# Nutritional properties, determination of phenolic compounds and antioxidant potential of Victoria amazonica (Poepp.) J.C. Sowerby petiole

**Sara Kethleen Soares de Loiola**
Universidade do Estado do Amazonas – UEA – EST

**Valdely Ferreira Kinupp**
Instituto Federal do Amazonas – IFAM

**Sergio Massayoshi Nunomura**
Instituto Nacional de Pesquisas do Amazonas – INPA

**Rita de Cassia Saraiva Nunomura**
Universidade Federal do Amazonas – UFAM

**Magno Perêa Muniz**
Instituto Nacional de Pesquisas do Amazonas – INPA

**Sergio Duvoisin Junior**
Universidade do Estado do Amazonas – UEA – EST

**Lílian Macedo Bastos**
Instituto Nacional de Pesquisas do Amazonas – INPA

**Rochelly Mesquita da Silva**
Universidade do Estado do Amazonas – UEA – EST

**Lorena Mota de Castro** (lorenamcastro.uea@gmail.com)
Universidade do Estado do Amazonas – UEA

**Patricia de Souza Pinto Hidalgo**
Universidade do Estado do Amazonas – UEA – EST

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# Abstract

This study reported for the first time the chemical profile of the petiole of *Victoria amazonica* (Poepp.) J.C. Sowerby, a non-conventional edible plant (NCEP), and demonstrated its nutritional and functional potential. Its proximate composition was determined by verifying the fiber, lipid, protein, ash, and carbohydrate contents in fresh samples. *V. amazonica* petiole has a high moisture content (97.62%), while has low amounts of protein, crude fiber and carbohydrates (3.10%, 1.81%, and 5.78%, respectively). At 19.5 kcal $g^{-1}$, this NCEP can be classified as a low-calorie food, especially due to low lipid content of 1.47%. Amongst the extracts and solvent-partitioned fractions, the ethyl acetate fraction showed the highest total phenolic content (25.47 GAE mg 100 $g^{-1}$) and antioxidant potential ($IC_{50}$: 13.67 μg $mL^{-1}$), which were evaluated using the Folin-Ciocalteu and DPPH methods, respectively. In comparison with well-known conventional fruits and plants, based on results obtained for the methanolic extract, the petiole showed a low total phenolic content (13.61 GAE mg 100 $g^{-1}$) and also an antioxidant potential ($IC_{50}$: 50.12 μg $mL^{-1}$) that was 10-fold higher than the gallic acid reference standard. Five phenolic acids were identified by dereplication using liquid chromatography coupled to mass spectrometry (LC-DAD-ESI-Q-TOF-MS). Gallic, ferulic, p-coumaric and 3,4-dihydroxybenzoic acids, as well the flavonoid quercetin-3-O-rhamnoside, were identified in methanolic extract. As a low-carb and low-fat food, this unconventional edible plant can be incorporated into diets with dietary restrictions and can be classified as a functional food since it is a source of bioactive compounds.


# Introduction

*Victoria amazonica* (Poepp.) J.C. Sowerby (Fig. 1), also known as the royal water lily, is an aquatic herbaceous plant, and the symbol of the Amazon region [1], though it can also be found in shallow lakes and waterways of the Guianas and the Pantanal biome in southern Brazil [2]. It belongs to the family Nymphaeaceae and, worldwide, it is probably one of the most well-known plants from the Amazon region [3, 4]. Despite being recognized primarily for its ornamental value and appreciated in numerous greenhouses around the world for its beauty and the peculiarity of its flowers, the species also has ecological, medicinal, and nutritional importance. Its flowers, rhizomes, stalks (petioles), stem and seeds can in fact be consumed [5]. Traditional populations living in Brazil consume a flour made from the dried seeds, which are starchy and are said to taste like popcorn [2, 6]. It is not by chance that its popular name in the region is “milho-da-água”, which literally translates as water maize.

The consumption of *V. amazonica*, considered a non-conventional edible plant (NCEP), in addition to being able to promote dietary diversification, can become a strategy for the maintenance of the forest, since this species develops in natural environments, without the need for inputs or the clearing of areas for agricultural production. Thus, its exploitation can be associated with environmental conservation [5]. The availability of nutritional information about these NCEPs can contribute to stimulating their consumption.

Located underneath the leaves of *V. amazonica*, the petiole is part of the basic constitution of the leaves' external morphology. It connects the limbus to the stem and can be consumed raw or cooked [3, 4, 7]. Currently, the commercial use of the petiole involves the manufacture of jelly from this part of this plant [8]. However, a characterization that reveals the nutritional value of *V. amazonica* petiole has not yet been performed. Thus, this study aimed to determine the proximate composition of the raw petiole, evaluate the antioxidant activity, total phenolic content of solvent-partitioned fractions of the methanolic extract, as well as determine the phenolic compounds in the methanolic extract using the dereplication technique in order to provide reliable data on its the potential nutritional and functional value.

# Materials And Methods

The material and methods section is described in the Supplementary Material.

# Results And Discussion

Centesimal composition

In Table 1, the chemical composition of the fresh *V. amazonica* petioles can be observed. The petioles showed a high moisture content (> 95%). In comparison, the *V. amazonica* seeds [9] have a water content (44.8%). Regarding the ash content, the petioles showed a high value (> 0.8%) in comparison with common fruit pulps from the Brazilian Amazon (< 0.5%) [10, 11]. In contrast to *V. amazonica*, other water lilies that belong to the Nymphaeaceae family, such as the red water lily (*Nymphaea x rubra*) and the zenkeri red tiger (*Nymphaea lotus Linn*), present low moisture (5.86–6.40%) and high ash content (22.69–27.36%) in their petioles [12, 13].

Table 1
Nutritional composition† of *Victoria amazonica* petiole

| **Parameter** | ***Victoria amazonica* petiole** | **% DRI* (100 g $d^{-1}$)** |
|---|---|---|
| Moisture (%) | 97.62 ± 0.02 | - |
| Ash (%) | 0.86 ± 0.08 | - |
| Lipids (%) | 1.47 ± 0.07 | 1.89-3.30[a] |
| Crude fiber (%) | 1.81 ± 0.06 | 4.76-7.24[b] |
| Protein (%) | 3.10 ± 0.02 | 5.54-6.74[c] |
| Carbohydrates (%) | 5.78 ± 0.06 | 4.45 |
| Energy (Kcal 100 $g^{-1}$)[d] | 48.78 | - |
| †Results as mean ± SD of measurements in triplicate | | |
| * Dietary reference intake (Institute of Medicine 2006) | | |
| [a] Obtained based on an Acceptable macronutrient distribution range (AMDR) based on a diet of 2,000 kcal/day | | |
| [b] Adequate intake (AI) | | |
| [c] Recommended dietary allowance (RDA) | | |
| [d] Calculated based on the method recommended by ANVISA [14] | | |

The total protein content of fresh petioles was similar to other NCEP species reported in the literature such as the leaves of *Xanthosoma taioba* (3.05 g 100 $g^{-1}$) [15] and *Amaranthus viridis* (2.11 g 100 $g^{-1}$) [16]. For adults of an average age, the recommended dietary allowance (RDA) for total protein varies between 46–56 g $d^{-1}$ [17]; therefore 100 g of fresh *V. amazonica* petiole provides a minimum of 5.54 g 100 $g^{-1}$ of the recommended daily protein intake, thus indicating that this part of the water lily is a poor source of protein.

As well as protein content, the petiole of *V. amazonica* has a low crude fiber content, especially when compared with the values found in *N. lotus* and *N. x rubra*, 15.10-18.16%, respectively. Values close to those obtained in this study (1.6–1.87 g 100 $g^{-1}$) were found in pulps of tropical fruits such as cubiu (*Solanum sessiliflorum* Dunal) and soursop (*Annona muricata* L.) [18]. Intakes of dietary fiber are related to a reduction in the risk of developing many diseases such as obesity, diabetes, and coronary heart diseases; and can improve blood pressure levels, serum lipid concentrations and immune function, besides helping to induce satiety [19, 20].

Carbohydrate content was the highest of the macronutrients found in *V. amazonica* petiole (> 5 g 100 $g^{-1}$), which is low compared to data obtained for other water lily such as *Nymphaea lotus* (51.31–80.96 g 100 $g^{-1}$) [21]. These values for total carbohydrates were in agreement with the range of values reported for other non-conventional plants such as perennial pigweed (*Amaranthus deflexus* L.), taro (*Colocasia esculenta*), and clove basil (*Ocimum gratissimum*), and other common vegetables [22]. Diets based on carbohydrate restriction are associated with the improvement of conditions such as type 2 diabetes, obesity and metabolic syndrome [23]. In this scenario, *V. amazonica* petiole emerges as an alternative food that can be used in diets with carbohydrate limitations.

A low average value of total lipids was found, this being the macronutrient least present in the *V. amazonica*. However, compared with other unconventional tropical fruits from Brazil, 0.24–1.17% [24], and the leaves of unconventional food plants (UFPs) consumed in the northeastern region of Brazil, 0.14–0.34% [22], the lipid content was lower in these than found in the *V. amazonica* petiole. The acceptable macronutrient distribution range (AMDR) of a macronutrient is the expression of nutrient ingestion recommendations as a percentage of total caloric intake [25], which, in this case, is the range of energy provided to an adult based on fat intake. Using the energy guideline of 2,000 kcal/day, it was possible to establish a recommended daily intake based on the AMDR of 20–35% of the energy provided based on fat intake. Therefore, in terms of fat, the energy provided by the Amazonian water lily petiole is low, which confirms the potential of this plant part in a diet with restriction of fat intake.

Calorimetric determination

Via the bomb calorimeter assay, it was possible to determine the caloric value of the fresh *V. amazonica* petiole sample, which was found to be 19.55 kcal 100 $g^{-1}$. In contrast with the applied system for estimating the energy value of foods using the Atwater factors [26], which is recommended by [14], it can be observed that this method overestimates the energy content (Table 1). According to [27], for certain applications, there are inaccuracies with the Atwater factor that are related to the digestibility of the macronutrients, more specifically in relation to the metabolizable energy value of any single food. The determination of the combustible energy content of food using adiabatic bomb calorimetry allows the measurement of single or mixed diets and can be applied to different kinds of foods. The energetic value of *V. amazonica* petiole is even lower than that the found in Mung bean (*Vigna radiata* L.; Family: Fabaceae) sprouts (30 kcal 100 $g^{-1}$), which is a low calorie functional food that is consumed worldwide [28].

Total phenolic contents and antioxidant activities

Total phenolic contents and antioxidant activity of the methanolic extract and solvent-partitioned fractions of *V. amazonica* petiole were determined and then compared with gallic acid, which is the reference standard. The results are shown in Table 2. For the total phenolic compound content, a significant difference between the samples is highlighted, and the ethyl acetate fraction derived from the methanolic extract showed the highest TPC, as also presented by [29] who observed a higher TPC in the ethyl acetate fraction of *Albizia myriophylla* bark methanolic extract. Based on the present data, it is possible to observe a relationship between TPC and antioxidant potential. Based on $IC_{50}$ value, the EtOAc fraction of the methanolic extract shows a higher antioxidant capacity, which is related to the significantly greater total phenolic content. By analyzing and comparing data published on lotus (*Nelumbo nucifera* Gaertn - Nymphaeaceae) rhizomes, it was found that total phenolic content of the extract reached values between 88.7–340.7 mg GAE 100 $g^{-1}$ [30], which corroborates the assumption that *V. amazonica* has a low content of this class of compounds as evidenced by high $IC_{50}$ value, since this is 10 times greater than the gallic acid reference standard.

Table 2

Results for antioxidant potential and total phenolic content (TPC)[†] for the methanolic extract and solvent-partition fractions of *V. amazonica* petiole

| **Sample** | **$IC_{50}$ ($\mu g\ mL^{-1}$)*** | **TPC** (GAE*** mg 100 $g^{-1}$)** |
|---|---|---|
| Chloroform fraction | $33.24 \pm 0.06^{c}$ | $2.33 \pm 0.15^{a}$ |
| Methanolic extract | $50.12 \pm 0.14^{d}$ | $13.61 \pm 0.01^{b}$ |
| Ethyl acetate fraction | $13.67 \pm 0.14^{b}$ | $25.47 \pm 0.57^{c}$ |
| Gallic acid (reference standard) | $4.53 \pm 0.24^{a}$ | - |
| [†]Results as mean ± SD of measurements in triplicate | | |
| [a,b,c,d] The values in the lines with different superscript letters are significantly different at $p \leq 0.05$, one-way ANOVA, Tukey's test | | |

* Inhibitory concentration required to obtain a 50% antioxidant effect.

** Total phenolic content.

***Gallic acid equivalent.

Dietary polyphenols, which occur in plants, have been widely reported, especially because of the health-promoting activities of these compounds and these include antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic, neuroprotective potential, antiadipogenic, and gut microbiota growth stimulus effects. Based on these findings, the consumption of foods containing polyphenols is encouraged in order to reduce the risk of chronic diseases [31]. Studies aiming to provide the characterization of phenolic compounds associated with the macronutrient profile and other micronutrients, especially with respect to non-conventional edible plants such as *Victoria amazonica*, allow better exploration of the potential of these plants for their integration in diets.

LC-DAD-ESI-Q-TOF-MS analysis of the constituents in the methanolic extract

The identification of polyphenols allows us to associate these compounds with antioxidant properties. The identification of phenolic compounds in the methanolic (MeOH) extract of the *V. amazonica* petiole was performed based on comparison with the retention time (RT) and *m/z* ratio of the patterns of flavonoids and phenolic acids in the positive and negative mode. Most of the compounds showed a higher response in negative mode, thus allowing a better detection of the metabolites. LC-DAD-ESI-Q-TOF-MS permitted the identification of five phenolic compounds, four of which are phenolic acids and one is a flavonol (Table 3). All compounds showed low error (≤ 6.8 ppm), which indicates the accuracy of the exact mass and molecular formula obtained.

Table 3
Phenolic profile of MeOH extract from *Victoria amazonica* using LC-MS in negative mode.

| No | RT (min) standard | m/z (M-H) standard | RT (min) sample | m/z (M-H) sample | Error (ppm) | Identification* |
|---|---|---|---|---|---|---|
| 1 | 2.9 | 169.0145 | 2.9 | 169.0132 | -15 | Gallic acid |
| 2 | 4.8 | 153.0182 | 4.7 | 153.0182 | 6.8 | Protocatechuic acid |
| 3 | 12.4 | 163.0398 | 12.3 | 163.0390 | -4.4 | p-Coumaric acid |
| 4 | 14.5 | 193.0503 | 14.4 | 193.0495 | -14.2 | Ferulic acid |
| 5 | 19.6 | 447.0922 | 19.8 | 447.0922 | 4.6 | Quercitrin hydrate |

* Gallic acid: 3,4,5-trihydroxybenzoic acid; protocatechuic acid: 3,4-dihydroxybenzoic acid; p-coumaric acid: 4-Hydroxycinnamic acid; ferulic acid: 4-Hydroxy-3-methoxycinnamic acid; and quercitrin hydrate: quercetin-3-O-rhamnoside

Protocatechuic acid has already been found in the leaves of *Victoria amazonica* [32]. The five compounds shown in Table 3 are reported herein for the first time in the petiole of *V. amazonica*. Secondary metabolites, such as phenolic acids and flavonoids, are notably beneficial as antioxidants [28]. The bioavailability of phenolic acids is largely investigated because of their health-promoting properties, which are mainly attributed to their antioxidant activity, second only to investigations of flavonoids [33].

Ferulic and p-coumaric acids are the two main representative of cinnamic acid derivatives and are found in various edible plants, especially cereal grains. With low toxicity and high absorptivity capacity, ferulic acid possesses many biological functions, such as the capacity to reduce cell damage, while also having antioxidant, anti-inflammatory, anticancer, immunostimulant properties, antidiabetic, antimicrobial and antithrombotic activities [34, 35]. Ferulic acid has a number of mechanisms of action in relation to antioxidant activity, including its capacity to bind to transition metals, inhibition of enzymes that catalyze the formation of free radicals through the cell respiration process. It acts as a hydrogen donor and, not only acts as a free radical scavenger, but also enhances scavenger enzyme activity [36–38]. Similarly, p-coumaric acid is also a considerably strong antioxidant due to its high capacity for scavenging free radicals, as well as the reduction of copper and iron ions [35, 39]. Available data indicate that the benzoic acid derivatives, gallic and protocatechuic acids, exert antioxidant and antidiabetic effects *in vitro*. When compared with protocatechuic acid, gallic acid presented DPPH radical scavenging that was significantly higher [40]; however, together, both phenolic acids interact in a synergic way and show a higher antioxidant capacity when analyzed individually [41].

The flavonol quercitrin hydrate (quercetin-3-O-rhamnoside) was identified in the methanolic extract of *V. amazonica* petiole. However, several compounds have been isolated from methanolic extracts of *V. amazonica* leaves, including anthocyanins [42], steroids, phenolic carboxylic acids, and chlorophylls [32]. In petals of *Victoria* species, Wu et al. [43] detected fourteen flavonoids, which included ten flavonols and four anthocyanins. The authors observed that different compositions of flavonoids result in different colors between the inner and outer petals. Quercitrin hydrate was reported for the first time in *V. amazonica*. Wound-healing properties, such as the increase of hydroxyproline content, re-epithelization of the injury site, significative reduction of C-reactive protein (CRP) level and decrease of the inflammatory process based on the reduction of the proinflammatory factor tumor necrosis factor-α (TNF-α), were reported for quercitrin hydrate by [44]. In addition, among the most common properties associated with phenolic compounds, such as antimicrobial, anti-inflammatory and antioxidant properties, the latter has been the most investigated, mainly due to the need to obtain substances that are capable of acting in the inhibition of oxidative stress caused by reactive oxygen species (ROS) [45]. Quercitrin hydrate had its antioxidant effect investigated by [46], and it was concluded that the mechanism of this activity occurs through direct participation in the scavenging of ROS and through the $Fe^{2+}$- binding. These achievements related to this compound, along with the presence of other polyphenols in *V. amazonica* that also act to attenuate and help prevent certain skin disorders [45], may explain the healing properties associated to this species, which, in folk medicine, is often used for this purpose [4].

Further studies should be developed in order to investigate the quantities of these phenolic compounds and their individual and associated contribution to antioxidant activity in *Victoria amazonica*.

# Conclusion

*Victoria amazonica* petiole is a low-calorie food with a high moisture and moderate mineral content. This non-conventional edible plant is a source of polyphenols that includes phenolic acids and flavonol, which contributes to its antioxidant activity, and thus its petiole can be classified as potential functional food. This study is the first to provide nutritional information about this aquatic plant, which is one that can be included in energy-restricted diets.

# Declarations

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DECLARATIONS

**Ethics Approval** This article does not contain any studies with human participants and/or animals performed by any of the authors.

**Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

**Conflict of Interest** The authors declare that they have no conflict of interest.

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# Figures

**Figure 1**

*Victoria amazonica* (Poepp.) J. C. Sowerby

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