Journal of Biotechnology and Biodiversity
journal homepage: *https://sistemas.uft.edu.br/periodicos/index.php/JBB/index*

Phenolic compounds from unconventional food plants occurring spontaneously in an agroforestry system

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INFO

Keywords
phenolic compounds
agroecological systems
unconventional food plants

ABSTRACT

In agroforestry systems, the interaction between different species recreates the activity of natural ecosystems, therefore, the herbaceous stratum tends to be diverse with occurrence of several spontaneous plants. This study aimed to survey leafy plant species with spontaneous occurrence of herbaceous extract present in an agroforestry system, identify the unconventional food plants (UFP), analyze biochemical characteristics and determine the total content of phenolic and flavonoid compounds of the species. A total of 33 species were collected across four seasonal collections, 14 of which were identified as UFP. Data analysis using ANOVA and the Scott-Knott test at 5% significance revealed significant differences among species for all variables evaluated. The average water content was 84%, while nitrogen content varied significantly, with a mean of 30.34%. The reducing sugar content was consistent across species, averaging 6.6%. The total phenolic content results were expressed on a dry weight basis ranged from 0.007 g GAE 100 g^{-1} for *Alternanthera tenella* to 0.144 g GAE 100 g^{-1} for *Emilia fosbergii*. The total flavonoid content ranged from 0.121 g QE 100 g^{-1} in *Sonchus oleraceus* to 1.071 g QE 100 g^{-1} in *Raphanus raphanistrum*, which, along with *Emilia fosbergii* (1.048 g QE 100 g^{-1}), exhibited the highest values of total flavonoid content. These findings suggest that the spontaneous herbaceous plants found in the agroforestry system are rich in bioactive compounds, offering potential for food diversification and contributing to local biodiversity conservation.

RESUMO

Palavras-chaves
compostos fenólicos
sistemas agroecológicos
plantas alimentícias não convencionais

Em sistemas agroflorestais, a interação entre diferentes espécies recria a atividade de ecossistemas naturais, portanto, o estrato herbáceo tende a ser diverso, com ocorrência de várias plantas espontâneas. Este estudo teve como objetivo fazer um levantamento de espécies vegetais folhosas com ocorrência espontânea do extrato herbáceo presente em um sistema agroflorestal, identificar as plantas alimentícias não convencionais, analisar características bioquímicas e determinar o teor total de compostos fenólicos e flavonoides das espécies. Um total de 33 espécies foram coletadas em quatro coletas sazonais, 14 das quais foram identificadas como plantas alimentícias não convencionais. A análise de dados usando ANOVA e o teste de Scott-Knott a 5% de significância revelou diferenças significativas entre as espécies para todas as variáveis avaliadas. O teor médio de água foi de 84%, enquanto o teor de nitrogênio variou significativamente, com uma média de 30,34%. O teor de açúcar redutor foi consistente entre as espécies, com média de 6,6%. Os resultados do conteúdo fenólico total foram expressos em peso seco e variaram de 0,007 g EAG 100 g^{-1} para *Alternanthera tenella* a 0,144 g EAG 100 g^{-1} para *Emilia fosbergii.* O conteúdo de flavonoides totais variou de 0,121 g EQ 100 g^{-1} para *Sonchus oleraceus* a 1,071 g EQ 100 g^{-1} para *Raphanus raphanistrum*, que, junto com a espécie *Emilia fosbergii* (1,048 g EQ 100 g^{-1}) exibiram os maiores valores de flavonóides totais. Essas descobertas sugerem que as plantas herbáceas espontâneas herbáceas encontradas no sistema agroflorestal são ricas em compostos bioativos, oferecendo potencial para diversificação de alimentos e contribuindo para a conservação da biodiversidade local.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

INTRODUCTION

A diversity diet is considered to be related with health benefits (Liu, 2013). Despite that, current society's diet has been reduced to 110 species, being consumed in large quantities varieties of wheat, potatoes, corn and rice, while there are between 12,500 to 15.00 edible plants on the planet (Rapoport and Drausal, 2001). In Brazil, there are more than 3,000 potential species of food plants still underexplored and many of them are considered weeds. Therefore, a valuable nutritional resource is wasted (Otero, 2019; Kinupp and De Barros, 2007).

In order to rescue and encourage their consumption, underutilized plants have received increasing attention. Kinupp and Lorenzi (2014), proposed the name "unconventional food plants" (UFP) that refers to that type of food species that have one or more parts with food potential but no common use. Some UFP are commonly considered as weed although they can be nutritious and good source of bioactive compounds with wide biological activity (Ghirardini et al., 2007; Kinupp and De Barros, 2008; Viana, 2015; Otero, 2019; De Carvalho et al., 2021).

Polyphenols are one of the groups of bioactive compounds that have attracted the attention for the functional food characteristics (Peisino et al., 2020). Phenolic compounds, such as flavonoids, are compounds related to the antioxidant activity that can have effective protection against free radicals, preventing from various diseases. Plants are the main source of these compounds (Brglez Mojzer et al., 2016; Santos-Buelga et al., 2019; Hernández-Rodríguez et al., 2019) and a diet made up of a variety of fruits and vegetables is based on a high intake of bioactive compounds with antioxidant activity (Pieniz et al., 2009). Rescuing and including the UFP in the routine diet, it will become even healthier. Although they do not have market value or are only commercialized on small scales (Leal et al., 2018), they are plants that are easy to produce or occur naturally (Kinuppi and Barros, 2007).

According to Leal et al. (2018) the urbanization and environmental restrictions are aspects that can inhibit the access to UFP, therefore sustainable production systems allied to a conscience for healthy eating can encourage the consumption and conservation of unconventional food plants. With the rise of new production systems, the agroforestry system is an example of great diversity of tree and also herbaceous species. The interaction between different species recreates the activity of natural ecosystems ensuring the balance and diversity of the agro-ecosystem (Steenbock et al., 2013). Thus, in this environment, the herbaceous strata tend to be diverse and to have an occurrence of a great number of different botanic families that might present potential as food or medicine (de Oliveira Júnior and Cabreira, 2012). In Brazil, agroforestry systems are widely adopted across all regions, serving as a significant or sole source of food production, particularly for traditional communities and family farmers. These systems are also increasingly present in urban and peri-urban settings, particularly in small and medium-sized cities, and are even found in metropolitan areas due to the expansion of urban agriculture (Santos et al., 2019; Shennan-Fárpon et al., 2022). Despite their prevalence, few studies explore the nutritional potential of spontaneous species within these systems, as well as the nutritional, medicinal, and pharmacological properties of their herbaceous components. Furthermore, there is a scarcity of detailed information regarding their chemical characteristics.

This research aims to investigate the spontaneous herbaceous plants occurring in an agroforestry system in Botucatu-SP to know the diversity and edibility of the species, as well as to know their content of phenolic compounds and flavonoids, which may indicate their antioxidant potential. Plants occurring in this area remain poorly studied from a floristic and chemical point of view and may also reflect the region's floristic richness and the potential for enriching the local diet.

MATERIAL AND METHODS

Management of the agroforestry system

The collections of samples were accomplished in the agroecological management area of the Agroecology Group of the Faculty of Agricultural Sciences (FCA) at Sao Paulo State University (UNESP) in Botucatu city in São Paulo state (S22º84'04.43''and W48º43'29.61''), where the development and management of an agroforestry system have been in operation since 2013.

The area has approximately 250m² and management activities occur weekly and consist of agroecological management employing techniques such as green manure, selective pruning and weeding, incorporation of biomass and straw, intercropping between companion plants, application of organic fertilizers, and other management techniques used in agroecological and agroforestry crops. Management activities aims to maintain the spontaneous species that occur there, contributing to the diversification of flora, microfauna, and macrofauna that exist in the

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

system.

Sample collection

In order to achieve a qualitative survey of herbaceous species, the method described by Filgueiras et al. (1994) was used, which consists of drawing lines in the direction of greater extension and walking through them collecting the present species.

The collections were carried out in periods that coincided with winter, spring, summer and autumn, respectively, totalling four collections throughout the year. The transects traced in the area were covered collecting all the spontaneous herbaceous species present that were in the reproductive period, with fertile material (flower or fruits), except species of the Poaceae family. The material for the biochemical analysis was separated, weighed and stored under refrigeration (-20 ºC) just after the collection until the moment of the biochemical analysis.

Identification of species

All species collected were preserved in the form of exsiccates and identified by comparison with materials already incorporated into the herbarium Irina Delanova Gemtchujnicóv - BOTU and by consulting the sites of herbarium with digitalized specimens (Tropicos.org). After being identified they were deposited in the herbarium and a list of spontaneous species was prepared.

Survey of unconventional food plants

Succeeding the identification of spontaneous species, a list of the ones identified as unconventional food plants was made by consulting their food potential in references such as scientific articles and books. The information regarding the edibility such as the common uses of those species present in this work was written in recognized literature, such as Kinupp and Lorenzi (2014), Kinupp and De Barros (2007), IBGE (1980) and Tanaka (1976).

Biochemical analysis

Biochemical analyses were performed on plant species collected in spring, seeking to homogenize the variations that quantitatively and qualitatively might influence the results. Frozen samples were lyophilized, weighed, ground in a cryogenic mill or by a mortar and pestle, and stored in a freezer at -80ºC. Each sample were composed of 100 grams of a mixture of leaves from three individuals of each species. The extract was performed using methanol (70%). All measurements were conducted in triplicates.

Water Content (%Water), Total Reducing Sugars (%TRS) and Total Proteins (%N)

Each sample was weighed just after collection and weighed again after lyophilization to obtain the percentage of water content by using the formula %WC (Water Content) = (Fresh Weight – Dry Weight) / Fresh Weight x 100. Determination of Total Reducing Sugars (%TRS) (hydrolyzed sucrose plus reducing sugars) with the previous hydrolysis of sucrose was analysed according to the Somogy-Nelson method (Nelson, 1944; Somogy, 1945). The determination of total protein was performed based on the total organic nitrogen content (%N), according to the Kjeldahl method (AOAC, 1995).

Total Phenolic Content (TPC) and Total Flavonoids Content (TFC)

Total Phenolic Content (TPC) was determined using spectrophotometric method described by Singleton e Rossi (1965), with absorbance reading performed at 725 nm. TPC amounts were calculated using a gallic acid calibration curve equation $y = 0{,}0119x + 0{,}1091$, $R^2 = 0{,}9834$. The concentration of gallic acid in each extract was calculated from the regression equation using their absorbance. Finally, these results were converted to the TPC as grams of gallic acid equivalents per 100 g sample (g GAE 100 g^{-1}). Total flavonoids content (TFC) was determined from an adaptation by Awad et al. (2000) and Popova et al. (2004), with absorbance measured at 425 nm. TFC amounts were calculated using a standard curve of quercetin solution $y = 0{,}0119x + 0{,}1091$, $R^2 = 0{,}9834$ and the results were expressed as grams of quercetin equivalents (g QE 100 g^{-1}). The concentration of quercetin in each extract was calculated from the regression equation using their absorbance.

Statistical Analysis

The results were submitted to analysis of variance and comparison of means for all variables evaluated. The analysis was performed by applying the Scott-Knott test, with a significance level of 5% ($p < 0.05$), using the computer statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

Species collected in the agroforestry system

Thirty-three herbaceous species of spontaneous occurrence were collected in a total of four collections carried out during a year. Data related to the family, species and identification number (BOTU) are presented in the list of species in the Table 1.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

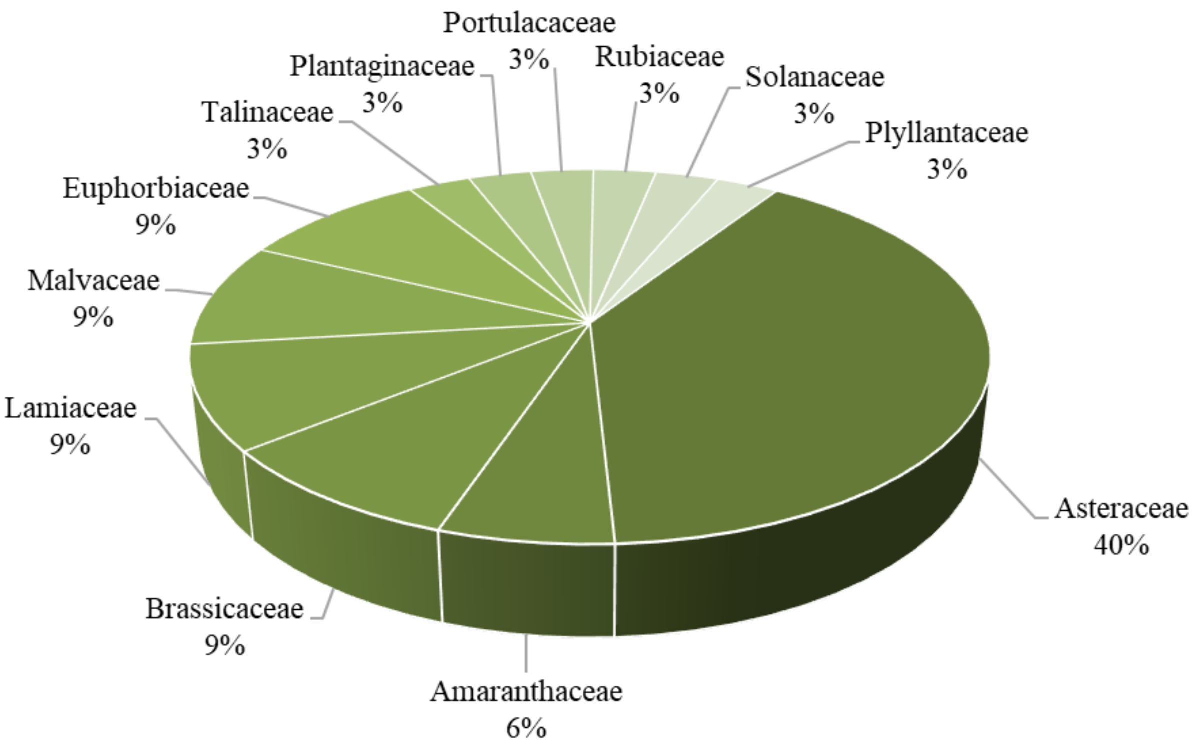
Table 1 - List of collected herbaceous plants of spontaneous occurrence in the agroforestry system by number, family, species, common name and identification number BOTU.

No.	Family	Species	Commom name	ID
1	Amaranthaceae	*Alternanthera tenella* Colla	apaga-fogo	32759
2	Amaranthaceae	*Amaranthus deflexus* L.	caruru	32837
3	Asteraceae	*Elephantopus mollis* Kunth	erva-de-colégio	32791
4	Asteraceae	*Acanthospermum australe* (Loefl.) Kuntze	carrapichinho	32766
5	Asteraceae	*Erechtites valerianifolius* (Link ex Spreng.) DC.	capiçoba	32959
6	Asteraceae	*Erechtites hieraciifolius* (L.) Raf. ex DC	serralha-brava	32960
7	Asteraceae	*Gnaphalium coarctatum* Willd.	erva-branca	32764
8	Asteraceae	*Emilia fosbergii* Nicolson	serralhinha	32765
9	Asteraceae	*Bidens pilosa* L.	picão-preto	32767
10	Asteraceae	*Cosmus sulphureus* Cav.	cosmos	32768
11	Asteraceae	*Cyrtocymura scorpioides* (Lam.) H. Rob.	erva-preá	32769
12	Asteraceae	*Sonchus oleraceus* L.	serralha	32777
13	Asteraceae	*Ageratum conyzoides* L.	mentrasto	32778
14	Asteraceae	*Tithonia diversifolia* (Hemsl.) A. Gray	margaridão	32783
15	Asteraceae	*Praxelis pauciflora* Kunth	botão-azul	32835
16	Brassicaceae	*Lepidium virginicum* L.	mastruço	32762
17	Brassicaceae	*Brassica rapa* L.	mostarda	32773
18	Brassicaceae	*Raphanus raphanistrum* L.	rábano	32774
19	Euphorbiaceae	*Euphorbia hirta* L.	erva-andorinha	32780
20	Euphorbiaceae	*Euphorbia heterophylla* L.	leitera	32769
21	Euphorbiaceae	*Euphorbia graminea* Jacq.	ponta-de-flecha	32832
22	Lamiaceae	*Marsypianthes chamaedrys* (Vahl) Kuntze	paracari	32770
23	Lamiaceae	*Leonurus sibiricus* L.	rubim	32776
24	Lamiaceae	*Leonotis nepetifolia* (L.) R. Br.	cordão-de-frade	32792
25	Malvaceae	*Sida cordifolia* L.	guanxuma	32790
26	Malvaceae	*Waltheria indica* L.	malva-veludo	32833
27	Malvaceae	*Triunfetta rhomboidea* Jacq.	malva-preta	32836
28	Phyllanthaceae	*Phyllantus tenellus* Roxb.	quebra-pedra	32834
29	Plantaginaceae	*Plantago australis* Lam.	tanchagem	32786
30	Portulacaceae	*Portulaca oleracea* L.	beldroega	32785
31	Rubiaceae	*Richardia brasiliensis* Gomes	mata-pasto	32786
32	Solanaceae	*Solanum americanum* Mill.	maria-pretinha	32756
33	Talinaceae	*Talinum paniculatum* (Jacq.) Gaertn.	beldroegão	32758

Among the thirty-three species collected, most belong to the Asteraceae family (40%) (Figure 1). This percentage demonstrates the importance of Asteraceae in plant cultivation, as they represent a large percentage of the total flora of angiosperms. They also have a cosmopolitan distribution, occurring spontaneously in both ruderal and agricultural areas (Roque and Bautista, 2008). Both families Brassicaceae, Lamiaceae, Euphorbiaceae and Malvaceae occurred with three species each.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

Figure 1 - Percentage of occurrence of botanical families of the species collected in the agroforestry system.



Studies in biodiversity shows the relation between agroforestry and biological conservation, besides high soil fertility and high diversity (Jacob et al., 2021). That is crucial to understand that preservation of biodiversity is related with the manager of natural resources (Hailu et al., 2021).

Unconventional food plants

Fourteen species (42.4%) were identified as unconventional food plants, determined by at least one citation in the literature. Most of the food species found in this study is part of the Asteraceae family. Table 2 shows the species of UFP collected over a year and their respective uses in cooking according to Kinupp and Lorenzi (2014), Kinupp and De Barros (2007), Tanaka (1976) and IBGE (1980).

Table 2 - Unconventional food plants collected over a year in the agroforestry system area by species, common name and culinary uses.

Species	**Common name**	**Culinary uses**
Alternanthera tenella Colla	apaga-fogo	cream, sauce, fried
Amaranthus deflexus L.	caruru	fried, braised, souffle
Bidens pilosa L.	picão-preto	risotto, braised, iced tea
Emilia fosbergii Nicolson	serralhinha	fresh or cooked leaves
Erechtites valerianifolius (Link ex Spreng.) DC.	serralha-brava	muffin, breaded flowers, sautéed
Erechtites hieraciifolius (L.) Raf. ex DC	capiçoba	with rice and chicken, braised
Sonchus oleraceus L.	serralha	salad, braised, cooked
Brassica rapa L.	mostarda	braised, breaded flowers
Lepidium virginicum L.	mastruço	braised, with meat, pate
Raphanus raphanistrum L.	rábano	braised, pickled fruit
Plantago australis Lam.	tanchagem	braised, breaded and salads
Portulaca oleracea L.	beldroega	braised, salad, sprouts
Solanum americanum Mill.	maria-pretinha	braised, soup, fruit jelly
Talinum paniculatum (Jacq.) Gaertn.	beldroegão	bread, pate, braised, salad

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

The fourteen species of unconventional food plants in this study can be found in the book "Plantas Alimentícias Não Convencionais (PANC) no Brasil", in Portuguese, a great reference on UFP, published in 2014. According to the author, these species can be consumed raw or cooked, in various types of culinary preparation, such as fresh, cooked or even mixed in homebread recipes once they present large leaf biomass.

Consumption of unconventional food plants

Most species can be consumed "in natura" (Kinupp and De Barros, 2008; Kinupp and Lorenzi, 2014), however some authors suggest preparing species before consumption to reduce antinutritional effects, mainly because cooking can reduce the percentage of phytates and tannins, substances that can impair nutrient absorption (Lola, 2009; Pompeu et al., 2014). Even with a rich nutritional profile, some species can still contain substances that are harmful to the animal organism. The genus *Erechtites*, for example, may have pyrrolizidine alkaloids known to be hepatotoxic (Knupp et al., 2016). Even so, *E. valerianifolius* and *E. hieraciifolius* are used in human food (Abdul et al., 2015). An extensive study carried out by the IBGE (1980) also shows that these species are consumed as leafy vegetables, especially its younger leaves, raw or cooked (Tanaka, 1976).

Many *Alternanthera* species are considered edible (Deladino et al., 2017). The species *A. tenella* has a nutritional profile that justifies its inclusion in our diet (Patil and Kore, 2015). In addition, the species is robust with high leaf biomass. From the same family, *A. deflexus* presents in its leaves a higher content of vitamin C compared to the content of this vitamin in the leaves of *T. paniculatum* and *Ipomoea batatas* (L.) Lam (Moura et al., 2020).

B. pilosa is a widely consumed plant, its young or mature leaves can be consumed as a vegetable with high antioxidant power, and are even sold in local markets in different countries (Manzanero-Medina et al., 2020). A recent study claims that their ingestion by rats and chickens caused no side effects, which may indicate that ingestion by humans may be similarly safe (Liang et al., 2020).

E. fosbergii is a species of great interest by the pharmaceutical and food industry, due to its health benefits, in line with its anti-inflammatory properties, in addition to antioxidants (Peisino et al., 2020). In addition to the leaves, its flowers can also be consumed (Kinupp and De Barros, 2014). Although, as same as *Erechtites* genus, the plant present pyrrolizidine alkaloids, secondary metabolite considered toxic for us (Freitas, 2020).

S. oleraceus is another widely used unconventional vegetable species, being one of the most used plants in the Mediterranean diet (Sánchez-Mata and Tardío, 2016). According to European regulations, the species can be classified as a "fiber source" as it presents 3 g of fiber per 100 g of leaves, in addition the species is a good source of lutein and vitamin A (Panfili et al., 2020).

P. australis is a known medicinal plant, but its use is also present in food. Both its leaves and seeds can be consumed (Terra and Viera, 2019). In this species, there is a high content of copper in the leaves (Kinupp and De Barros, 2008). However, studies show that species of the *Plantago* genus can be bioindicators of environmental pollution, as they can accumulate toxic metals (such as aluminum) in their tissues. Therefore, for safe consumption, the growing environment must be adequate and safe (Tinkov et al., 2016).

The fruits of *S. americanum,* known in Brazil as "maria pretinha" are consumed when ripe, a source of vitamins and antioxidants (Yuan et al., 2018). However, its leaves can also be consumed, showing itself as a promising leaf vegetable, mainly due to its iron and protein content (Kinupp and De Barros, 2008; Cáceres and Cruz, 2019).

From the Brassicaceae family, *R. raphanistrum* is considered a wild radish that has very tender leaves and large leaf biomass. The consumption of its leaves is common in salads or stir-fries, presenting a slight spicy taste. Still, all parts of the plant can be consumed in different ways (Iyda et al., 2019).

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

From the species *T. paniculatum*, leaves, stems and shoots can be used (Moura et al., 2020; Menezes et al., 2021). It is a plant rich in potassium (K), sodium (Na), magnesium (Mg). According to the National Health Surveillance Agency (Brazil), the Dietary Reference Intakes (DRI) recommends the intake of 1 g of calcium per day (BRASIL, 2005). Thus, when consuming 100 g of the edible parts of this plant (1.06 g of calcium), the individual reaches the DRI. The same is true for the consumption of *A. deflexus* (1.13 g of calcium). Therefore, these species are seen as a good source of calcium (Moura et al., 2020).

Biochemical analyses

Water Content (% WC), Total Reducing Sugars (% TRS) and Total Protein (% N)

The percentage of Water Content (% WC), Total Reducing Sugars (% TRS) and protein, expressed as a percentage of nitrogen (%N) are shown in Table 3. The analysis of variance and the Scott-Knott test at a 5% significance level showed significant differences among the samples. While all % WC means were statistically distinct, the % TRS and % N contents exhibited groupings with some statistically similar means, highlighting patterns of homogeneity among certain species.

The % WC ranged from 78.16% in *S. americanum* to 90.36% in *E. valerianifolius* leaves, with the remaining nine samples displaying distinct intermediate values. The average water content across all samples was approximately 83%. This marked variability in water content highlights the physiological and structural differences among the sampled species, reflecting their specific adaptations to environmental conditions or inherent anatomical traits. The relatively lower water content observed in *S. americanum* may be associated with its thinner, more delicate leaf structure, which potentially facilitates faster water loss under certain environmental conditions. Conversely, the higher water content in *E. valerianifolius* likely indicates greater succulence or tissue capacity to retain moisture.

The wide range of water content observed also suggests a potential influence of ecological or agronomic factors, such as soil water availability, microclimatic conditions, or growth stage at the time of collection. Understanding this variability is crucial for applications involving these plants, including post-harvest management and their potential uses in food or other industries, where moisture content directly impacts quality and processing.

Table 3 - Water Content (% WC), Total Reducing Sugars (% TRS) and Total Proteins (% N) of unconventional food plants collected during spring in the agroforestry system.

Species	% WC	% N	% TRS
Alternanthera tenella Colla	79.78 j	25.71 c	5.33 b
Amaranthus deflexus L.	82.82 g	43.54 a	3.69 c
Erechtites valerianifolius (Link ex Spreng.) DC.	90.36 a	28.60 c	8.57 a
Bidens pilosa L.	86.92 c	33.78 b	8.11 a
Emilia fosbergii Nicolson.	84.27 e	23.56 d	8.55 a
Erechtites hieraciifolius (L.) Raf. ex DC.	81.21 i	25.20 c	6.08 b
Sonchus oleraceus L.	83.41 f	29.96 c	8.76 a
Raphanus raphanistrum L.	82.56 h	37.52 b	4.53 c
Plantago australis Lam.	84.93 d	18.90 e	4.25 c
Solanum americanum Mill.	78.16 k	39.76 a	7.71 a
Talinum paniculatum (Jacq.) Gaertn.	88.67 b	27.25 c	7.07 a

Means followed by the same letter in the columns do not differ statistically according to Scott-Knott test ($p < 0.05$)

The percentage of total reducing sugars (TRS) varied significantly among the analyzed species, with some forming statistically similar groups. TRS values ranged from 8.76% in *S. oleracea* to 3.69% in *A. deflexus*, with the highest levels observed in *E. valerianifolius* (8.57%), *E. fosbergii* (8.55%),

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

and *B. pilosa* (8.11%). This grouping suggests that certain species share similar metabolic profiles, potentially influenced by genetic traits or environmental factors. The elevated carbohydrate content in these species highlights their potential as a source of readily available energy, emphasizing their nutritional value and broader applications.

Carbohydrates are the most abundant macronutrients in fruits and vegetables, and their concentration significantly influences the physical, chemical, and sensory characteristics of vegetables, such as flavor, aroma, and texture, due to their sweet taste. According to Chitarra and Chitarra (2005), the average concentration of simple sugars in vegetables typically ranges between 2% and 5%. However, the data show that unconventional vegetables can serve as excellent energy sources, with many species exceeding this range. Notably, species within the Asteraceae family, such as *E. fosbergii* (8.72%), *E. hieraciifolius* (8.71%), *S. oleraceus* (8.60%), and *B. pilosa* (8.56%), exhibited particularly high TRS percentages. In contrast, species such as *R. raphanistrum*, *P. australis*, and *A. deflexus* presented the lowest TRS percentages, with *A. deflexus* showing a minimal value of 2.98%, classifying it as a low-energy index plant.

The protein content (%N) in the leaves of these vegetables varied significantly, ranging from 18.90% in *P. australis* to 43.54% in *A. deflexus*, with species forming five statistically distinct groups (A to E). In comparison, Kinupp and De Barros (2008) reported similar protein values for *T. paniculatum* (21.85%) and *E. valerianifolius* (23%), but lower values for *B. pilosa* (21.27%), *S. americanum* (29.90%), and *P. australis* (14.50%).

Our analysis found *S. americanum* to have the second-highest protein content (39.76%), aligning with the findings of Kinupp and De Barros (2008), where this species had the highest protein content (29.90%) among those studied. This result highlights the species' potential as a protein source since most of the analyzed species demonstrated high protein content, with values comparable to or exceeding those reported in the Brazilian Food Composition Table (NEPA/UNICAMP, 2013) for conventional vegetables such as mustard (28.57%) and spinach (33.33%). These findings underscore the nutritional richness of these unconventional vegetables, offering promising alternatives for dietary protein sources and contributing to food security and nutritional diversity.

Total Flavonoid Content (TFC) and Total Phenolic Content (TPC)

TFC and TPC are show in Table 4. The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) varied significantly among the species, forming distinct groups according to the Scott-Knott test at 5% significance.

Table 4 –Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) of unconventional food plants collected during spring in the agroforestry system.

Species	TFC (g QE 100 g-^{1})	TPC (g GAE 100 g-^{1})
Alternanthera tenella Colla	0.903 b	0.007 d
Amaranthus deflexus L.	0.464 d	0.019 d
Erechtites valerianifolius (Link ex Spreng.) DC.	0.693 c	0.018 d
Bidens pilosa L.	0.314 d	0.080 b
Emilia fosbergii Nicolson.	1.048 a	0.144 a
Erechtites hieraciifolius (L.) Raf. ex DC	0.887 b	0.019 d
Sonchus oleraceus L.	0.774 c	0.034 c
Raphanus raphanistrum L.	1.071 a	0.020 d
Plantago australis Lam.	0.338 d	0.019 d
Solanum americanum Mill.	0.121 e	0.022 d
Talinum paniculatum (Jacq.) Gaertn.	0.358 d	0.043 c

Means followed by the same letter in the columns do not differ statistically according to the Scott-Knott test ($p < 0.05$)

For TFC, the values ranged from 1.071 g QE 100 g^{-1} in *R. raphanistrum* to 0.121 g QE 100 g^{-1} in *S. americanum*. *R. raphanistrum* (1.071 g QE 100 g^{-1}) and *E. fosbergii* (1.048 g QE 100 g^{-1}) exhibited the highest flavonoid levels, significantly surpassing the other species. In contrast, *S. americanum* showed the lowest TFC (0.121 g QE 100 g^{-1}), high-

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

lighting substantial differences in flavonoid biosynthesis across species.

For TPC, values ranged from 0.144 g GAE 100 g^{-1} in *E. fosbergii* to 0.007 g GAE 100 g^{-1} in *A. tenella*. *E. fosbergii* had the highest phenolic content (0.144 g GAE 100 g^{-1}), followed by *B. pilosa* (0.080 g GAE 100 g^{-1}), forming a separate statistical group. These results suggest that *E. fosbergii* is a particularly rich source of phenolic compounds, which may confer significant antioxidant properties. In contrast, *A. tenella* had the lowest TPC (0.007 g GAE 100 g^{-1}), indicating limited phenolic accumulation in this species. Notably, for some species, our study is the first to investigate their phenolic and flavonoid content.

In line with previous research, *S. oleraceus* was shown to have a high TFC, with an average content of 0.62 g QE 100 g^{-1} in our study, which aligns with the findings of Xia et al. (2011) and Petropoulos et al. (2018), who reported high flavonoid content in *S. oleraceus*. Similarly, *R. raphanistrum* presented high flavonoid values (1.071 g QE 100 g^{-1}), consistent with Shin et al. (2015) and Iyda et al. (2019), who noted high phenolic content in radishes, particularly quercetin and kaempferol.

A. tenella showed intermediate flavonoid content (0.903 g QE 100 g^{-1}), consistent with findings by Deladino et al. (2017), who identified flavones, including glucopyranosil-vitexin and vitexin, as major compounds in the species. This plant also has potential medicinal uses, such as antibacterial and antioxidant activities (Reis et al., 2015), and could be a source of natural bioactive compounds or food colorants (Sakuta, 2014).

T. paniculatum, a species with both food and medicinal uses, showed TPC similar to that reported by Menezes et al. (2021) (358 mg GAE 100 g^{-1}), supporting the idea that solvent combinations, such as methanol/water, enhance phenolic compound extraction.

There are no previous studies on the flavonoid content of *A. deflexus*, which showed 0.464 g QE 100 g^{-1} and 0.019 g GAE 100 g^{-1}. However, other *Amaranthus* species are known to be rich in phenolic compounds and flavonoids (Viana et al., 2015), and cooking methods have been shown to influence their antioxidant activity (Han and Xu, 2014; Roy et al., 2021).

P. australis demonstrated lower levels of both TPC (0.019 g GAE 100 g^{-1}) and TFC (0.338 g QE 100 g^{-1}). The high antioxidant activity of *Plantago* species is well-documented, with beneficial medicinal properties (Zhang et al., 2021; Kujawska and Luczaj, 2015), even though this species showed lower averages compared to others.

S. americanum, often cited as edible (Ray et al., 2020), showed the lowest flavonoid content (0.121 g QE 100 g^{-1}) and phenolic content (0.022 g GAE 100 g^{-1}). While most studies on *S. nigrum* focus on fruits, more research is needed on the leaves of *S. americanum* for potential consumption.

Both *Erechtites* species (*E. hieracifolius* and *E. valerianifolius*) had low TPC values (0.019 and 0.018 g GAE 100 g^{-1}, respectively), but *E. hieracifolius* had a higher TFC (0.887 g QE 100 g^{-1}) compared to *E. valerianifolius* (0.693 g QE 100 g^{-1}), indicating their potential as sources of flavonoid-rich extracts.

The content of phenolic compounds in plants is strongly linked to their antioxidant activity. Phenols, including flavonoids and other polyphenols, are well-known for their ability to scavenge free radicals and neutralize reactive oxygen species (ROS), which are often associated with oxidative stress and various diseases. These compounds possess antioxidant properties due to their hydroxyl groups, which can donate electrons to free radicals, thereby stabilizing them and preventing cellular damage.

In the context of the spontaneous species occurring in agroforestry systems, high phenolic content often correlates with higher antioxidant activity. This is because phenolic compounds, especially flavonoids, are one of the primary groups responsible for the antioxidant activity in plants (Rice-Evans et al., 1997; Larson, 1988). When plants accumulate more phenols, it typically results in a more potent ability to combat oxidative stress. As a result, these species may be particularly valuable for their potential health benefits, as they could offer protection against diseases related to oxidative damage, such as cardiovascular diseases, cancer, and neurodegenerative disorders (Kris-Etherton et al., 2002).

From these spontaneous species, we can expect a diverse range of antioxidant potentials, given the variation in phenolic content observed. Species like *R. raphanistrum* and *E. fosbergii*, with their high phenolic content, are particularly promising for antioxidant applications in functional foods, dietary supplements, or even pharmaceutical products. On the other hand, species with lower phenolic content may still contribute valuable antioxidants, but their use may be more complementary, supporting the idea that agroforestry systems can offer a spectrum of plant-based antioxidants for various applications.

Overall, despite being considered weeds, many of the species studied represent valuable food sources. These plants have both food and medicinal potential, with bioactive compounds that may promote health benefits. Further research into the nutritional aspects, processing methods, and functional uses of these species is necessary to enhance

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v13n3.20316

their potential as functional foods and encourage their consumption.

CONCLUSIONS

With the survey of spontaneous species carried out inside an agroforestry system, we noticed that among the various species that can be considered weed, we can find valuable resources as food. The agroforestry system has a great diversity of spontaneous herbaceous species and many are edible plants presenting bioactive compounds with good benefits for health. Furthermore, the species have different types of culinary preparations reported in the literature.

Further studies may encourage more consumption of these plants since they can promote a more diversified diet. In addition, the cultivation/management of agroforestry system can promote access to these resources, popularizing access to them, corroborating to the rescue and multiplication of this species.

ACKNOWLEDGMENTS

Aknowledgements to Professor Giuseppina Pace Pereira Lima for supervising the biochemical analysis. The authors thanks to the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) for the financial support and scholarship.

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