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Bioactive Compounds Profiling and Nutritional Composition of Three Species from the Amaranthaceae Family†

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Abstract: In this work, the chemical and nutritional composition of three Amaranthaceae species (*Alternanthera sessilis*, *Dicliptera chinensis*, and *Dysphania ambrosioides*) was studied. The results showed a differential flavonoid content in the three species: *A. sessilis* and *D. ambrosioides* showed similar flavonoid contents (15.1 ± 0.6 and 15.1 ± 0.1 mg/g extract, respectively), followed by *D. chinensis* (11.4 ± 0.1 mg/g extract). On the other hand, the nutritional results showed a high protein content in all species (16.9− 13.9 ± 0.1 g/100 g dw) and revealed the presence of organic acids, such as oxalic and succinic acid. Therefore, bioactive compounds, together with protein and organic acids, could be of great value to the food industry.

Keywords: medicinal plants, phenolic compounds, nutritional value, phytochemistry

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1. Introduction

Several plant species have played an important role in traditional medicine worldwide, as humans have been using plants as a natural remedy for a multitude of diseases for 60,000 years [1]. In particular, the plants from Amaranthaceae family biosynthesize several bioactive compounds with beneficial biological activities, including essential oils, betalains, terpenoids, and phenolic compounds [2]. Different phytochemical studies have verified the different biological activities associated with the plant extracts belonging to this family, such as antioxidant, antidiabetic, antitumor, antibacterial, anti-inflammatory, among others [3].

Specially, three Amaranthaceae species, namely: *Alternanthera sessilis* (L.) R.Br. ex Dc, *Dicliptera chinensis* (L.) Juss. and *Dysphania ambrosioides* (L.) Mosyakin & Clemants, have been little explored in terms of their phytochemical valorization. *A. sessillis* has been used in traditional Malaysian medicine, both as an infusion and as food, while in China, its leaves have even been used for the treatment of eye and skin diseases, snake bites, and wound healing [4]. *D. chinensis*, has a major distribution in southern China, Bangladesh, northern India, and Vietnam [5], where it was traditionally used with detoxifying and diuretic purposes, thanks to the production of organic acids, flavonoids, terpenoids, steroids, and polysaccharides [6]. Finally, *D. ambrosioides*, distributed throughout South America, is known to be used in traditional medicine as a remedy for parasitic diseases, and it is still currently used to treat parasitosis because the presence of ascaridol [7].

Due to the health-enhacing potential attributed to Amaranthaceae species, in this work the nutritional characterization and chemical composition, in terms of phenolic compounds, will be carried out. As a result, this research could be considered as the

starting point for a more targeted search for bioactive compounds [8] biosynthesized by these underexplored plant species.

2. Materials and Methods

2.1. Plant Material and Nutritional and Chemical Characterization

The samples proceeding from the Amaranthaceae species involved in this work, *A. sessilis*, *D. chinensis*, and *D. ambrosioides*, were thoroughly washed, air-dried, crushed, and sieved to obtain plant homogenates, which were stored at -80 °C until use.

The nutritional characterization (ashes, proteins, lipids, and carbohydrates, as well as energy) of the three plants was carried out following the methodology adapted previously [9]. The determinations were carried out by duplicate, and results were expressed in terms of percentage of composition for ashes, proteins, lipids, and carbohydrates, whereas energy was expressed as the mean ± standard deviation (SD) in kcal/100 g dry weight (dw).

The chemical composition (total sugars, fatty acids, and organic acids) was evaluated following the methodology also described by Barros et al. (2013) [9]. The determinations were performed in duplicate, and the results were expressed as the mean ± SD in g/100 g dw for total sugars and organic acids composition, whereas fatty acids were expressed as the relative percentage of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs).

2.2. Sample Extraction and Determination of Phenolic Compounds

For the determination of phenolic compounds, 1 g of each sample was macerated using 50 mL of ethanol/water (80:20 v/v) as solvent. This mixture was stirred at room temperature for 1 h and then filtered. This process was repeated twice, and extracts were collected and concentrated at 40 °C in a rotary evaporator, to remove the alcoholic fraction. The aqueous phase was frozen and freeze-dried. For the identification of phenolic compounds, a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA) was used, following a previous methodology [10]. The determination was performed by diode array detector (DAD) and mass spectrometry (MS) (LTQ XL mass spectrometer, Thermo Finnigan, San Jose, CA, USA) working in negative mode. Once identified and quantified, compounds were grouped by their parental skeleton, being expressed as luteolin derivatives (LD), apigenin derivatives (AD), kaempferol derivatives (KD), quercetin derivatives (QD), and isorhamnetin derivatives (ID), in mg/g dw.

3. Results and Discussion

3.1. Nutritional Characterization

The results for the nutritional composition of Amaranthaceae plants are shown in **Figure 1**. The inorganic content of all plants, represented by the ashes, showed similar values, being higher than those of other plants of the same family from the genus *Amaranthum* [11] For proteins, *D. chinensis* (16.8 g/100 g dw) and *A. sessilis* (16.1 g/100 g dw) showing similar values, higher than those of *D. ambrosioides* (13.9 g/100 g dw). These values are comparable to other species such as *Chenopodium quinoa* (quinoa), and they were also higher than other cereals such as wheat, maize, or rice [12] With respect to lipids, the values for *A. sessilis* (0.74 g/100 g dw) were very low compared with the other species, which ranged between 1.1 – 1.8 g/100 g dw, being comparable to the lipid content of fruits and vegetables [12]. Regarding the carbohydrate content, the results were also very similar between the three species: 68.8 g/100 g dw for *D. chinensis*, 73.2 g/100 g dw for *A. sessilis*, and 71.5 g/100 g dw for *D. ambrosioides*, being in accordance with the carbohydrate contents of *C. quinoa* and other cereals, as well as other foods, such as chocolate, flour or bread [12] Finally, the energy value did not vary much either, ranging from 350 - 365 kcal/100 g dw, with *A. sessilis* being the species with the highest energy intake.

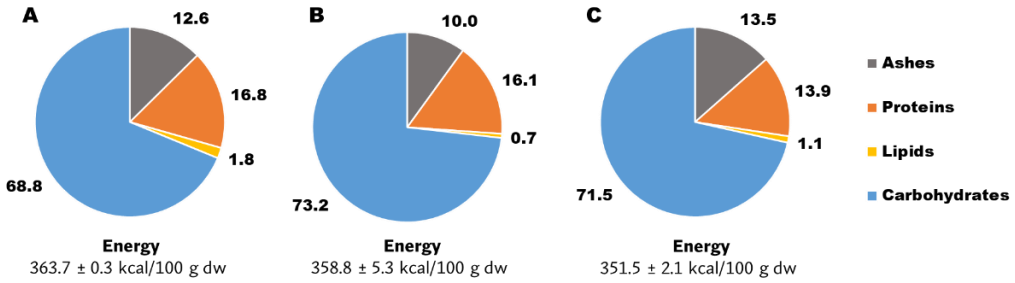

Figure 1. Nutritional characterization (ashes, proteins, lipids, and carbohydrates), and energy determination of three Amaranthaceae plants: **A)** *A. sessilis*, **B)** *D. chinensis* and **C)** *D. ambrosioides*. The results for nutrient content were expressed as relative content in percentage, whereas energy was expressed as mean ± SD, in kcal/100 g dw.

3.2. Chemical Characterization

The results for the chemical characterization of Amarathaceae species, in terms of total sugars, organic acids, and fatty acids contents are shown in **Figure 2**. The results for free sugars were, in decreasing order, 6.33 g/100 g dw for *D. chinensis*, 4.13 g/100 g dw for *A. sessilis*, and 0.34 g/100 g dw for *D. ambrosioides*. In this case, *A. sessilis* has the most similar content to that estimated for *C. quinoa* (2 - 3 g/100 g dw) [12].

Organic acids were detected in all three plant species studied, with a total content of ~9.13 g/100 g dw for *D. chinensis*, 8.43 g/100 g dw for *A. sessilis*, and 5.43 g/100 g dw for *D. ambrosioides*. Oxalic acid stood out as the organic acid present in the highest concentrations in all species, especially in the case of *A. sessilis* (data not shown). This acid has been associated with reduced dietary Ca^{2+} availability and various kidney diseases [13]. Succinic acid and fumaric acid were also detected in *D. chinensis*, although in lower proportions. Both acids are in high demand by the food, cosmetic and pharmaceutical industries [14]. In addition, in the case of *C. quinoa*, previous data showed the presence of oxalic, citric and fumaric acid [15], revealing a similar profile for this functional food in terms of organic acids content.

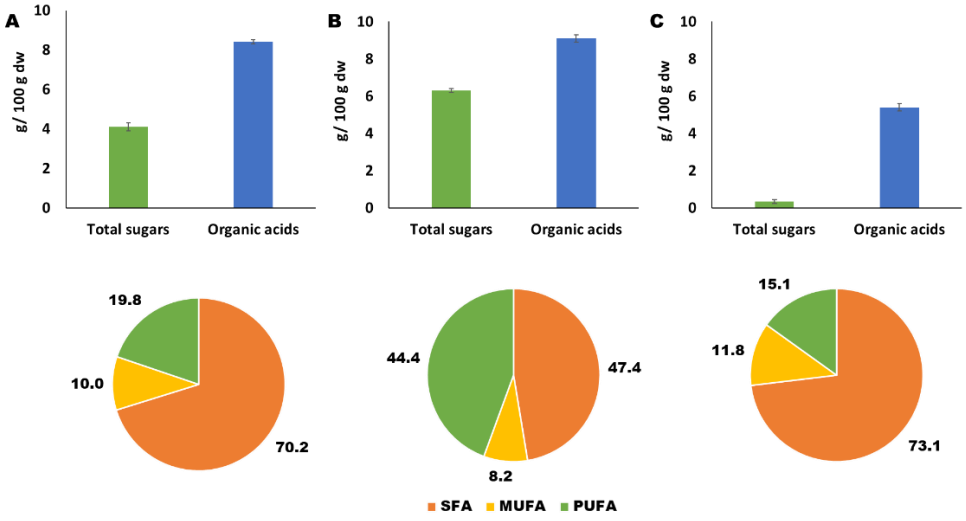

Figure 2. Chemical characterization (total free sugar, organic acids, and fatty acids contents) of three Amaranthaceae plants: **A)** *A. sessilis*, **B)** *D. chinensis*, and **C)** *D. ambrosioides*. Results for total sugars and organic acids were expressed as g/100 g dw, vertical bars indicate standard deviation. The results for fatty acids content were expressed as relative abundance, in percentage. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Concerning the relative abundance of fatty acids in Amaranthaceae plants (pie charts in **Figure 2**) SFAs, MUFAs, and PUFAs were detected in these species. According to the data obtained, the plant with the highest amount of SFA was *D. ambrosioides* with 73.1% of the total fatty acids (**Figure 2A**), followed by *A. sessilis* with 70.2% and *D. chinensis* with 47.4%. As for the MUFA, all plants exhibited very similar values between them, ranging 8.23 – 11.8%. Finally, the results for PUFAs showed that *D. chinensis* had the highest abundance with 44.4% (**Figure 2B**), while *D. ambrosioides* and *A. sessilis* presented a similar abundance (19.8% and 15.1% respectively). According to data, the most abundant fatty acid in the three species was hexadecanoic acid, with the range of abundance in the percentage of total fatty acids in the three plants being 32 - 40%. A previous study identified the fatty acids of *A. sessilis,* in which the second most abundant fatty acid was hexadecanoic acid [16]. Regarding the high content in PUFAs for *D. chinensis*, and due to the beneficial properties associated with these bioactive compounds as antioxidant and cardioprotective agents, it is suggested that this species presents the healthiest chemical profile.

3.3. Determination of phenolic compounds

The phenolic profiling of hydroethanolic extracts of Amaranthaceae plants was performed by UPLC-DAD-ESI/MS, revealing that flavonoids were the most abundant family of phenolic compounds in these species. The results for the determination of phenolic compounds are shown in **Figure 3**. In the case of *A. sessilis* extracts, luteolin derivatives (LD) were the most abundant compounds, with concentrations of 9.7 mg/g extract, being luteolin-8-*C*-(rhamnosyl)ketodeoxyhexoside the most prevalent derivative (**Figure 3A**). In a lesser extent, apigenin derivatives (AD), kaempferol derivatives (KD), and quercetin derivatives (QD) were also reported (<1.8 mg/g extract) (**Figure 3A**). This plant has two varieties distinguished by their color, red and green, and previous studies have shown that the red variety has a better nutritional composition, a higher content of phenolic compounds, and a greater antioxidant capacity [12]. Thus, the phenolic composition of *A. sessilis* has been seen to be affected by the variety employed.

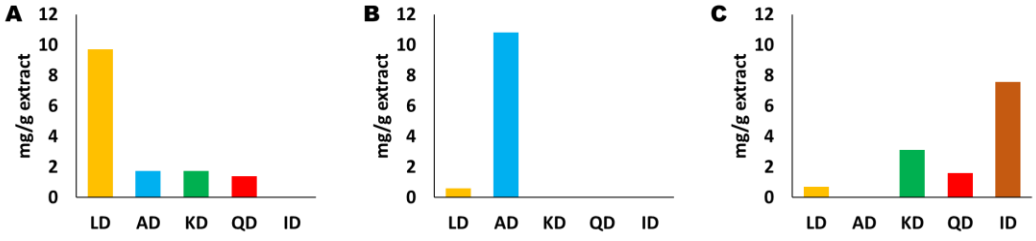

Figure 3. Identification of phenolic compounds (fatty acids, organic acids and tocopherols) of three plants belonging to the Amantharaceae family: A) *A. sessilis,* B) *D. chinensis* and C) *D. ambrosioides*. LD: luteolin derivatives, AD: apigenin derivatives, KD: kaempferol derivatives, QD: quercetin derivatives, and ID: isorhamnetin derivatives.

With respect to *D. chinensis*, the extracts essentially contained different ADs, accounting for 10.82 mg/g extract (**Figure 3B**), being apigenin 6-C-glucoside-8-C-arabinoside the most prevalent compound. This is the first time, to the best of our knowledge, that the phenolic profiling of *D. chinensis* is determined, being spotted as a potential natural source of apigenin, which have been largely characterized as a bioactive compound [17].

Finally, the results for *D. ambrosioides* (**Figure 3C**) showed that this was the only species presenting isorhamnetin derivatives (ID), which were the most abundant compounds in the hydroethanolic extracts (7.58 mg/g extract), and isorhamnetin-3-O-rutinoside were quantified as the main phenolic compound. Besides IDs, LDs, KDs, and QDs were also identified in this species in lower concentrations, and lacking ADs (**Figure 3C**). In previous studies on *D. ambrosioides*, the highest concentration of phenolic compounds extracted was obtained by methanolic extracts, with 87.7 ± 1.4 μg of gallic acid equivalents/mg

extract and 57 ± 1.4 μg quercetin equivalents/mg extract. Moreover, the same authors identified quercetin as the most abundant phenolic compound on *D. ambrosioides* [18], which is in accordance with our results, since quercetin-*O*-rhamnosyl-pentoside was the second most abundant compound in the hydroethanolic extracts. This suggests a critical role of solvent on the extraction of phenolic compounds from Amaranthaceae plants.

4. Conclusions

In this work, the determination of nutritional and chemical characterization, as well as the phenolic profiling of three Amaranthaceae species largely used in tradicional medicine was developed. In this regard, similar chemical profiles were obtained for all species, with comparable inorganic, protein, lipid, and carbohydrate contents. The results on fatty acid composition revealed that *D. chinensis* showed the healthier profile with a high proportion of PUFAs. Finally, the determination of phenolic compounds suggested a species-dependent biosynthesis of these compounds, being luteolin derivatives, apigenin derivatives, and isorhamnetin derivatives the most prevalent phytoconstituents on *A. sessilis*, *D. chinensis*, and *D. ambrosioides*, respectively. Overall, our results shed light on the characterization of these species from a nutritional point of view and suggested that Amaranthaceae species can be considered as sources of bioactive compounds to be applied in the food, cosmetic, and pharmacological industries.

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Conflicts of Interest: The authors declare no conflict of interest.

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