TPC (mg GAE/g)
Infusion	10	47.89 ± 0.89 [a]	3.16 ± 0.08 [a]
Decoction	10	45.48 ± 0.67 [b]	4.72 ± 0.11 [b]

Different letters represent statistically significant differences according to Student's t-test for $p < 0.05$.

It was observed that both extraction methods were effective in extracting phenolic compounds, with similar IC_{50} values (mg/mL). However, decoction was able to extract a higher amount of phenolic compounds than infusion and showed greater antioxidant activity. Since both methods are traditionally used for tea preparation, as noted by Mota *et al.* (2014) and Cerqueira *et al.* (2020), if *P. ornatus* Codd. were to be used by the population for antioxidant purposes, the decoction would be the recommended method.

Table 2 presents the results obtained for the reflux extract and its lyophilized form. As expected, the lyophilized extract showed the highest antioxidant activity (6.85 ± 0.60 mg/mL) and the highest TPC (23.21 ± 0.60 mg/g). As previously mentioned, the goal of this method was to obtain a concentrated extract to be used in the *A. salina* toxicity test. The yield of the extract after lyophilization was 64.63%.

Table 2 - Evaluation of Antioxidant Capacity (DPPH•) and Total Polyphenol Content (TPC) of *Plectranthus ornatus* extracts.

Extract Type	Concentration (% w/v)	DPPH (IC_{50} mg/mL)	TPC (mg GAE/g)
Reflux	5	21.74 ± 0.51 [b]	7.13 ± 0.28 [b]
Freeze drying	1	6.85 ± 0.60 [a]	23.21 ± 0.60 [a]

Different letters represent statistically significant differences according to Student's t-test for $p < 0.05$.

The results show that all extracts demonstrated the ability to scavenge the DPPH• radical, with the best result obtained from the lyophilized extract (IC_{50} = 6.85 mg/mL). In addition, all processes showed a strong correlation between total phenolic content and antioxidant activity (Table 3).

The literature provides limited information on the phenolic compound content and antioxidant activity of aqueous extracts of this species. Rodrigues *et al*. (2021) reported an IC_{50} of 32.21 µg/mL for *P. ornatus* Codd. extracts obtained by ethanol maceration at a concentration of 0.05 mg/mL for 72 hours. Although this result indicates greater antioxidant activity, such superiority is expected, given the higher extraction efficiency of organic solvents like ethanol compared to water (Andrade *et al*., 2020). However, the true value of the present study lies in the validation of the safety and effectiveness of aqueous extraction methods, which more accurately reflect traditional preparation practices such as teas or infusions. By confirming both the presence of bioactive compounds and the absence of toxicity at tested concentrations, this work strengthens the scientific basis for the traditional use of *P. ornatus* and highlights the potential of optimizing low-cost, accessible extraction techniques.

Table 3 - Bivariate correlation between Antioxidant Capacity (DPPH•) and Total Polyphenol Content (TPC) of *Plectranthus ornatus* extracts from different methods.

Infusion	**DPPH•**	**TPC**
DPPH•	-	r =0.873; p=0.001
TPC	r =0.873; p=0.001	-
Decoction		
DPPH•	-	r =0.887; p=0.000
TPC	r =0.887; p=0.000	-
Reflux		
DPPH•	-	r =0.923; p=0.000
TPC	r =0.923; p=0.000	-
Freeze drying		
DPPH•	-	r =0.962; p=0.000
TPC	r =0.962; p=0.000	-

Morais *et al.* (2013) evaluated the total phenolic content (TPC) of various medicinal plant extracts, including *P. ornatus* Codd. These extracts were prepared by ethanol maceration for 7 days at a concentration of 10 mg/mL. The results indicated that the plant has a high phenolic content (151.47 mg GAE/g plant), which is significantly higher than the values obtained in the present study. Again, this can be attributed to ethanol's greater extraction capacity for phenolic compounds. The DPPH• scavenging capacity in their study was expressed with an IC_{50} of 2.63 mg/mL.

Andrade *et al.* (2020) investigated phenolic compounds in organic extracts (methanol, ethyl acetate, and acetone) at 20 mg/mL (w/v), obtained via sonication for 1 hour, as well as in aqueous extracts at 10 mg/mL obtained through microwave extraction and later lyophilization. The authors used a bioassay-guided method for phytochemical investigation and successfully isolated phenolic

compounds, although they were not quantified. Antioxidant activity was evaluated using DPPH• radical scavenging, but only the organic methanolic extracts showed the best results.

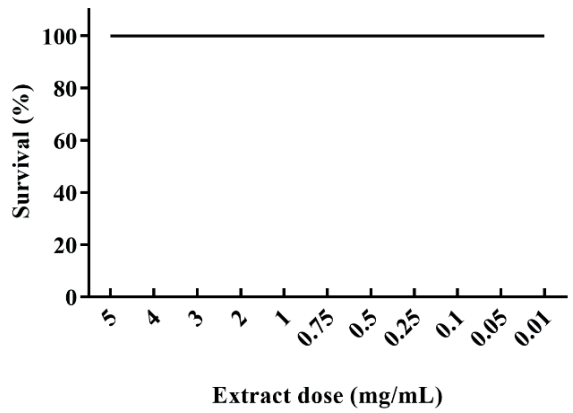
Matias *et al.* (2019) characterized the phenolic compounds of several *Plectranthus* species in aqueous, methanolic, and acetone extracts. They also evaluated antioxidant activity using the DPPH• radical scavenging, with methanolic extracts showing the highest activity, confirming that this solvent is more efficient at extracting polyphenols. Among the aqueous extracts tested, only *P. madagascariensis* showed significant antioxidant activity. However, the authors noted that, unlike organic extracts, aqueous extracts did not contain diterpenes and were considered non-toxic.

Brito *et al*. (2018) evaluated extracts from various *Plectranthus* species traditionally used in folk medicine, preparing a decoction at 10 mg/mL for 10 minutes. The extracts were lyophilized and tested for DPPH• activity and toxicity. Although *P. ornatus* Codd. was not included in their study, the decoction of *P. zuluensis* showed high DPPH• radical scavenging capacity, with an IC_{50} of 80 µg/mL and no toxic activity.

Both Andrade *et al.* (2020) and Matias *et al.* (2019) confirm that the organic extracts contain more phenolic compounds compared to the aqueous ones. However, organic extracts require solvent removal and often contain diterpenes, which are compounds capable of producing cardiotoxins and neurotoxins (Thawabteh e*t al*., 2021), rendering them potentially toxic to humans. It is important to emphasize that, although organic solvents are highly efficient in extracting phenolic compounds, they are not typically used in homemade tea preparations. Therefore, aqueous extracts are more practical, cost-effective, and safer for human consumption.

The findings from Andrade *et al*. (2020) and Matias *et al.* (2019) that aqueous extracts do not extract diterpenes and are non-toxic are supported by the *A. salina* assay conducted in this study. In our tests, concentrations of 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 mg/mL were evaluated, and no toxicity was observed (Figure 2). Consequently, it was not possible to calculate the LD_{50}. Extracts with LD_{50} above 1 mg/mL are considered non-toxic; between 0.5 and 1 mg/mL, low toxicity; between 0.1 - 0.5 mg/mL, mild toxicity; and between 0 - 0.1 mg/mL, high toxicity (Silva *et al*., 2021).

Figure 2 - Survival of *A. salina* exposed to increasing doses of *Plectranthus ornatus* lyophilized aqueous extract.



To date, this is the first study to investigate the differential aqueous extraction of *Plectranthus ornatus* Codd., analyzing total phenolic content and antioxidant activity at concentrations typically used in homemade teas, along with their potential toxicity. Although limited by time, which unabled HPLC analysis of individual phenolic compounds and LD_{50} determination, the findings provide a promising foundation for future research. The data found highlights the plant's potential as a safe and natural antioxidant source, encouraging further studies to fully characterize its bioactive compounds and therapeutic applications.

CONCLUSION

Based on the tests performed, it can be concluded that tea made from *Plectranthus ornatus* Codd., popularly known as "Boldinho," contains phenolic compounds with significant antioxidant activity. Aqueous extraction methods, commonly used in traditional preparations, proved effective for obtaining these bioactive compounds, while freeze-drying preserved their chemical stability and redox potential. Furthermore, the toxicity assay revealed no toxic effects at the tested concentrations, suggesting the extract is *in vivo* safe. Nonetheless, future studies should explore higher concentrations to establish potential toxic thresholds and further elucidate the extract's safety profile. Overall, this study demonstrates the potential of combining traditional knowledge with modern techniques, highlighting a novel technological approach to aqueous extracts that enhances both efficacy and safety.

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