**Fernanda Anselmo Moreira**

# Avaliação sazonal do potencial biológico de extratos de *Merostachys neesii* Rupr. (Poaceae: Bambusoideae)


# Seasonality of the biological potential of extracts of *Merostachys neesii* Rupr. (Poaceae: Bambusoideae)

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências Biológicas, na Área de Botânica.

Orientadora: Prof[a] Dr[a] Cláudia Maria Furlan

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*“Dedico esse trabalho a minha amada mãe, Lidia”*

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Summary

# Introduction: an overview of bamboos

## Bambusoideae: taxonomic and biological aspects, distribution, and importance

Poaceae, popularly known as grasses, is a large family of monocots, which is located in the clade of the Commelinids and belongs to Poales (Byng et al. 2016). It is a monophyletic family with 12 recognized subfamilies, equally monophyletic: Anomochlooideae, Aristidoideae, Arundinoideae, Bambusoideae, Chloridoideae, Danthonioideae, Micrairoideae, Oryzoideae, Panicoideae, Pharoideae, Puelioideae, and Pooideae (GPWG II 2012; Soreng et al. 2015, 2017).

Subfamilies Anomochlooideae, Pharoideae, and Puelioideae are basal lineages within Poaceae, while the others are distributed in two clades, known by the acronyms BOP and PACMAD. Bambusoideae, Oryzoideae, and Pooideae are included in the BOP clade, whereas Panicoideae, Arundinoideae, Chlorodoideae, Micrairoideae, Aristidoideae, and Danthonoideae are in the PACMAD clade. Both clades are responsible for most of the Poaceae diversity (GPWG II 2012; Jones et al. 2014; Soreng et al. 2015, 2017). A huge number of grasses species (more than 4,500 species) show the $C_4$ photosynthetic carbon fixation. PACMAD clade includes plants with only the Calvin-Benson cycle ($C_3$) or $C_4$ metabolism, while BOP clade and basal lineages exhibit only the $C_3$ metabolism (Bouchenak-Khelladi et al. 2010; GPWG II 2012).

Poaceae is among the botanical families with the largest number of species (Hodkinson 2018). It is estimated that there are approximately 11,506 species distributed into 768 genera (Soreng et al. 2017). In Brazil, it was registered 1,493 species of Poaceae (about 13%) and 225 genera distributed throughout its territory (Poaceae in Flora do Brasil 2020a).

This family has a cosmopolitan distribution and can be found in a wide variety of terrestrial habitats (Linder et al. 2018), being responsible for approximately 40% of the planet's vegetation cover (Gibson et al. 2009). Economically, Poaceae has a huge economic value, since this family comprises some of the greatest global importance crops, as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.). Besides, other grasses stand out in the economic sector for their relevance as biofuels, such as sugarcane (*Saccharum officinarum* L.); as building materials (bamboos); and as forage species and weeds (Hodkinson 2018). Some grasses are also used in traditional medicine, including lemongrass (*Cymbopogon citratus* (DC.) Stapf) (Avoseh et al. 2015; Ekpenyong et al. 2015), barley (*Hordeum vulgare* L.), maize, oat (*Avena sativa* L.), rice, sugarcane, and wheat (Di Stasi et al. 2002; Said et al. 2002; Benítez et al. 2010; Alzweiri et al. 2011; Cakilcioglu et al. 2011; Hajdu and Hohmann 2012).

Bambusoideae Luerss. is the subfamily of Poaceae comprising bamboos. This subfamily, as mentioned earlier, is inserted in the BOP clade, being Pooideae its sister-group and Oryzoideae the sister-group of the clade formed by Bambusoideae plus Pooideae (Sungkaew et al. 2009). Some

Bambusoideae's synapomorphies are the loss of the first leaf in the seedlings, leaf mesophyll with strongly asymmetrically invaginated arm cells, and presence of relatively broad pseudopetiolate leaves, normally with fusoid cells flanking the vascular bundles (GPWG 2001; Clark et al. 2015). Bamboos exhibit a wide geographical distribution containing representatives in all continents, except Europe and Antarctica, as shown in figure 1. They are found in latitudes from 46°N to 47°S and at altitudes ranging from sea level to 4,300 meters high (Judziewicz et al. 1999; BPG 2012; Clark et al. 2015). They occupy a range of habitats, mainly tropical and temperate forest areas (GPWG 2001; Clark et al. 2015). Indeed, bamboos constitute the only group of plants in Poaceae that have diversified in this type of environment (BPG 2012).

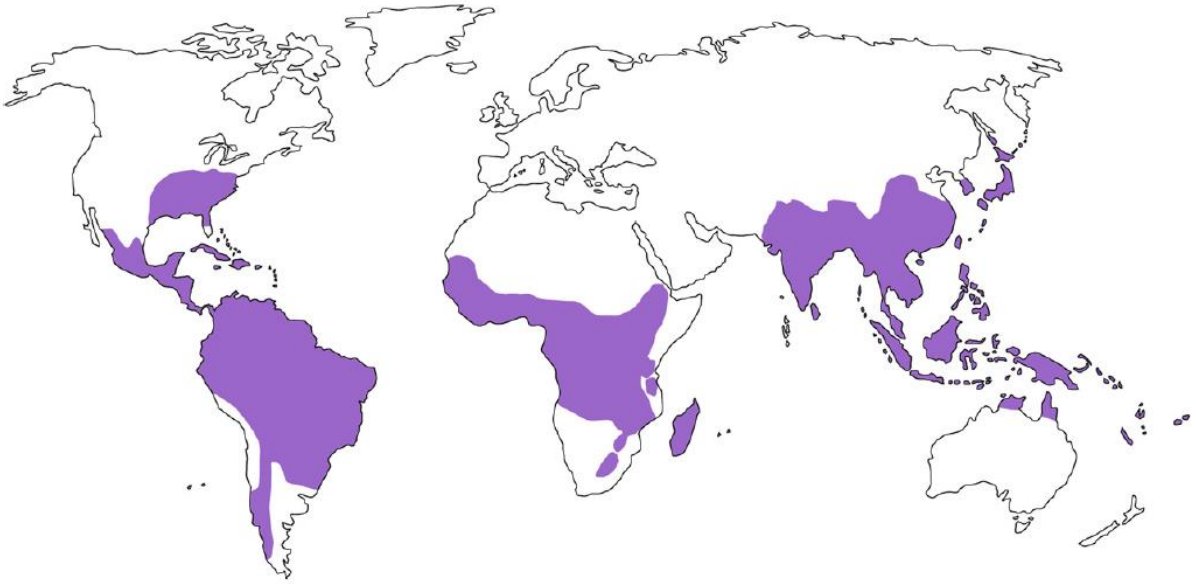

**Figure 1:** Distribution of Bambusoideae in the world (Kelchner and BPG 2013).

Bamboos have two distinct morphological habits: woody and herbaceous. Woody bamboos have a well-developed rhizome system, strongly lignified culms, complex vegetative branching, culm leaves protecting the young shoot, foliage leaves containing an outer ligule, bisexual spikelets, and normally exhibit gregarious monocarpic flowering (Judziewicz et al. 1999; BPG 2012; Clark et al. 2015). This type of flowering is characterized by the simultaneous blossom of individuals of the same population, at relatively regular intervals of time, followed by their perishing. In other words, they present only one flowering event in their life cycle (Judziewicz et al. 1999). These flowering cycles can range from 7 to 120 years. Therefore, bamboos are found more frequently in their vegetative stage (Kelchner and BPG 2013).

Herbaceous bamboos, on the other hand, present poorly lignified culms, simple vegetative branching, unisexual spikelets, usually do not exhibit culm leaves and outer ligule and have a

seasonal flowering pattern. Moreover, these bamboos also have crenate (olyroid) silica bodies, except for *Buergersiochloa* Pilg. (Judziewicz et al. 1999; BPG 2012; Clark et al. 2015).

Bamboos, mainly woody bamboos, constitute an important plant element in the composition of many tropical and temperate forests (Judziewicz et al. 1999). They provide food and shelter for a wide variety of animal groups (invertebrates, amphibians, reptiles, birds, and mammals), some of which are of interest for conservation (Bystriakova et al. 2004; Song et al. 2011; Campos 2016). Besides serving as shelter and food resources, these plants can also afford several other ecological benefits. For instance, well-developed rhizome, which help to stabilize the soil around it; dense foliage protecting the soil by the rainfall interception; significant leaf litter deposition; and thick culms are some features present in this group of plants that help to prevent soil erosion and landslides and to protect river banks and water sources (Bystriakova et al. 2004; Ben-Zhi et al. 2005; Song et al. 2011). They also play a major role in the rehabilitation of degraded land and in carbon sequestration (Ben-Zhi et al. 2005; Nath et al. 2009; Song et al. 2011; Wang et al. 2013). Another relevant ecological aspect regarding bamboos is that they may influence the forest structure and dynamic (Bystriakova et al. 2004). Lima et al. (2012) reported that bamboo-dominated environments have lower species density of seedlings, saplings, and adult plants and higher seedlings and saplings recruitment, adult trees mortality, and small-sized trees prevalence, demonstrating that environments with bamboo overabundance are more dynamic than those not dominated by bamboos. Massive flowering followed by the die-off of woody bamboos alters environmental conditions and recruitment in forests as well (Budke et al. 2010).

Concerning the socioeconomic viewpoint, bamboos are also extremely relevant. Since ancient times, humans have used these multipurpose plants. Nowadays, the marketing of bamboos and their products has become an important source of income, especially in poor communities, contributing to their development and poverty reduction (Filgueiras and Gonçalves 2004, Yuming et al. 2004, Song et al. 2011; Mukul and Rana 2013). Hundred of uses are attributed to bamboos (Judziewicz et al. 1999; Chongtham et al. 2011), such as biofuel, construction of houses, bridges and furniture (Vogtländer et al. 2010), household utensils, handicrafts (Mukul and Rana 2013), musical instruments, papermaking, ornamental purposes, as well as being widely used as food resources for domestic animals (Judziewicz et al. 1999) and human (Nongdam and Tikendra 2014), and for medicinal purposes (Yuming et al. 2004; Choudhury et al. 2012). Several species have economic value and bamboos have been considered a potential substitute for timber (Song et al. 2011).

It is estimated that there are 1,670 species of bamboos and 125 genera (Soreng et al. 2017). According to molecular data, these genera are distributed in three tribes: Arundinarieae, represented by woody bamboos of temperate climate; Bambuseae, covering woody bamboos of tropical climate;

and Olyreae, contemplating the herbaceous bamboos (Sungkaew et al. 2009; Kelchner and BPG 2013; Soreng et al. 2015, 2017). These tribes are responsible for approximately 35%, 58%, and 7% of bamboo species, respectively (Soreng et al. 2017). Regarding Bambuseae, it is possible to observe two distinct lineages: Neotropical and Paleotropical woody bamboos (Kelchner and BPG 2013; Wysocki et al. 2015; Soreng et al. 2015, 2017).

Molecular data revealed that Olyreae is a sister-group of Bambuseae (tropical woody bamboos), while Arundinarieae (temperate woody bamboos) is a sister-group of the clade formed by Olyreae plus Bambuseae; therefore, the woody habit is paraphyletic. To date, it is not known for sure whether the woody habit appeared twice independently in Bambusoideae or appeared only once in a common ancestor of this subfamily and, at some point in the evolutionary history of the bamboos, this habit was lost in Olyreae (Sungkaew et al. 2009; Wysocki et al. 2015).

Bamboos belonging to Arundinarieae can be found in temperate regions, both in the New and Old World, presenting a relatively Laurasian distribution pattern. Bambuseae occurs in the tropics, being the Paleotropical woody bamboos situated in the tropical areas of the Old World, while the Neotropical woody bamboos are distributed in the New World. Olyreae, on the other hand, is restricted to the New World, with few exceptions outside this scope (Wysocki et al. 2015).

In Brazil, 34 genera and approximately 259 native species have been reported, of which 175 are endemic. These data represent about 16% of the world's bamboo diversity. Bambusoideae can be found in all regions of the Brazilian territory (Poaceae in Flora do Brasil 2020b), being Atlantic Rain Forest undoubtedly the Brazilian biome with the greatest bamboo richness, followed by the Amazon region and the Brazilian Savannah (Cerrado) (Filgueiras and Gonçalves 2004; Greco et al. 2015).

Species from the three tribes can be found in Brazil, being Bambuseae the most representative, 24 genera and 187 species (166 natives and 129 endemics). Arundinarieae, in Brazil, is represented only by cultivated species (Poaceae in Flora do Brasil 2020b). Regarding Olyreae, Brazil constitutes the center of diversity of this tribe, especially the Atlantic Rain Forest, from the Southern Bahia to the Northern Espírito Santo (Longhi-Wagner 2012). These data show that Brazil has a great diversity of bamboos, the largest in the New World (Filgueiras and Gonçalves 2004). However, further studies on the diversity of Bambusoideae, especially in the Atlantic Rain Forest and Amazonia, are needed for a better knowledge of bamboo diversity in Brazil, as in some regions this group has been poorly investigated (Longhi-Wagner 2012).

**Aims of the present study**

Brazil has the largest bamboo diversity in the New World, but phytochemical and pharmacological studies of native species are scarce. In addition, bamboos constitute an important

plant resource that, among other applications, has been used in traditional Chinese medicine for centuries.

Furthermore, Brazil is an important diversity center for many bamboo genera, among them, *Merostachys* Spreng. *Merostachys* is one of the Neotropical woody bamboo genera (Bambuseae) popularly known as *taquara*. Some vegetative characteristics of this genus are the presence of short pachymorph rhizomes forming clumps; scandent habit; usually it has hollow culms (Judziewicz et al. 1999); branch complement containing 3 to 340 sub-branches (Shirasuna and Filgueiras 2013). As in other woody bamboo genera, it presents a gregarious monocarpic life cycle (Judziewicz et al. 1999). The interval between blooms is not yet clear for this genus, but for some species, it varies between 30 to 34 years (Sendulsky 1995; Guerreiro 2014).

*Merostachys* comprises 53 species (Ruiz-Sanchez et al. 2018), with distribution in Southern Mexico, Central and South America. Species of *Merostachys* occur at the borders of and inside forests at altitudes ranging from sea level up to 1,500 meters (Judziewicz et al. 1999). In Brazil, there are 46 species described, being 44 endemics. They are distributed in all regions of the country, being found at Cerrado, Amazon, and Atlantic Rain Forest (*Merostachys* in Flora do Brasil 2020a). The species of Brazilian occurrence are still very poorly studied, constituting a field of research to be explored in several areas (Shirasuna and Filgueiras 2013).

*Merostachys neesii* Rupr. is an endemic species occurring in the Atlantic Rain Forest in the South and Southeast regions of the country (*Merostachys* in Flora do Brasil 2020b). Some relevant vegetative characteristics for the identification of this species are: branches with 2 to 3 black and swollen nodes; presence of 4 to 10 leaves per branch; absence of apical fimbriae in culm and branch sheaths, a rare feature in the genus (da Mota et al. 2009); culms with 10-20 m in height and 10-35 mm in diameter; branch complement with 11 to 50 branches; and glabrous culm leaf with brown and shiny adaxial face and brown to vinaceous and opaque abaxial face. Its life cycle is around 30 to 33 years (Shirasuna and Filgueiras 2013). Figure 2 shows some of the vegetative characteristics of *M. neesii* mentioned above.

This species is popularly known as *taquara-lisa* or *taquara-poca* (Gavilanes et al. 1992). Until recently, it was in the list of presumably extinct plants of the São Paulo' flora. However, in recent studies, the presence of a large population of *M. neesii* was verified in the Serra do Mar State Park in Santa Virgínia, SP (Filgueiras and Shirasuna 2009) and in the PEFI (Fontes do Ipiranga State Park, São Paulo, SP) where it was found in 52 points. In this last location, most individuals were fertile in 2012 (Shirasuna and Filgueiras 2013).


**Figure 2:** Details of some vegetative characters of *Merostachys neesii* Rupr. A- Branch complement; B- Detail of the node; C- Culm leaf; and D- Foliage leaves.

The chemical importance of bamboos and its therapeutic and food uses in Asia, and the absence of phytochemical, nutritional, and pharmacological studies of *M. neesii*, an endemic Brazilian species, were important for the species selection. *M. neesii* was chosen to carry out this work, contributing to the phytochemical knowledge of the Brazilian native flora. Furthermore, this study aimed to help in the search for new sources of bioactive compounds and to understand how seasonality can affect secondary metabolites production.

Our initial premises were: (1) Asian bamboos have been used for millennia in folk medicine and as food resource; (2) Asian bamboos are a good source of important nutritional components; (3) phenolic compounds are the main class of secondary metabolites reported for Asian bamboos; and (4) this class of secondary metabolites may have its quantities affected by the seasonal variations of the environment. Therefore, our hypotheses are:

1- Brazilian native bamboos, like the Asian bamboos, have medicinal and nutritional potential;

2- Seasonality influences the phenolic contents, especially flavonoids, of *M. neesii*, which will result in differences in the bioactivity of its extracts.

*M. neesii* was collected (authorization number CNPq 010006/2015-0) in the PEFI with the assistance of the Ph.D. Maria Tereza Grombone-Guaratini, researcher of the Institute of Botany of São Paulo (IBt-SP). A voucher specimen (Furlan70) was deposited at SPF Herbarium of the University of São Paulo (São Paulo, Brazil). Leaves and culms (culms plus branches) of four *M. neesii* clumps were collected in the dry (August/2016, n=3; and August/2017, n=4) and rainy (March/2017, n=3; and February/2018, n=4) seasons; each clump represents one replica. Figure 3 shows the location of PEFI as well as the harvest sites of the four clumps (**C1**-**C4**): **C1**:

23°38’33.09” S - 46°37’30.82” W; **C2**: 23°38’31.36” S - 46°37’16.68” W; **C3**: 23°38’32.09” S - 46°37’16.16” W; and **C4**: 23°38’31” S - 46°37’29” W.

PEFI is located in Southeast region of the municipality of São Paulo (23°38’08” - 23°40’18” S and 46°36’48” - 46°38’00” W). It is currently a *sui generis* Conservation Unit. Its vegetation is characterized as belonging to the Atlantic Rain Forest, with the predominant occurrence of typical species of Ombrophilous Dense Forest in the regeneration stage (Shirasuna and Filgueiras 2013). According to Shirasuna and Filgueiras (2013), nine species of *Merostachys* occur in the PEFI, and in 2012 most of the culms of *M. neesii* were fertile. Plant material was harvested in a population of *M. neesii* that, after the flowering event (2012), perished and regenerated in 2015.

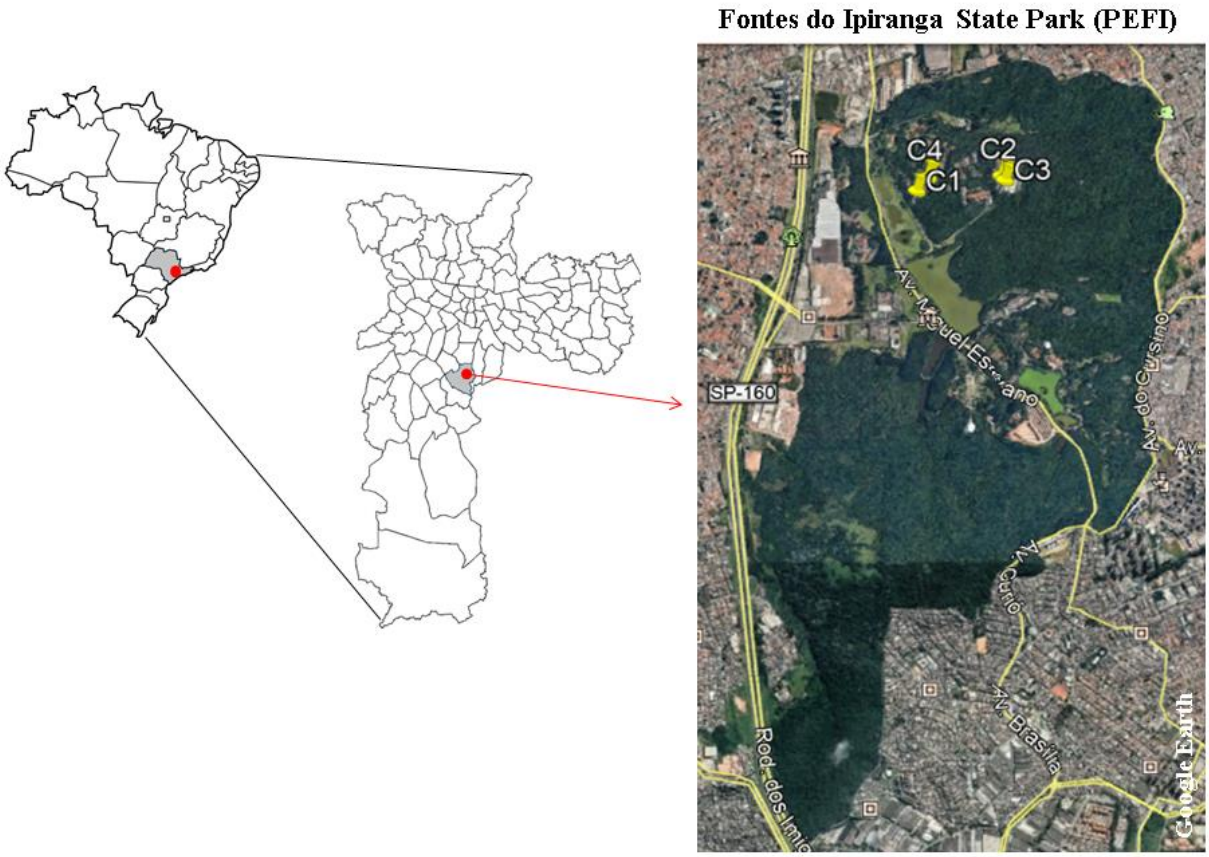


**Figure 3:** Location of the Fontes do Ipiranga State Park (PEFI), São Paulo/SP. Yellow signs indicate the harvesting points (C1-C4) of *Merostachys neesii*.

The objectives of this study were:

- Characterize and quantify the major nutritional components of leaves and culms of *M. neesii*;
- Characterize and quantify the major flavonoids of leaves and culms of *M. neesii*;
- Analyze the antioxidant and anticholinesterase potential of these extracts;
- Evaluate the seasonal influence on the composition and contents of nutrients and flavonoids

of *M. neesii* extracts;

- Evaluate the seasonal influence on antioxidant and anticholinesterase potential of leaves and culms extracts of *M. neesii*.

This thesis is divided into three chapters as follow:

- Chapter 1 – Major constituents of *Merostachys neesii*: a Brazilian endemic bamboo species.
- Chapter 2 – Nutritional value of *Merostachys neesii*: seasonal implications.
- Chapter 3 – Biological potential of *Merostachys neesii*: seasonal implications.

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# Chapter 1 – Major constituents of *Merostachys neesii*: a Brazilian endemic bamboo species

## 1. Introduction

Bambusoideae comprises more than 1,600 bamboo species divided into 125 genera (Soreng et al. 2017) distributed through tropical and temperate forests (Clark et al. 2015). Bamboo has an enormous range of uses in Asia, where it has been used for food and medicinal purposes for millennia (Choudhury et al. 2012; Nongdam and Tikendra 2014).

Bamboo shoots are appreciated in Asian cuisine and have been considered as a health beneficial food due to their nutritional composition (presence of various amino acids, vitamins and minerals, high fiber content and low-fat level) and the presence of bioactive compounds (phytosterols and phenolic compounds) (Chongtham et al. 2011). Bamboo shoots are also used to improve digestion, prevent and cure cancer and cardiovascular problems, and for the treatment of jaundice (Kumar et al. 2017). Additionally, leaves are used for treating fever, cough, leprosy, lung inflammation, osteoporosis, and osteoarthritis (Sangeetha et al. 2015).

Phenolic compounds are the main constituents described for bamboo species. For example, phenolic acids as chlorogenic, *p*-coumaric, caffeic, ferulic, and gallic acids (Gong et al. 2015; Goyal et al. 2017; Pande et al. 2018), coumarins, such as scopoletin, scopolin, and umbelliferone (Wang et al. 2013), and flavonoids, as vitexin, isovitexin, orientin, isoorientin, and tricin (Lv et al. 2011; Van Hoyweghen et al. 2012; Tanaka et al. 2014). Furthermore, triterpenes have also been reported in bamboos (friedelin and lupenone) (Jiao et al. 2007; Choi et al. 2013). All these compounds exhibit pharmacological activity, demonstrating the medicinal potential of bamboos.

Brazil has one of the largest bamboo diversity in the world. There is the occurrence of about 259 native species (several endemic) and 34 genera in Brazil (Poaceae in Flora do Brasil 2020), being behind China (Das et al. 2008; Canavan et al. 2017) in the number of species. Also, Brazil is the diversity center for some genera, among them *Merostachys* Spreng. *Merostachys* comprises 53 species (Ruiz-Sanchez et al. 2018); 46 of them occur in Brazil, and 44 are endemic (*Merostachys* in Flora do Brasil 2020).

Although bamboos have ancient medicinal importance and there are reports of different groups of nutrients and bioactive compounds in these plants, few species have been chemically studied more broadly, that is, besides their phenolic composition. Studies of chemical characterization using native bamboo species are even scarcer. Thus, this study aimed to characterize the major constituents of leaves and culms of *Merostachys neesii* Rupr., an endemic species of the Atlantic Rain Forest, and contribute to the chemical knowledge of Brazilian biodiversity.

## 2. Material and Methods

### 2.1 Plant material

Leaves and culms (culms plus branches) of *M. neesii* were harvested at the Fontes do Ipiranga State Park (PEFI), located in São Paulo/Brazil. Plant material was identified by Ph.D. Maria Tereza Grombone-Guaratini, researcher of the Institute of Botany of São Paulo (IBt-SP), and a voucher specimen (Furlan70) was deposited at SPF Herbarium of the University of São Paulo (São Paulo, Brazil).

### 2.2 Extraction procedures

Plant material was frozen, freeze-dried, powdered, and submitted to three extraction processes: serial maceration, infusion, and hot methanol followed by a partition with water and chloroform. Serial maceration (70 g $L^{-1}$) was carried out using hexane and 70% ethanol for 21 days each, with solvent exchanges every two days, at room temperature, and constant stirring. Infusion (8 g $L^{-1}$) was performed for 20 minutes. Hexane, hydroethanol, and aqueous extracts of leaves (**HE-L**, **EE-L**, and **AE-L**) and culms (**HE-C**, **EE-C**, and **AE-C**) were concentrated under reduced pressure at 45ºC using a rotary evaporator, freeze-dried, and kept at room temperature until analysis.

Hot methanol extraction (**HME**) was performed following the procedures of Lisec et al. (2006), with modifications. Fifty milligrams of freeze-dried, milled plant material were extracted using 1,400 µL of pre-cooled methanol (-20°C). Also, 60 µL of ribitol (0.2 mg $mL^{-1}$) were added (the internal standard used in sugar quantification - see chapter 2). The material was briefly stirred and incubated at 70°C and 950 rpm for 10 min. Afterward, the extract was centrifuged (10 min at 11,000 *g*), the supernatant was transferred to glass tubes, and 1,500 µL of ultrapure water (at 4°C) and 750 µL chloroform (at -20°C) were added. After stirring, the material was centrifuged again, the polar phases of leaves (**HME-L**) and culms (**HME-C**) were collected, 100 µL were transferred to inserts and dried under vacuum.

Hexane extracts (nonpolar constituents analysis) and polar phase of hot methanol extracts (primary metabolites analysis, especially) were analyzed by gas chromatography coupled to electron impact mass spectrometry (GC-EIMS) while hydroethanol and aqueous extracts (flavonoids analysis) by high performance liquid chromatography coupled to electrospray ionization high-resolution mass spectrometry (HPLC-ESI/HRMS) and high performance liquid chromatography coupled to diode array detector (HPLC-DAD). Figure 1.1 shows a flowchart with all extracts obtained from *M. neesii*.

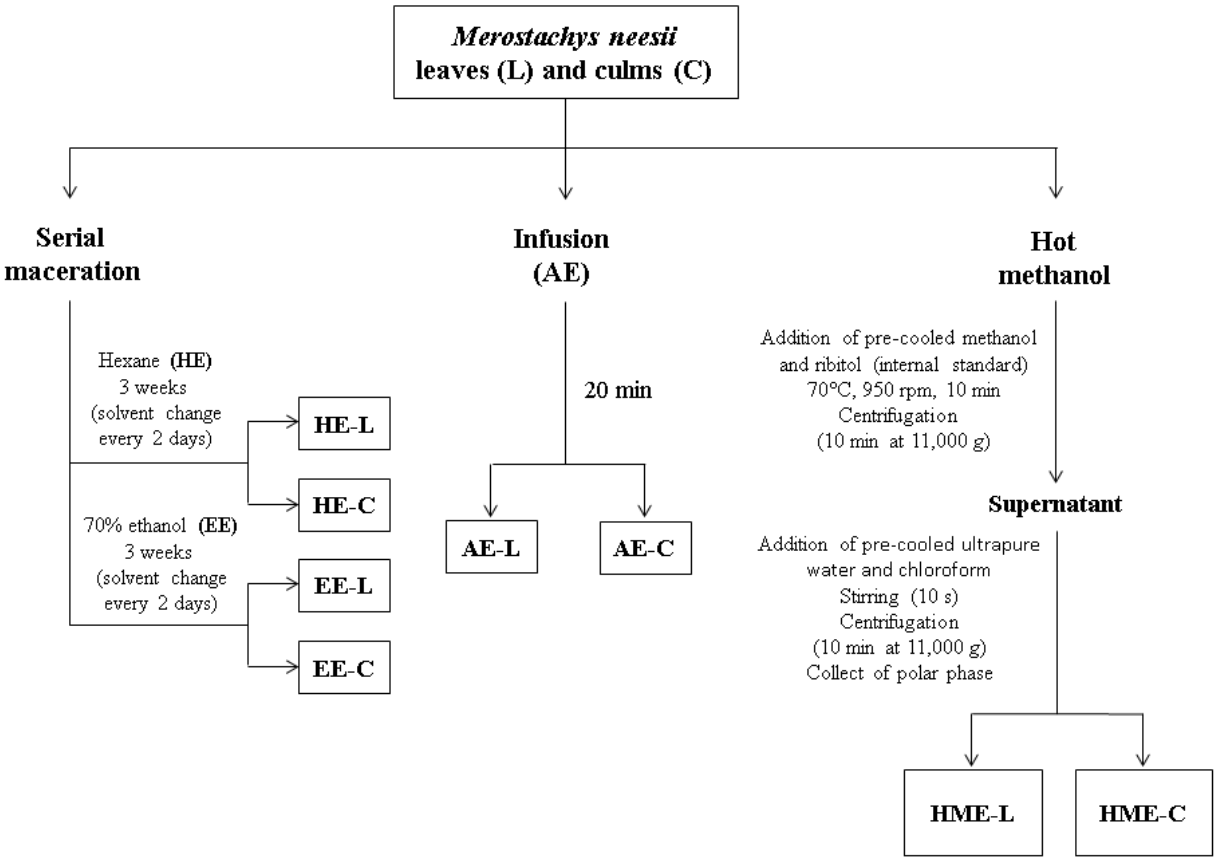



**Figure 1.1:** Flowchart of extraction processes of leaves and culms from *Merostachys neesii* Rupr.

## 2.3 Chemical analyzes

### 2.3.1 Analysis by GC-EIMS

**- Polar compounds analysis**

For the analysis of polar compounds (mainly monosaccharides, disaccharides, sugar alcohols, amino acids, and organic acids), polar phases of hot methanol extract were analyzed by GC-EIMS according to Nagai (2017). Polar phase aliquots, previously transferred to inserts and dried under vacuum, were derivatized using a two-step procedure: methoxymation and trimethylsilylation. Methoximation was performed to prevent the ring formation of sugars. During this process, the sugar is reduced and the carbonyl group in the beta-position is stabilized, forming two stereoisomers. On the other hand, trimethylsilylation enables greater volatilization of polar compounds, resulting in the formation of trimethylsilyl (TMS) derivatives (Roessner et al. 2000). Thus, samples were dissolved in 28 µL of methoxyamine hydrochloride (Sigma-Aldrich) in pyridine (20 mg $mL^{-1}$) and heated at 37°C for 2h. After methoxymation, the derivatives were trimethylsilylated by adding 48 µL of MSTFA (*N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide, Sigma-Aldrich) and heating the mixture for 30 min at 37°C (Nagai 2017).

The constituents present in the polar phase were analyzed by GC-EIMS (6850 Network GC System-Agilent/Agilent 5975 VL MSD) equipped with a VF-5ms column (Agilent, length 30 m, ID 250 μm, 0.25 μm film thickness) and a pre-column (0.25 mm x 10 m), with helium as carrier gas at a flow rate of 1 mL $min^{-1}$ and 1 μL of injection volume (splitless mode). The initial oven temperature was adjusted to 70ºC for 5 min, increasing 5ºC $min^{-1}$ to a final temperature of 295ºC. The injector, quadrupole, and ion source temperatures were of 230ºC, 150ºC, and 200ºC, respectively. MS detection was carried out with electron ionization (EI) at 70 eV, operating in the full-scan acquisition mode ranging between 50-600 *m/z* at 2.66 scan $s^{-1}$ (Nagai 2017).

Compound identification was carried out by comparing its retention time, Linear Retention Index (LRI), and mass fragmentation pattern to commercial standards and literature data. For each compound, LRI was determined using an *n*-alkane standard mixture (C8-C20 from Sigma-Aldrich and C21-C28, except the C27 homologue, from PolyScience) and calculated according to Viegas and Bassoli (2007) using the following equation:

$$LRI = 100 * \left(\frac{t_c - t_n}{t_{n+1} - t_n} + n\right)$$

Where $t_c$, retention time of compound; $t_n$, retention time of previous *n*-alkane; $t_{n+1}$, retention time of posterior *n*-alkane; and n, carbon number of the previous *n*-alkane.

Regarding mass fragmentation pattern, experimental data were compared with NIST digital library spectra (v2.0, 2008), European Mass Bank database (https://massbank.eu/MassBank/Index), Golm Metabolome Database (GMD) (http://gmd.mpimp-golm.mpg.de/), Scifinder, NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/), and literature. When the Match value provided by the NIST library was above 800 and it was not possible to compare LRI and mass fragmentation patterns with data in different databases, the first hit suggestion was accepted. Match value is an indication of similarity between the unknown spectrum (*i.e.*, experimental data) and the known spectrum of the library. Match values above 900, between 800-900, from 700 to 800, and below 600 are considered excellent, good, fair, and poor, respectively (NIST 2008).

**- Nonpolar compounds analysis**

For the analysis of nonpolar compounds, hexane extracts were solubilized in hexane (1 mg $mL^{-1}$) and analyzed by GC-EIMS. One hundred microliters of hexane extract and 10 μL of nonadecane (1 mg $mL^{-1}$ in dichloromethane, the internal standard used for quantification – see chapter 2) were transferred to an insert, dried under vacuum, and derivatized. Derivatization consisted of dissolving previously dried aliquots of the hexane extract in 25 μL of pyridine and adding 25 μL of BSTFA (*N,O-bis*-(trimethylsilyl)-trifluoroacetamide, Sigma-Aldrich) – the derivatizing agent used in the formation of TMS derivatives; then, the material was heated at 70°C for 1 h. After derivatization, the constituents present in the hexane extract were analyzed by GC-

EIMS (6850 Network GC System-Agilent/Agilent 5975 VL MSD) equipped with a HP-5MS capillary column (5% phenyl, 95% polydimethylsiloxane – Agilent, length 30 m, ID 250 µm, 0.25 µm film thickness), with helium as carrier gas at a flow rate of 1 mL $min^{-1}$ and 1 µL of injection volume (split 10:1 mode). The initial oven temperature was adjusted to 150ºC for 6 min, increasing 5ºC $min^{-1}$ to a final temperature of 325ºC. The injector, quadrupole, and ion source temperatures were of 320ºC, 150ºC, and 230ºC, respectively. MS detection was carried out with electron ionization (EI) at 70 eV, operating in the full-scan acquisition mode ranging between 50-800 *m/z* at 2.66 scan $s^{-1}$ (Sala-Carvalho et al. 2019).

Compound identification was performed by comparing the LRI and mass fragmentation pattern. For each compound, LRI was determined using an *n*-alkane standard mixture (C8-C20 from Sigma-Aldrich and the alkanes C21-C26, C28, C30, C32, C36, C38, and C40 from PolyScience) and calculated according to Viegas and Bassoli (2007). Regarding mass fragmentation pattern, experimental data were compared with NIST digital library spectra (v2.0, 2008), European Mass Bank database (https://massbank.eu/MassBank/Index), Golm Metabolome Database (GMD) (http://gmd.mpimp-golm.mpg.de/), Scifinder, and NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/). When the Match value provided by the NIST library spectra was above 800 and it was not possible to compare LRI and mass fragmentation patterns with data in databases, the first hit suggestion was accepted.

### 2.3.2 Analysis of flavonoids by HPLC-ESI/HRMS and HPLC-DAD

For the analysis of flavonoids, hydroethanolic and aqueous extracts were solubilized in 80% MeOH (5 mg $mL^{-1}$), filtered (PTFE 0.45 µm), and analyzed by HPLC-ESI/HRMS. The liquid chromatograph (Shimadzu, Kyoto, Japan) was equipped with a controller (CBM-20A), two pumps (model LC-20AD), an automatic injector (SIL-20AHT), a column oven (CTO-20A), and a UV/visible detector (SPD-20A). Chromatographic separation was achieved by using a Kinetex C-18 column (Phenomenex, 100 A, 100 x 1 mm, 2.6 µm PFP) at 40°C, with a solvent flow rate of 0.2 mL $min^{-1}$ directly infused into the mass spectrometer, and 5 µL of injection volume. The mobile phase consisted of 0.1% formic acid and acidified acetonitrile (0.1% formic acid, acidified-ACN) starting with 0% of acidified-ACN at 0 min, increasing to 10% (0-10 min), isocratic for 10 min (10-20 min), raising from 10% to 15% (20-25 min), isocratic for 10 min (25-35 min), ranging from 15% to 30% (35-50 min), increasing from 30% to 45% (50-71 min), reaching 100% at 90 min, and isocratic for 2 min. Separated compounds were monitored at 340 nm. The mass spectrometer (BrukerMicrOTOF-QII) operated in positive mode and nitrogen was used as nebulizer (4 Bar) and dried gas (flow of 8 L $min^{-1}$). The capillary voltage was set at 4,500 V and drying temperature to 200°C. The collision and the quadrupole energy were set to 12 and 6 eV, respectively. RF1 and RF2 funnels were programmed to 400 and 200 Vpp, respectively, and the monitored mass range was 100-1000 kDa.

MS was previously calibrated using sodium formate. All data were processed using Data Analysis 4.3 software (Bruker). This analysis was performed at the Department of Fundamental Chemistry, Chemistry Institute, University of São Paulo, under the supervision of Ph.D. Lydia F. Yamaguchi and Ph.D. Massuo Jorge Kato.

To assist in compound identification, the extracts were also analyzed under similar conditions using a HPLC-DAD (LC1260 – Agilent Technologies) equipped with a Kinetex C-18 column (Phenomenex, 100 A, 100 x 4.6 mm, 2.6 µm) at 40°C, with solvent flow rate of 0.2 mL $min^{-1}$, and 5 µL of injection volume. The mobile phase consisted of 0.1% formic acid and acetonitrile (ACN) starting with 0% of ACN at 0 min, increasing to 10% (0-10 min), isocratic for 10 min (10-20 min), increasing from 10% to 15% (20-25 min), isocratic for 10 min (25-35 min), ranging from 15% to 30% (35-50 min), increasing from 30% to 45% (50-71 min), raising from 45% to 70% (71-80 min), reaching 100% at 81 min, and isocratic for 4 min. Separated compounds were monitored at 340 nm.

Compounds identification was performed by comparing fragmentation of the precursor $[M+H]^+$ = *m/z* of protonated molecular ion mass recorded during MS analysis with literature; and by comparing the retention time and UV/visible spectra of commercial standards analyzed under the same conditions.

## 3. Results and discussion

### 3.1 Analysis of polar metabolites by GC-EIMS

Thirty-five compounds were detected by the GC-EIMS and it was possible to identify 27 constituents (plus ribitol, the internal standard) as TMS or TMS/MeOX derivatives. These compounds include soluble sugars (monosaccharide, disaccharide, and sugar alcohol), amino acids, organic acids, phenolic acids, and catecholamines (Table 1.1, Figure 1.2). Chromatograms of **HME-L** and **HME-C** are shown in supplementary material 1 (Figure S1.1). Moreover, mass spectra of the detected compounds (Figure S1.4-S1.13) and a table containing the 10 most abundant fragments of these compounds followed by their respective abundances (Table S1.1) are also shown in supplementary material 1.

**Table 1.1:** Constituents detected by GC-EIMS in polar phase of hot methanol extracts of leaves (**HME-L**) and culms (**HME-C**) from *Merostachys neesii* Rupr.

| Peak | RT (min) | Suggestion | TMS/ MeOX | Match | LRI | LRI (Nagai 2017) | LRI (Mass Bank) | LRI (GMD) | HME-L | HME-C |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 12.173 | Oxalic acid | 2 | 761 | 1120.5 | - | - | 1118.3 | ✓ | ✓ |
| **2** | 13.140 | NI-1 | - | - | 1152.9 | - | - | - | ✓ | ✓ |
| **3** | 16.274 | Serine | 2 | 932 | 1261.3 | - | - | 1254.2 | ✓ | ✓ |
| **4** | 16.646 | Glycerol | 3 | 930 | 1274.6 | 1274.5 | 1264.7 | 1262.3 | ✓ | ✓ |
| **5** | 16.724 | NI-2 | - | - | 1277.3 | - | - | - | ✓ | ✓ |
| **6** | 17.833 | Succinic acid | 2 | 914 | 1317.5 | 1314.1 | 1303.7 | 1310.7 | ✓ | ✓ |
| **7** | 18.149 | Glyceric acid | 3 | 916 | 1329.4 | 1329.0 | 1318.9 | 1319.9 | ✓ | ✓ |
| **8** | 18.540 | Itaconic acid | 2 | 902 | 1344.0 | - | - | 1339.1 | ✓ | ✓ |
| **9** | 20.078 | Mesaconic acid | 2 | 867 | 1401.7 | - | 1389.3 | - | ✓ | - |
| **10** | 20.598 | NI-3 | - | - | 1422.4 | - | - | - | ✓ | ✓ |
| **11** | 22.208 | Malic acid | 3 | 921 | 1486.0 | 1484.9 | 1478.0 | 1479.3 | ✓ | ✓ |
| **12** | 22.622 | Erythritol | 4 | 918 | 1502.5 | - | - | 1493.3 | ✓ | - |
| **13** | 23.046 | 5-oxoproline | 2 | 943 | 1520.2 | 1519.7 | 1519.7 | 1521.7 | ✓ | ✓ |
| **14** | 23.178 | GABA | 3 | 859 | 1525.7 | 1524.5 | 1526.6 | 1527.5 | ✓ | - |
| **15** | 23.465 | Erythronic acid | 4 | 892 | 1537.7 | - | - | 1545.9 | ✓ | - |
| **16** | 23.889 | Threonic acid | 4 | 933 | 1555.5 | - | - | 1545.9 | ✓ | ✓ |
| **17** | 27.443 | Arabitol | 5 | 931 | 1710.3 | - | - | 1707.6 | ✓ | - |
| **18** | 27.548 | Ribitol (internal standard) | 5 | 918 | 1715.1 | 1715.8 | 1710.9 | 1712.7 | ✓ | ✓ |
| **19** | 27.888 | NI-4 | - | - | 1731.0 | - | - | - | ✓ | - |
| **20** | 28.236 | Aconitic acid | 3 | 906 | 1747.1 | - | 1734.5 | 1741.1 | ✓ | ✓ |
| **21** | 29.243 | Azelaic acid | 2 | 890 | 1794.0 | - | - | 1790.7 | ✓ | ✓ |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **22** | 29.617 | Citric acid | 4 | 872 | 1811.9 | 1811.3 | 1803.9 | 1803.9 | ✓ | ✓ |
| **23** | 30.649 | Fructose [1] | 5/1 | 932 | 1862.3 | 1861.3 | 1855.5 | 1853.9 | ✓ | ✓ |
| **23a** | 30.847 | | | 900 | 1872.1 | 1870.5 | 1864.3 | 1863.1 | | |
| **24** | 31.000 | Galactose | 5/1 | 940 | 1879.5 | 1870.5 | 1874.8 | 1876.1 | ✓ | ✓ |
| **25** | 31.151 | Glucose [2] | 5/1 | 933 | 1886.9 | 1885.4 | 1881.6 | 1880.5 | ✓ | ✓ |
| **25a** | 31.503 | | | 940 | 1904.3 | 1903.6 | 1902.6 | 1899.1 | | |
| **26** | 31.743 | Mannitol | 6 | 885 | 1916.6 | - | - | 1913.2 | ✓ | ✓ |
| **27** | 31.878 | Sorbitol | 6 | 867 | 1923.5 | - | - | 1919.7 | ✓ | ✓ |
| **28** | 32.399 | Gallic acid | 4 | 865 | 1950.2 | - | - | 1945.9 | ✓ | ✓ |
| **29** | 33.762 | NI-5 | - | - | 2020.8 | - | - | - | ✓ | ✓ |
| **30** | 34.673 | Dopamine | 4 | 908 | 2069.5 | - | - | 2076.8 | ✓ | ✓ |
| **31** | 34.818 | *Myo*-inositol | 6 | 922 | 2077.3 | 2076.0 | 2083.6 | 2080.2 | ✓ | ✓ |
| **32** | 36.053 | Norepinephrine | 5 | 854 | 2145.1 | - | - | 2148.3 | ✓ | - |
| **33** | 38.759 | NI-6 | - | - | 2300.2 | - | - | - | ✓ | ✓ |
| **34** | 39.807 | NI-7 | - | - | 2363.6 | - | - | - | ✓ | ✓ |
| **35** | 43.766 | Sucrose | 8 | - | 2615.0 | - | 2614.8 | 2623.0 | ✓ | ✓ |

RT (min): retention time in minutes; TMS: trimethylsilyl; MeOX: *O*-methyloxime; Match: NIST02 library; LRI: Linear Retention Index; GMD: Golm Metabolome Database; NI: not identified; GABA: gamma-aminobutyric acid.

[1] Fructose stereoisomers produced by methoximation reaction.

[2] Glucose stereoisomers produced by methoximation reaction.

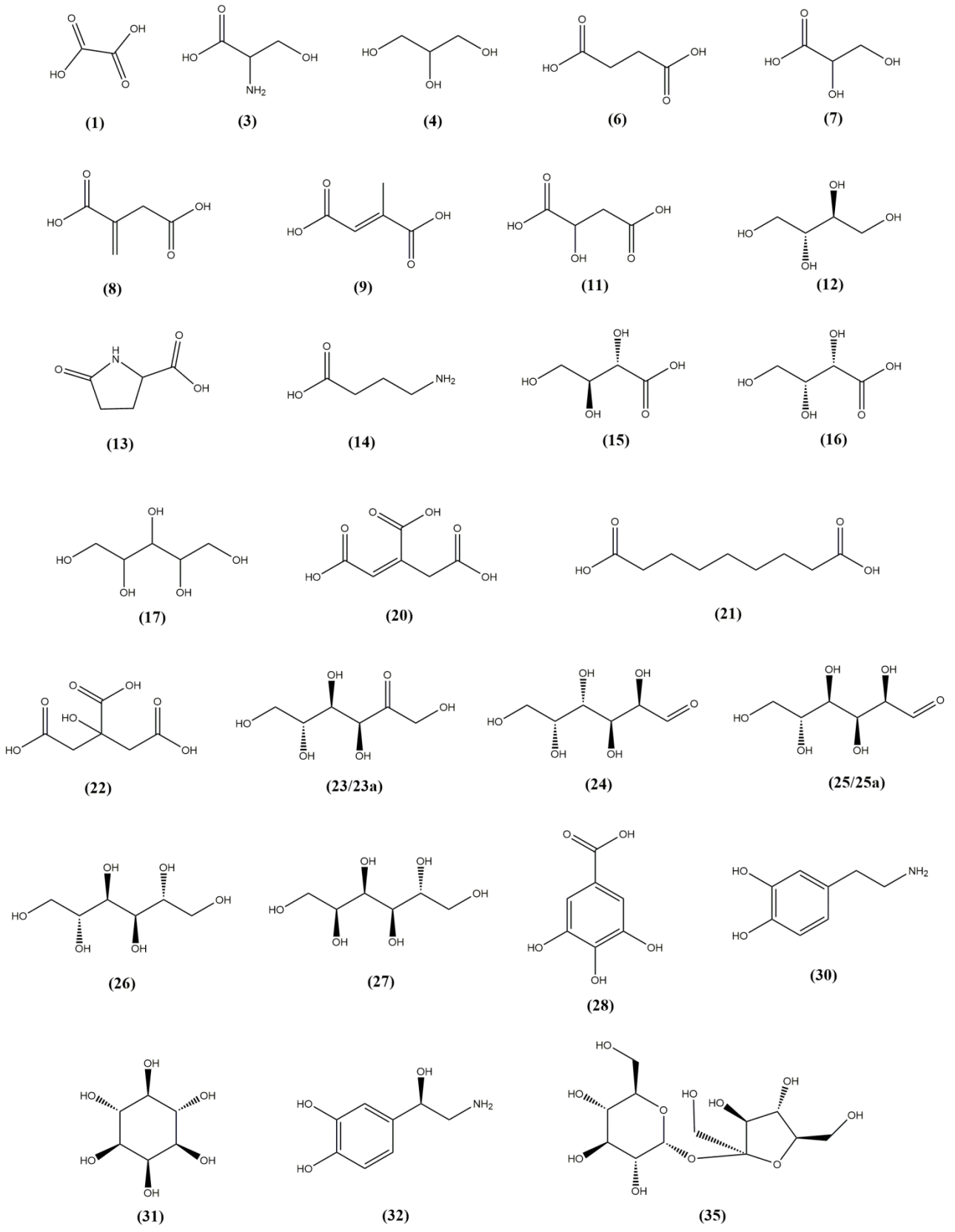



**Figure 1.2:** Compounds identified in the polar phase of the hot methanol extract (**HME**) of leaves and culms of *Merostachys neesii* Rupr. by GC-EIMS. Numbers below each chemical structure correspond to those shown in Table 1.1.

Both organs (leaf and culm) showed similar chemical profiles. Culms showed absence (or peak below detection) of mesaconic acid, erythritol, GABA, erythronic acid, arabitol, NI-4, and norepinephrine (Table 1.1).

Carbohydrates are produced through photosynthesis and perform various functions in plants, such as energy source for growth and development, signaling molecules, osmotic adjustment, carbon transport molecules, and precursors in the biosynthesis of a huge range of compounds. The most common soluble sugars in plant tissues are sucrose, maltose, glucose, and fructose (Halford et al. 2011). For leaves and culms of *M. neesii*, fructose (**23** and **23a**), glucose (**25** and **25a**), and sucrose (**35**) were the major soluble sugars (Figure S1.1). Leaves and culms of *Phyllostachys aureosulcata* McClure (Knott et al. 2017) and culms of *Sasa palmata* (Burb.) E.G. Camus (Magel et al. 2005) also showed these saccharides as the predominant sugars. In the present work, galactose (**24**) was also detected, but as a minority constituent when compared to other saccharides.

Additionally, six sugar alcohols were detected: glycerol (**4**), erythritol (**12**), arabitol (**17**), mannitol (**26**), sorbitol (or glucitol, **27**), and *myo*-inositol (**31**) (Table 1.1). Sugar alcohols (or polyols) play roles in osmotic control in plants submitted to abiotic stress. Moreover, they are also an important form of carbon storage and transport, although to a lesser extent compared to sucrose and its derivatives (Noiraud et al. 2001; Merchant and Richter 2011). These sugars have lower energy value than sucrose, so they have become an alternative sweetener. Another advantage is that sugar alcohols are non-cariogenic; however, its consumption should be done sparingly due to its laxative properties (Ghosh and Sudha 2012; Grembecka 2015). Chemically, sugar alcohols are the reduced form of sugars (aldoses and ketoses). These compounds may have three or more carbon atoms, all bearing a hydroxyl group. They can be acyclic (also known as alditols) or cyclic (cyclitols). Glycerol, erythritol, arabitol, mannitol, and sorbitol are examples of alditols with three, four, five, six, and six carbon atoms, respectively. *Myo*-inositol, in turn, is a six-carbon atoms cyclitol. Erythritol, sorbitol, and *myo*-inositol were also found in corn grains (*Zea mays* L.) (Farag et al. 2019) and inositol and mannitol in leaves of *Poa pratensis* L. and *Cynodon transvaalensis* x *C. dactylon* (Du et al. 2011), all species belonging to Poaceae.

Amino acids are responsible for many functions for living beings; besides, they are building blocks of proteins and precursors of a great range of molecules of great physiological importance (Wu 2009). Bamboos, especially their shoots, feature a wide variety of amino acids, especially tyrosine (Chongtham et al. 2011). However, in the present study, only one amino acid (serine, **3**) and two amino acid derivatives (5-oxoproline, **13**; and gamma-aminobutyric acid, or GABA, **14**) were detected. The low amino acid detection in *M. neesii* leaves and culms may be due to the use of freeze-dried material rather than fresh material.

Organic acids result from incomplete oxidation of assimilates produced during the photosynthetic process (Igamberdiev and Eprintsev 2016). They can be found in a wide range of fruits and vegetables and play a crucial role in maintaining the quality, stability, acceptability, and nutritional value in foodstuffs (Shui and Leong 2002). Eleven organic acids were identified in *M. neesii*. For leaves, the main organic acids were aconitic (**20**), malic (**11**), itaconic (**8**), and citric (**22**); while for culms were aconitic, malic, citric, and oxalic acids (**1**) (Figure S1.1). Few studies have characterized the organic acid profile in bamboo, even bamboo shoots being part of the Asian cuisine. Kozukue et al. (1983) found oxalic, citric, malic, succinic, and fumaric acids in shoots of *Phyllostachys pubescens* J.Houz.

Gallic acid (or 3,4,5-trihydroxybenzoic acid, **28**) is a fairly widespread polyphenolic compound in plants that can be found in its free form or as a derivative. The strong antioxidant capacity and prooxidant action demonstrated by gallic acid provides a wide range of biological activities, such as antiproliferative activity against various cancer cell lines, anti-inflammatory, antimicrobial, antiviral, antiallergic, antiarteriosclerosis, and neuroprotective, hepatoprotective, and nephroprotective effects (Badhani et al. 2015; Choubey et al. 2015). This compound was described in the leaves of *Merostachys riedeliana* Rupr. ex Döll (Jose et al. 2016), *Bambusa textilis* McClure (Liu et al. 2016), and *B. balcooa* Roxb. (Goyal et al. 2017), and shoots of *Dendrocalamus asper* (Schult.) Backer, *D. strictus* (Roxb.) Nees, and *B. tulda* Roxb. (Pandey and Ojha 2013).

Two catecholamines were identified in *M. neesii*, dopamine (**30**) and norepinephrine (or noradrenaline, **32**) (Table 1.1). Catecholamines are a class of biogenic amines with a 3,4-dihydroxy-substituted phenyl ring. Although they are well known as neurotransmitters in mammals, these compounds are also found in several flowering plant families (Kulma and Szopa 2007; Schenck and Maeda 2018), including plants from the human diet, such as banana, orange, tomato, spinach, pea, and avocado (Briguglio et al. 2018), and some species of Poaceae, for instance *Poa annua* L., *P. cookii* (Hook.f.) Hook.f., and *P. kerguelensis* (Hook.f.) Steud. (Hennion et al. 2012). In plants, it has been suggested that catecholamines are hydrosoluble antioxidants, possibly involved in the plant stress response. Further, they can interact with phytohormones and affect plant growth and development, as well as influencing plant flowering and sugar metabolism (Kulma and Szopa 2007). This is the first report of catecholamines in Bambusoideae.

### 3.2 Analysis of nonpolar metabolites by GC-EIMS

Analysis of hexane extracts of leaves (**HE-L**) and culms (**HE-C**) of *M. neesii* by GC-EIMS led to the detection of 43 constituents in the nonpolar extracts, and it was possible to identify 27 compounds (plus nonadecane, the internal standard) (Table 1.2; Figure 1.3). Chromatograms of hexane extracts are shown in supplementary material 1 (Figure S1.2).

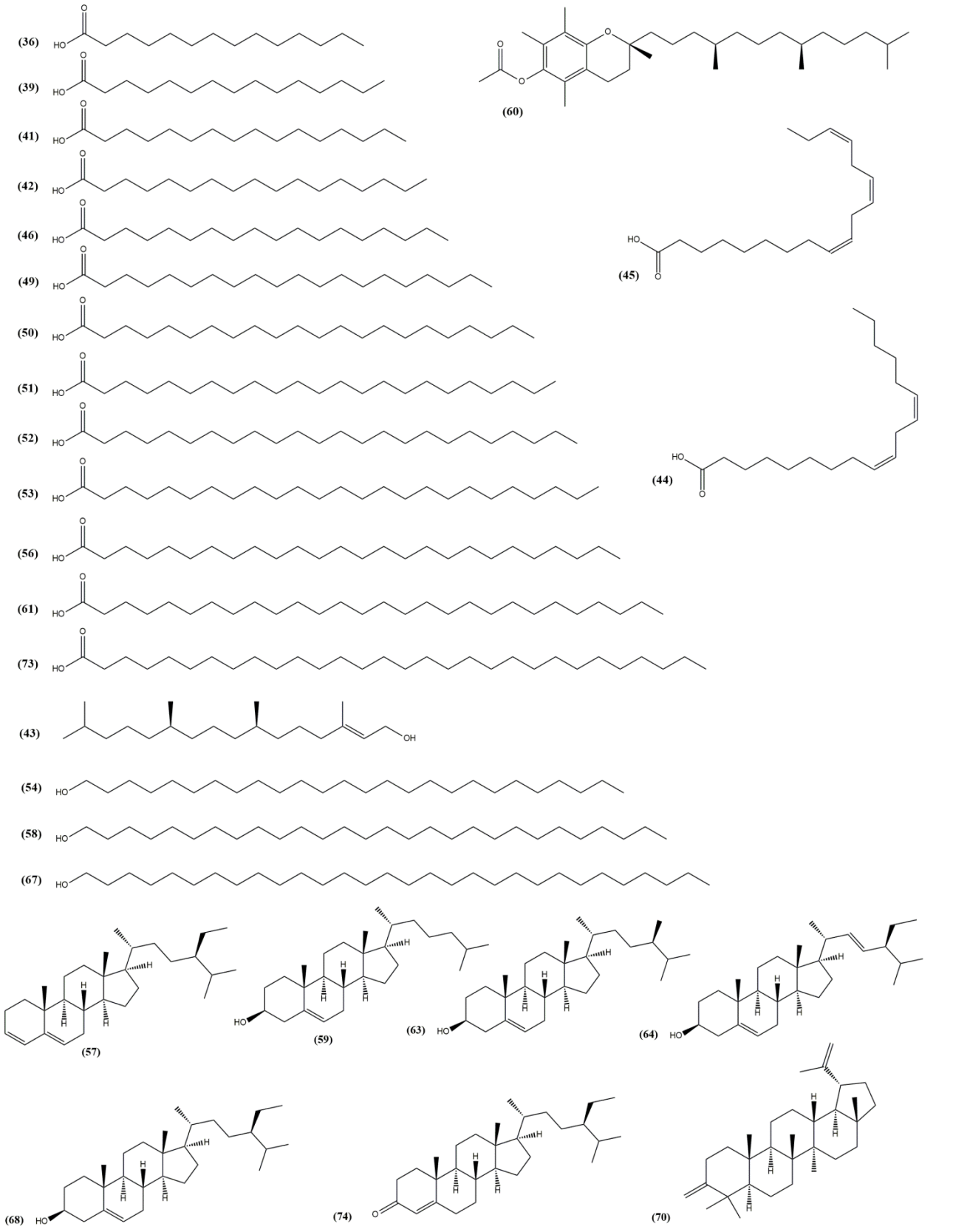



**Figure 1.3:** Compounds identified in the hexane extracts (**HE**) of *Merostachys neesii* Rupr. leaves and culms by GC-EIMS. Numbers below each chemical structure correspond to those shown in Table 1.2.

Among the constituents present in hexane extracts are: 15 fatty acids, three primary alcohols, six sterols (5 phytosterols and cholesterol), one triterpene, *α*-tocopherol acetate, and phytol. Mass spectra of all detected compounds are shown in supplementary material 1 (Figure S1.14-S1.24). Additionally, a table containing data of the 10 most abundant fragments of these compounds followed by their respective abundances are also present in the supplemental material 1 (Table S1.2).

Fatty acids play different functions in plants, such as energy source, components of cell membranes, precursors of cuticular wax and plant defense. The most common fatty acids found in plants are palmitic (16:0) and stearic (18:0), saturated fatty acids, and oleic (18:1), linoleic (18:2), and linolenic (18:3), unsaturated fatty acids (Lim et al. 2017). In the human body, fatty acids are also extremely relevant as they integrate the structure of cell membranes and are also energy source and precursors of eicosanoids (*e.g*. prostaglandins) which are molecules with regulatory functions within the body (Calder 2015). Humans are also capable of synthesizing fatty acids, except those known as essential fatty acids. Essential fatty acids (ω-6 and ω-3 series derived from linoleic and linolenic acids, respectively) are those that the human body is unable to synthesize but are necessary for growth and development, besides promoting health benefits (Das 2006).

Fatty acid was the main group of compounds detected in the hexane extracts of *M. neesii* (15 compounds). Culms showed higher fatty acid diversity than leaves (Figure S1.2 and Table 1.2). In general, the most abundant fatty acids in both leaves and culms were palmitic acid (or hexadecanoic acid, **41**), linoleic acid (or 9,12-octadecadienoic acid, **44**), and α-linolenic acid (or 9,12,15-octadecatrienoic acid, **45**). Except for pentacosanoic acid (25:0, **53**), hexacosanoic acid (26:0, **56**), octacosanoic acid (28:0, **61**), and triacontanoic acid (30:0, **73**), the others were also reported in *Phyllostachys pubescens* shoot oil (Lu et al. 2010b). Moreover, palmitic acid was one of the major constituents in the methanol extract of *P. pubescens* culms (Mu et al. 2004).

Three primary alcohols, namely 1-hexacosanol (**54**), 1-octacosanol (**58**), and 1-triacontanol (**67**) were identified in the hexane extracts (Table 1.2).

Phytol (**43**) was detected in both leaves and culms of *M. neesii* (Table 1.2). This compound is an acyclic diterpene present in the structure of chlorophyll, tocopherols, and vitamin K. Free phytol is a product from chlorophyll degradation (Hussain et al. 2013).

**Table 1.2:** Constituents detected by GC-EIMS in hexane extracts of leaves (**HE-L**) and culms (**HE-C**) from *Merostachys neesii* Rupr.

| Peak | RT (min) | Suggestion | TMS | Match | LRI | LRI (Mass Bank) | LRI (GMD) | HE-L | HE-C |
|---|---|---|---|---|---|---|---|---|---|
| 36 | 12.826 | Myristic acid (14:0) | 1 | 897 | 1853.8 | - | 1845.8 | - | ✓ |
| 37 | 13.502 | NI-8 | - | - | 1884.6 | - | - | ✓ | ✓ |
| 38 | 13.872 | Nonadecane (internal standard) | - | 858 | 1901.5 | - | 1900.0 | ✓ | ✓ |
| 39 | 14.930 | Pentadecanoic acid (15:0) | 1 | 885 | 1952.6 | - | 1945.5 | ✓ | ✓ |
| 40 | 16.741 | NI-9 | - | - | 2042.9 | - | - | ✓ | - |
| 41 | 16.920 | Palmitic acid (16:0) | 1 | 930 | 2052.1 | 2038.4 | 2045.4 | ✓ | ✓ |
| 42 | 18.772 | Heptadecanoic acid (17:0) | 1 | 892 | 2150.1 | 2142.9 | 2144.6 | ✓ | ✓ |
| 43 | 19.392 | Phytol | 1 | 898 | 2183.9 | 2168.1 | 2170.9 | ✓ | ✓ |
| 44 | 19.964 | Linoleic acid (18:2) | 1 | 950 | 2215.8 | 2209.6 | 2209.0 | ✓ | ✓ |
| 45 | 20.087 | Linolenic acid (18:3) | 1 | 946 | 2222.5 | 2215.6 | - | ✓ | ✓ |
| 46 | 20.541 | Stearic acid (18:0) | 1 | 928 | 2248.9 | 2236.0 | 2243.5 | ✓ | ✓ |
| 47 | 23.574 | NI-10 | - | - | 2430.3 | - | - | - | ✓ |
| 48 | 23.658 | NI-11 | - | - | 2435.5 | - | - | ✓ | - |
| 49 | 23.828 | Arachidic acid (20:0) | 1 | 855 | 2446.2 | - | 2453.8 | - | ✓ |
| 50 | 26.856 | Behenic acid (22:0) | 1 | 883 | 2643.7 | 2637.5 | 2650.6 | - | ✓ |
| 51 | 28.287 | Tricosanoic acid (23:0) | 1 | - | 2742.4 | - | 2731.1 | - | ✓ |
| 52 | 29.670 | Lignoceric acid (24:0) | 1 | 836 | 2841.3 | 2836.3 | 2824.4 | ✓ | ✓ |
| 53 | 31.007 | Pentacosanoic acid (25:0) | 1 | 797 | 2940.1 | - | 2937.2 | - | ✓ |
| 54 | 31.115 | 1-hexacosanol | 1 | 811 | 2948.4 | - | - | - | ✓ |
| 55 | 31.761 | NI-12 | - | - | 2997.8 | - | - | - | ✓ |
| 56 | 32.304 | Hexacosanoic acid (26:0) | 1 | 815 | 3039.8 | - | 3036.3 | ✓ | ✓ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 57 | 32.970 | Stigmasta-3,5-diene | - | 814 | 3091.4 | - | - | ✓ | ✓ |
| 58 | 33.647 | 1-octacosanol | 1 | 826 | 3145.8 | - | - | - | ✓ |
| 59 | 33.786 | Cholesterol | 1 | 832 | 3157.3 | - | 3188.1 | - | ✓ |
| 60 | 34.256 | α-tocopherol acetate | - | 851 | 3195.2 | - | 3200.0 | ✓ | ✓ |
| 61 | 34.788 | Octacosanoic acid (28:0) | 1 | - | 3239.1 | 3237.0 | 3232.0 | - | ✓ |
| 62 | 34.845 | NI-13 | - | - | 3243.8 | - | - | ✓ | - |
| 63 | 35.107 | Campesterol | 1 | 917 | 3265.6 | - | 3298.2 | ✓ | ✓ |
| 64 | 35.493 | Stigmasterol | 1 | 934 | 3297.5 | - | 3319.3 | ✓ | ✓ |
| 65 | 35.573 | NI-14 | - | - | 3304.2 | - | - | ✓ | - |
| 66 | 35.798 | NI-15 | - | - | 3323.2 | - | - | ✓ | - |
| 67 | 36.021 | 1-triacontanol | 1 | 772 | 3342.1 | - | - | ✓ | ✓ |
| 68 | 36.189 | *β*-sitosterol | 1 | 904 | 3356.3 | - | 3385.8 | ✓ | ✓ |
| 69 | 36.362 | NI-16 | - | - | 3370.2 | - | - | ✓ | - |
| 70 | 36.378 | Lupenone | - | 815 | 3372.5 | - | - | - | ✓ |
| 71 | 36.642 | NI-17 | - | - | 3394.5 | - | - | - | ✓ |
| 72 | 36.795 | NI-18 | - | - | 3407.7 | - | - | - | ✓ |
| 73 | 37.118 | Triacontanoic acid (30:0) | 1 | - | 3435.3 | - | 3432.5 | ✓ | - |
| 74 | 37.333 | *β*-sitostenone | - | 802 | 3453.9 | - | - | ✓ | ✓ |
| 75 | 37.657 | NI-19 | - | - | 3481.8 | - | - | ✓ | ✓ |
| 76 | 37.832 | NI-20 | - | - | 3496.8 | - | - | - | ✓ |
| 77 | 38.093 | NI-21 | - | - | 3519.4 | - | - | ✓ | ✓ |
| 78 | 39.516 | NI-22 | - | - | 3644.7 | - | - | - | ✓ |

RT (min): retention time in minutes; TMS: trimethylsilyl; Match: NIST02 library; LRI: Linear Retention Index; GMD: Golm Metabolome Database; NI: not identified.

*α*-Tocopherol acetate (**60**) was found in *M. neesii* leaves and culms (Table 1.2). This compound is the esterified form of *α*-tocopherol. *α*-Tocopherol along with seven other compounds constitutes the vitamin E forms. This vitamin is lipid-soluble and exhibits potent antioxidant activity, among other beneficial health effects (Hussain et al. 2013).

Lupenone (**70**), a lupane-type triterpenoid, was found in **HE-C** (Table 1.2). Triterpenes are formed from the tail-tail union of two farnesyl diphosphate (FPP) molecules, synthesized by the mevalonic acid biosynthetic pathway (MEV), giving rise to squalene, the precursor of triterpenes and steroids (Dewick 2009). Lupenone has been aroused interest for medicinal purposes due to its therapeutic potential for several diseases. Some pharmacological activities that have been reported for this secondary metabolite are anti-inflammatory, antiviral - herpes simplex virus (HSV-1 and HSV-2), African swine fever virus (ASFV), and chikungunya (CHIKV) - anti-diabetic, and anticancer (Xu et al. 2017). Besides, lupenone has shown moderate inhibition activity against the BACE1 (*β*-site amyloid precursor protein cleaving enzyme 1), whose increased levels are found in people with Alzheimer's disease (Koirala et al. 2017). This pentacyclic triterpene has been described in several plant families, including Poaceae (Xu et al. 2017). Lupenone was found in some bamboo species, such as *Phyllostachys nigra* var. *henonis* (Mitford) Rendle (Jiao et al. 2007; Lu et al. 2010a) and *P. pubescens* (Ma et al. 2011).

Cholesterol (**59**) was also found only in **HE-C** (Table 1.2). This sterol is widespread in plants, but generally present in low amounts (Dewick 2009; Sonawane et al. 2016). In plants, cholesterol is biosynthesized from cycloartenol and is an important precursor of many metabolites (Sonawane et al. 2016). Cholesterol has been previously reported in *Pleioblastus amarus* (Keng) Keng f., *Phyllostachys pubescens*, *Dendrocalamus latiflorus* Munro, and *Phyllostachys praecox* C.D.Chu & C.S.Chao shoots (Lu et al. 2009).

As well as cholesterol, phytosterols are another group of sterol (compounds derived from triterpene) featuring the 1,2-cyclopentanoperhydrophenanthrene ring system; however, they differ due to substitutions at C-4 (ring A) and C-24 (side chain), unsaturation of the rings and side chain, and conjugation of the C-3 alcoholic hydroxyl group with fatty acids, phenolic acids, and carbohydrates (Uddin et al. 2015; Moreau et al. 2018). Functionally, like cholesterol, phytosterols act to stabilize the lipid bilayer of cell membranes (Uddin et al. 2015). They are widely distributed in plants and constitute the main sterol in these organisms (Dewick 2009), being campesterol, stigmasterol, and sitosterol the most common phytosterols (Uddin et al. 2015; Moreau et al. 2018). Phytosterols perform several benefits to human health. They help to reduce serum cholesterol levels and the risk of cardiovascular diseases and exhibit atherosclerotic, antioxidant, anti-inflammatory and, anticancer activities. Also, they may help to prevent Alzheimer's disease, for example, stigmasterol showed activity on reducing the amyloidogenic processing (Uddin et al. 2015).

In total, five phytosterols were detected in leaves and culms of *M. neesii*: stigmasta-3,5-diene (**57**), campesterol (**63**), stigmasterol (**64**), *β*-sitosterol (**68**), and *β*-sitostenone (**74**) (Table 1.2). *β*-sitosterol was the most abundant phytosterol for both leaves and culms (Figure S1.2). Phytosterols have also been described as constituents of other bamboo species, for example, stigmasterol, campesterol, and *β*-sitosterol in *Phyllostachys pubescens* shoot skins (Tanaka et al. 2013); stigmastanol, ergosterol, stigmasterol, campesterol, and *β*-sitosterol in *Pleioblastus amarus*, *Phyllostachys pubescens*, *Dendrocalamus latiflorus*, and *Phyllostachys praecox* shoots (Lu et al. 2009), and *Phyllostachys pubescens* shoot oil (Lu et al. 2010b); stigmasterol and *β*-sitosterol in *Bambusa bambos* (L.) Voss leaves (Sriraman et al. 2015); and *β*-sitosterol in *Sasa palmata* leaves (Zulkafli et al. 2014).

### 3.3 Analysis of flavonoids by HPLC-ESI/HRMS

Flavonoids are an important group of polyphenols widely distributed in plants. These compounds exhibit various pharmacological activities, such as antioxidant, anti-inflammatory, anticancer, hepatoprotective, antibacterial, antiviral, and anticholinesterase. Furthermore, they are considered nutraceutical compounds because of the many health-promoting benefits and reducing the risk of various illnesses (Kumar and Pandey 2013; Terahara 2015; Panche et al. 2016). Flavonoids have a 15-carbon atoms skeleton, where two benzene rings (A- and B-ring) are bonded through three carbon atoms forming a heterocyclic pyran ring (ring C). According to the pattern of C-ring oxidation and substitution, different classes are formed, including flavones, flavonols, chalcones, flavanols, flavanones, anthocyanins, and isoflavonoids (Kumar and Pandey 2013). Flavonoids have often been reported as a major constituent in bamboo species, mainly flavones *C*-glycosides derived from apigenin and luteolin and *O*-glycosides derived from tricin (Park et al. 2007; Van Hoyweghen et al. 2012; Wang et al. 2012; Liu et al. 2016).

By analyzing the UV/visible spectra of all compounds present in the extracts analyzed by HPLC-DAD, it was possible to detect 10 flavonoids (compounds from **79** to **88**, figure 1.4), all belonging to the flavone group. In general, the UV/visible spectrum of flavonoid is characterized by the presence of two maximum absorption peaks (bands I and II). Band I is associated with the B-ring cinnamoyl system and for flavones has a maximum absorption peak between 300 and 350 nm. On the other hand, band II is associated with the A-ring benzoyl system and absorbs in the region between 240 and 280 nm (Mabry et al. 1970).

To obtain more structural information regarding the flavones detected in this work, the extracts were also analyzed by HPLC-ESI/HRMS. This technique has been described as a suitable tool for the identification of this group of phenolic compounds without prior isolation (Cuyckens and Claeys 2004; Ferreres et al. 2007). Chromatograms of the hydroethanol and aqueous extracts obtained at 340 nm are shown in supplementary material 1 (Figure S1.3).

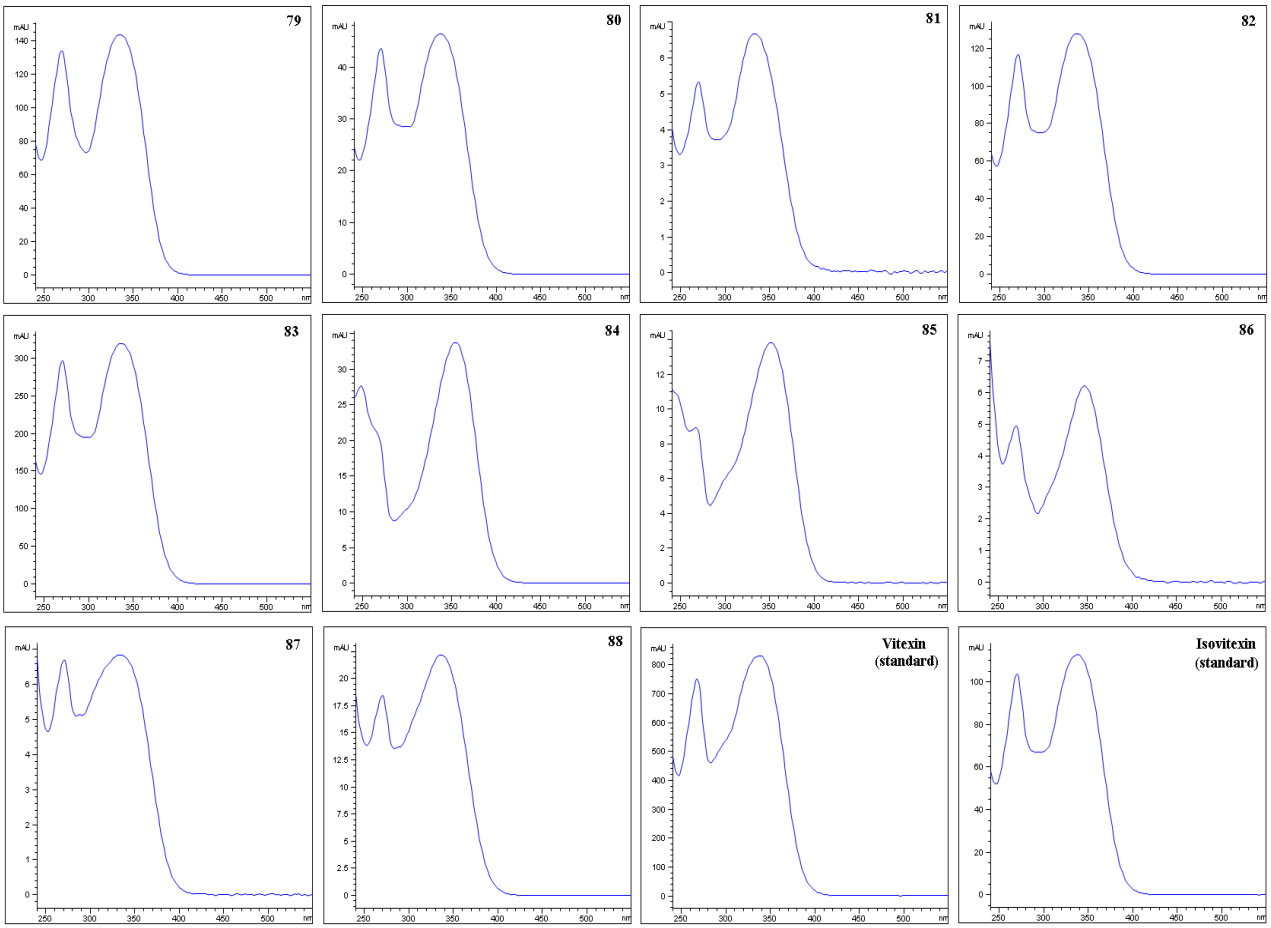



**Figure 1.4:** UV/visible spectra of the flavonoids detected in the hydroethanol and aqueous extracts of *Merostachys neesii* Rupr. leaves and culms by HPLC-DAD (340 nm). Numbers correspond to those shown in Table 1.3.

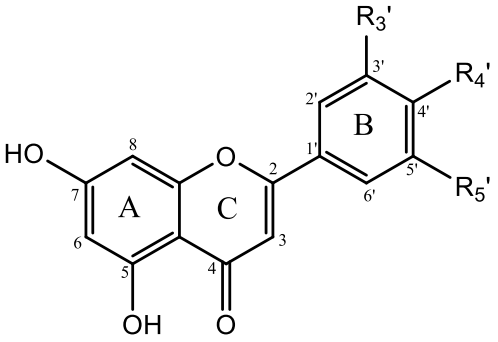
The low collision energy (12 eV) used during HPLC-ESI/HRMS analysis allowed the observation of the precursor ion $[M+H]^+$ of the flavones present in the *M. neesii* extracts, as well as its main fragments. Thus, it was possible to determine the aglycone and in some cases its substituents (hexose or pentose). Data on retention time (RT) and the main ion fragments are summarized in Table 1.3. Mass spectra ($MS^+$) of the flavonoids are shown in supplementary material 1 (Figure S1.25-S1.27).

**Table 1.3:** Flavonoids detected by HPLC-ESI/HRMS in hydroethanol (**EE**) and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) from *Merostachys neesii* Rupr.

| Peak | RT (min) | Suggestion | MW [gmol$^{-1}$] | Main fragments | EE-L | EE-C | AE-L | AE-C |
|---|---|---|---|---|---|---|---|---|
| 79 | 32.9 | Apigenin *O*-hexoside *C*-hexoside | 595 | 617.1473 $[M+Na]^+$; 595.1684 $[M+H]^+$; 475.3254 $[M+H-120]^+$; 433.1133 $[M+H-162]^+$; 415.0955 $[M+H\text{-hexose-}H_2O]^+$; 313.0711 $[M+H\text{-hexose-}120]^+$ | ✓ | ✓ | ✓ | ✓ |
| 80 | 34.0 | Apigenin *O*-pentoside *C*-hexoside | 565 | 587.1342 $[M+Na]^+$; 565.1574 $[M+H]^+$; 433.1143 $[M+H-132]^+$; 415.0957 $[M+H\text{-pentose-}H_2O]^+$; 313.0727 $[M+H\text{-pentose-}120]^+$ | ✓ | ✓ | ✓ | ✓ |
| 81 | 35.4 | Isovitexin | 433 | 455.0970 $[M+Na]^+$; 433.1148 $[M+H]^+$; 415.1088 $[M+H-H_2O]^+$; 397.0955 $[M+H-2H_2O]^+$; 379.0782 $[M+H-3H_2O]^+$; 367.0738; 337.0721; 313.0706 $[M+H-120]^+$; 283.0615 $[M+H-150]^+$ | ✓ | ✓ | ✓ | ✓ |
| 82 | 43.6 | Apigenin *O*-hexoside *C*-pentoside | 565 | 587.1426 $[M+Na]^+$; 565.1590 $[M+H]^+$; 403.1048 $[M+H-162]^+$; 385.0841 $[M+H\text{-hexose-}H_2O]^+$; 367.0738 $[M+H\text{-hexose-}2H_2O]^+$; 313.0707 $[M+H\text{-hexose-}90]^+$ | ✓ | ✓ | ✓ | ✓ |
| 83 | 45.2 | Apigenin *O*-pentoside *C*-pentoside | 535 | 557.1309 $[M+Na]^+$; 535.1501 $[M+H]^+$; 403.1053 $[M+H-132]^+$; 385.0844 $[M+H\text{-pentose-}H_2O]^+$; 367.0741 $[M+H\text{-pentose-}2H_2O]^+$; 313.0707 $[M+H\text{-pentose-}90]^+$ | ✓ | ✓ | ✓ | ✓ |
| 84 | 47.8 | Tricin derivative 1 | 679 | 701.5025 $[M+Na]^+$; 679.5198 $[M+H]^+$; 653.1800; 566.4354; 453.3500; 331.2600; 209.1673 | ✓ | ✓ | ✓ | ✓ |
| 85 | 60.1 | Tricin derivative 2 | 331 | 331.0856 $[M+H]^+$ | ✓ | ✓ | - | - |
| 86 | 60.6 | Tricin derivative 3 | 527 | 549.1446 $[M+Na]^+$; 527.1605 $[M+H]^+$; 331.0852 | - | ✓ | - | - |
| 87 | 67.0 | Tricin derivative 4 | 569 | 591.1463 $[M+Na]^+$; 569.1652 $[M+H]^+$; 331.0811 | - | ✓ | - | - |
| 88 | 67.5 | Tricin derivative 5 | 569 | 591.1505 $[M+Na]^+$; 569.1672 $[M+H]^+$; 331.0822 | - | ✓ | - | - |

RT (min): retention time in minutes; MW: molecular weight.

Fragmentation data suggest the flavones present in *M. neesii* are derived from apigenin and tricin (Figure 1.5). Apigenin is a flavone with two hydroxyls groups in the A-ring (C5 and C7) and one in the B-ring (C4'), while tricin also has these three hydroxyl groups in the same positions plus two methoxyls in the B-ring (C3' and C5'). Regarding apigenin derivatives detected in *M. neesii*, all are *C*-glycosides, and some of them also have an *O*-glycosylation.




| Aglycone | $R_{3'}$ | $R_{4'}$ | $R_{5'}$ |
|---|---|---|---|
| Apigenin | H | OH | H |
| Tricin | $OCH_3$ | OH | $OCH_3$ |

**Figure 1.5:** Structural representation of flavonoid aglycones found in leaves and culms of *Merostachys neesii* Rupr.

Glycosylated flavonoids are the form commonly found in plants, especially *O*-glycosides. Flavonoid *O*-glycosides exhibit the glycosidic moieties bonded to the oxygen of the hydroxyl group of the aglycone. These glycosylations may occur mainly at positions 7, 4', and 3. Moreover, sugar substituents may also occur bonded directly to a carbon atom (C6 and C8 positions) of the flavonoid backbone, namely *C*-glycosides (Ferreres et al. 2007; Guo et al. 2013b). Flavonoid *C*-glycosides can be divided into mono- and di-*C*-glycosides, and *O*,*C*-glycosides. In this last group, *O*-glycosylation may occur in the phenolic hydroxyl group or the hydroxyl group of the sugar from the *C*-glycosyl residue (Cuyckens and Claeys 2004; Ferreres et al. 2007).

By analyzing the fragmentation pattern, it is possible to differ *O*-glycosides, *C*-glycosides, and *O*,*C*-glycosides. In all cases, the precursor ion $[M+H]^+$ is produced. For flavonoid *O*-glycosides, monosaccharide residues are eliminated resulting in the $[M+H-162]^+$, $[M+H-146]^+$, and $[M+H-132]^+$ ions for hexoses, deoxyhexoses, and pentoses, respectively. In this way, it is possible to determine the monosaccharide group present in the flavonoid (Cuyckens and Claeys 2004).

In flavonoid mono-*C*-glycosides, the main fragments are related to cross-ring cleavages of the sugar moiety and loss of water molecules. The water loss is much more pronounced in 6-*C*-glycosides than in 8-*C*-glycosides. This is because the loss of water in 6-*C*-glycosides may occur between the 2"-hydroxyl group of the saccharide and the 5- or 7-hydroxyl groups of the aglycone. Whereas for 8-*C*-glycosides elimination of water can occur only between 2"-hydroxyl group of the sugar and the 7-hydroxyl group. Concerning to sugar cross-ring cleavages, the main products ions are $[M+H-120]^+$, $[M+H-150]^+$, $[M+H-90]^+$, and $[M+H-134]^+$ for hexoses and $[M+H-90]^+$, [M+H-

60]$^+$, and [M+H-104]$^+$ for pentoses. Additionally, the elimination of monosaccharide residues (*i.e.* losses of 162 amu for hexoses and 132 amu for pentoses) is either absent or present in low abundance (Cuyckens and Claeys 2004; Cavaliere et al. 2005).

Regarding flavonoid di-*C*-glycosides, mass spectra are more complex. Losses of water molecules (from three to six) and cross-ring cleavages of the sugar are rather common, but generally, there are no ions related to the elimination of monosaccharide residues. Finally, for flavonoid *O*,*C*-glycosides (for both *O*-glycosylation in phenolic hydroxyl and sugar hydroxyl from *C*-glycosyl residue), it is common to observe both ions from the loss of sugar residue from *O*-glycosylation and from sugar residue plus the loss of a water molecule ([M+H-180]$^+$ and [M+H-150]$^+$ for hexose and pentose, respectively). When comparing the two ion fragments, the second one is more abundant in *C*-glycosides with *O*-glycosylation in the sugar hydroxyl group (Cavaliere et al. 2005). Figure 1.6 shows a scheme of some of these cleavages cited above.

It is noteworthy that sodium [M+Na]$^+$ and potassium [M+K]$^+$ adducts are generally detected in first-order mass spectra obtained with ESI in the positive mode (Cuyckens and Claeys 2004).

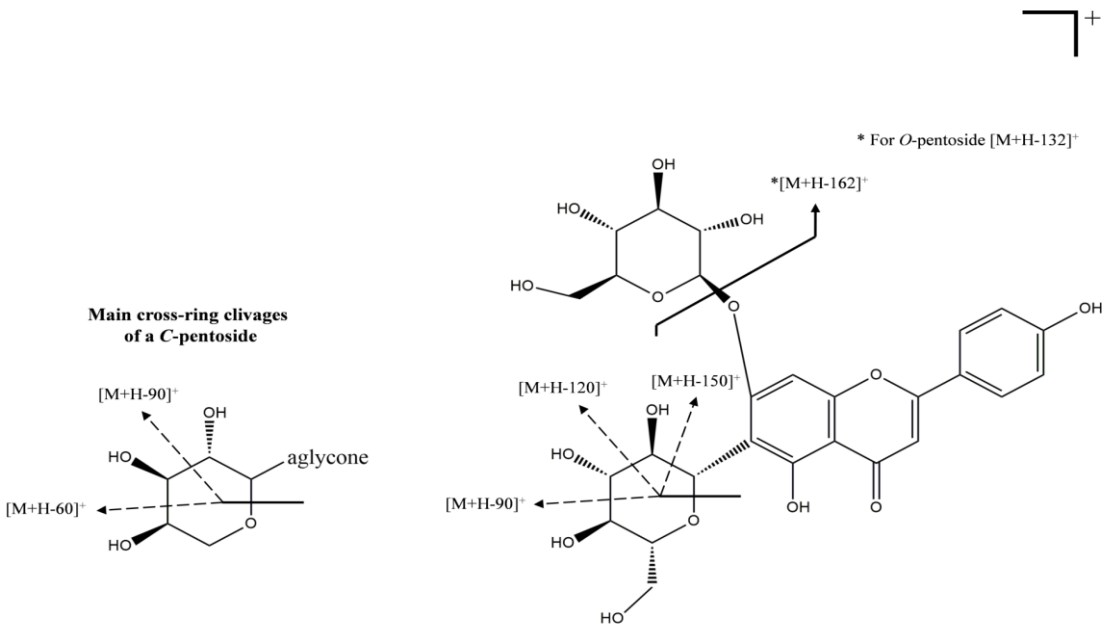



**Figure 1.6:** Scheme showing the loss of the *O*-glycoside moiety and main cross-ring cleavages of a *C*-hexose. Adaptated from: Cuyckens and Claeys (2004) and Guo et al. (2013b).

In the present work, it was not possible to determine the position of the *C*-glycosylations (C6 or C8) for any of the apigenin derivatives detected (constituents from **79** to **83**), except for isovitexin (**81**), whose UV/visible spectrum was compared with a commercial standard (Figure 1.4). Although water loss fragments are more pronounced in 6-*C*-glycosides than in 8-*C*-glycosides, as mentioned above, in *M. neesii* extracts was found only one of these isomers, so no comparison was

possible. Nevertheless, the UV/visible spectra of these flavonoids are more similar to isovitexin (6-*C*-glycoside) than to vitexin (8-*C*-glycoside) (Figure 1.4). UV/visible spectrum of vitexin has a shoulder (302 nm) in Band I that is absent in isovitexin. Thus, it was assumed that *C*-glycosylations may be in position 6. It was not possible to determine the position of *O*-glycosylations.

The flavonoid **79** was partially identified as apigenin *O*-hexoside *C*-hexoside ($[M+H]^+$ = *m/z* 595.1684). The fragment at *m/z* 475.3254 (28%, $[M+H-120]^+$) is a typical loss of *C*-linked hexose and the base peak at *m/z* 433.1133 refers to the loss of a hexose ($[M+H-162]^+$), indicating an *O*-glycosylation. These two ion fragments show the presence of two hexose units, allowed the determination of apigenin. Additionally, fragments showing *m/z* 415.0955 (29%) and 313.0711 (11%) indicate loss of one water molecule and 120 amu, respectively, both after loss of the *C*-linked hexose residue. Apigenin *O*-hexoside *C*-hexoside has already been described in other bamboo species, including *Sasa veitchii* (Carrière) Rehder (Van Hoyweghen et al. 2012) and *Bambusa textilis* (Liu et al. 2016).

Flavonoid **80** was suggested to be apigenin *O*-pentoside *C*-hexoside ($[M+H]^+$ = *m/z* 565.1574). The base peak at *m/z* 433.1143 refers to the loss of a pentose ($[M+H-132]^+$), which indicates an *O*-glycosylation. In addition, ions at *m/z* 415.0957 (20%, $[M+H\text{-pentose-}H_2O]^+$) and 313.0727 (10%, $[M+H\text{-pentose-}120]^+$) indicate the presence of a *C*-hexose. Again, apigenin was determined as the aglycone portion. Apigenin *O*-pentoside *C*-hexoside has also been already reported in Bambusoideae (Lv et al. 2011; Liu et al. 2016; Qiu and Zhang 2019).

Flavonoid **81** was described as isovitexin (apigenin 6-*C*-glucoside, $[M+H]^+$ = *m/z* 433.1148). Identification was performed by analyzing the UV/visible spectrum (Figure 1.4), the mass fragmentation pattern and comparing it with data in the literature. Fragmentation of the base peak resulted in fragments related to water loss (*m/z* 415.1088, 397.0955, and 379.0782) and diagnostic of a *C*-hexose ($[M+H-120]^+$ and $[M+H-150]^+$), confirming this compound as an apigenin-derivative mono-*C*-glycoside. Moreover, the fragmentation pattern was very similar to that found in the literature for isovitexin analyzed by UHPLC-ESI-QTOF-MS-MS with similar collision energy (10 eV) (Guo et al. 2013b) to that employed in this study (12 eV). The UV/visible spectrum was also compared to that of isovitexin. This flavone has been often reported for several bamboo species (Lv et al. 2011; Ni et al. 2012; Van Hoyweghen et al. 2012; Wang et al. 2012; Guo et al. 2013a; Tanaka et al. 2014; Liu et al. 2016, Qiu and Zhang 2019; Zhao et al. 2019).

Peak **82** was tentatively identified as apigenin *O*-hexoside *C*-pentoside ($[M+H]^+$ = m/z 565.1590). The base peak of this flavonoid at *m/z* 403.1048 ($[M+H-162]^+$) indicates the loss of an *O*-hexose. Fragments at *m/z* 385.0841 ($[M+H\text{-pentose-}H_2O]^+$), 367.0738 ($[M+H\text{-pentose-}2H_2O]^+$), and 313.0707 ($[M+H\text{-pentose-}90]^+$) characterized the presence of a *C*-pentose. Qiu and Zhang (2019) analyzed the chemical profile of *Bambusa multiplex* (Lour.) Raeusch. ex Schult. by UHPLC-

DAD-QTOF-MS (negative mode) and detected a compound ($[M-H]^-$ = *m/z* 563.1404) that exhibited ions at *m/z* 401 and 311; however, the authors did not propose an identification. On the other hand, Dong et al. (2014) detected 91 flavonoids in rice, among them a flavonoid ($[M+H]^+$ = *m/z* 565.1554) that was suggested as *C*-pentosyl-apigenin *O*-hexoside. This apigenin derivative also presented fragments at *m/z* 403, 367, and 313, among others.

The fifth apigenin derivative (**83**) found in *M. neesii* was partially identified as apigenin *O*-pentoside *C*-pentoside ($[M+H]^+$ = *m/z* 535.1501). The fragment at *m/z* 403.1053 (base peak, $[M+H-132]^+$) is formed by a typical loss of an *O*-linked pentose. Furthermore, ions at *m/z* 385.0844 (12%, $[M+H\text{-pentose-}H_2O]^+$), 367.0741 (10%, $[M+H\text{-pentose-}2H_2O]^+$), and 313.0707 (3%, $[M+H\text{-pentose-}90]^+$) suggests the presence of another pentose unit, but bonded to a carbon atom. Apigenin *O*-pentoside *C*-pentoside with *O*-glycosylation either in the phenolic hydroxyl group or in the *C*-linked pentose hydroxyl group has already been described in bamboo species (Van Hoyweghen et al. 2012).

Regarding flavonoids **84** to **88**, all showed a fragment at *m/z* 331, suggesting that these compounds are tricin derivatives (MW 330); however, it was not possible to determine the substituents. Thus, these compounds were only identified as tricin derivatives, another flavone commonly found in several bamboo species (Park et al. 2007; Kim et al. 2009; Lv et al. 2011; Van Hoyweghen et al. 2012; Guo et al. 2013a; Tanaka et al. 2014; Van Hoyweghen et al. 2014; Liu et al. 2016; Zhao et al. 2019).

For both hydroethanol and aqueous extracts, the major flavonoids in leaves and culms were constituents **83** (apigenin derivative) and **84** (tricin derivative), respectively. The second most abundant flavonoid was constituent **80** (apigenin derivative) for both organs (Figure S1.3). Thus, flavonoid profile data for leaves and culms of *M. neesii* corroborate those described for other bamboo species, which mainly present *C*-glycosides derivatives of the flavones apigenin and luteolin and derivatives of tricin.

## 4. Conclusions

In general, studies of chemical characterization of bamboo are focused on the nutritional aspects of bamboo shoots. Native bamboos are even more poorly studied, especially *Merostachys*, whose center of diversity is located in Brazil. So far only three other species of this genus (*M. riedeliana*, *M. pluriflora* Munro ex E.G.Camus, and *M. magellanica* Send.) have been chemically studied.

From the 88 compounds detected in *M. neesii*, 64 were partial identified. Chemical profile of *M. neesii* corroborates those obtained for other bamboo species, and most of the compounds identified in this work are described for the first time in this genus. Moreover, hydroethanol extracts

showed several phenolic compounds besides flavonoids. Although sometimes in smaller quantities than flavonoids, it demonstrates that *M. neesii* polar extracts are quite complex and need further studies. In conclusion, *M. neesii* showed the presence of several compounds that may have nutritional and/or medicinal value.

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**Supplementary material 1**

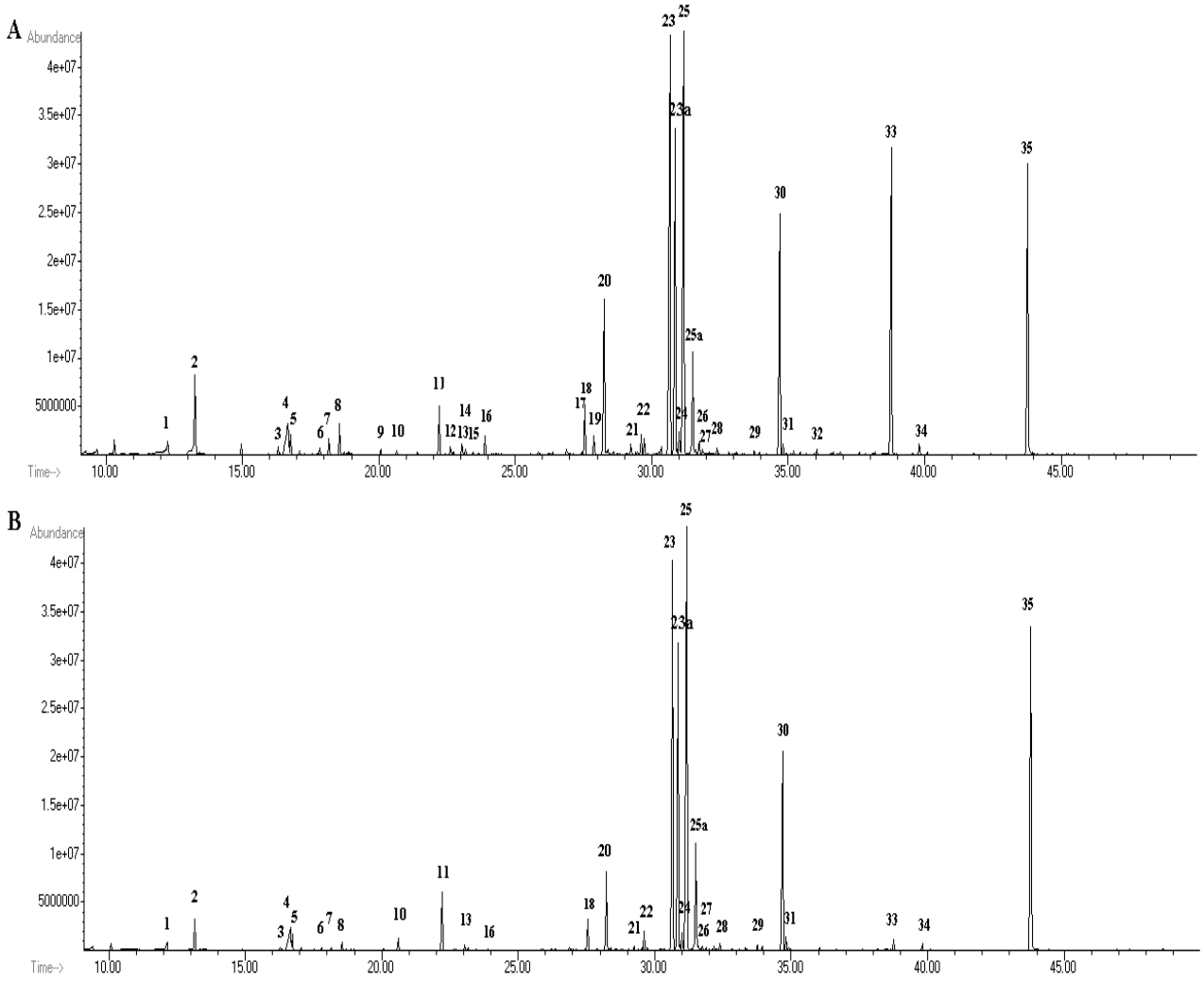


**Figure S1.1:** GC-EIMS chromatograms of polar phases of hot methanol extract of *Merostachys neesii* Rupr. A) **HME-L**; and B) **HME-C**. Numbers above each peak correspond to those shown in Table 1.1. HME-L: polar phase of hot methanol extract of leaves; HME-C: polar phase of hot methanol extract of culms.

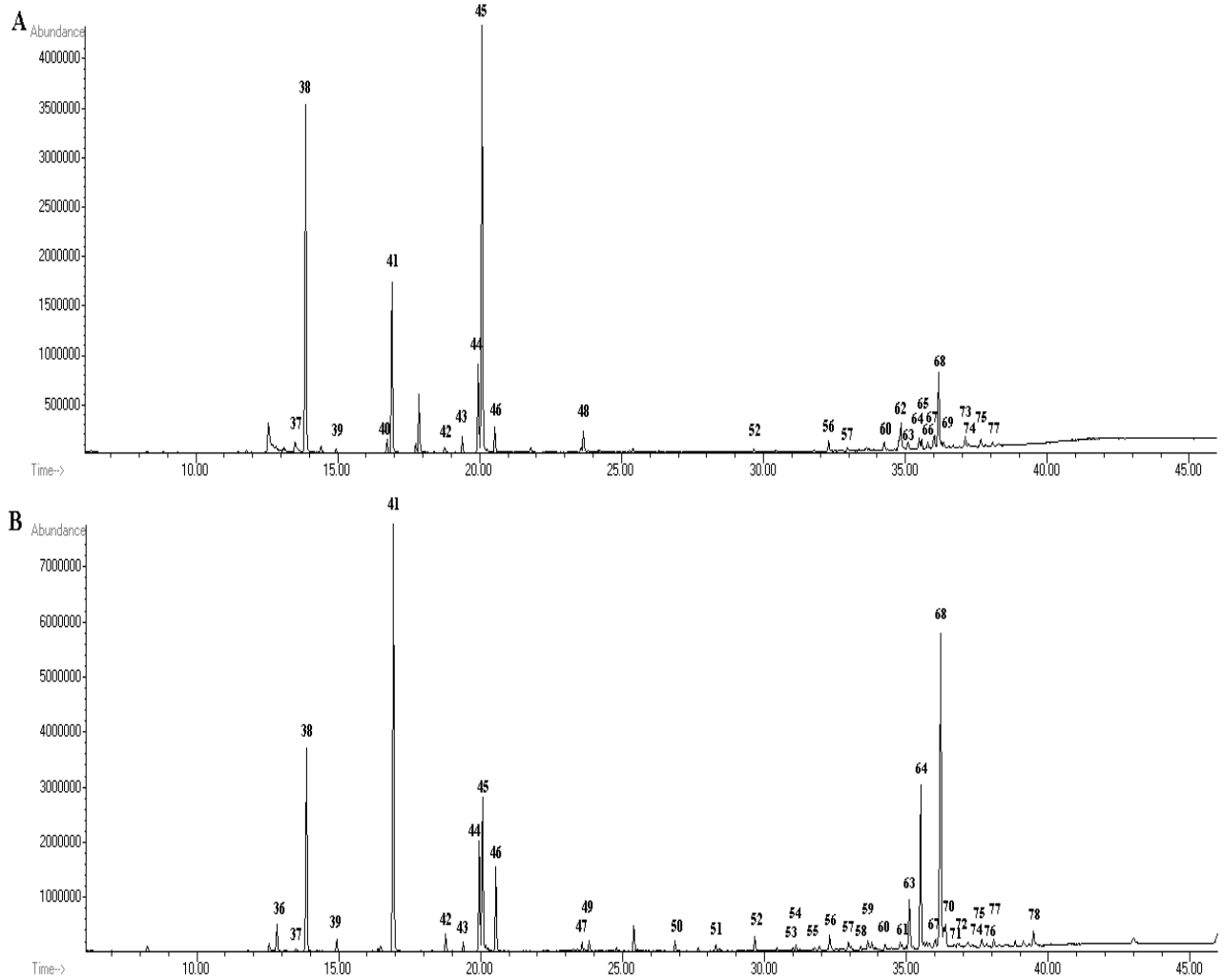


**Figure S1.2:** GC-EIMS chromatograms of hexane extracts of *Merostachys neesii* Rupr. A) **HE-L**; and B) **HE-C**. Numbers above each peak correspond to those shown in Table 1.2. HE-L: hexane extract of leaves; HE-C: hexane extract of culms.

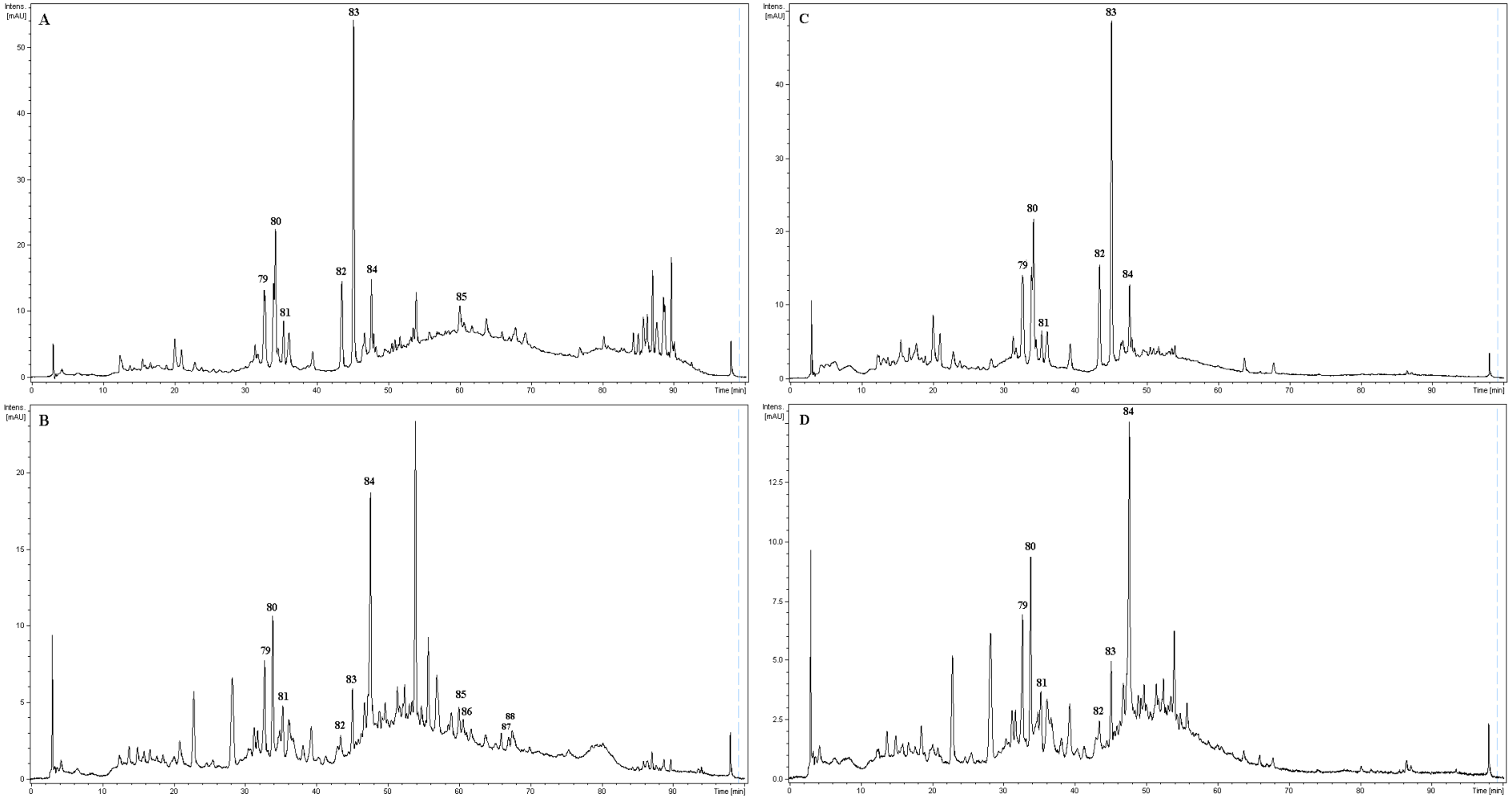


**Figure S1.3:** HPLC-ESI/HRMS chromatograms of hydroethanol and aqueous extracts of *Merostachys neesii* Rupr. obtained at 340 nm. A) **EE-L**; B) **EE-C**; C) **AE-L**; and D) **AE-C**. Numbers above each peak correspond to those shown in Table 1.3. EE-L: hydroethanol extract of leaves; EE-C: hydroethanol extract of culms; AE-L: aqueous extract of leaves; AE-C: aqueous extract of culms.

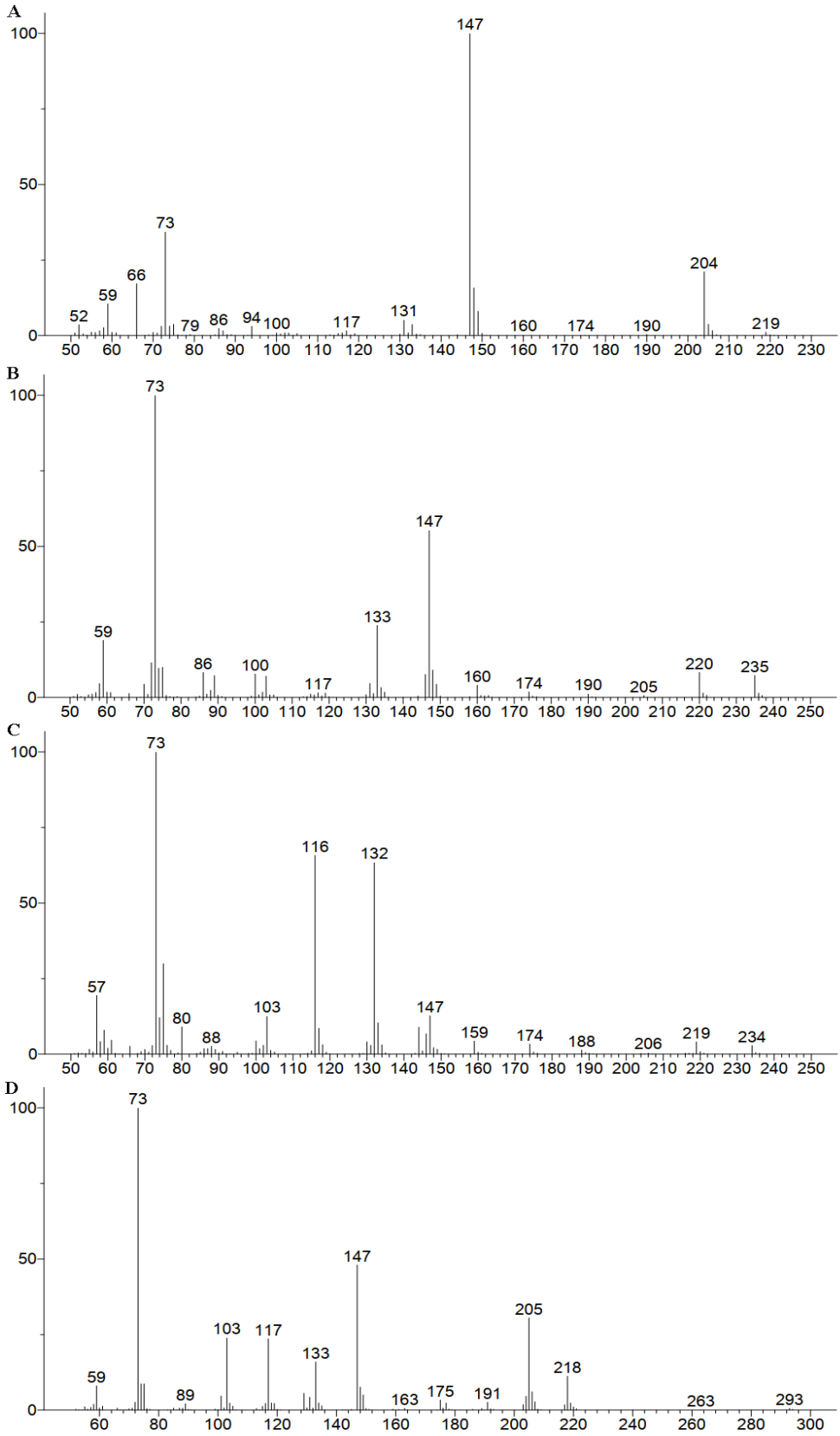


**Figure S1.4:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Oxalic acid TMS derivative (**1**); B) NI-1 (**2**); C) Serine TMS derivative (**3**); and D) Glycerol TMS derivative (**4**).

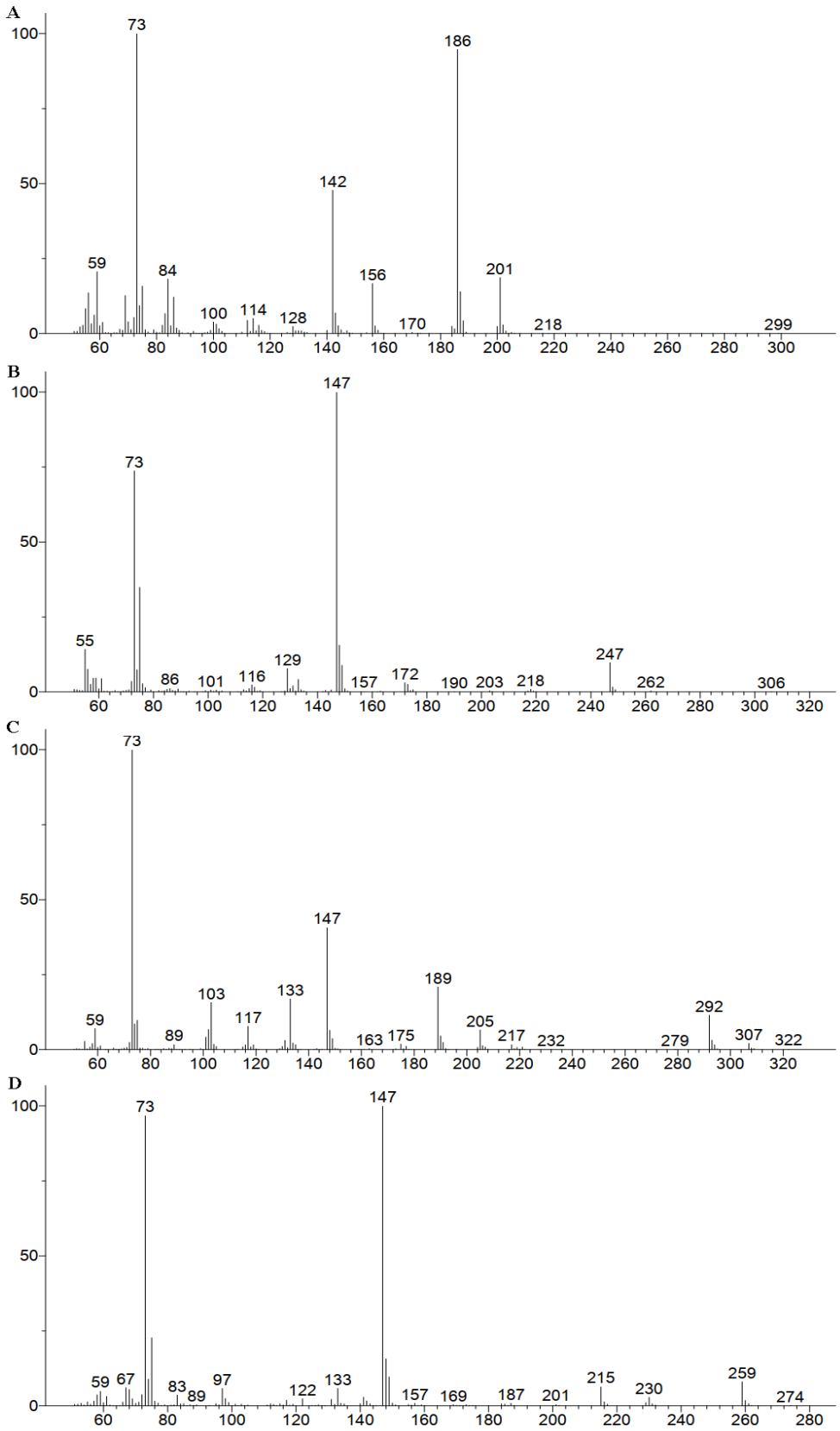


**Figure S1.5:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) NI-2 (**5**); B) Succinic acid TMS derivative (**6**); C) Glyceric acid TMS derivative (**7**); and D) Itaconic acid TMS derivative (**8**).

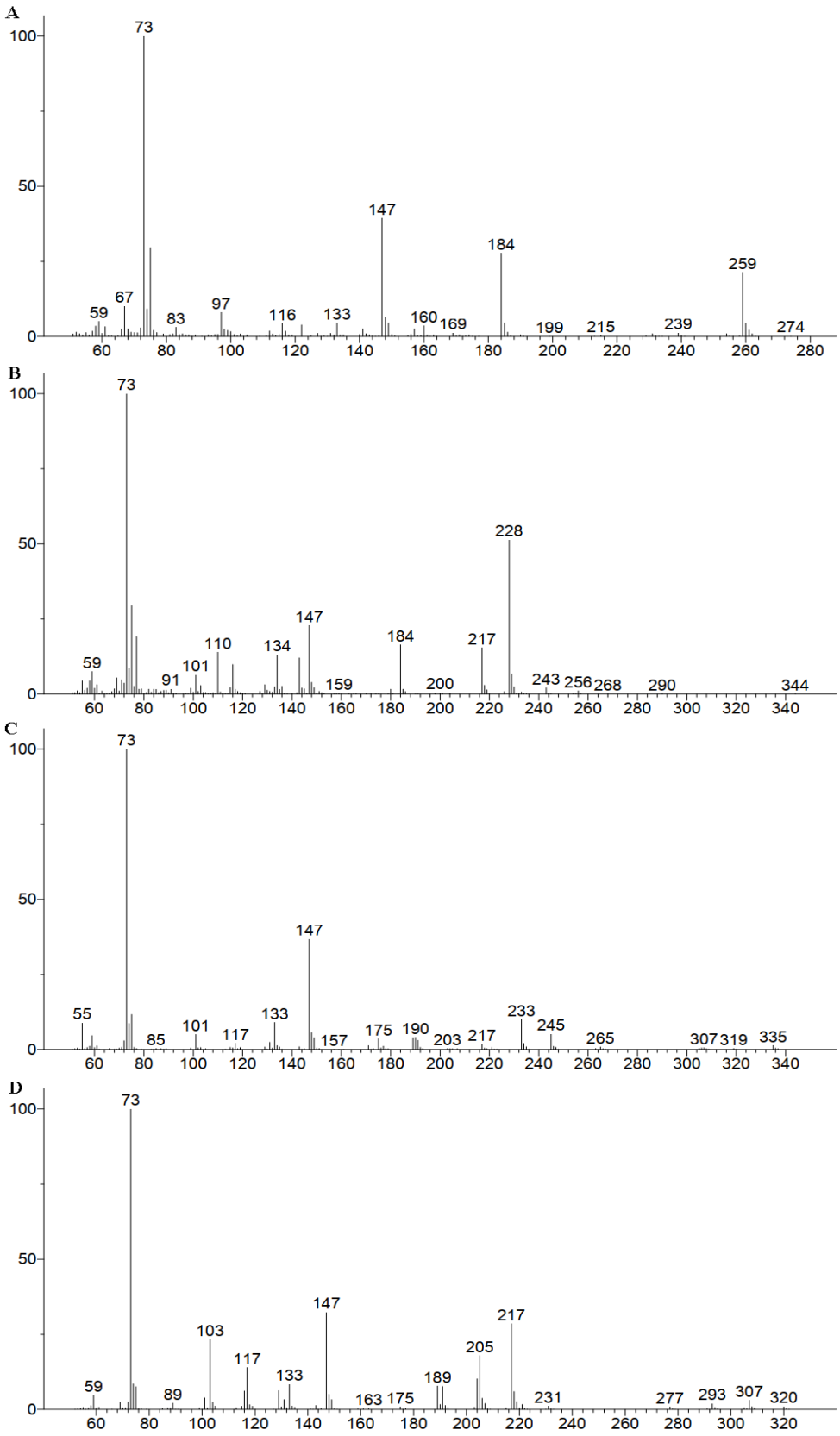


**Figure S1.6:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Mesaconic acid TMS derivative (**9**); B) NI-3 (**10**); C) Malic acid TMS derivative (**11**); and D) Erythritol TMS derivative (**12**).

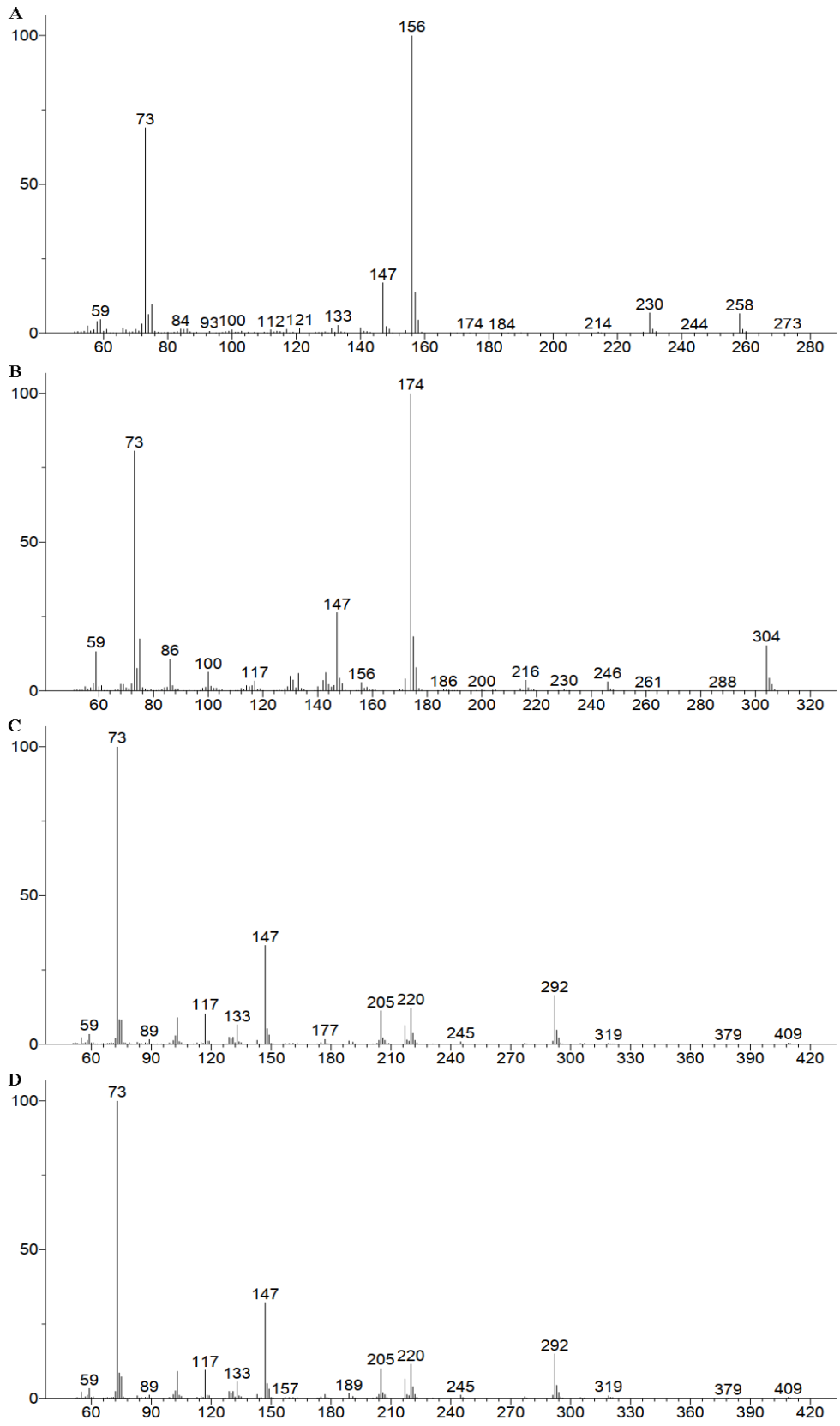


**Figure S1.7:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) 5-oxoproline TMS derivative (**13**); B) GABA TMS derivative (**14**); C) Erythronic acid TMS derivative (**15**); and D) Threonic acid TMS derivative (**16**).

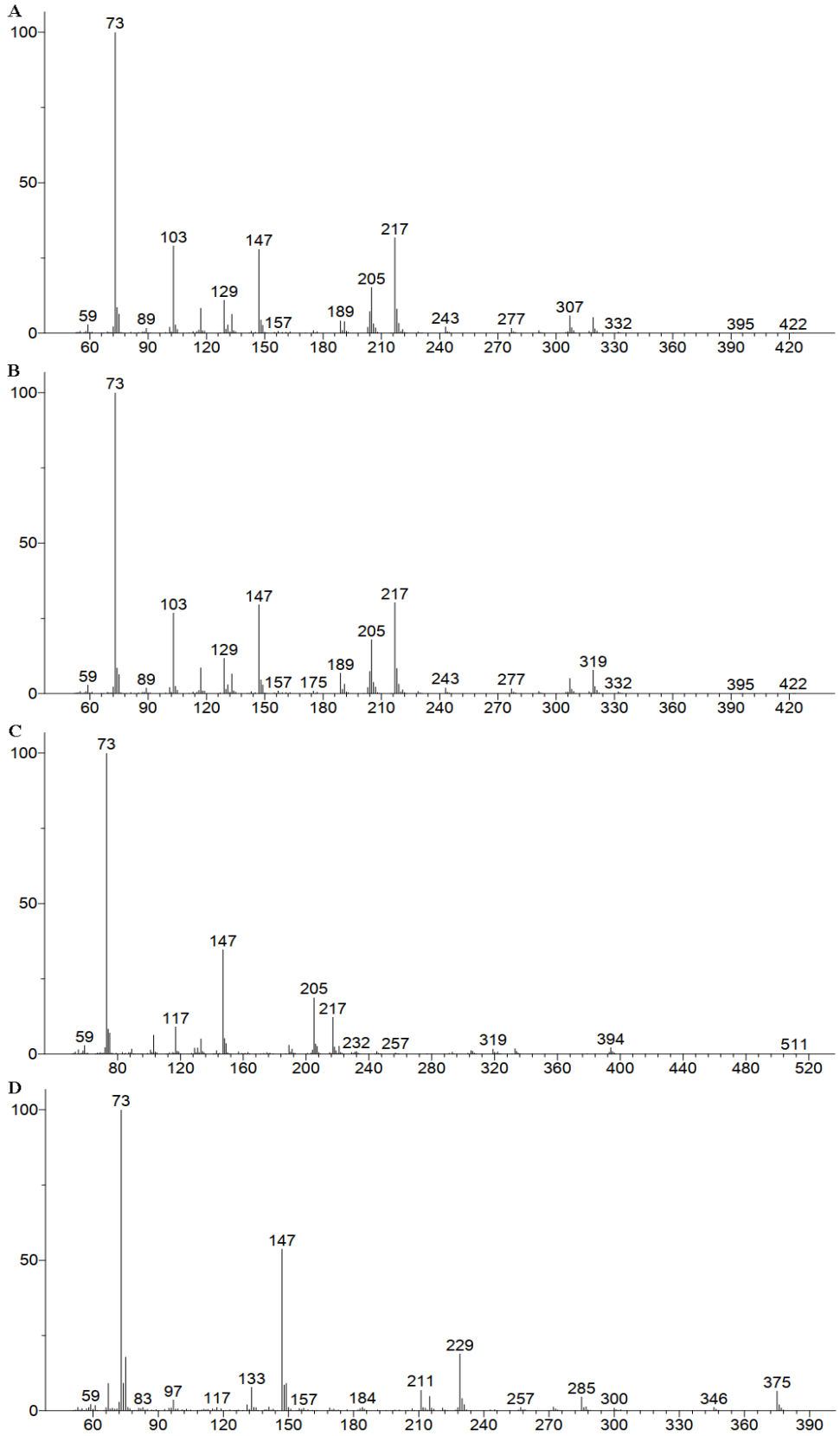


**Figure S1.8:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Arabitol TMS derivative (**17**); B) Ribitol TMS derivative (internal standard, **18**); C) NI-4 (**19**); and D) Aconitic acid TMS derivative (**20**).

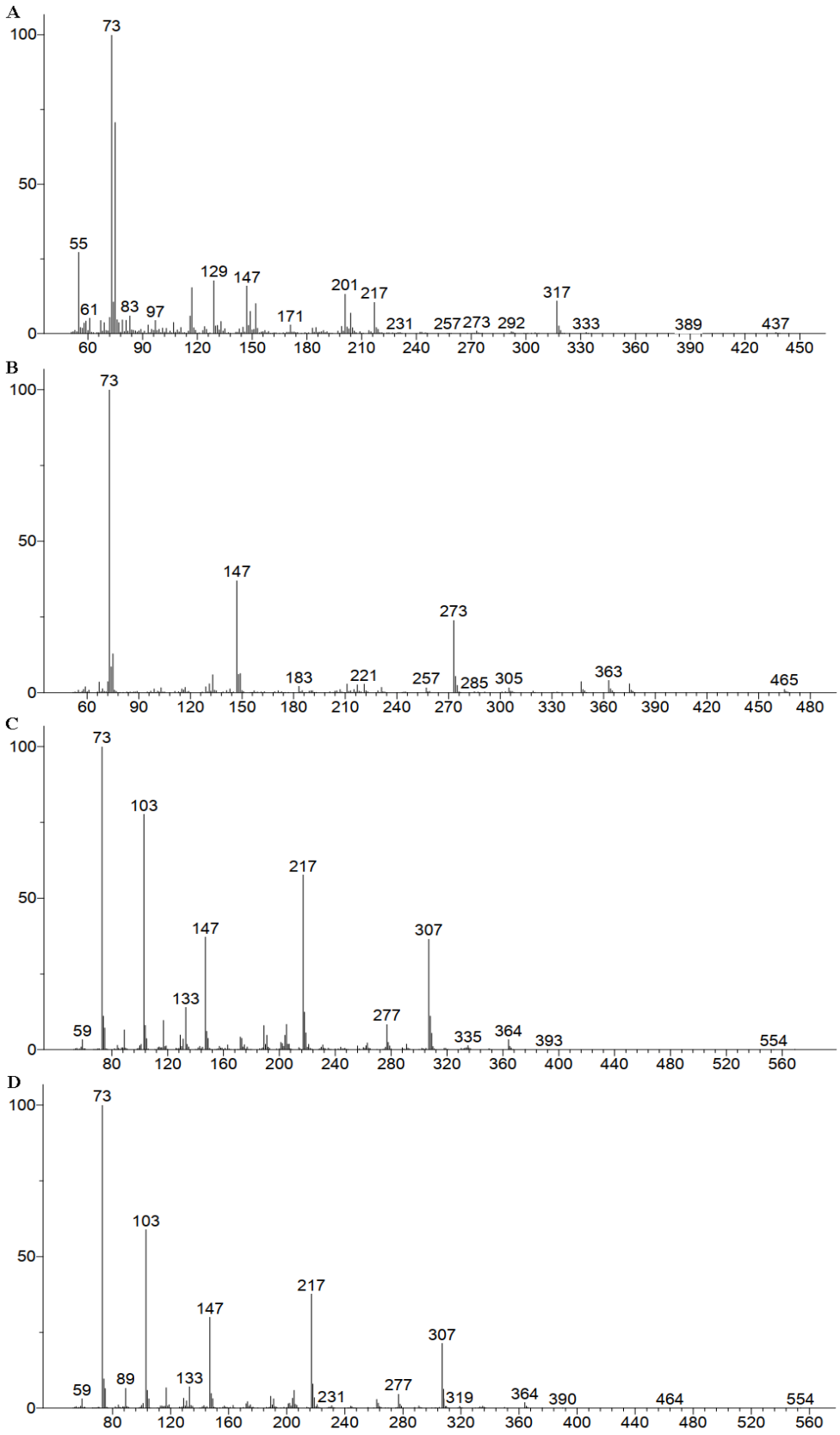


**Figure S1.9:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Azelaic acid TMS derivative (**21**); B) Citric acid TMS derivative (**22**); C) Fructose TMS/MeOX derivative (**23**); and D) Fructose TMS/MeOX derivative (**23a**).

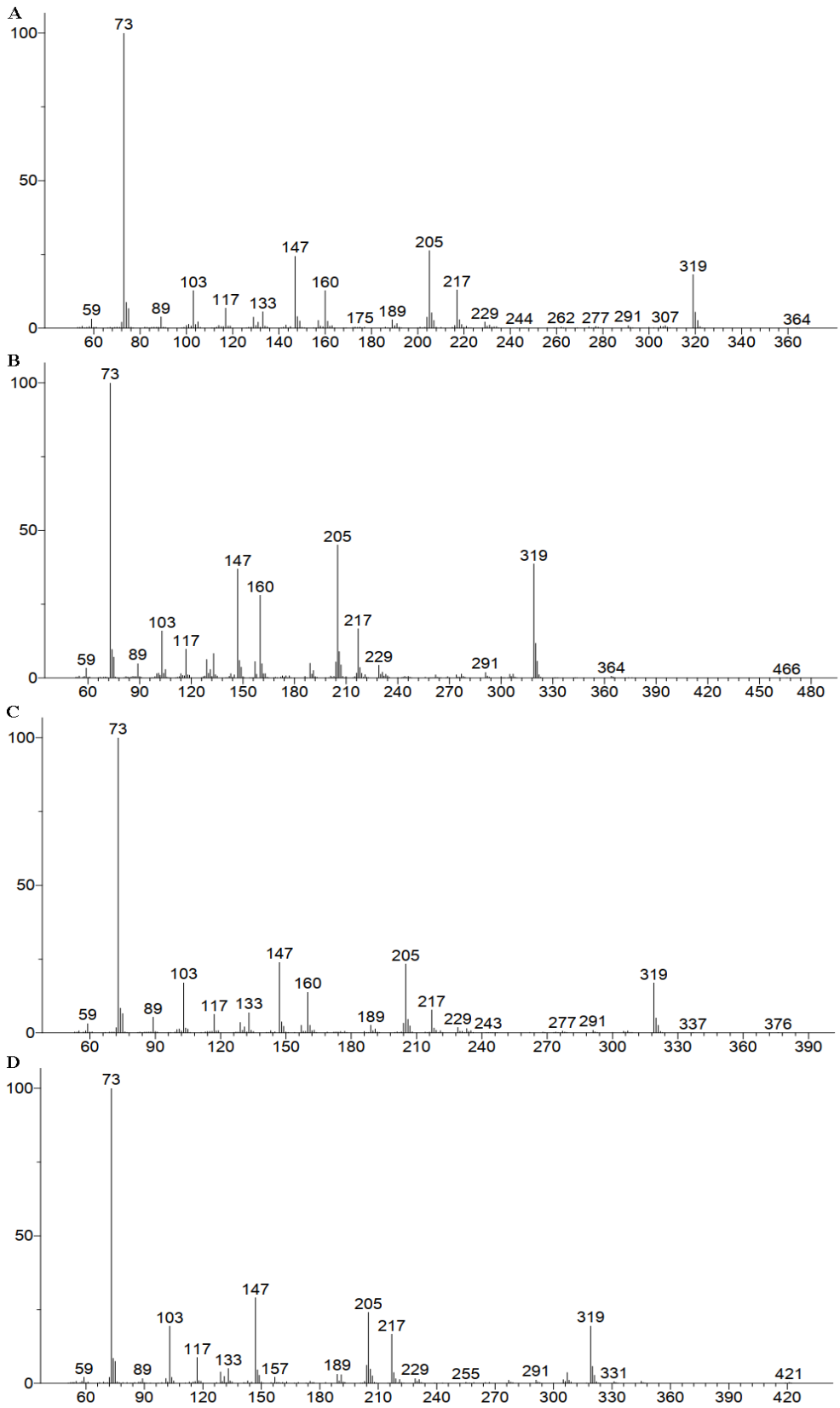


**Figure S1.10:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Galactose TMS/MeOX derivative (**24**); B) Glucose TMS/MeOX derivative (**25**); C) Glucose TMS/MeOX derivative (**25a**); and D) Mannitol TMS derivative (**26**).

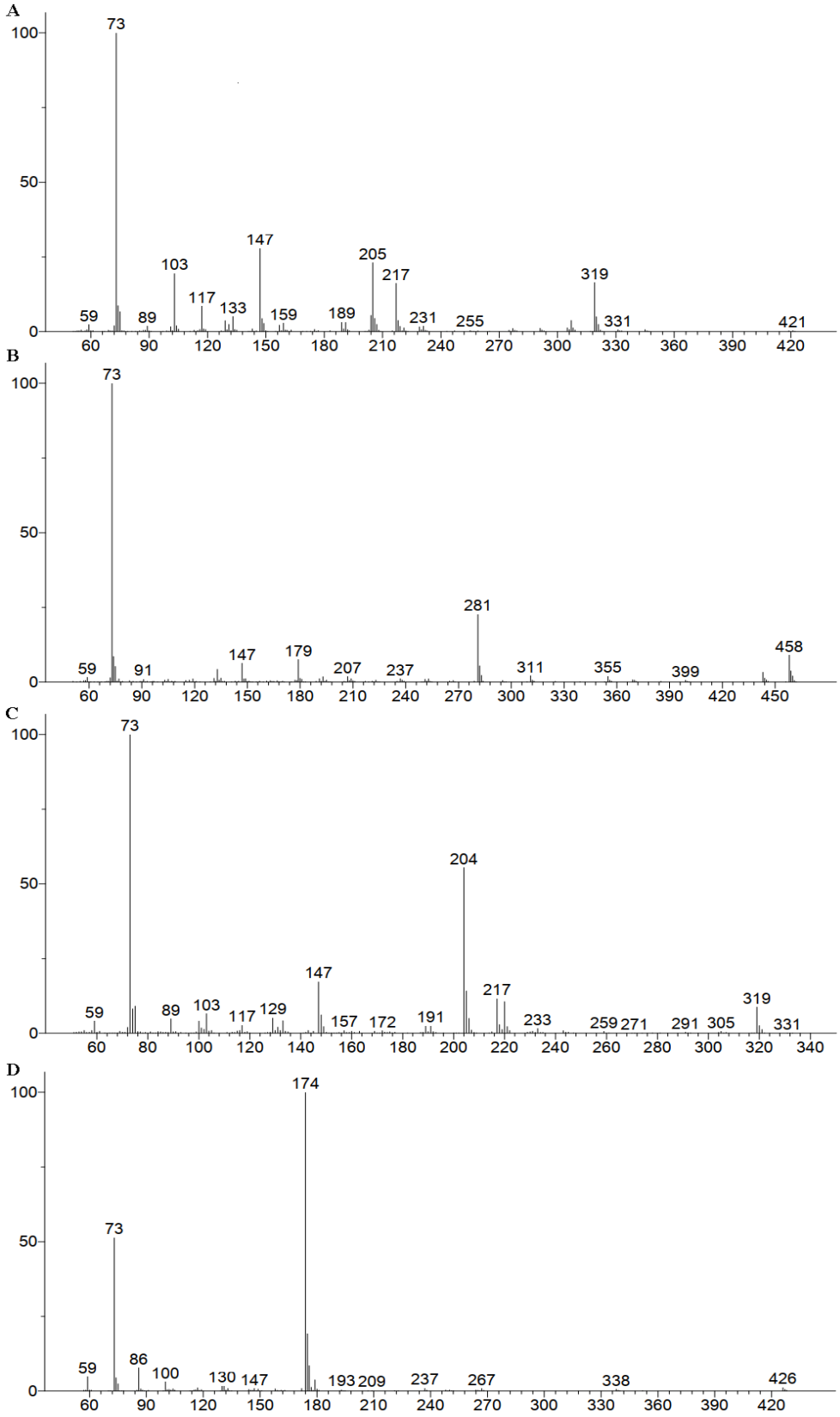


**Figure S1.11:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Sorbitol TMS derivative (**27**); B) Gallic acid TMS derivative (**28**); C) NI-5 (**29**); and D) Dopamine TMS derivative (**30**).

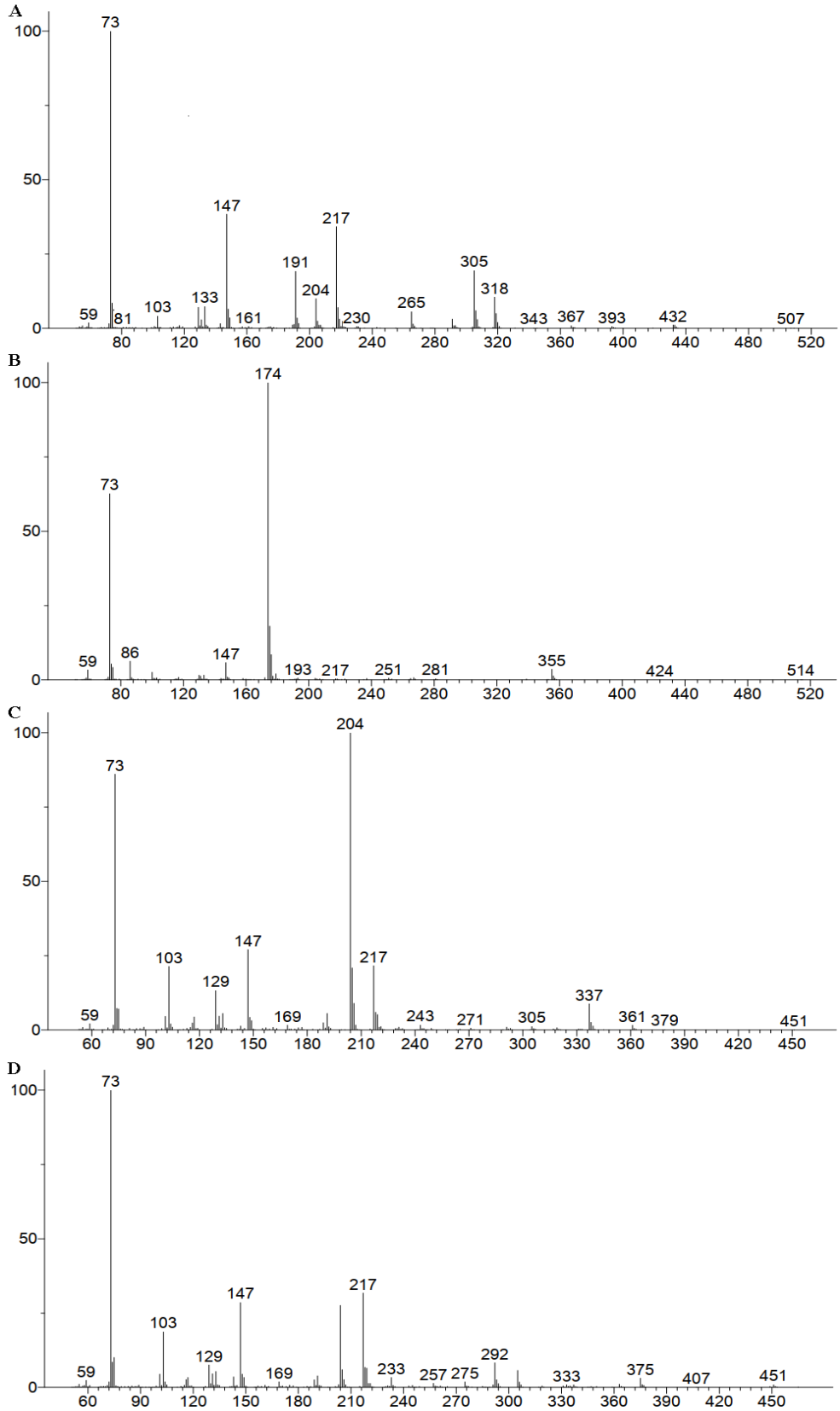


**Figure S1.12:** Mass spectra of compounds detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) *Myo*-inositol TMS derivative (**31**); B) Norepinephrine TMS derivative (**32**); C) NI-6 (**33**); and D) NI-7 (**34**).

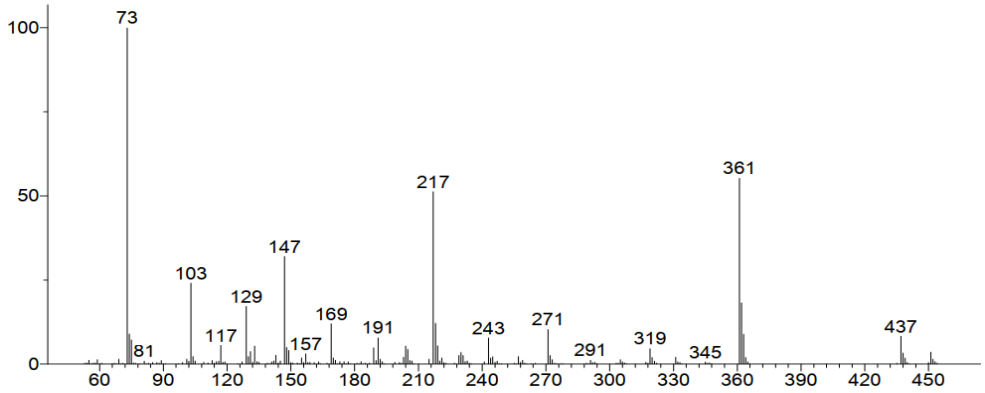


**Figure S1.13:** Mass spectra of sucrose TMS derivative (**35**) detected in the polar phase of the hot methanol extract of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS.

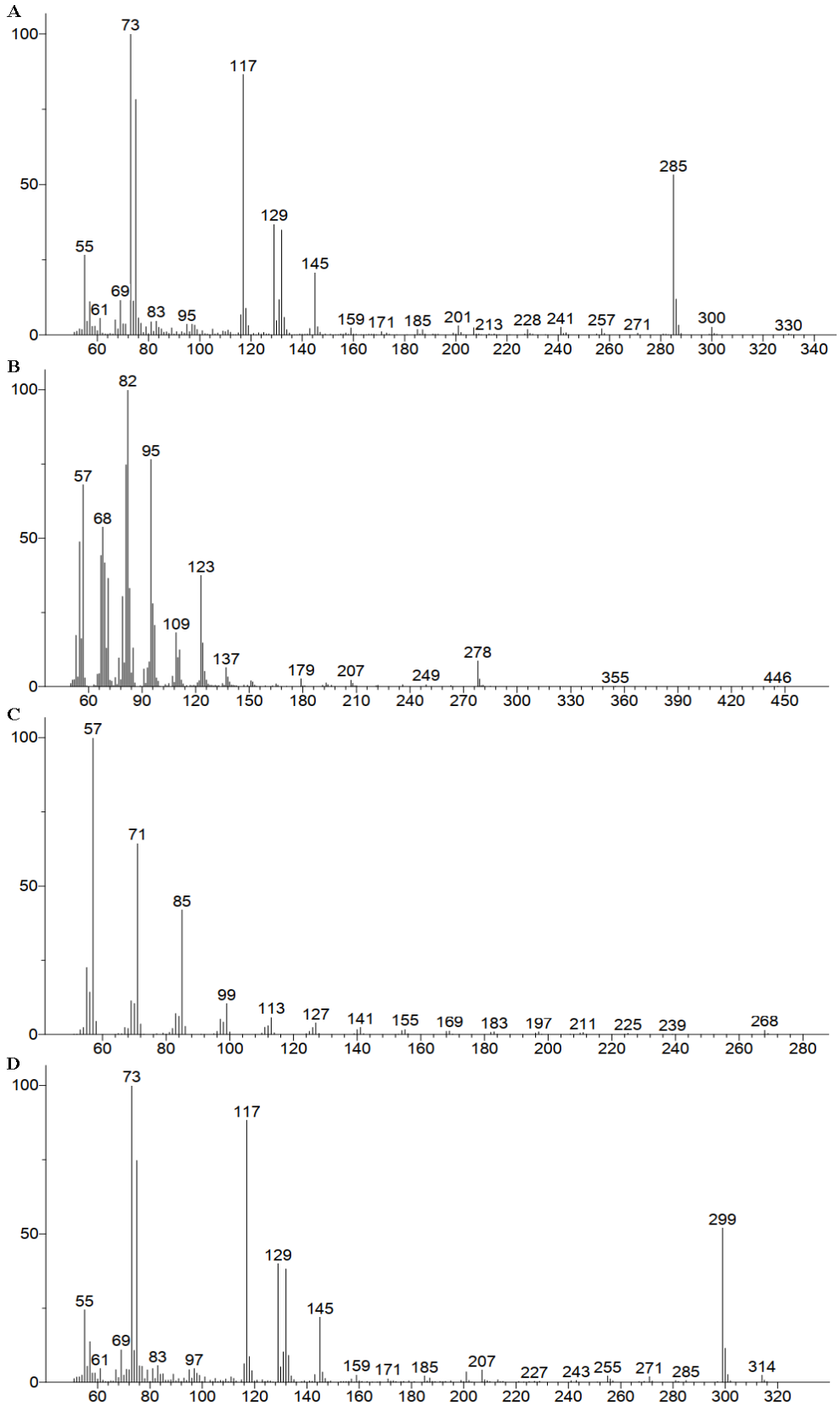


**Figure S1.14:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Myristic acid TMS derivative (**36**); B) NI-8 (**37**); C) Nonadecane (internal standard, **38**); and D) Pentadecanoic acid TMS derivative (**39**).

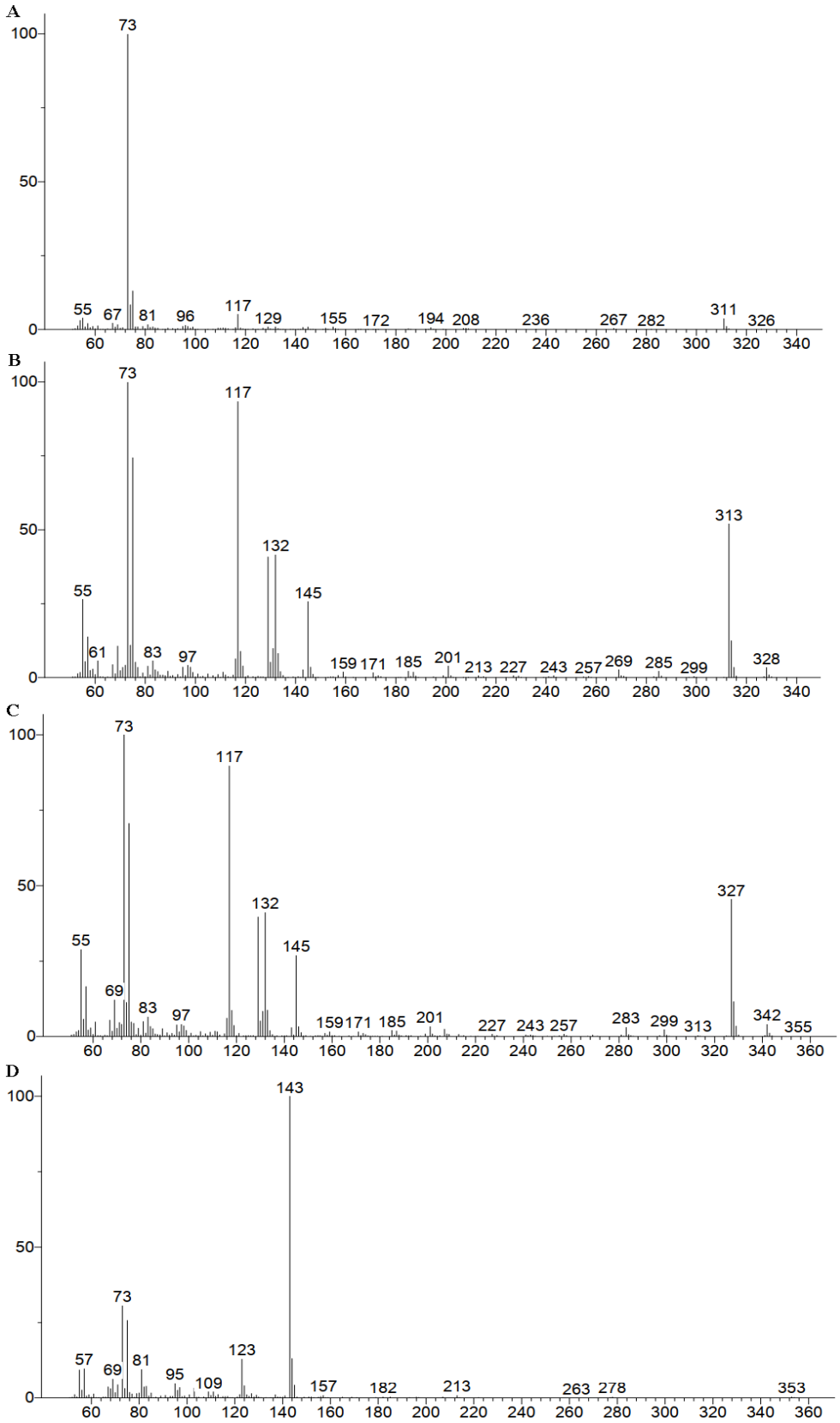


**Figure S1.15:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) NI-9 (**40**); B) Palmitic acid TMS derivative (**41**); C) Heptadecanoic acid TMS derivative (**42**); and D) Phytol TMS derivative (**43**).

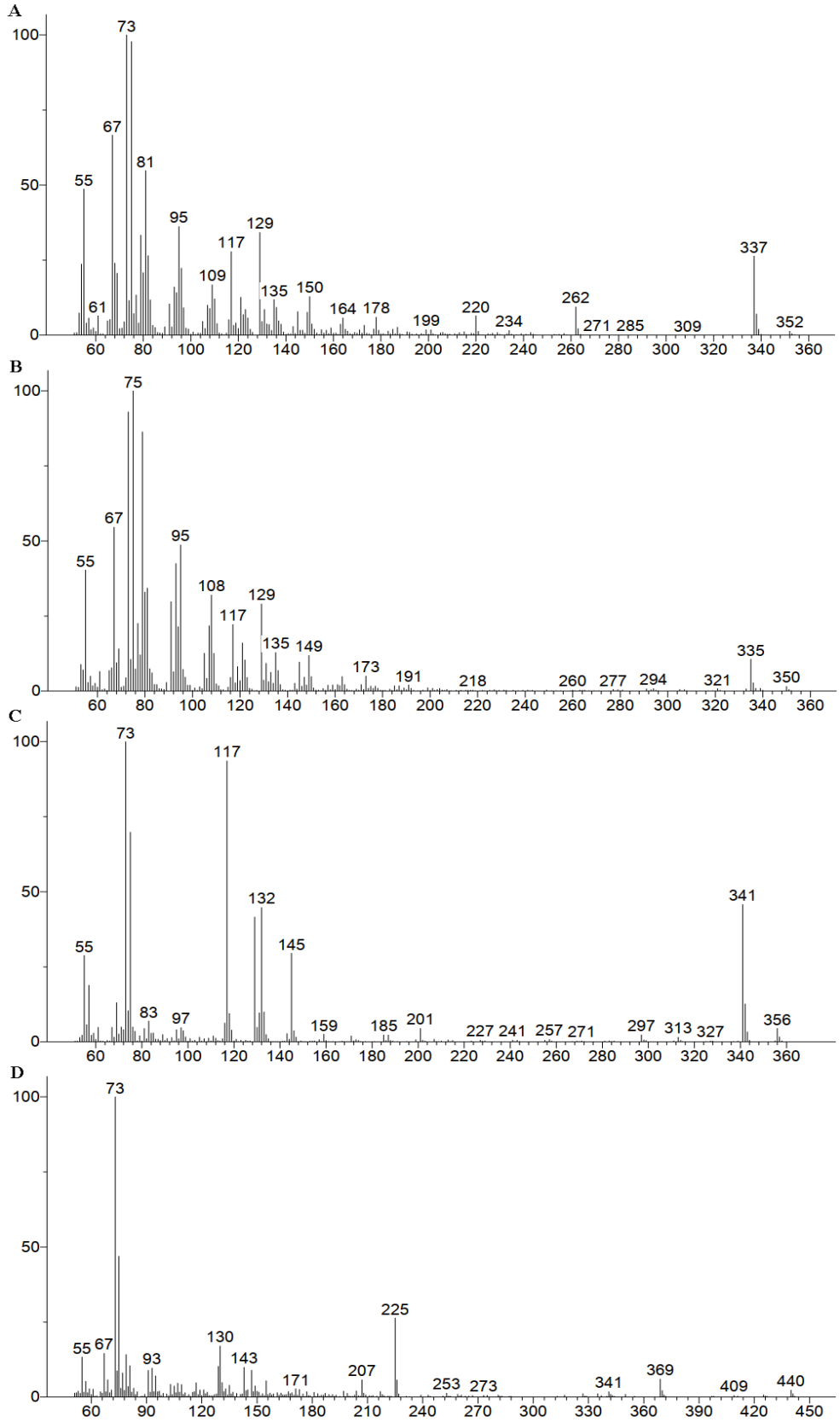


**Figure S1.16:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Linoleic acid TMS derivative (**44**); B) Linolenic acid TMS derivative (**45**); C) Stearic acid TMS derivative (**46**); and D) NI-10 (**47**).

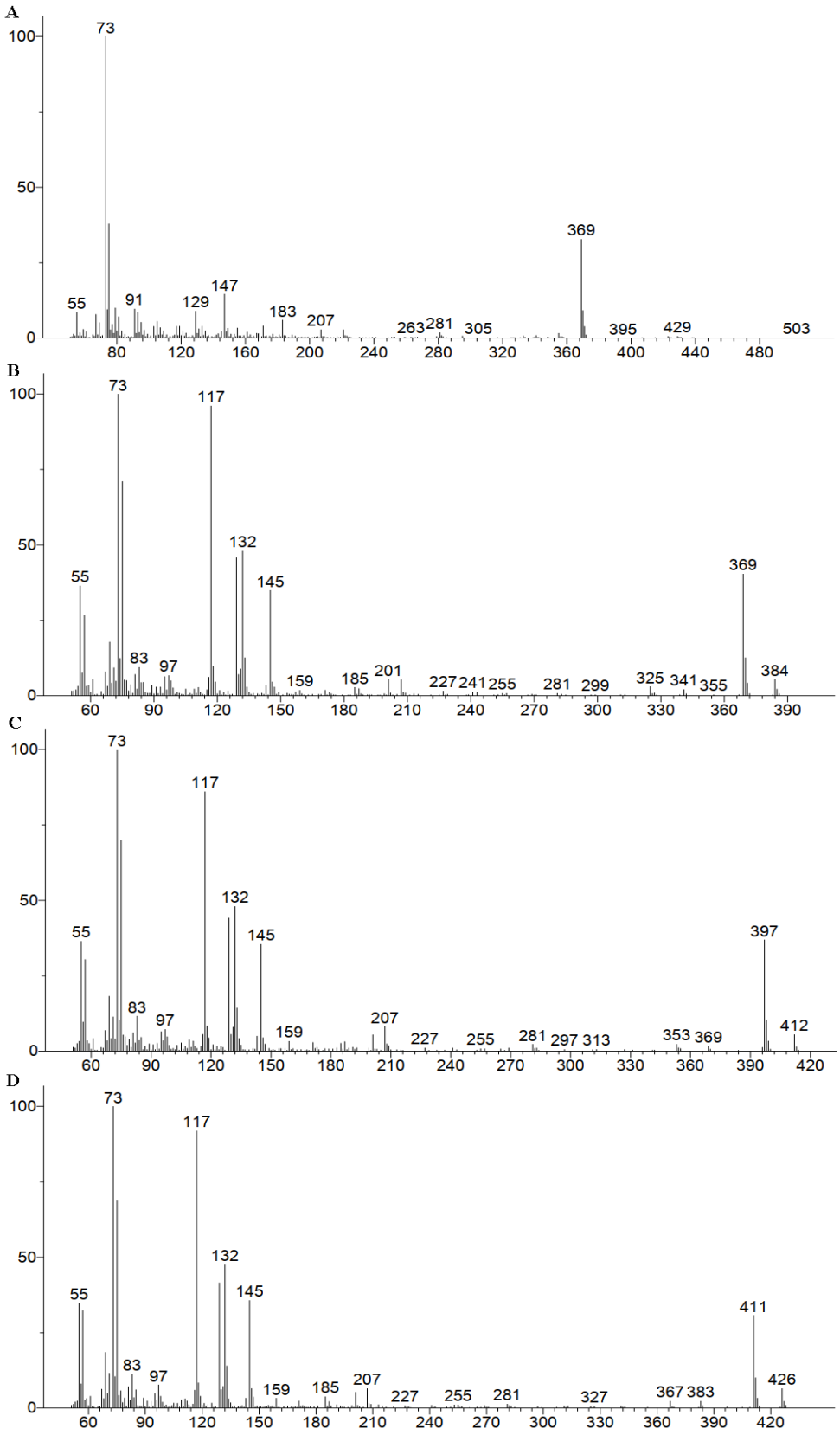


**Figure S1.17:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) NI-11 (**48**); B) Arachidic acid TMS derivative (**49**); C) Behenic acid TMS derivative (**50**); and D) Tricosanoic acid TMS derivative (**51**).

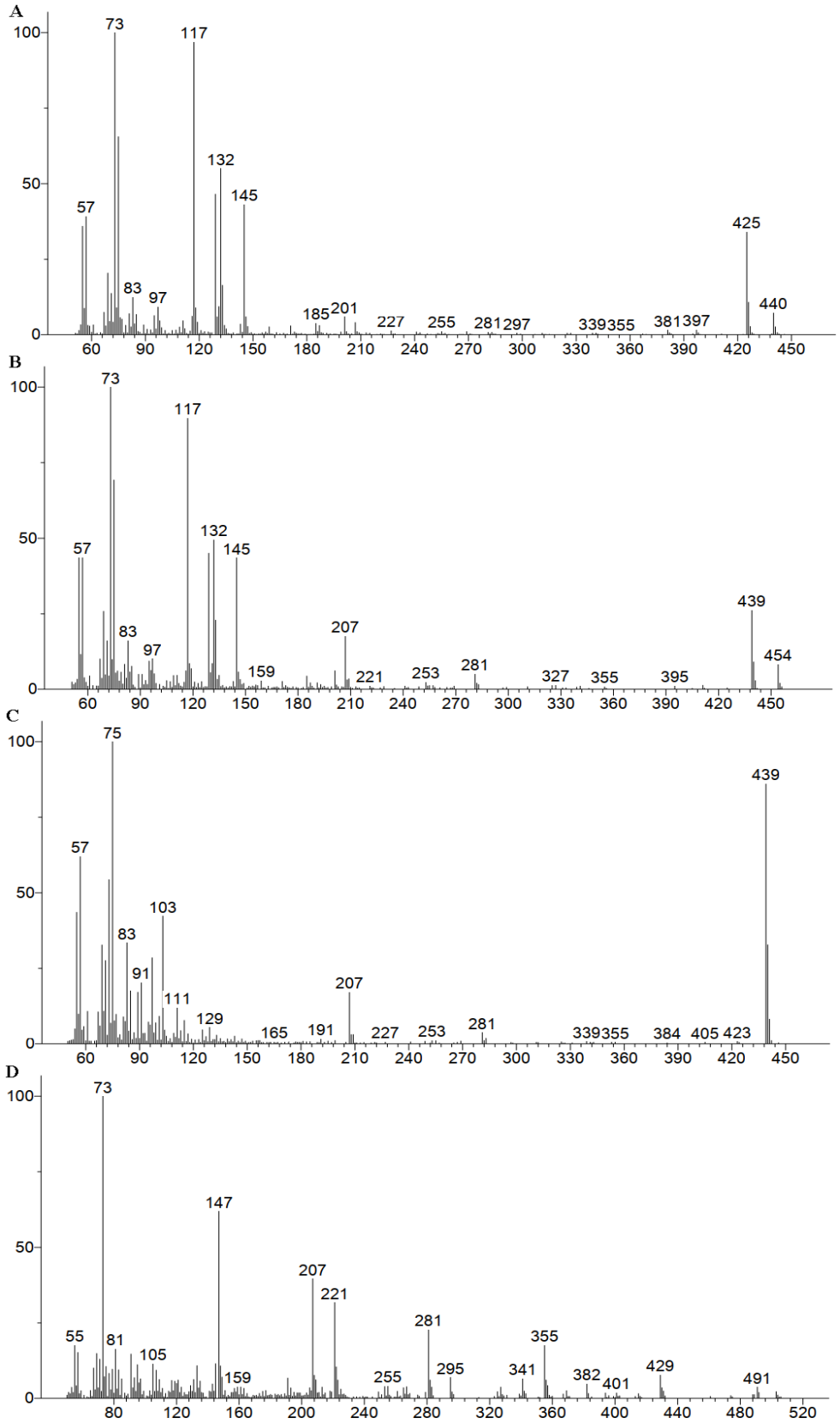


**Figure S1.18:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Lignoceric acid TMS derivative (**52**); B) Pentacosanoic acid TMS derivative (**53**); C) 1-hexacosanol TMS derivative (**54**); and D) NI-12 (**55**).

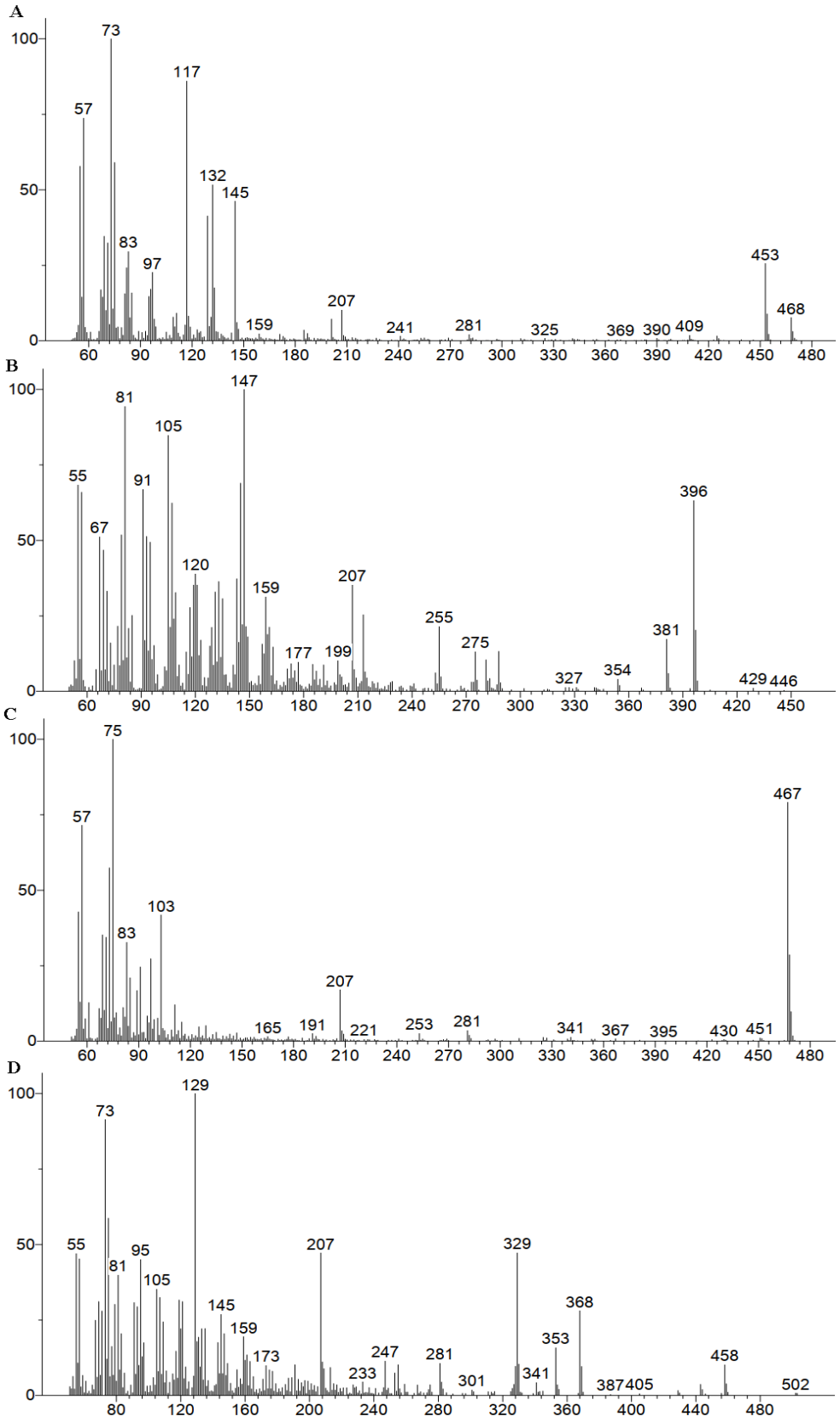


**Figure S1.19:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Hexacosanoic acid TMS derivative (**56**); B) Stigmata-3,5-diene (**57**); C) 1-octacosanol TMS derivative (**58**); and D) Cholesterol TMS derivative (**59**).

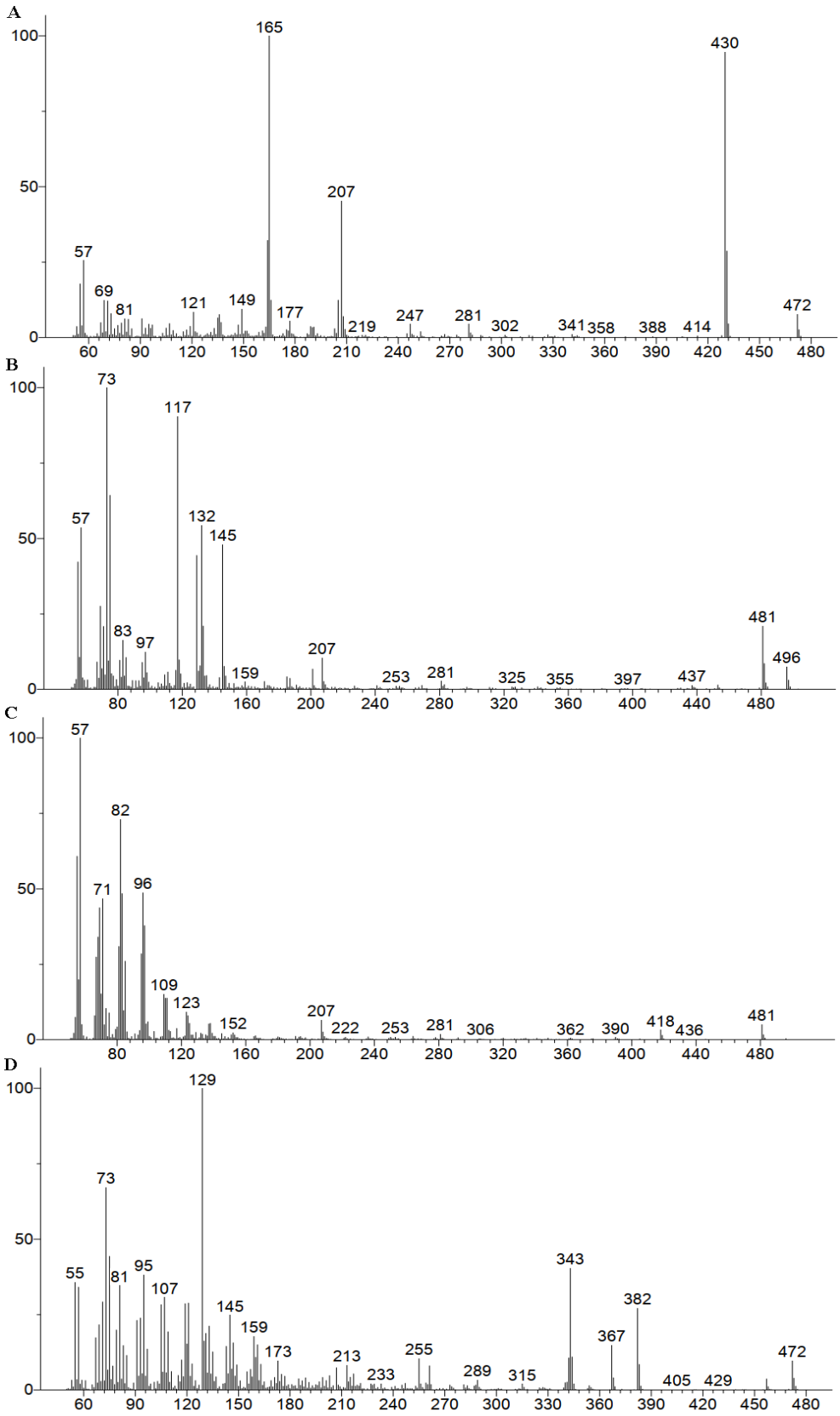


**Figure S1.20:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) α-Tocopherol acetate (**60**); B) Octacosanoic acid TMS derivative (**61**); C) NI-13 (**62**); and D) Campesterol TMS derivative (**63**).

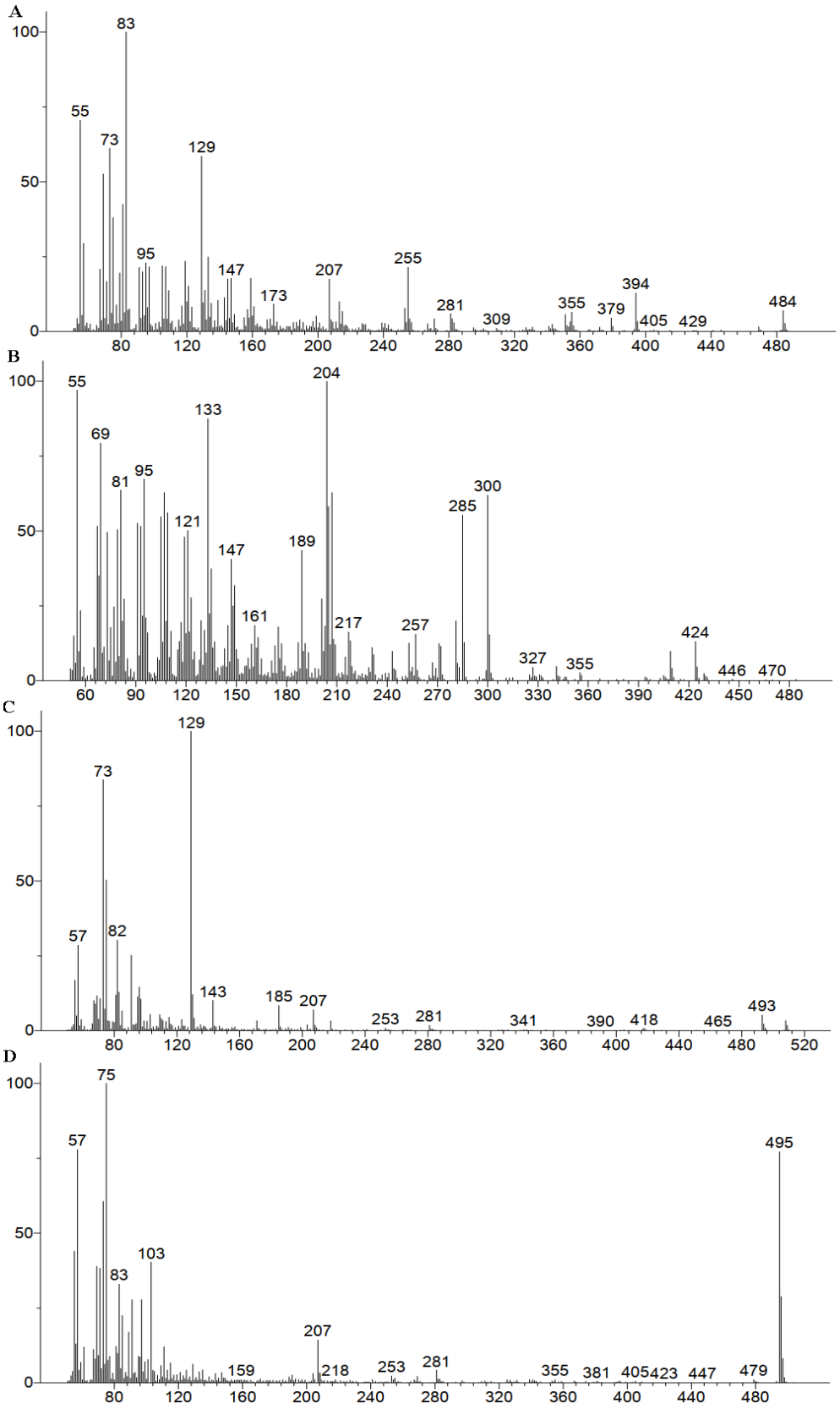


**Figure S1.21:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) Stigmasterol TMS derivative (**64**); B) NI-14 (**65**); C) NI-15 (**66**); and D) 1-triacontanol TMS derivative (**67**).

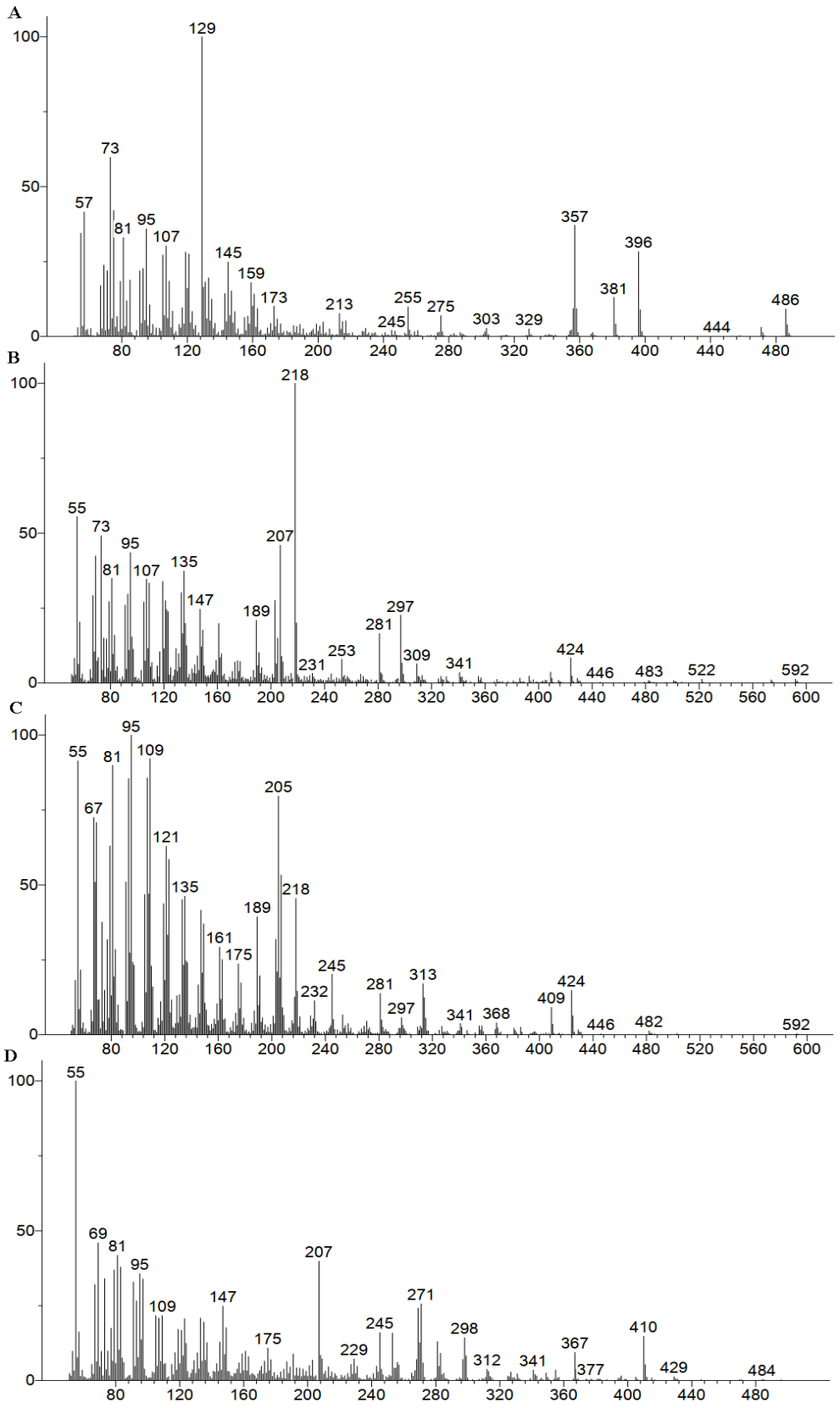


**Figure S1.22:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) *β*-sitosterol TMS derivative (**68**); B) NI-16 (**69**); C) Lupenone (**70**); and D) NI-17 (**71**).

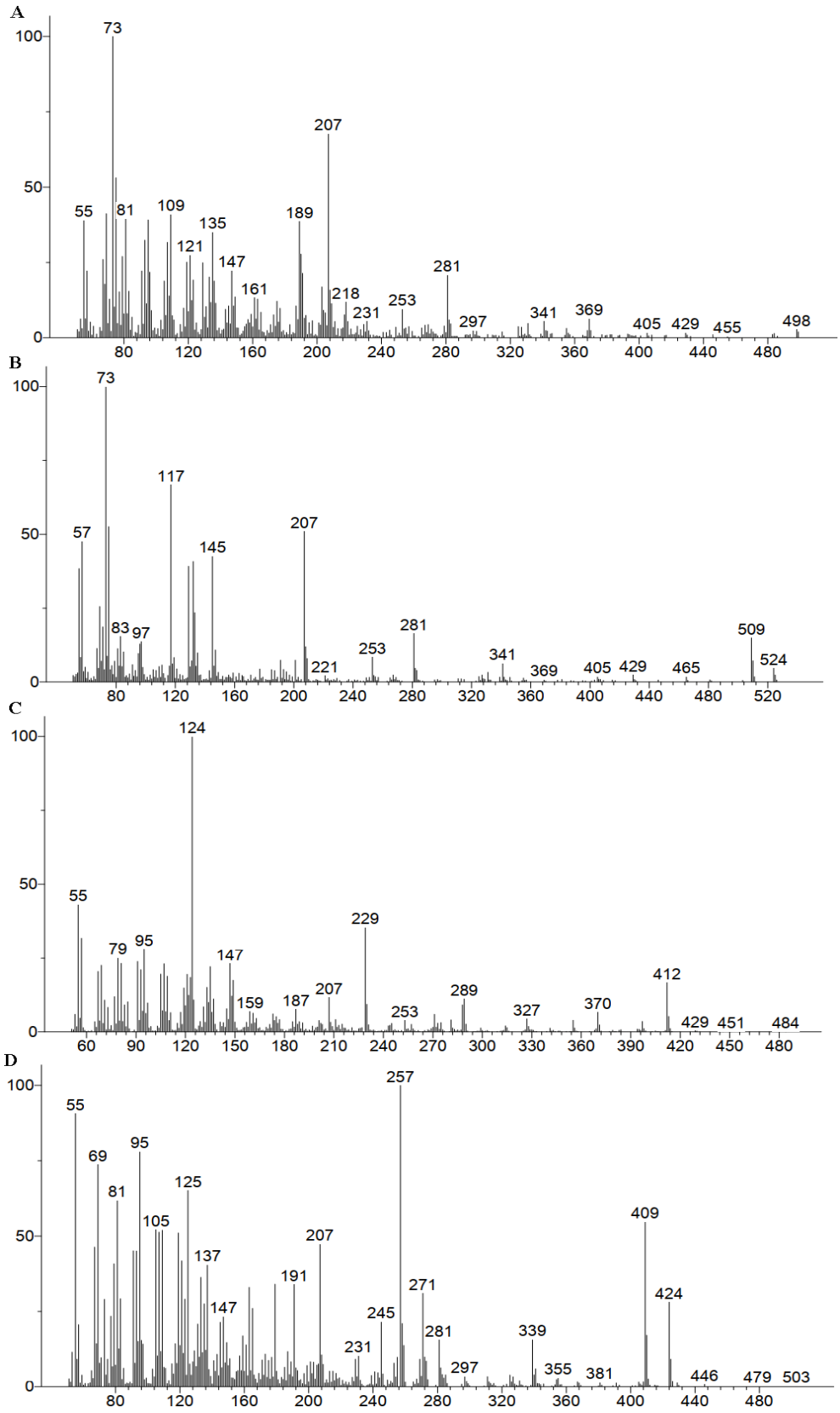


**Figure S1.23:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) NI-18 (**72**); B) Triacontanoic acid TMS derivative (**73**); C) *β*-sitostenone (**74**); and D) NI-19 (**75**).

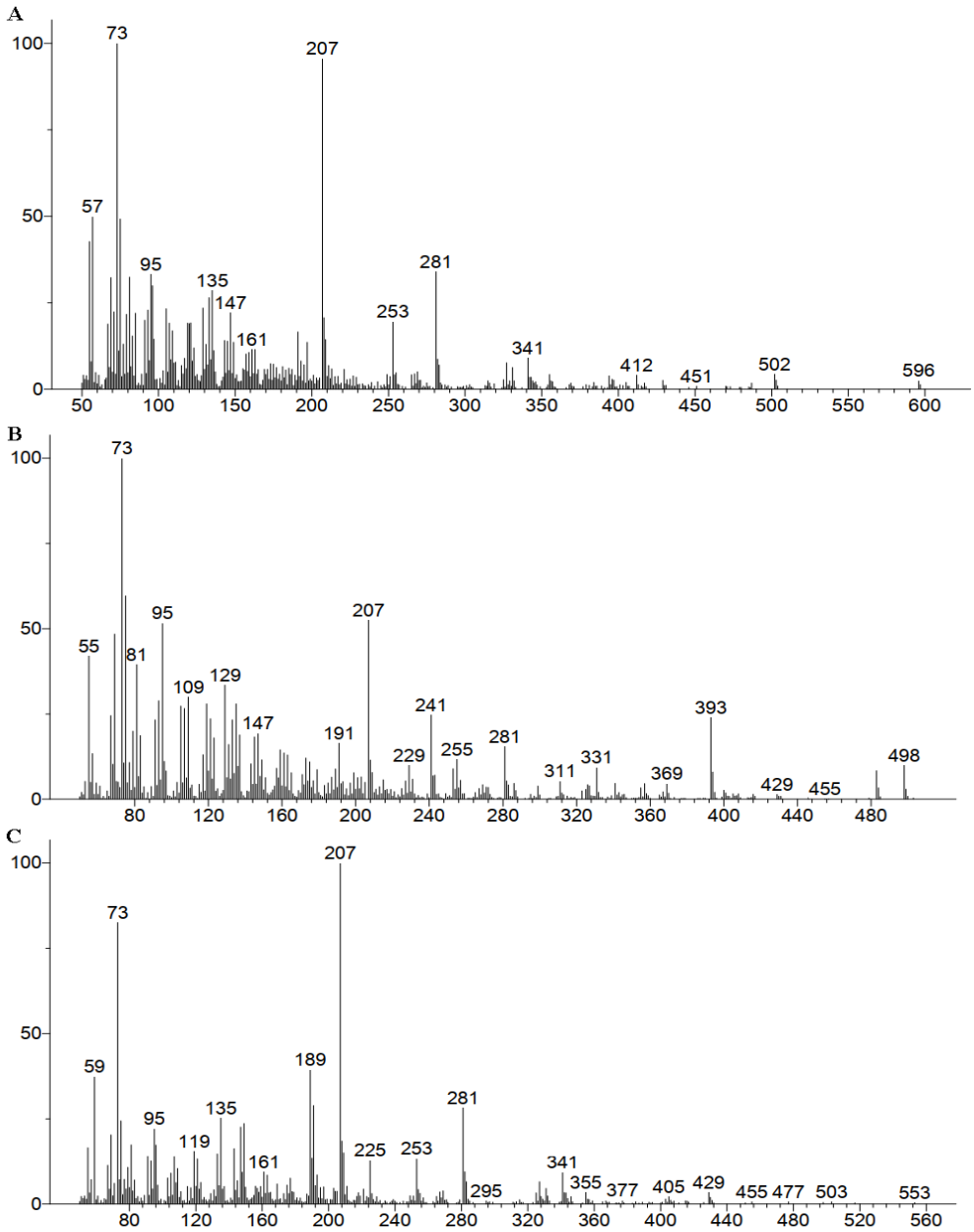


**Figure S1.24:** Mass spectra of compounds detected in the hexane extract of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr. analyzed by GC-EIMS. A) NI-20 (**76**); B) NI-21 (**77**); and C) NI-22 (**78**).

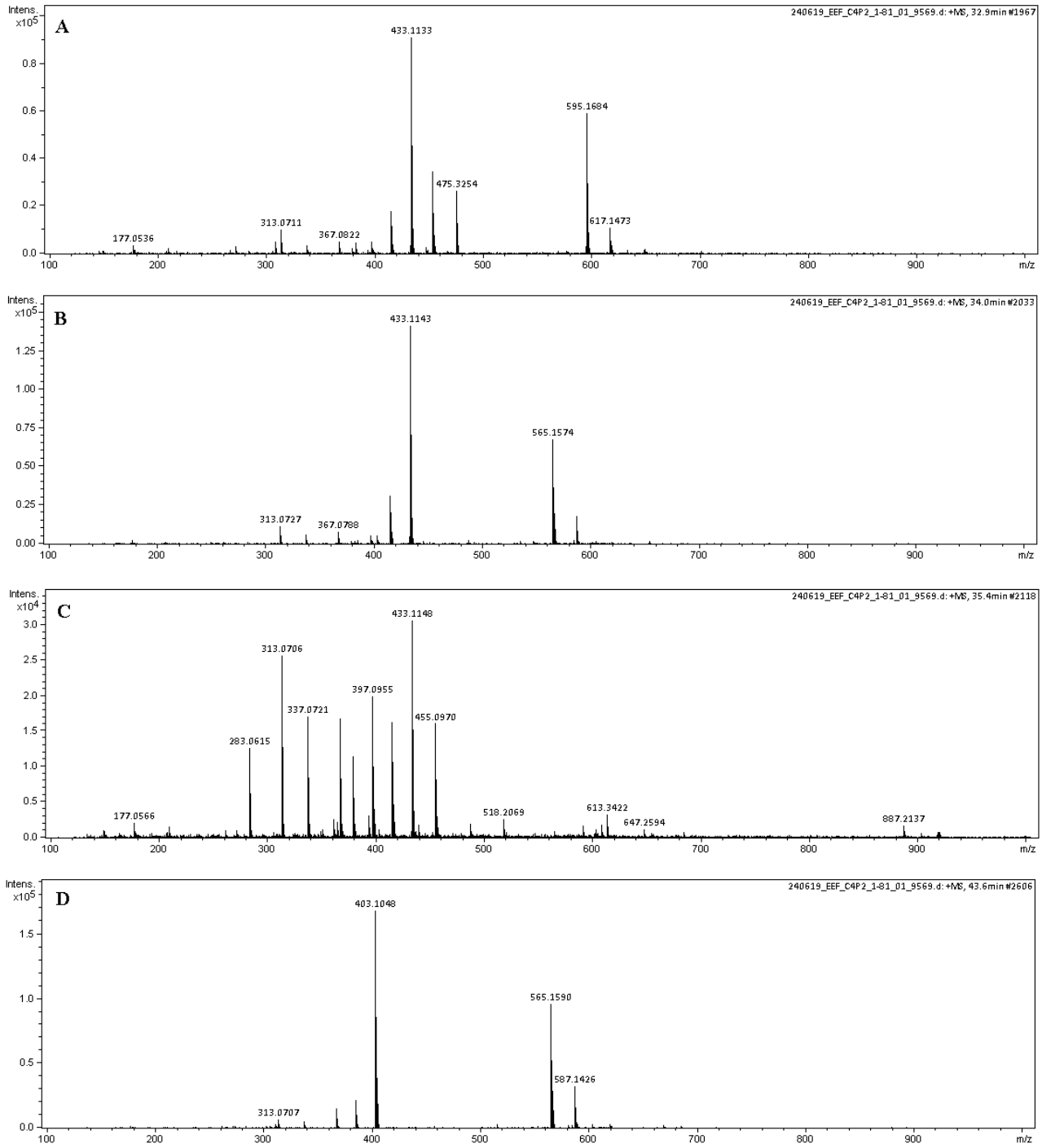


**Figure S1.25:** Mass spectra ($MS^+$) of flavonoids detected in the hydroethanol (**EE**) and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) of *Merostachys neesii* Rupr. analyzed by HPLC-ESI/HRMS. A) Apigenin *O*-hexoside *C*-hexoside (**79**); B) Apigenin *O*-pentoside *C*-hexoside (**80**); C) Isovitexin (**81**); and D) Apigenin *O*-hexoside *C*-pentoside (**82**).

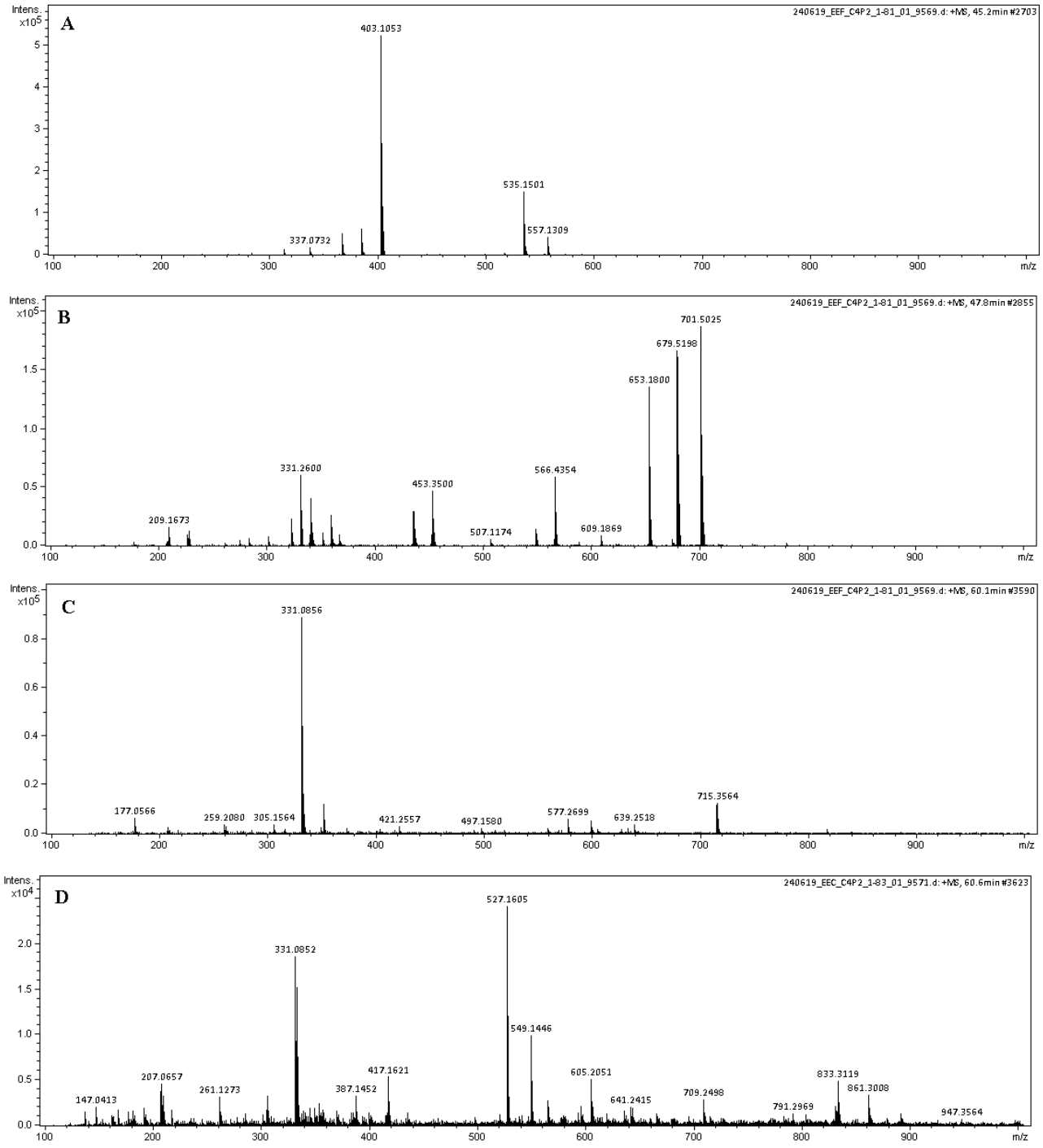


**Figure S1.26:** Mass spectra ($MS^+$) of flavonoids detected in the hydroethanol (**EE**) and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) of *Merostachys neesii* Rupr. analyzed by HPLC-ESI/HRMS. A) Apigenin *O*-pentoside *C*-pentoside (**83**); B) Tricin derivative 1 (**84**); C) Tricin derivative 2 (**85**); and D) Tricin derivative 3 (**86**).

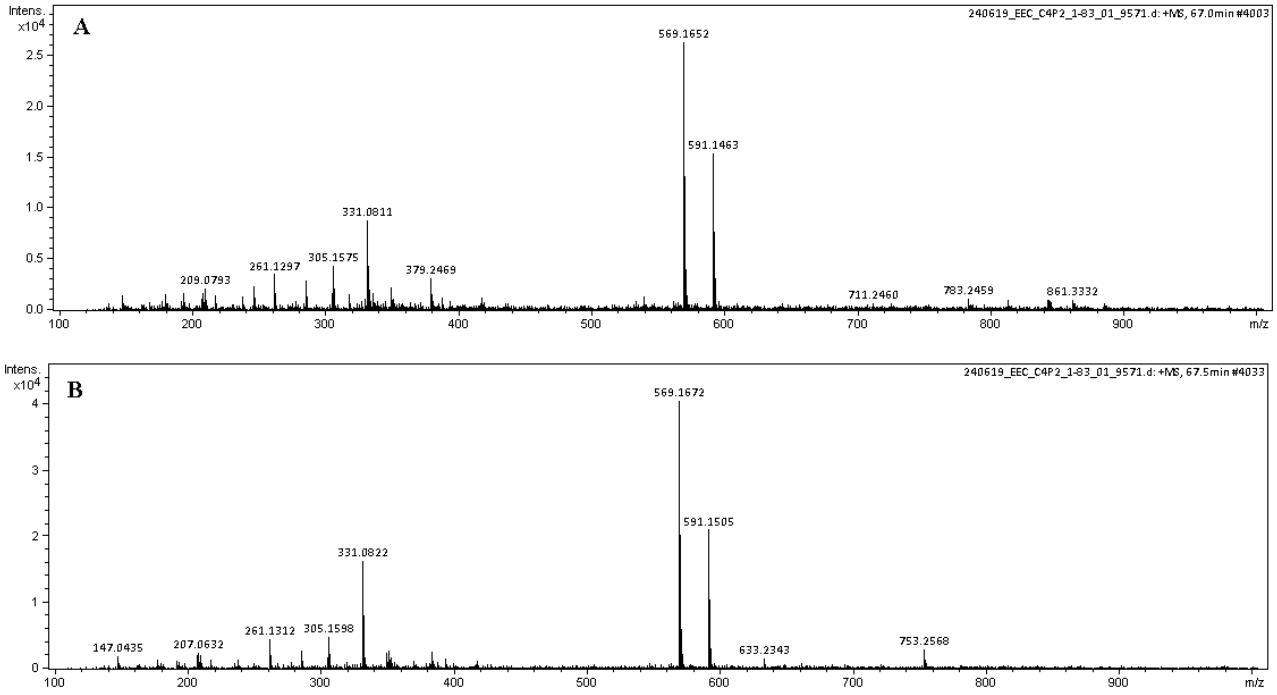


**Figure S1.27:** Mass spectra ($MS^+$) of flavonoids detected in the hydroethanol (**EE**) and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) of *Merostachys neesii* Rupr. analyzed by HPLC-ESI/HRMS. A) Tricin derivative 4 (**87**); and B) Tricin derivative 5 (**88**).

**Table S1.1:** Mass fragments and their respective relative abundances of the constituents detected by GC-EIMS in the polar phase of hot methanol extracts of leaves (**HME-L**) and culms (**HME-C**) of *Merostachys neesii* Rupr.

| Peak | RT (min) | Suggestion | Fragments with relative abundances: *m/z* (intensity, %) |
|---|---|---|---|
| 1 | 12.173 | Oxalic acid TMS derivative | 147 (100); 73 (35); 204 (21); 66 (16); 148 (15); 59 (11); 149 (7); 131 (5); 52 (4); 75 (4) |
| 2 | 13.140 | NI-1 | 73 (100); 147 (55); 133 (23); 59 (19); 72 (11); 75 (11); 74 (10); 148 (9); 100 (8); 86 (8) |
| 3 | 16.274 | Serine TMS derivative | 73 (100); 116 (66); 132 (63); 75 (30); 57 (19); 147 (13); 103 (13); 74 (12); 133 (10); 80 (9) |
| 4 | 16.646 | Glycerol TMS derivative | 73 (100); 147 (48); 205 (31); 103 (24); 117 (24); 133 (16); 218 (11); 74 (9); 75 (9); 59 (8) |
| 5 | 16.724 | NI-2 | 73 (100); 186 (95); 142 (48); 59 (21); 201 (19); 84 (18); 156 (17); 75 (16); 187 (14); 56 (14) |
| 6 | 17.833 | Succinic acid TMS derivative | 147 (100); 73 (74); 75 (35); 148 (16); 55 (14); 247 (10); 149 (9); 129 (8); 56 (8); 74 (7) |
| 7 | 18.149 | Glyceric acid TMS derivative | 73 (100); 147 (41); 189 (21); 133 (17); 103 (16); 292 (11); 75 (10); 74 (9); 117 (8); 59 (7) |
| 8 | 18.540 | Itaconic acid TMS derivative | 147 (100); 73 (97); 75 (23); 148 (16); 149 (10); 74 (9); 259 (8); 215 (6); 67 (6); 133 (6) |
| 9 | 20.078 | Mesaconic acid TMS derivative | 73 (100); 147 (39); 75 (30); 184 (28); 259 (21); 67 (10); 74 (9); 97 (8); 148 (6); 59 (5) |
| 10 | 20.598 | NI-3 | 73 (100); 228 (51); 75 (29); 147 (23); 77 (19); 184 (16); 217 (15); 110 (14); 134 (13); 143 (12) |
| 11 | 22.208 | Malic acid TMS derivative | 73 (100); 147 (37); 75 (12); 233 (10); 133 (9); 55 (9); 74 (9); 148 (6); 245 (5); 101 (5) |
| 12 | 22.622 | Erythritol TMS derivative | 73 (100); 147 (32); 217 (29); 103 (23); 205 (18); 117 (14); 204 (10); 74 (8); 133 (8); 189 (8) |
| 13 | 23.046 | 5-oxoproline TMS derivative | 156 (100); 73 (69); 147 (17); 157 (14); 75 (10); 230 (7); 258 (7); 74 (6); 59 (5); 158 (4) |
| 14 | 23.178 | GABA TMS derivative | 174 (100); 73 (81); 147 (26); 175 (18); 75 (17); 304 (15); 59 (13); 86 (11); 176 (8); 74 (8) |
| 15 | 23.465 | Erythronic acid TMS derivative | 73 (100); 147 (33); 292 (14); 117 (12); 220 (11); 205 (11); 103 (9); 74 (8); 75 (8); 217 (7) |
| 16 | 23.889 | Threonic acid TMS derivative | 73 (100); 147 (32); 292 (15); 220 (12); 205 (10); 117 (10); 103 (9); 74 (9); 75 (7); 217 (7) |
| 17 | 27.443 | Arabitol TMS derivative | 73 (100); 217 (32); 103 (29); 147 (28); 205 (15); 129 (11); 74 (9); 117 (8); 218 (8); 204 (7) |
| 18 | 27.548 | Ribitol TMS derivative (internal standard) | 73 (100); 147 (28); 217 (28); 103 (26); 205 (17); 129 (11); 74 (9); 117 (9); 218 (8); 204 (7) |
| 19 | 27.888 | NI-4 | 73 (100); 147 (35); 205 (19); 217 (12); 117 (9); 74 (8); 75 (7); 103 (6); 148 (5); 133 (5) |

| | | | |
|---|---|---|---|
| 20 | 28.236 | Aconitic acid TMS derivative | 73 (100); 147 (54); 229 (19); 75 (18); 74 (9); 67 (9); 149 (9); 148 (9); 133 (8); 211 (7) |
| 21 | 29.243 | Azelaic acid TMS derivative | 73 (100); 75 (71); 55 (27); 129 (18); 147 (16); 117 (15); 201 (13); 317 (11); 217 (11); 74 (11) |
| 22 | 29.617 | Citric acid TMS derivative | 73 (100); 147 (37); 273 (24); 75 (13); 74 (9); 149 (6); 148 (6); 133 (6); 274 (5); 363 (4) |
| 23 | 30.649 | Fructose TMS/MeOX derivative[1] | 73 (100); 103 (78); 217 (58); 147 (37); 307 (37); 133 (14); 218 (12); 74 (11); 308 (11); 117 (10) |
| 23a | 30.847 | Fructose TMS/MeOX derivative[1] | 73 (100); 103 (59); 217 (38); 147 (30); 307 (21); 74 (10); 218 (8); 133 (7); 89 (7); 117 (7) |
| 24 | 31.000 | Galactose TMS/MeOX derivative | 73 (100); 205 (26); 147 (24); 319 (18); 217 (13); 103 (13); 160 (13); 74 (9); 117 (7); 75 (7) |
| 25 | 31.151 | Glucose TMS/MeOX derivative[2] | 73 (100); 205 (45); 319 (39); 147 (37); 160 (28); 217 (17); 103 (16); 320 (12); 117 (10); 74 (10) |
| 25a | 31.503 | Glucose TMS/MeOX derivative[2] | 73 (100); 147 (24); 205 (23); 103 (17); 319 (17); 160 (14); 74 (8); 217 (8); 133 (7); 75 (7) |
| 26 | 31.743 | Mannitol TMS derivative | 73 (100); 147 (30); 205 (24); 319 (20); 103 (19); 217 (17); 117 (9); 74 (8); 75 (7); 204 (6) |
| 27 | 31.878 | Sorbitol TMS derivative | 73 (100); 147 (30); 205 (24); 103 (19); 319 (19); 217 (17); 74 (9); 117 (9); 75 (8); 320 (6) |
| 28 | 32.399 | Gallic acid TMS derivative | 73 (100); 281 (23); 458 (9); 74 (9); 179 (8); 147 (6); 282 (5); 75 (5); 133 (4); 459 (4) |
| 29 | 33.762 | NI-5 | 73 (100); 204 (55); 147 (17); 205 (14); 217 (12); 220 (11); 75 (9); 319 (9); 74 (8); 103 (7) |
| 30 | 34.673 | Dopamine TMS derivative | 174 (100); 73 (51); 175 (19); 176 (8); 86 (8); 59 (5); 74 (4); 179 (4); 100 (3); 75 (2) |
| 31 | 34.818 | *Myo*-inositol TMS derivative | 73 (100); 147 (38); 217 (34); 305 (20); 191 (19); 318 (11); 204 (10); 74 (8); 133 (7); 129 (7) |
| 32 | 36.053 | Norepinephrine TMS derivative | 174 (100); 73 (63); 175 (18); 176 (8); 86 (6); 147 (6); 74 (5); 75 (4); 355 (4); 59 (3) |
| 33 | 38.759 | NI-6 | 204 (100); 73 (86); 147 (27); 217 (22); 103 (21); 205 (21); 129 (13); 206 (9); 337 (9); 74 (7) |
| 34 | 39.807 | NI-7 | 73 (100); 217 (32); 147 (29); 204 (28); 103 (19); 75 (10); 74 (8); 292 (8); 129 (8); 218 (7) |
| 35 | 43.766 | Sucrose TMS derivative | 73 (100); 361 (55); 217 (51); 147 (32); 103 (24); 362 (18); 129 (17); 218 (12); 169 (12); 271 (10) |

RT (min): retention time in minutes; TMS: trimethylsilyl; MeOX: *O*-methyloxime; NI: not identified; GABA: gamma-aminobutyric acid.
[1] Fructose stereoisomers produced by methoximation reaction. [2] Glucose stereoisomers produced by methoximation reaction.

**Table S1.2:** Mass fragments and their respective relative abundances of the constituents detected by GC-EIMS in the hexane extracts of leaves (**HE-L**) and culms (**HE-C**) of *Merostachys neesii* Rupr.

| Peak | RT (min) | Suggestion | Fragments with relative abundances: *m/z* (intensity, %) |
|---|---|---|---|
| 36 | 12.826 | Myristic acid TMS derivative | 73 (100); 117 (87); 75 (78); 285 (53); 129 (37); 132 (35); 55 (27); 145 (21); 286 (12); 131 (12) |
| 37 | 13.502 | NI-8 | 82 (100); 95 (77); 81 (75); 57 (68); 68 (54); 55 (49); 67 (44); 69 (42); 123 (38); 71 (36) |
| 38 | 13.872 | Nonadecane (internal standard) | 57 (100); 71 (64); 85 (42); 55 (23); 56 (14); 69 (11); 99 (11); 70 (10); 83 (7); 84 (6) |
| 39 | 14.930 | Pentadecanoic acid TMS derivative | 73 (100); 117 (90); 75 (72); 299 (51); 129 (40); 132 (36); 55 (25); 145 (23); 57 (13); 300 (11) |
| 40 | 16.741 | NI-9 | 73 (100); 75 (15); 74 (8); 117 (6); 55 (4); 311 (4); 54 (3); 67 (2); 57 (2); 81 (2) |
| 41 | 16.920 | Palmitic acid TMS derivative | 73 (100); 117 (97); 75 (74); 313 (56); 132 (42); 129 (42); 145 (26); 55 (26); 314 (14); 57 (13) |
| 42 | 18.772 | Heptadecanoic acid TMS derivative | 73 (100); 117 (92); 75 (72); 327 (46); 132 (42); 129 (38); 55 (30); 145 (28); 57 (16); 328 (12) |
| 43 | 19.392 | Phytol TMS derivative | 143 (100); 73 (33); 75 (26); 144 (13); 123 (13); 55 (10); 57 (10); 81 (10); 69 (6); 95 (5) |
| 44 | 19.964 | Linoleic acid TMS derivative | 73 (100); 75 (98); 67 (67); 81 (55); 55 (49); 95 (36); 129 (34); 79 (33); 117 (28); 82 (26) |
| 45 | 20.087 | Linolenic acid TMS derivative | 75 (100); 73 (93); 79 (86); 67 (55); 95 (49); 93 (42); 55 (40); 81 (34); 80 (33); 108 (32) |
| 46 | 20.541 | Stearic acid TMS derivative | 73 (100); 117 (94); 75 (70); 341 (46); 132 (45); 129 (42); 145 (30); 55 (29); 57 (19); 69 (13) |
| 47 | 23.574 | NI-10 | 73 (100); 75 (47); 225 (26); 130 (17); 67 (15); 79 (14); 55 (13); 81 (10); 129 (10); 143 (10) |
| 48 | 23.658 | NI-11 | 73 (100); 75 (39); 369 (29); 183 (21); 147 (12); 79 (11); 74 (11); 91 (11); 129 (10); 93 (10) |
| 49 | 23.828 | Arachidic acid TMS derivative | 73 (100); 117 (83); 75 (70); 132 (47); 369 (43); 129 (42); 145 (35); 55 (32); 57 (23); 69 (17) |
| 50 | 26.856 | Behenic acid TMS derivative | 73 (100); 117 (87); 75 (70); 132 (51); 129 (42); 397 (42); 145 (40); 55 (33); 57 (28); 69 (19) |
| 51 | 28.287 | Tricosanoic acid TMS derivative | 73 (100); 117 (92); 75 (69); 132 (48); 129 (41); 145 (36); 55 (35); 57 (32); 411 (31); 69 (18) |
| 52 | 29.670 | Lignoceric acid TMS derivative | 73 (100); 117 (97); 75 (65); 132 (55); 129 (46); 145 (43); 57 (39); 55 (36); 425 (34); 69 (20) |
| 53 | 31.007 | Pentacosanoic acid TMS derivative | 73 (100); 117 (90); 75 (69); 132 (50); 129 (50); 57 (44); 55 (44); 145 (44); 439 (26); 69 (26) |
| 54 | 31.115 | 1-hexacosanol TMS derivative | 75 (100); 439 (86); 57 (62); 73 (54); 55 (43); 103 (42); 83 (34); 440 (33); 69 (33); 97 (28) |
| 55 | 31.761 | NI-12 | 88 (100); 101 (73); 57 (67); 73 (60); 55 (51); 89 (36); 71 (34); 69 (31); 83 (24); 207 (24) |

| | | | |
|---|---|---|---|
| 56 | 32.304 | Hexacosanoic acid TMS derivative | 73 (100); 117 (86); 57 (74); 75 (59); 55 (58); 132 (52); 145 (46); 129 (41); 69 (35); 71 (32) |
| 57 | 32.970 | Stigmasta-3,5-diene | 147 (100); 81 (94); 105 (85); 145 (69); 55 (69); 91 (67); 57 (66); 396 (63); 107 (62); 79 (52) |
| 58 | 33.647 | 1-octacosanol TMS derivative | 75 (100); 467 (79); 57 (72); 73 (57); 55 (43); 103 (42); 69 (35); 71 (34); 83 (33); 468 (29) |
| 59 | 33.786 | Cholesterol TMS derivative | 129 (100); 73 (91); 75 (59); 329 (47); 207 (47); 55 (47); 57 (45); 95 (45); 81 (40); 105 (35) |
| 60 | 34.256 | $\alpha$-tocopherol acetate | 165 (100); 430 (95); 207 (45); 164 (32); 431 (29); 57 (26); 55 (18); 69 (13); 166 (12); 205 (12) |
| 61 | 34.788 | Octacosanoic acid TMS derivative | 73 (100); 117 (87); 75 (62); 132 (53); 57 (52); 145 (49); 129 (44); 55 (42); 69 (27); 481 (25) |
| 62 | 34.845 | NI-13 | 57 (100); 82 (73); 55 (61); 96 (49); 83 (48); 71 (47); 69 (44); 97 (38); 68 (34); 81 (31) |
| 63 | 35.107 | Campesterol TMS derivative | 129 (100); 73 (67); 75 (44); 343 (41); 95 (38); 55 (36); 81 (35); 57 (34); 107 (31); 71 (29) |
| 64 | 35.493 | Stigmasterol TMS derivative | 83 (100); 55 (70); 129 (62); 73 (56); 69 (53); 81 (42); 75 (36); 57 (27); 255 (25); 133 (23) |
| 65 | 35.573 | NI-14 | 204 (100); 55 (97); 133 (88); 69 (80); 95 (67); 81 (64); 107 (63); 207 (63); 300 (62); 205 (58) |
| 66 | 35.798 | NI-15 | 129 (100); 73 (84); 75 (50); 82 (30); 57 (29); 91 (25); 55 (17); 96 (15); 83 (13); 130 (12) |
| 67 | 36.021 | 1-triacontanol TMS derivative | 75 (100); 57 (75); 495 (66); 73 (60); 55 (47); 103 (40); 71 (40); 69 (38); 83 (33); 97 (30) |
| 68 | 36.189 | $\beta$-sitosterol TMS derivative | 129 (100); 73 (61); 57 (42); 75 (42); 95 (36); 357 (35); 55 (34); 81 (34); 107 (29); 119 (28) |
| 69 | 36.362 | NI-16 | 218 (100); 55 (56); 73 (49); 207 (46); 95 (44); 69 (42); 135 (38); 81 (35); 107 (35); 119 (34) |
| 70 | 36.378 | Lupenone | 95 (100); 109 (92); 55 (91); 81 (90); 107 (86); 93 (85); 205 (80); 67 (73); 69 (71); 121 (63) |
| 71 | 36.642 | NI-17 | 55 (100); 207 (53); 69 (47); 81 (47); 73 (38); 83 (38); 97 (36); 79 (35); 95 (34); 67 (33) |
| 72 | 36.795 | NI-18 | 73 (100); 207 (68); 75 (53); 69 (41); 109 (41); 81 (40); 55 (39); 95 (39); 189 (39); 135 (35) |
| 73 | 37.118 | Triacontanoic acid TMS derivative | 73 (100); 117 (84); 75 (62); 57 (57); 132 (53); 145 (53); 129 (45); 55 (41); 207 (32); 69 (26) |
| 74 | 37.333 | $\beta$-sitostenone | 124 (100); 55 (43); 229 (34); 57 (33); 95 (27); 79 (26); 91 (25); 81 (24); 147 (24); 107 (24) |
| 75 | 37.657 | NI-19 | 55 (100); 257 (92); 95 (84); 69 (79); 81 (70); 207 (69); 125 (66); 109 (59); 119 (54); 67 (54) |
| 76 | 37.832 | NI-20 | 73 (100); 207 (96); 57 (50); 75 (49); 55 (43); 281 (34); 95 (33); 81 (32); 69 (32); 96 (30) |
| 77 | 38.093 | NI-21 | 73 (100); 207 (77); 75 (44); 55 (41); 69 (40); 95 (40); 135 (31); 81 (29); 133 (24); 129 (24) |
| 78 | 39.516 | NI-22 | 207 (100); 73 (86); 189 (42); 59 (36); 191 (32); 281 (31); 147 (25); 75 (24); 135 (24); 149 (23) |

RT (min): retention time in minutes; TMS: trimethylsilyl; NI: not identified.

# Chapter 2 - Nutritional value of *Merostachys neesii*: seasonal implications

## 1. Introduction

Bambusoideae is the Poaceae subfamily that comprises the bamboos. These giant grasses have a wide geographic distribution and they are found mainly in temperate and tropical forests (Clark et al. 2015). Bamboos are evergreen, fast-growing, large producers of biomass, and a multipurpose plant, all favorable characteristics from the economic point of view (Halvorson et al. 2010). In Asia, region with the largest bamboo biodiversity in the world (Bystriakova and Kapos 2006), these plants are considered commodities (Hu et al. 2000; Basumatary et al. 2015), being used for different purposes, as wood for building, furniture manufacturing, papermaking, handicraft (Yuming et al. 2004; Borah et al. 2008), prevention of soil erosion, as medicinal (Honfo et al. 2015), and as food resource for humans (Hossain et al. 2015) and animals (Sharma and Borthakur 2008; Mekuriaw et al. 2011).

Bamboos, especially their shoots (juvenile and tender culms of bamboo), have been used as food by humans for hundreds of years (Chongtham et al. 2011; Nirmala et al. 2018). Bamboo shoot is a very popular food item in the traditional cuisine and it is consumed worldwide, especially in Asian countries. The high content of water, protein and dietary fiber, low-fat levels, and the presence of vitamins and various amino acids and minerals make bamboo shoots a health food (Satya et al. 2010; Chongtham et al. 2011; Nongdam and Tikendra 2014). The high fiber content of bamboo shoots has made of this forest plant a great source of fiber. These products containing concentrated dietary fiber are well accepted by consumers and many companies have added these bamboo fibers in different types of food and beverage products (Chongtham et al. 2011; Felisberto et al. 2017). Nevertheless, it is worth to be aware that bamboos may also contain toxic compounds, for instance, taxiphyllin, a cyanogen glycoside (Satya et al. 2010; Nongdam and Tikendra 2014). Cyanogen glycoside has already been reported in *Dendrocalamus asper* (Schult.) Backer, *D. strictus* (Roxb.) Nees, *Bambusa tulda* Roxb. (Pandey and Ojha 2013), *Chimonobambusa callosa* (Munro) Nakai, *B. cacharensis* R.B.Majumdar, *B. tuldoides* Munro, *B. manipureana* H.B.Naithani & N.S.Bisht, *B. nutans* Wall. ex Munro, *B. oliveriana* Gamble, *D. giganteus* Munro, *D. hamiltonii* Nees & Arn. ex Munro, *D. hookeri* Munro, *D. manipureanus* H.B. Naithani & N.S.Bisht, *Melocanna baccifera* (Roxb.) Kurz, *Schizostachyum dullooa* Gamble R.B.Majumdar shoots, among others (Waikhom et al. 2013).

Besides its high nutritional value, the bamboo shoot also contains bioactive compounds, such as phytosterols and phenolic compounds. These compounds, although not considered as nutrients per se, are important because they promote several health benefits (Chongtham et al. 2011).

Different studies have reported that bamboo leaves and culms also contain phytosterols and phenolic compounds (Tanaka et al. 2014; Gong et al. 2015; Sriraman et al. 2015; Tongco et al. 2016; Gagliano et al. 2018; Wani et al. 2019), as well as a lot of other traditional nutrients, demonstrating that these plant organs, as well as the shoots, also have potential as a source of bioactive compounds. Indeed, in China, a standardized extract, AOB (antioxidant of bamboo leaves), obtained from leaves of *Phyllostachys* spp. is used as a food antioxidant, authorized by the Chinese Ministry of Health (Lu et al. 2005). Studies on the use of AOB as a food additive have reported that it reduces the levels of acrylamide, a toxic compound found in heat-treated food as potato crisps, French fries, and fried chicken wings, without adversely affecting the sensory properties of the product, demonstrating its efficacy as a food additive (Zhang et al. 2007a, 2007b). In addition, studies about acute and subchronic toxicity, reproductive, and teratogenicity of AOB have demonstrated that its use as a food additive is safe (Lu et al. 2005, 2006). Phenolic compounds, such as hydroxycinnamic derivatives, coumaric lactone, and flavones *C*-glycosides, are the main constituents in AOB (Lu et al. 2006).

Bamboo leaves are also important in livestock. They have been used as fodder for centuries (Chongtham et al. 2011), mainly during the dry season when crops, normally used as forage, are scarce (Sahoo et al. 2010; Mekuriaw et al. 2011). In some places, small twigs of bamboo are also used to feed animals (Mekuriaw et al. 2011).

Brazil is an important center of bamboo diversity. The Amazon Rain Forest and Atlantic Rain Forest are the Brazilian biomes with the largest number of species, being many of them endemic (Greco et al. 2015). Atlantic Rain Forest is recognized as a hotspot of biodiversity, presenting a high rate of endemism (Myers et al. 2000). However, although Brazil has a great diversity of bamboo species and there are several studies on the health benefits and food resource for humans and livestock provided by Asian bamboos, studies using Brazilian native bamboo species are incipient. Thus, this study aimed to analyze the nutritional composition of *Merostachys neesii* Rupr. leaves and culms, an endemic bamboo species from Brazil, and the implications of seasonality on its potential as fodder and/or food additive.

## 2. Material and Methods

### 2.1 Plant material

Leaves and culms (culms plus branches) from four *M. neesii* clumps (C1-C4) were harvested in the dry (August/2017, n=4) and rainy (February/2018, n=4) seasons at Fontes do Ipiranga State Park (PEFI), São Paulo, Brazil (see figure 3 in Introduction: an overview of bamboos). Each clump represents one replica. Plant material was identified by Ph.D. Maria Tereza

Grombone-Guaratini, researcher of the Institute of Botany of São Paulo (IBt-SP). A voucher specimen (Furlan70) was deposited at the Herbarium of the University of São Paulo (SPF).

### 2.2 Climate data

Data of precipitation, air temperature, solar irradiation, and relative air humidity three months before and after the harvested periods (August/2017 and February/2018) were obtained at the Meteorological Station of the Institute of Astronomy, Geophysics and Atmospheric Sciences of USP (IAG-USP).

### 2.3 Chemical analysis

Plant material was previously separated in leaves and culms (culms plus branches). Then, they were frozen, freeze-dried, ground, and kept at room temperature until they were analyzed.

**- Protein**

For the quantification of proteins, 200 mg of freeze-dried ground plant material were extracted using 2 mL of ultrapure water. The extract was centrifuged at 10,000 *g* and 4°C for 10 min and the supernatant was collected and stored at -80°C until analysis (Esposito and Domingos 2014). The protein content was determined by the Bradford method using 40 µL of extract and 200 µL of Bradford reagent (Sigma-Aldrich) diluted in ultrapure water (4:1) (water was used as negative control). The reaction mixture was stirred for 1 min and the absorbance was measured at 590 nm using a Synergy $H^1$ microplate reader (BioTek, Inc.). All samples and controls were made in methodological triplicate. Results were compared with a standard curve of bovine serum albumin (BSA) in ultrapure water (10 to 200 µg $mL^{-1}$) and expressed as a percentage.

**- Nitrogen and crude protein (CP)**

Nitrogen content was determined by elemental analysis based on the Pregl-Dumas method. Ten milligrams of the freeze-dried ground plant material (leaves and culms) were analyzed using an elemental analyzer (Perkin-Elmer 2400 series II), in which the samples were submitted to combustion in a pure oxygen atmosphere and the gases were quantified by a thermal conductivity detector (TDC). Crude protein content was estimated by multiplying the nitrogen content by 6.25 (Halvorson et al. 2010). All samples were made in duplicate. Results were expressed as a percentage.

**- Soluble sugar (SS)**

For the determination of soluble sugars, obtaining the polar phase of hot methanol extract of leaves and culms was carried out as described in item 2.2 of chapter 1. These extracts were analyzed by GC-EIMS (see Chapter 1, item 2.3.1 – polar compounds analysis). For the quantification, areas of all compounds identified as sugar were compared with the area of the ribitol (internal standard). Results were expressed as mg of compound per g of dry weight (mg $g^{-1}$) and

presented as mean ± standard deviation (n=4). All sugars detected were combined to obtain the total soluble sugar (TSS) content. Results were expressed as a percentage.

**- Lipid**

For the determination of the total lipid content, hexane extracts of leaves and culms were obtained as described in item 2.2 of chapter 1. These extracts were analyzed by GC-EIMS (see Chapter 1, item 2.3.1 – nonpolar compounds analysis). All compounds identified as fatty acids were quantified (areas of the fatty acids were compared with the area of the nonadecane - internal standard), expressed as a percentage, and the values were combined to obtain the total lipid content.

**- Fibers and lignins**

The neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose, and cellulose contents were determined by extracting of the soluble material in boiling detergent solutions according to Hatfield et al. (1994), with modifications. Briefly, 250 mg of the freeze-dried ground plant material (leaves and culms) were extracted using 15 mL of neutral detergent solution (18.61 g of EDTA-Na (ethylenediaminetetraacetic acid disodium salt dehydrate), 6.81 g of sodium tetraborate ($Na_2B_4O_7.10H_2O$), 4.56 g of sodium phosphate dibasic anhydrous ($Na_2HPO_4$), 30 g of SDS, and 10 mL of 2-ethoxyethanol added to 1 L of water) at 100°C for 1 h. The residue was transferred to previously weighed Gooch crucibles and was washed with hot water and acetone. After washing, the residue was dried at 100°C for 2 h and weighed to calculate the percentage of neutral detergent fiber (NDF) using the following equation:

NDF % = $((W_{r1} - W_e) \times 100)/S$, where $W_{r1}$, $W_e$, and S correspond to weight of the crucible plus residue obtained after extraction with neutral detergent, weight of the empty crucible, and weight of dry plant material (sample), respectively.

Then, the NDF was extracted using 20 mL of acid detergent solution (20 g of CTAB (cetyltrimethylammonium bromide) and 24.5 mL of sulfuric acid added to 1 L of water) at 100°C for 1h and subsequently washed with hot water and acetone. After that, the residue was dried at 100°C for 2 h and weighed to calculate the percentage of acid detergent fiber (ADF) using the following equation:

ADF % = $((W_{r2} - W_e) \times 100)/S$, where $W_{r2}$, $W_e$, and S correspond to weight of the crucible plus residue obtained after extraction with acid detergent, weight of the empty crucible, and weight of dry plant material, respectively.

Next, the ADF was hydrolyzed using a sulfuric acid solution (640 mL sulfuric acid adjusted to 1 L with water) at room temperature for 3 h. After hydrolysis, the residue was washed, dried at 100°C for 2 h, weighed, and submitted to combustion at 450°C for 2 h. The percentage of acid detergent lignin (ADL) was calculated using the following equation:

ADL % = (($W_a$ - $W_c$) x 100)/S, where $W_a$, $W_c$, and S were weight of the residue after hydrolysis with sulfuric acid, weight of the residue after combustion, and weight of dry plant material, respectively.

Hemicellulose and cellulose contents were calculated by the difference between ADF and NDF and between ADL and ADF, respectively.

**- Ash**

Ash content was determined using the combustion residue of the material plant from fiber and lignin analysis.

**- Total phytosterol**

For the determination of the total phytosterol content, hexane extracts of leaves and culms were obtained as described in item 2.2 of chapter 1. These extracts were analyzed by GC-EIMS (see Chapter 1, item 2.3.1 – nonpolar compounds analysis). All compounds identified as phytosterols were quantified (areas of the phytosterols were compared with the area of the nonadecane - internal standard), expressed as a percentage, and the values were combined to obtain the total phytosterol content.

**- Phenolic compounds**

For quantification of phenolic compounds, 40 mg of the freeze-dried ground leaves and culms were extracted with 10 mL of 80% MeOH at 70°C for 1 h and regular stirring, using a dry bath. The material was filtered and the supernatant adjusted to 10 mL. All phenolic compounds quantifications were performed using a microplate reader.

Total phenolic content was determined by the Folin-Ciocalteu method according to the procedures performed by Furlan et al. (2015). In each well of a 96-well microplate were added 190 μL of ultrapure water, 10 μL of the Folin-Ciocalteu reagent (Sigma-Aldrich), 50 μL of the extract (80% methanol was used as the negative control), and 50 μL of 10% sodium carbonate solution. The reaction mixture was homogenized, incubated for 30 min at 40°C, and the absorbance measured at 760 nm. All samples and controls were made in methodological triplicate. Results were compared with a standard curve of gallic acid (5 to 80 μg $mL^{-1}$) and expressed as a percentage.

Flavonoid content was determined using the aluminum chloride method modified from Santos and Furlan (2013). An aliquot de 100 μL of the extract (80% methanol was used as the negative control) was mixed with 100 μL of 5% aluminum chloride solution. The reaction mixture was homogenized and the absorbance determined at 420 nm. All samples and controls were made in methodological triplicate. Results were compared with a standard curve of quercetin (3.6 to 84 μg $mL^{-1}$) and expressed as a percentage.

Total tannin content was determined by the protein precipitation-BSA method described by Waterman and Mole (1994) and adapted to 96-well microplate by Anselmo-Moreira et al. (2019). An aliquot of 175 µL of BSA solution (bovine serum albumin 1 mg $mL^{-1}$ in acetate buffer 0.2 M, pH 5.0) was added to 50 µL of the extract (80% methanol was used as the negative control). After 10 min, the material was centrifuged at 10,621 *g* for 10 min. The supernatant was discarded and 200 µL of the 1% SDS reagent (sodium dodecyl sulfate) were added to the precipitate. After stirring, 75 µL of 0.01 M ferric chloride reagent were added. The reaction mixture was homogenized, transferred to a 96-well microplate, incubated for 15 min at room temperature, and the absorbance was determined at 520 nm. All samples and controls were made in methodological triplicate. Results were compared with a standard curve of tannic acid (0.2 to 2.4 mg $mL^{-1}$) and expressed as a percentage.

**- Cyanogenic glycoside**

Cyanogenic glycoside detection was performed by the picric acid test according to Hogg and Ahlgren (1942), with modifications. One hundred and fifty milligrams of minced fresh leaves were transferred to stoppered test tube containing 10 mL of water, 6 drops of dichloromethane, and a suspended strip of filter paper saturated with a picric acid solution (25 g of sodium carbonate ($Na_2CO_3$) and 5 g of picric acid to 1 L of water). Then, the test tube was sealed and incubated in water-bath at 70°C for 2 h. Change in the coloration of the paper filter indicates the presence of cyanogenic glycosides.

### 2.4 Statistical analysis

The data were presented as mean ± standard deviation (n=4). Normality (Shapiro-Wilk test) and homoscedasticity (*F*-test for equality of two variances) of the data were tested using R software (version 3.5.1). Data showing normal distribution were analyzed by the two-sample *t*-test (equal variance) or Welch two-sample *t*-test (unequal variance). When required, data transformation ($log_{10}$) was performed. For non-parametric data, means were compared using the Wilcoxon rank test (for two samples). All tests were compared at a significance level of 95%.

## 3. Results and discussion

### 3.1 Nutritional potential of *Merostachys neesii*

Bamboos have been used as medicine and food for a long time (Nirmala et al. 2018). The nutritional quality of bamboos has attracted increasing attention from the population due to their potential use in the food industry as food supplements, especially due to their antioxidant potential and high fiber content (Gong et al. 2015; Felisberto et al. 2017).

In addition, these plants may also be an alternative fodder, especially in periods when commonly used foods are less abundant. Chemical composition analysis of forage species provides

relevant information on their nutritional value, constituting an initial step in the evaluation of species for animal feed. In general, fodders exhibiting around 30% of dry matter, 40% of organic matter digestibility, and less than 10% of total ash in the dry matter are usually considered good forages. Moreover, forages with higher contents of crude protein, N-free extract (total carbohydrates excluding the crude fiber), and ether extract and low amounts of crude fiber are also preliminary indications that help to evaluate the forage quality (Mandal and Gautam 2012).

Although there are studies on nutritional composition and phytochemical characterization of different bamboo species, most of them refer to Asian bamboos, with few studied American bamboo species. Table 2.1 shows the chemical composition data of *M. neesii* leaves and culms collected during rainy and dry seasons.

Protein is an essential macronutrient for proper body growth and maintenance in all living organisms. Protein requirements in humans and animals vary according to different factors (age, pregnant and lactating female, and so on) (Chongtham et al. 2011). Leaves and culms of *M. neesii* showed 0.08 and 0.02 g/100 g of protein, respectively. Nirmala et al. (2007) evaluated nutritional aspects in juvenile and 10-day-old bamboo shoots of different species (*Bambusa bambos* (L.) Voss, *B. tulda*, *Dendrocalamus asper*, and *D. hamiltonii*). The authors found protein levels ranged from 3.1 to 3.7 and from 2.2 to 2.6 g/100 g of fresh weight, respectively, and there was a reduction with the age of bamboo shoot. Mature culms of *M. neesii* contain a much lower protein amount than bamboo shoots of other species. The nutritional composition may vary among species and with the age. Food reserves are consumed during the growth of bamboo shoots resulting in changes in the chemical composition (Nirmala et al. 2007).

**Table 2.1:** Nutritional (protein, crude protein, total soluble sugar, total lipid, NDF, ADF, ADL, hemicellulose, cellulose, ash, total phytosterol, total phenol, and total flavonoid) and antinutritional (total tannin and cyanogenic glycoside) composition (%) of *Merostachys neesii* Rupr. leaves and culms harvested in the dry (August/2017, n=4) and rainy (February/2018, n=4) seasons.

| **Composition (%)** | **Leaves** | | | **Culms** | | |
|---|---|---|---|---|---|---|
| | **Dry season** | **Rainy season** | **Averages** | **Dry season** | **Rainy season** | **Averages** |
| Protein | 0.09 ± 0.06 | 0.07 ± 0.01 | 0.08 ± 0.04 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| CP | 18.63 ± 0.69 | 18.91 ± 1.22 | 18.77 ± 0.93 | 6.23 ± 0.88 | 5.14 ± 0.45 | 5.69 ± 0.87 |
| TSS | $^{np}$1.07 ± 0.44 | 1.30 ± 0.33 | 1.19 ± 0.38 | 1.36 ± 0.25 | 0.94 ± 0.37 | 1.15 ± 0.37 |
| TL | **20.55 ± 4.03** $^{b}$ | **31.01 ± 4.54** $^{a}$ | 25.78 ± 6.86 | 34.31 ± 2.67 | 36.00 ± 13.09 | 35.16 ± 8.79 |
| NDF | 65.22 ± 2.24 | 64.29 ± 4.09 | 64.76 ± 3.10 | 80.51 ± 2.08 | 83.11 ± 2.71 | 81.81 ± 2.63 |
| ADF | $^{np}$59.07 ± 2.88 | 55.79 ± 3.98 | 57.43 ± 3.66 | 76.96 ± 2.60 | 80.31 ± 2.76 | 78.63 ± 3.06 |
| ADL | $^{np}$11.89 ± 2.48 | 10.54 ± 0.90 | 11.22 ± 1.87 | 12.65 ± 1.34 | 12.31 ± 2.25 | 12.48 ± 1.72 |
| HC | 6.15 ± 1.52 | 8.50 ± 1.78 | 7.32 ± 1.98 | 3.35 ± 0.79 | 2.80 ± 0.78 | 3.18 ± 0.83 |
| CEL | $^{np}$39.30 ± 2.46 | 39.90 ± 2.61 | 39.60 ± 2.37 | 55.76 ± 3.16 | 58.04 ± 3.19 | 56.90 ± 3.18 |
| Ash | $^{np}$6.50 ± 3.35 | 4.41 ± 1.18 | 5.45 ± 2.58 | 7.21 ± 1.18 | 8.81 ± 4.67 | 8.01 ± 3.27 |
| Total phytosterol | $^{np}$**3.48 ± 0.66** $^{b}$ | **5.90 ± 1.26** $^{a}$ | 4.69 ± 1.59 | 21.28 ± 7.65 | 16.12 ± 2.09 | 18.70 ± 5.88 |
| Total phenol | 2.10 ± 0.41 | 2.06 ± 0.64 | 2.08 ± 0.50 | **1.11 ± 0.08** $^{a}$ | **0.63 ± 0.16** $^{b}$ | 0.87 ± 0.28 |
| Total flavonoid | **0.20 ± 0.01** $^{b}$ | **0.23 ± 0.02** $^{a}$ | 0.22 ± 0.02 | **0.15 ± 0.03** $^{a}$ | **0.11 ± 0.01** $^{b}$ | 0.13 ± 0.03 |
| Total tannin | ND | ND | ND | ND | ND | ND |
| CG* | ND | ND | ND | - | - | - |

* Qualitative detection method (absence/presence). - : not evaluated.

ND: not detected; CP: crude protein; TSS: Total soluble sugar; TL: Total lipid; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; HC: hemicellulose; CEL: cellulose; CG: cyanogenic glycoside. Data are presented as the mean ± standard deviation (n=4). Data were compared between seasons for the same organ. Bold data and different letters represent significantly different means ($P$<0.05). Parameters indicated with a superscript *np* ($^{np}$) had non-parametrical distribution. Averages were calculated with the data obtained for both seasons (n=8).

Crude protein (CP) is one of the main nutritional components to evaluate forage quality. In general, plants having less 10% of CP are considered of low quality. Values between 10-15% and above 15% are considered acceptable and of good quality, respectively (Bhandari et al. 2015). The mean CP level in the leaves of *M. neesii* was 18.8%. CP content in bamboo leaves is quite variable among species. In general, bamboo leaves have CP contents above 10%. Halvorson et al. (2010) found CP levels in leaves of different bamboo species (*Phyllostachys aureosulcata* McClure, *P. bambusoides* Siebold & Zucc., *P. bissetii* McClure, *P. dulcis* McClure, *P. flexuosa* Rivièri & C. Rivièri, *P. mannii* Gamble, *P. nuda* McClure, *P. rubromarginata* McClure, *Semiarundinaria fastuosa* (Mitford) Makino, and *Arundinaria gigantea* (Walter) Muhl.) ranging from 14.8 to 17.9%. Bhardwaj et al. (2018) also evaluated the CP content in bamboo leaves (*Dendrocalamus hamiltonii*, *D. asper*, *Melocanna baccifera*, *Phyllostachys aurea* Rivièri & C. Rivièri, *P. bambusoides*, and *P. pubescens* J.Houz.) and observed similar results for other species (from 12.2 to 17.2%). Antwi-Boasiako et al. (2011) found slightly higher values for *Oxytenanthera abyssinica* (A.Rich.) Munro (19.4%), *Bambusa ventricosa* McClure (19.0%), *B. vulgaris* var. *vulgaris* (18.4%), and *B. vulgaris* var. *vittata* (18.7%). On the other hand, some species contain smaller amounts of CP (<10%), such as *B. balcooa* Roxb. (Bhandari et al. 2015; Andriarimalala et al. 2019) and *B. bambos* (Bhandari et al. 2015). Concerning culms, *M. neesii* presented 5.7% of CP. In *Guadua* spp., an American bamboo genus, CP levels ranged from 1.4% to 3.8% (Sánchez-Echeverri et al. 2014). In the present study, the content of CP in culms was lower than leaves. Dierenfeld et al. (1982) found that leaves of *Phyllostachys aureosulcata* (13.4%) contained crude protein content 4.5 times higher than culms (3.0%).

Carbohydrate is another essential nutrient in food. Generally, carbohydrate content in food crops is reported as total non-structural carbohydrate and determined using proximal analysis (Hoffman et al. 2001). For bamboos, the same pattern was followed in many works, so the results obtained in the present study are not comparable with literature. However, some studies have mentioned that bamboo leaves are a good source of carbohydrates (Sahoo et al. 2010; Antwi-Boasiako et al. 2011); in fact, some species have similar levels (about 30%) to plants used for grazing, for example, *Pennisetum purpureum* Schumach. (Antwi-Boasiako et al. 2011). The total soluble sugar (TSS) contents of leaves (1.2%) and culms (1.2%) were similar. Ten soluble sugars were identified in leaves and eight of them in culms of *M. neesii* (Figure 2.1).

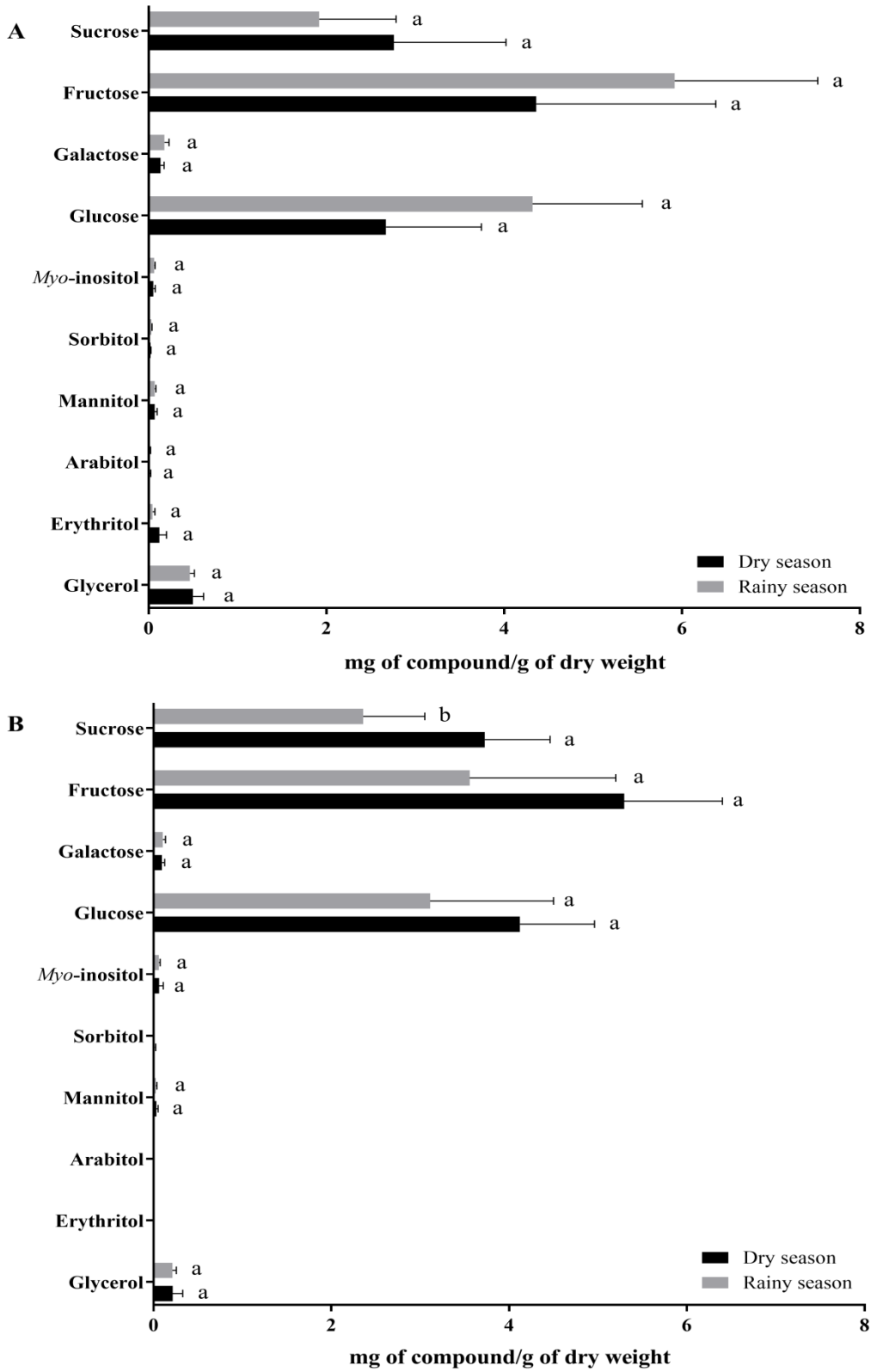


**Figure 2.1:** Content of sugars (mg of compound per g of dry weight, mg $g^{-1}$ DW) detected by GC-EIMS in *Merostachys neesii* Rupr. harvested in the dry and rainy seasons. (A) Leaves and (B) Culms. Data are presented as mean ± standard deviation (n=4). Different letters represent significantly different means ($P < 0.05$).

Among the sugars detected, there were three monosaccharides (glucose, galactose, and fructose), one disaccharide (sucrose), five acyclic sugar alcohols (glycerol, erythritol, arabitol, mannitol, and sorbitol), and a cyclitol (*myo*-inositol). In both leaves and culms, the main carbohydrates were fructose, glucose, and sucrose (Figure 2.1), corroborating data reported for culms of *Sasa palmata* (Burb.) E.G.Camus (Magel et al. 2005), *Bambusa blumeana* Schult.f., *B. vulgaris*, and *Gigantochloa scortechinii* Gamble (Abd Latif et al. 1992).

Lipid is another important nutrient in evaluating the nutritional quality of foodstuff. As observed for carbohydrates, lipid content in the present study was estimated differently from other studies. In these works, results were presented as ether extract (or crude fat) obtained by Soxhlet extraction or, mainly, by proximal analysis. On the other hand, the present study analyzed the fatty acids content by GC-EIMS. Fatty acids are an important source of energy, among other functions (Calder 2015). Basically, they can be divided into saturated (only single bonds between carbon atoms) and unsaturated (at least one double bond between carbon atoms). Humans are capable of synthesizing fatty acids, except those known as essential fatty acids (linoleic and linolenic acids) (Das 2006). Some saturated fatty acids have been associated with a higher risk of cardiovascular diseases due to evidence that they increase, for example, LDL (Low Density Lipoproteins) and total cholesterol levels. On the other hand, low to moderate consumption of polyunsaturated fatty acids (PUFA, those with more than one double bond between carbon atoms), such as linoleic, has been associated with lower LDL and total cholesterol concentrations and, consequently, lower incidence of cardiovascular problems (Calder 2015).

As mentioned earlier, bamboo shoots have high nutrient content but low fat. This feature is interesting from a nutritional point of view for humans. On the other hand, for forage purposes, very low amounts of crude fat are not interesting, for example, for dairy cattle, as they are an important source of vitamins D, E, and K, among other functions (Bhardwaj et al. 2018). In *M. neesii*, the hexane extracts of leaves and culms showed 25.8% and 35.2% of total fatty acids in their chemical composition, respectively (Table 2.1). Moreover, leaves had around three times more PUFA (19.7%) than saturated fatty acids (6.1%). Culms, on the other hand, exhibited more similar amounts of saturated (19.3%) and unsaturated (15.9%) fatty acids, with a slight predominance of saturated fatty acids (Figure 2.2). Antwi-Boasiako et al. (2011) found low lipid values (Soxhlet extraction) in *Bambusa vulgaris* var. *vittata* (1.6%), *B. vulgaris* var. *vulgaris* (1.5%), *B. ventricosa* (1.5%), and *Oxytenanthera abyssinica* (1.5%). Sahoo et al. (2010) evaluated the nutritional composition of leaves of 12 bamboo species and found the ether extract ranging between 1.4% in *Dendrocalamus strictus* and 4.7% *D. hamiltonii*. Felisberto et al. (2017) analyzed lipid content in young bamboo culm (around 36-month-old) flours of three species (*Dendrocalamus asper*,

*Bambusa tuldoides*, and *Bambusa vulgaris*) and found values below 1%. According to the authors, low lipid level in young bamboo culms is beneficial for its use as a source of starch and fiber.

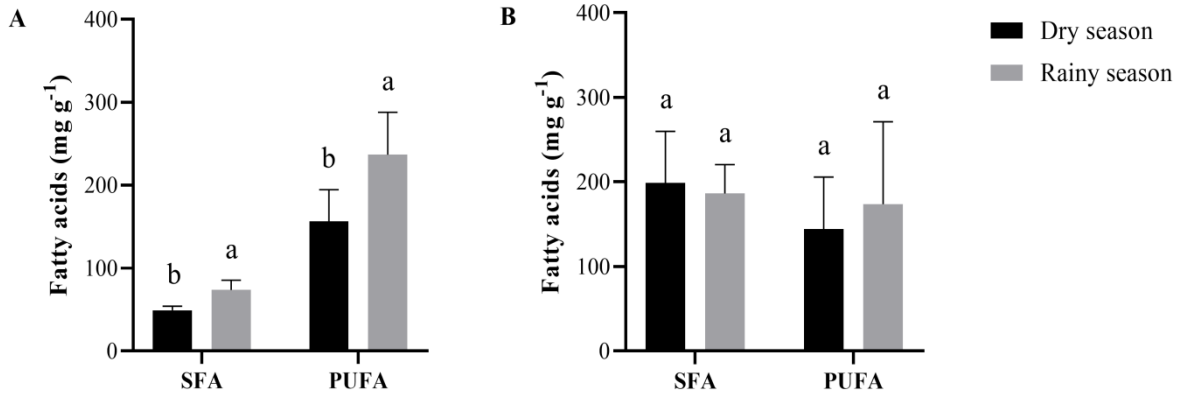


**Figure 2.2:** Content of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) (mg of compound per g of dry extract, mg $g^{-1}$) detected by GC-EIMS in *Merostachys neesii* Rupr. harvested in the dry and rainy seasons. (A) Leaves and (B) Culms. Data are presented as mean ± standard deviation (n=4). Different letters represent significantly different means ($P < 0.05$).

Regarding cell wall constituents, *M. neesii* leaves had lower NDF and ADF contents than culms. ADL levels were similar in both organs. On the other hand, leaves presented the highest hemicellulose content but the lowest cellulose content. For leaves, the NDF content (64.8%) of *M. neesii* was similar to obtained for *Phyllostachys aureosulcata* (Dierenfeld et al. 1982) and to some species evaluated by Bhandari et al. (2015). ADF amount (57.4%) was higher than the reported for other bamboo species (Khanal and Subba 2001; Halvorson et al. 2010; Bhandari et al. 2015; Andriarimalala et al. 2019). ADL data (11.2%) corroborates those obtained by Andriarimalala et al. (2019). Sahoo et al. (2010) evaluated the hemicellulose content, a structural polysaccharide present in the plant cell wall, of some bamboo species; they observed the average value of 29%. Khanal and Subba (2001) found superior value for *Arundinaria intermedia* Munro (34.5%). These results point to a less amount of hemicellulose in *M. neesii* leaves (7.3%). Cellulose amount (39.6%), another structural polysaccharide, was comparable to those found by Sahoo et al. (2010). For culms, contents of NDF, ADF, ADL, hemicellulose, and cellulose were 81.8%, 78.6%, 12.5%, 3.2%, and 56.9%, respectively. In culms of *Phyllostachys aureosulcata*, NDF, ADL, hemicellulose, and cellulose contents were 83.6%, 7.6%, 23.6%, and 38.2%, respectively (Dierenfeld et al. 1982).

In terms of fiber and lignin, for forage quality, plants with high NDF (> 60%), ADF (> 45%), and ADL (> 20%) are considered as a low-quality product. NDF (mainly hemicellulose,

cellulose, and lignin) is negatively related to the feed voluntary intake and availability energy, *i.e.* higher NDF levels reduce feed intake and energy content. On the other hand, ADF (cellulose and lignin) and ADL (primarily lignin) fractions represent lower digestibility (Bhandari et al. 2015). Regarding the presence of fiber in the human diet, this nutrient has aroused great interest due to the benefits proportionate by its ingestion, such as protection against cardiovascular diseases, type II diabetes, obesity, and colon cancer. Thus, food with high dietary fiber content is desirable to reduce some of the major health problems that affect especially the Western population (Slavin 2013). Dietary fibers include lignin and nonstarch polysaccharides (cellulose, hemicellulose, pectin, gums, and mucilage) that are not absorbed by humans or digested by enzymes present in the human gastrointestinal tract (Yang et al. 2017). In the present study, it was observed that leaves and culms of *M. neesii* showed high content of cell wall constituents, demonstrating that although for forage purposes this species has low fiber quality; it could have potential as a source of fiber in the food industry. Felisberto et al. (2017) verified that flours produced from young bamboo culms have potential as a source of fiber (total and insoluble fiber content greater than 60 g/100 g) and starch.

Ash level is a good indicator of the content of minerals present in plants (Mandal 1997; Andriarimalala et al. 2019). However, special attention should be given to bamboos. In general, monocots accumulate larger amounts of silica than eudicots, especially Poaceae species (Roselló et al. 2015). Studies on the chemical composition of ash of bamboo leaves revealed the presence of Si as one of the major elements (Lux et al. 2003; Roselló et al. 2015). Hence, high ash content does not necessarily indicate higher amounts of nutritionally important minerals. In the present study, the ash content of leaves was 5.5%. In a previous study, it was described that the ash content of *Arundinaria intermedia* leaves was 7.3% (Khanal and Subba 2001). Halvorson et al. (2010) also reported ash content below 10% for leaves of bamboo species. Culms of *M. neesii* exhibited 8.0% of ash content, greater than the one found for *Phyllostachys aureosulcata* (1.5%) (Dierenfeld et al. 1982).

Phytosterol, a biologically active non-nutrient, was detected in the hexane extract of both leaves (4.7%) and culms (18.7%) of *M. neesii*. For these two plant parts, the major phytosterols were *β*-sitosterol, stigmasterol, and campesterol (see Chapter 1, compounds **68**, **64**, and **63**, respectively). In qualitative studies using phytochemical screening, phytosterol was detected in the leaves of *Gigantochloa levis* (Blanco) Merr. (Tongco et al. 2016) and leaves, culms, and shoots of *Bambusa balcooa* (Wani et al. 2019). Moreover, Sriraman et al. (2015) detected stigmasterol (0.035% w/w) and *β*-sitosterol (0.028% w/w) in *Bambusa bambos* leaves. Phytosterols are tetracyclic compounds (1,2-cyclopentanoperhydrophenanthrene structure) with a side chain at C17. They exhibit physiological function and similar structure to cholesterol (Miras-Moreno et al. 2016), differing from this mainly regarding to substitutions in the C4 (ring A) and C24 (side chain),

unsaturation of the rings and side chain, and conjugation of the C3 alcoholic hydroxyl group with fatty acids, phenolic acids, and carbohydrates (Moreau et al. 2018). Phytosterols are largely distributed in plants, but in low amounts (Miras-Moreno et al. 2016). There are over 250 phytosterols described and the most common are campesterol, stigmasterol, and sitosterol (Moreau et al. 2018). These compounds can reduce plasma cholesterol levels in animals and have antitumor, anti-inflammatory, and antidiabetic activities. Due to their beneficial effects, some institutions, such as the US Food and Drug Administration (FDA) and European Union Scientific Committee, have allowed the addition of phytosterol in food products (Ogbe et al. 2015; Miras-Moreno et al. 2016).

Phenolic compounds, such as phenolic acids, coumarins, flavonoids, and tannins, are characterized by having at least one hydroxyl group attached to an aromatic ring (Zhang and Tsao 2016). With more than 8,000 known structures, phenolic compounds are widely widespread in plants and exhibit a range of biological activities, such as anti-inflammatory, antibacterial, and antiviral; besides, they can prevent degenerative diseases, among them cancer, diabetes, heart and neurodegenerative diseases. Many of these biological effects are mainly a result of their strong antioxidant capacity (El Gharras 2009; Tsao 2010; Shahidi and Ambigaipalan 2015). The antioxidant properties of phenolic compounds have also awakened the interest of the food industry. Growing concern about synthetic food additives and preservatives has increased the demand for natural antioxidants (Brewer 2011; Shahidi and Ambigaipalan 2015).

Flavonoids constitute an important group of phenolic compounds. They are widely distributed in plants and are found in all plant parts (Panche et al. 2016). These phenolics are based on a 15-carbon atoms skeleton organized into two benzene rings (ring A and ring B) linked by a heterocyclic pyran ring (ring C) (Kumar and Pandey 2013). With over 6,000 compounds identified (Panche et al. 2016), the different flavonoid sub-groups, such as flavones (main group found in bamboos), flavonols, isoflavonoids, among others, vary according to the substitution and oxidation pattern of the C-ring (Kumar and Pandey 2013). They are responsible for several health-beneficial and biological activities (Panche et al. 2016), and although they are not considered nutrients, these bioactive compounds are relevant in the human diet (Procházková et al. 2011).

As mentioned before, phenolic compounds, especially hydroxycinnamic acid derivatives, coumarins, and flavonoids (mainly flavones *C*-glycosides), have been described in bamboos (Van Hoyweghen et al. 2012; Wang et al. 2013). Hydromethanolic extracts of *M. neesii* leaves and culms showed 2.1% and 0.87% of total phenol content, respectively. Regarding the total flavonoid content, leaves and culms presented 0.22% and 0.13%, respectively. Due to the well-known antioxidant activity of bamboos, many studies have determined the content of total phenol and flavonoid in bamboo extracts, but most of them studied Asian bamboos.

Leaves showed higher contents than the culms of both classes of phenolic compounds. The same pattern was also observed for *Sasa quelpaertensis* Nakai (Ko et al. 2018). The methanol extract of *Bambusa arundinacea* Willd. leaves showed 1.46% and 0.67% of total phenol and flavonoid, respectively (Macwan et al. 2010). Hydroethanolic extract of leaves from *Gigantochloa levis* exhibited 0.08% of total phenol (Tongco et al. 2016). Shang et al. (2014) determined the total flavonoid content of hydroethanolic extract at 200°C of *Phyllostachys nigra* (Lodd. ex Lindl.) Munro leaves and found 657 mg QE 100 $g^{-1}$ (about 0.66%). Choi et al. (2018) investigated the total flavonoid content in the ethyl acetate fraction of extracts of *Phyllostachys nigra* var. *henonis* (Mitford) Rendle culms observing a range from 0.74 to 0.96%. Extract of leaves and culms from *Sasa palmata* presented total phenolic content of 1.21% and 0.35%, respectively (Kurosumi et al. 2007). The results obtained for *M. neesii* showed that this species is also a good source of phenolic compounds. However, it is noteworthy that although the quantifications performed in these works used the same colorimetric methods and standards (with slight modifications) used in the present study, the extraction methods were different.

Tannins are also widespread polyphenolic compounds in plants. They are mainly known to have the property of binding to the protein, precipitating them (Barbehenn and Constabel 2011). They can be classified into hydrolyzable tannins (gallotannins and ellagitannins) and condensed tannins or proanthocyanidins (prodelphinidins and procyanidins). Some potential beneficial health effects have been reported for these compounds, such as antioxidant, cardioprotective, neuroprotective, antithrombotic, immunomodulatory, anti-inflammatory, antimicrobial, antidiabetic, and anticancer (Smeriglio et al. 2017; Rauf et al. 2019). Tannins have long been considered as antinutrients in forage as they affect nutrient availability by forming complexes with protein, minerals, vitamins, and starch. However, it is currently known that the presence of tannins in feed plants does not necessarily negatively affect nutrient digestibility; it depends on the animal and the molecular structure, origin, and amount of tannin. Indeed, depending on these factors, tannins may even be beneficial to cattle (Naumann et al. 2017). Bamboos generally do not have significant amounts of tannins (Halvorson et al. 2010). In the present study, tannins were detected neither in leaves nor in culms of *M. neesii*, corroborating data reported for other bamboo species where these compounds were not present at significant levels (Khanal and Subba 2001; Bhardwaj et al. 2018).

Finally, cyanogenic glycosides, unlike tannins, are common in bamboos and constitute an important group of antinutrients (Choudhury et al. 2010; Satya et al. 2010). These compounds are produced from amino acids and contain at least one unit of sugar, usually glucose. Plants that synthesize cyanogenic glycosides contain these compounds and their specific catabolic enzymes ($\beta$-glycosidases) in separated cell compartments, avoiding autotoxicity. After wounding, these enzymes get in contact with cyanogenic glycosides, hydrolyzing them and resulting in the release of

hydrogen cyanide (HCN), a highly toxic compound (Gleadow and Møller 2014). To verify the presence of cyanogenic glycosides in *M. neesii*, a qualitative analysis was performed to detect these compounds in fresh leaves. Significant amounts of cyanogenic glycosides were not detected for the leaves of this species. Bhandari et al. (2015) evaluated cyanogenic glycoside content in 14 bamboo species and only *Bambusa balcooa*, *Dendrocalamus giganteus*, *D. hamiltonii*, and *Melocanna baccifera* had acceptable levels, *i.e.* up to 0.1 mg $g^{-1}$. On the other hand, all species investigated by Bhardwaj et al. (2018) (*D. hamiltonii*, *D. asper*, *Melocanna baccifera*, *Phyllostachys aurea*, *P. bambusoides*, and *P. pubescens*) were above acceptable levels. Interestingly some species studied by Bhandari et al. (2015), which presented low levels of cyanogenic glycosides, were also evaluated by Bhardwaj et al. (2018); however, in the second work the contents of these antinutrients were above acceptable, indicating that environmental factors may influence the content of these secondary metabolites. Indeed, Bhardwaj et al. (2018) found an increase in cyanogenic glycoside content with advancing growth season (from November to February).

### 3.2 Implications of seasonal variation on nutritional potential of *Merostachys neesii*

PEFI is located in São Paulo city (Brazil), whose climate is designated Cwa (subtropical humid), according to the Koppen classification. The climate is characterized by a dry winter and a humid summer. Thus, there are two well-defined seasons: dry season (from April to September) and rainy season (from October to March) (http://www.estacao.iag.usp.br/seasons/index.php#). Monthly data of total precipitation, air temperature, solar irradiation, and relative air humidity from May/2017 to April/2018 are shown in Figure 2.3. Precipitation was high from October/2017 to March/2018 (ranging from 124.6 to 222.2 mm), except in February/2018 (62.5 mm) when precipitation was below average. On the other hand, the driest months were mainly from June/2017 to September/2017 (ranging from 2.2 to 102.0 mm). Temperature was lower from May/2017 to August/2018 (mean value of 17°C) and started to increase in September/2017, maintaining an average temperature of 21°C (from October/2017 to March/2018). Then, in April/2018, it started to decrease again. A similar pattern was observed for solar irradiation, from May/2017 to August/2017 the average radiation was 13 MJ $m^{-2}$ while from September/2017 to March/2018 was around 18.8 MJ $m^{-2}$. Relative humidity remained relatively constant (around 80%), except for September/2017 (about 70%) when there was a marked reduction (data not shown).

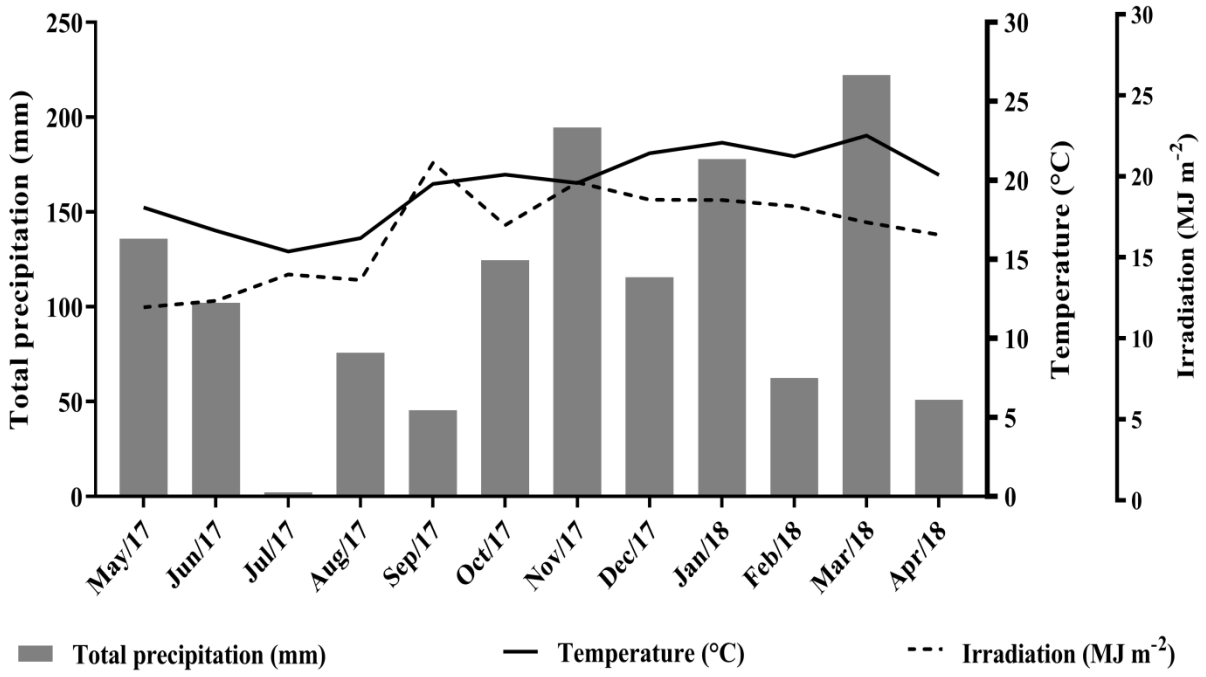


**Figure 2.3:** Meteorological data of the Fontes do Ipiranga State Park (PEFI), São Paulo/Brazil, during the studied period. Data were obtained from the Meteorological Station of the Institute of Astronomy, Geophysics and Atmospheric Sciences of USP (IAG-USP).

For all the meteorological data evaluated in this study, data referring to the 30 days before the harvest (including the harvest day) were considered to verify if there was a significant difference between the seasons. The rainy season had significantly higher precipitation, air temperature, and solar irradiation than the dry season (Figure 2.4). There was no significant difference in relative air humidity between seasons. Mean values of total precipitation, air temperature, solar irradiation, and relative air humidity in the dry season were 24.4 mm, 16°C, 13 MJ $m^{-2}$, and 76%, respectively. For the rainy season, these data corresponded to 177.9 mm, 22°C, 18 MJ $m^{-2}$, and 81%, respectively.

Although there was a significant difference for precipitation, temperature, and solar irradiation between seasons, none of the nutritional parameters here evaluated (protein, crude protein, total sugar, NDF, ADF, ADL, hemicellulose, cellulose, and ash) showed significant alteration, for both leaves and culms, except for lipid. Leaves showed higher lipid content in the rainy season than in the dry season (Table 2.1). Bhardwaj et al. (2018) observed that the ether extract content (lipid content) in bamboo leaves of some species reduced from November to February.

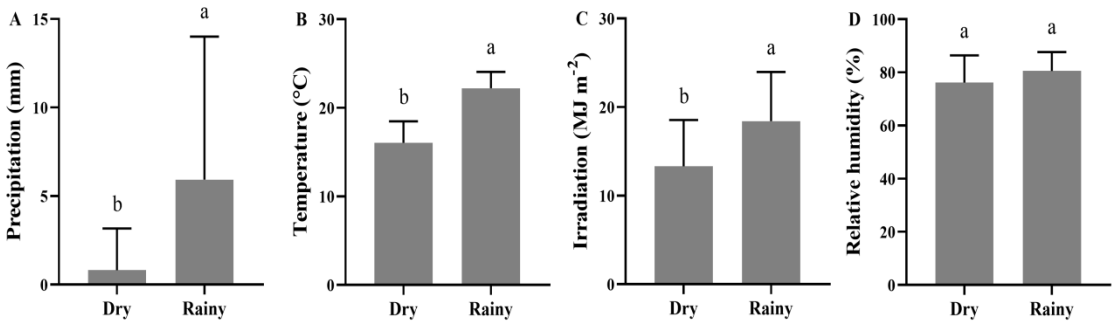


**Figure 2.4:** Meteorological data of 30 days before plant harvest. (A) Precipitation, (B) Temperature, (C) Solar irradiation, and (D) Relative air humidity. Data were obtained from the Meteorological Station of the Institute of Astronomy, Geophysics and Atmospheric Sciences of USP (IAG-USP). Different letters represent significantly different means ($P < 0.05$).

Contrary to the findings of this study, seasonal fluctuations in the content of many nutrients were found for other bamboo species. Okahisa et al. (2006) evaluated the glucose and starch levels in *Phyllostachys pubescens* culms harvested from a bamboo plantation forest in Yawata (Japan). The authors found that glucose concentration was normally higher during spring and summer as compared with autumn and winter. For starch, the pattern was opposite; there was a lesser amount of starch in summer than in winter. Yayota et al. (2009) reported that the crude protein content in leaves from *Pleioblastus argenteostriatus* f. *glaber* exhibited no significant difference between spring and autumn. Nevertheless, dry matter and lignin contents were higher in the autumn while NDF content was higher in the spring. Besides, ruminal degradability was lower during the autumn and this result was related to higher lignin content in this season. Halvorson et al. (2010) assessed several nutritional components in 10 bamboo species and found that total non-structural carbohydrates decreased from mid-summer to winter, while crude protein, ADL, ADF, and ash increased. Cellulose content was the only component that did not vary among seasons.

Despite total soluble sugar content showed no significant difference, the same was not observed for each sugar constituent. In the dry season, culms presented significantly higher content of sucrose than in the rainy season. Besides, still for culms, sorbitol was detected only in the dry season (Figure 2.1B).

Sugars display different functions in stressed plants. They can act as signaling molecules, energy sources, and compatible solutes (Rosa et al. 2009). Compatible solutes are usually small molecules of low molecular weight, neutral, soluble, and are not toxic in high concentrations. The main groups of osmolites are polyamines, betaines, saccharides, sugar alcohols, and amino acids, especially proline which is the main compatible solute (Singh et al. 2015). It has been observed that

during drought stress, among other stresses, there is an increase in the amount of compatible solutes in plant tissues to protect membrane integrity, stabilize proteins and enzymes, ROS scavenging, and maintain cell turgor by adjusting cellular osmotic, protecting the cell apparatus from damage caused by dehydration (Singh et al. 2015; Sami et al. 2016). Sucrose and sorbitol, which increased in culms during the dry season, are examples of compatible osmolytes (Seki et al. 2007).

Regarding bioactive compounds in *M. neesii*, total phytosterol, phenol, and flavonoid contents showed seasonal changes (Table 2.1). Seasonality is an important environmental factor that can affect both the quantity and the quality of compounds produced by plants. Different classes of secondary metabolites are affected according to the time of year in which the plant is harvested (Gobbo-Neto and Lopes 2007).

Total phytosterol content in leaves during the rainy season (5.9%) was higher than in the dry season (3.5%). Culms did not show a significant difference between seasons. Lu et al. (2009) evaluated the total phytosterol content in *P. pubescens* shoots in three seasons (summer, spring, and winter). They observed the highest phytosterol content during spring. In *Rauvolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae) was observed higher phytosterol content during summer; the authors suggested that temperature may have influenced this variation (Dey and Pandey 2014).

Concerning phenolic compounds, total phenol content was affected only in culms, which showed higher content in the dry season (1.1%) than in the rainy season (0.63%). Seasonality also affected the flavonoid content of leaves and culms, but in the opposite way. Leaves exhibited a high amount of flavonoids during the rainy season (dry: 0.20%; rainy: 0.23%), while culms showed the highest content of flavonoids during the dry season (dry: 0.15%; rainy: 0.11%). Overall, these results are different from those described in the literature for bamboo species.

In *Sasa quelpaertensis*, total phenol content in leaves and culms were lower during spring (May) and showed no significant difference until early-autumn (September). However, the amount of total phenol in leaves began to increase during the autumn and presented the highest content in winter. On the other hand, there was a reduction in total phenol in culms in this same period. A similar pattern was observed for total flavonoid content (Ko et al. 2018). Higher total phenol and flavonoid content in leaves during autumn-winter than spring-summer was also observed for *Sasa argenteostriata* (Ni et al. 2012), *Pleioblastus kongosanensis* f. *aureostriatus* Muroi & Yu. Tanaka, and *Shibataea chinensis* Nakai (Ni et al. 2014).

A possible explanation for these opposite results is that *Sasa*, *Shibatea*, and *Pleioblastus* genera belong to the Arundinarieae tribe, while *Merostachys* belongs to Bambuseae tribe (Soreng et al. 2017). Another explanation is that the locality where these plants occur (temperate climate) the climate conditions are quite different from those found in the region where *M. neesii* was harvested.

## 4. Conclusions

Leaves of *M. neesii* showed concentrations of nutrients similar to other bamboo species reported as potential sources of fodder. Also, both leaves and culms showed bioactive compounds, namely phytosterols and phenolic compounds. Nevertheless, while leaves had a higher content of phenolic compounds, culms were richer in phytosterols.

Seemingly, seasonality did not affect the nutrient content of leaves and culms, except for lipid; however, secondary metabolites were significantly affected.

It was observed higher production of lipids (both saturated and polyunsaturated fatty acids), phytosterols, and flavonoids in leaves during the rainy season. On the other hand, there was a reduction in the total phenol and total flavonoid contents in culms in this season.

Based on the results presented here, *M. neesii* could be considered as a potential source of supplementary forage, as well as a source of bioactive compounds. Furthermore, results suggest that the best season to harvest this plant is during the rainy season when there is a high content of phenolic compounds, lipids, and phytosterols in the leaves.

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# Chapter 3 – Biological potential of *Merostachys neesii*: seasonal implications

## 1. Introduction

Reactive oxygen species (ROS) includes radical ($OH^{\bullet}$ and $O_2^{\bullet-}$) and non-radical molecules ($H_2O_2$ and $OH^-$) which in eukaryotic organisms are mainly formed by the partial reduction of oxygen during the mitochondrial oxidative phosphorylation process (Ray et al. 2012; Lushchak 2014). In plants, chloroplasts also represent an important source of ROS production (Mittler 2017). In addition to mitochondria and chloroplasts, ROS may also be produced in the cytosol, peroxisomes, endoplasmic reticulum, lysosomes, and plasma membrane. Reactive nitrogen species (RNS), such as nitric oxide ($NO^{\bullet}$) produced from *L*-arginine, is another important group of naturally occurring reactive species in living organisms (Di Meo et al. 2016). ROS and RNS act on different biological processes beneficial to living beings, such as signaling, gene expression, and combat to infectious agents (Fransen et al. 2012; Lushchak 2014). Nevertheless, at moderate or high levels, they can cause damage to important biomolecules, such as DNA, lipids, and proteins (Pisoschi and Pop 2015; Di Meo et al. 2016).

Living organisms have antioxidant systems that help eliminate or reduce the negative effects of reactive species. These antioxidants can be classified as enzymatic and non-enzymatic. In the first group several enzymes are listed, as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx), while the second group is composed mainly by vitamins C and E, glutathione, melatonin, uric acid, polyamines, bilirubin, carotenoids, and polyphenols (Lobo et al. 2010; Birben et al. 2012; Mirończuk-Chodakowska et al. 2018). Humans obtain some of these non-enzymatic antioxidants only in their diet through the consumption of fruits, vegetables, and dietary supplements (Lushchak 2014; Mirończuk-Chodakowska et al. 2018). Nevertheless, there are situations where there is an imbalance in the production and elimination system of reactive species leading to an increase in ROS and RNS concentrations and promoting cellular damage; this oxidant/antioxidant imbalance in favor of oxidants is known as oxidative stress (Birben et al. 2012; Pisoschi and Pop 2015). Oxidative stress is associated with aging as well as several pathologies such as cancer, diabetes, atherosclerosis, inflammatory, and cardiovascular and neurodegenerative diseases (Lobo et al. 2010; Birben et al. 2012; Rahal et al. 2014; Pisoschi and Pop 2015).

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders affecting millions of elderly people around the world. AD is an incurable disease associated with loss of memory, cognitive deficit, and behavioral changes (Kumar and Singh 2015; Hung and Fu 2017). Currently, it is not known exactly what causes AD and the pathogenesis is not yet fully understood as well (Tramutola et al. 2017). However, some neuropathological changes observed in AD are

extracellular deposition of *β*-amyloid in diffuse and neuritic plaques, hyperphosphorylated Tau protein accumulating intracellularly as neurofibrillary tangles, acetylcholine deficiency, microgliosis, widespread neuronal loss, and neuroinflammation (Reitz and Mayeux 2014; Hung and Fu 2017). Moreover, increasing evidence has shown that oxidative stress plays an important role in the onset and progression of AD (Wang et al. 2014; Tramutola et al. 2017). In AD patients, increased levels of oxidative stress have been reported when compared to control people of the same age. This oxidative imbalance has been observed through increased levels of lipid peroxidation, oxidative protein modification, increased oxidative damage to DNA and RNA, reduced levels of non-enzymatic antioxidants (Wang et al. 2014) as well as reduced enzymatic antioxidant activity (Wang et al. 2014; Tramutola et al. 2017). Different therapeutic approaches covering the different targets involved in AD pathology have been developed, including acetylcholinesterase inhibitors, which is an important therapeutic form in the treatment of AD, and antioxidant therapy (Kumar and Singh 2015).

Bamboos are giant grasses with enormous socioeconomic relevance, especially in Asia, where people have been using these plants to treat illness for centuries (Sangeetha et al. 2015; Nirmala et al. 2018). Bamboos are well known for their antioxidant activity. In China, it was authorized the use of a standardized bamboo leaf extract (AOB) as a food antioxidant (Lu et al. 2005). The main constituents described in this bamboo extract are phenolic compounds, among them flavonoids (Lu et al. 2006). Flavonoids are plant secondary metabolites with several described biological activities, including strong antioxidant activity (Procházková et al. 2011) and acetylcholinesterase inhibitory activity (Uriarte-Pueyo and Calvo 2011).

Biotic and abiotic factors, including seasonality, can affect the content of bioactive compounds produced by plants (Gobbo-Neto and Lopes 2007). Studies have shown that concentration of secondary metabolites and the intensity of biological activities of plant extracts were affected by seasonality (Chaves et al. 2013; Sartor et al. 2013; Zhu et al. 2013; de Alencar Filho et al. 2017). Thus, studies on seasonal changes in bioactive compound content and biological activity are important to determine the best time for plant harvest; however, few medicinal species have been evaluated regarding their chemical composition and pharmacological potential along different seasons.

*Merostachys* Spreng. is a genus of woody bamboo (Bambuseae tribe) whose diversity center is located in Brazil (Judziewicz et al. 1999). The presence of phenolic compounds, such as phenolic acids and flavonoids, was described for this genus (Jose et al. 2016; Gagliano et al. 2018). Regarding the biological potential, it has been reported antioxidant activity for *M. pluriflora* Munro ex E.G. Camus (Gagliano et al. 2018), phytotoxic activity for *M. riedeliana* Rupr. ex Döll (Jose et al. 2016), and antioxidant, phytotoxic, and anticholinesterase activities for *M. magellanica* Send.

(Grombone-Guaratini et al. 2012). *M. neesii* Rupr. is an endemic species of the Atlantic Rain Forest and there is no study on its major chemical constituent and biological potential.

Assuming that Brazil has a huge biodiversity of bamboos with few species studied on their chemical composition and biological potential, together with the seasonal influence on the amounts of bioactive molecules, the aims of this study were: (*i*) to evaluate the antioxidant and anticholinesterase potential of *M. neesii* leaves and culms; (*ii*) to investigate the effects of seasonality on these biological activities as well as on the main chemical constituents of this species; and (*iii*) to verify if there is a correlation between chemical composition and biological activity.

## 2. Material and Methods

### 2.1 Plant material and extraction procedures

Leaves and culms (culms plus branches) of four *M. neesii* clumps were collected in the dry (August/2016, n=3; and August/2017, n=4) and rainy (March/2017, n=3; and February/2018, n=4) seasons at the Fontes do Ipiranga State Park (PEFI), located in São Paulo/Brazil.

Extraction procedures were carried out as described in chapter 1 (item 2.2). Briefly, plant material was frozen, freeze-dried, powdered, and submitted to two extraction processes: 1. serial maceration (75 g $L^{-1}$) using hexane and 70% ethanol for 21 days each, with solvent exchanges every two days, at room temperature, and constant stirring; and 2. infusion (8 g $L^{-1}$) for 20 minutes. Hexane, hydroethanol, and aqueous extracts of leaves (**HE-L**, **EE-L**, and **AE-L**) and culms (**HE-C**, **EE-C**, and **AE-C**) were concentrated under reduced pressure at 45ºC using a rotary evaporator and freeze-dried for yield calculation.

Extract yield was calculated according to the following equation:

% Yield = (W1 x 100) / W2, where W1 and W2 correspond to the weight of the freeze-dried extract and the weight of the dry plant material used in the extraction, respectively.

### 2.2 Determination of the major constituents of *Merostachys neesii*

Hexane extracts were analyzed by GC-EIMS as described in chapter 1 (item 2.3.1 - nonpolar compounds analysis). For the quantification of nonpolar constituents, areas of the compounds were compared with the area of the nonadecane (internal standard). Results were expressed as mg $g^{-1}$ of the dry extract.

Hydroethanol and aqueous extracts were analyzed by HPLC-DAD as described in chapter 1 (item 2.3.2). For quantification, the areas of the flavonoids (see chapter 1, Table 1.3) were compared with a standard curve of vitexin (3 to 120 µg $mL^{-1}$) analyzed under the same conditions of the samples. Results were expressed as mg equivalent to vitexin (VE) per g of dry extract (mg $g^{-1}$ VE).

**2.3 Determination of antioxidant activity**

For all antioxidant assays, the antioxidant potential of the leaves and culms extracts was performed in methodological triplicate using a Synergy $H^1$ microplate reader (BioTek, Inc.). Results were expressed as mean ± standard deviation of the effective concentration to achieve 50% ($EC_{50}$) of the antioxidant activity. $EC_{50}$ values were calculated using a linear regression equation between the well concentration and the antioxidant activity percentage (% AA) of each sample.

**- DPPH radical scavenging capacity assay**

DPPH radical scavenging activity was determined according to the method described by Furlan et al. (2015). A 0.20 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution was prepared and 200 µL were mixed with 20 µL of the extracts diluted in 10% DMSO (15.6 to 1000 µg $mL^{-1}$). After an incubation period of 20 min at room temperature, the absorbance was measured at 515 nm. Trolox (12.5 to 200.0 µg $mL^{-1}$) and 10% DMSO were used as positive and negative controls, respectively. Percentage of antioxidant activity (% AA) was calculated using the following formula:

% AA = [($Abs_{nc}$ – $Abs_{sample}$) / $Abs_{nc}$] x 100, where $Abs_{nc}$ is the absorbance of the negative control and $Abs_{sample}$ is the absorbance of the sample.

**- ABTS radical scavenging capacity assay**

ABTS radical scavenging activity was determined according to Santos et al. (2016). ABTS radical solution was prepared by mixing the stock solutions of 7 mM ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and 2.6 mM potassium persulfate (1:1); then, it was kept in the dark at room temperature for 12 to 16 h before use. At the beginning of the test, 1 mL of the ABTS radical solution was diluted in 30 mL of methanol. A 280 µL aliquot of this $ABTS^+$ solution was added to 20 µL of the extracts diluted in 10% DMSO (15.6 to 1000 µg $mL^{-1}$). After an incubation period of 2 h at room temperature, the absorbance was evaluated at 734 nm. Trolox (10 to 200 µg $mL^{-1}$) and 10% DMSO were used as positive and negative controls, respectively. Percentage of antioxidant activity (% AA) was calculated using the following formula:

% AA = [($Abs_{nc}$ – $Abs_{sample}$) / $Abs_{nc}$] x 100, where $Abs_{nc}$ is the absorbance of the negative control and $Abs_{sample}$ is the absorbance of the sample.

**- Iron (III) reduction to iron (II) (FRAP) assay**

Iron (III) reduction to iron (II) activity was evaluated according to Furlan et al. (2015). FRAP reagent was obtained by combining 2.5 mL of 10 mM TPTZ (2,4,6-tris (2-pyridyl) s-triazine), 2.5 mL of 20 mM ferric chloride, and 25 mL of 38 mM acetate buffer (pH 3.6). For each 10 µL of the extracts diluted in 10% DMSO (15.6 to 1000 µg $mL^{-1}$) were added 25 µL of ultrapure water and 265 µL of the FRAP reagent. After an incubation period of 30 min at 37°C, the absorbance was detected at 595 nm. Trolox (10 to 200 µg $mL^{-1}$) and 10% DMSO were used as

positive and negative controls, respectively. Percentage of antioxidant activity (% AA) was calculated using the following formula:

% AA = ($Abs_{sample}$ / $Abs_{pc}$) x 100, where $Abs_{sample}$ is the absorbance of the sample and $Abs_{pc}$ is the absorbance of the maximum concentration of the positive control.

**- Oxygen Radical Absorbance Capacity (ORAC) assay**

Oxygen radical absorbance capacity was evaluated according to Santos et al. (2016). Sodium fluorescein solution (8 nM) was freshly prepared in phosphate buffer solution (PBS) (75 mM, pH 7.0). A 150 µL aliquot of this solution was mixed with 25 µL of the extracts diluted in PBS (3.9 to 250 µg $mL^{-1}$). The reaction mixture was incubated for 30 minutes at 37°C. Then, 25 µL of 75 mM AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride) in phosphate buffer were added. Fluorescence (excitation: 485 nm; emission 528 nm) was measured every 1 min for 2 h. Trolox (3.13 to 100 µM) and PBS were used as positive and negative controls, respectively. The antioxidant capacity was based on the calculation of the area under the curve (AUC), by the use of the formula:

AUC = 1+ $f_1/f_0$+ $f_2/f_0$+ $f_3/f_0$+… $f_i/f_0$, where $f_0$ is the initial fluorescence reading at 0 min and $f_1$ is the fluorescence reading at time 1. The net AUC was calculated by subtracting the AUC of the negative control from the AUC of the sample. Percentage of antioxidant activity (% AA) was calculated using the following formula:

% AA = (Net $AUC_{sample}$ / Net $AUC_{pc}$) x 100, where Net $AUC_{sample}$ is the net AUC of the sample and Net $AUC_{pc}$ is the net AUC of the maximum concentration of the positive control.

**2.4 Determination of anticholinesterase activity**

For the acetylcholinesterase (AChEI) and butyrylcholinesterase (BChEI) inhibition assays, it was used a stock solution (4.93 U $mL^{-1}$) of acetylcholinesterase (AChE) from *Electrophorus electricus* (electric eel, type VI-S freeze-dried powder 987 U $mg^{-1}$) and a stock solution (14 U $mL^{-1}$) of butyrylcholinesterase (BChE) from equine serum (140 U $mg^{-1}$), both dissolved in 50 mM Tris/HCl buffer (pH 8.0). Results were expressed as inhibitory concentration to reach 50% inhibition ($IC_{50}$). $IC_{50}$ values were calculated using a nonlinear logarithmic regression equation between the well concentration and the enzyme inhibition percentage of each sample.

Anticholinesterase activity was evaluated according to Mathew and Subramanian (2014), based on Ellman's method (Ellman et al. 1961), with modifications. At the time of the test, 5 mM DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) in Tris/HCl buffer (50 mM, pH 8.0) containing 0.1 M sodium chloride and 0.02 M magnesium chloride, 18 mM acetylthiocholine iodide (ATCI) in ultrapure water, and further AChE-dilution (0.13 U $mL^{-1}$) in buffer 50 mM Tris/HCl (pH 8.0) containing 0.1% BSA (bovine serum albumin, fraction V, pH 5.0-5.6) were prepared. A 20 µL aliquot of the extract diluted (31.25 to 2,000 µg $mL^{-1}$) in 50 mM Tris/HCl buffer (pH 8.0), 100 µL

of DTNB solution, 40 µL of 50 mM Tris/HCl buffer (pH 8.0), and 20 µL of diluted enzyme were added. The reaction mixture was incubated for 15 min at 25°C. After that, 20 µL of ATCI were added and absorbance was measured (at 412 nm) at time zero ($t_0$) and every 5 min for 20 min. Neostigmine (15.6 to 2,000 ng $mL^{-1}$) and 50 mM Tris/HCl buffer (pH 8.0) were used as positive and negative controls, respectively. The absorbance of the sample and controls were obtained by subtracting the absorbance in 20 min from the absorbance in the $t_0$.

The same procedures were performed by BChE inhibition, except for further BChE-dilution (0.30 U $mL^{-1}$), ATCI was replaced by 18 mM butyrylthiocholine iodide (BTCI) in ultrapure water, and neostigmine (125 to 8,000 ng $mL^{-1}$).

Percentage of AChE or BChE inhibition (% AChEI or % BChEI) was calculated using the following formula:

% AChEI or % BChEI = $100 - ((Abs_{sample} / Abs_{nc}) \times 100$, where $Abs_{sample}$ is the absorbance of the sample and $Abs_{nc}$ is the absorbance of the negative control.

### 2.5 Statistical analysis

The data were presented as mean ± standard deviation. Normality (Shapiro-Wilk test) and homoscedasticity (Barlett test) of the data were tested using R software (version 3.5.1). Data showing normal distribution were analyzed by one-way ANOVA followed by *post-hoc* Tukey test (equal variance) or one-way Welch followed by *post-hoc* Games-Howell test (unequal variance). When required, data transformation ($log_{10}$) was performed. For non-parametric data, data were compared by the Kruskal-Wallis rank test followed by a *post-hoc* Dunn test. All tests were compared at a significance level of 95%.

Additionally, principal component analyses (PCA), using Fitopac 2.1 software, were performed to summarize results and identify possible patterns of biological activities and chemical composition in different seasons, as well as patterns between activities and chemical composition.

## 3. Results and discussion

### 3.1 Meteorological data during harvest time

As discussed in chapter 2 (item 3.2), the climate of São Paulo (Brazil), where the PEFI is placed, exhibits a dry winter and a humid summer. Figure 3.1 shows monthly data of total precipitation, air temperature, and solar irradiation from May/2016 to April/2018. Rainy season (from October to March) presented higher total precipitation, temperature, and solar irradiation than the dry season (from April to September). Relative humidity was approximately constant (about 80%, data not shown). In January/2017, it rained 67% more (368 mm) than the historical average for the same period (219 mm). The means of total precipitation, temperature, and solar irradiation of the rainy period for the first and second years of harvesting were 179 mm, 21.6°C, and 19.2 MJ $m^{-2}$

and 150 mm, 21.4°C, and 18.4 MJ $m^{-2}$, respectively. The same data for the dry periods of the first and second years of harvesting were 73 mm, 17.5°C, and 14.0 MJ $m^{-2}$ and 91 mm, 17.7°C, and 14.5 MJ $m^{-2}$, respectively.

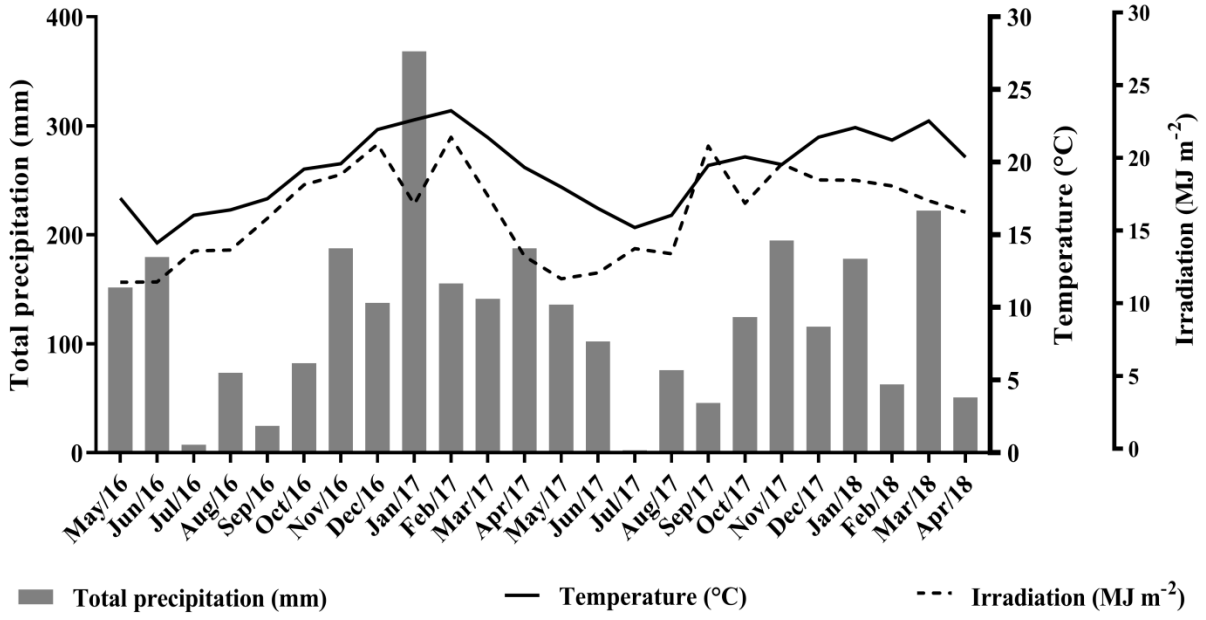


**Figure 3.1:** Meteorological data of the Fontes do Ipiranga State Park (PEFI), São Paulo/Brazil, during the studied period. Data were obtained from the Meteorological Station of the Institute of Astronomy, Geophysics and Atmospheric Sciences of USP (IAG-USP).

Meteorological data of 30 days before harvesting is shown in figure 3.2. Although the 30-day cumulative precipitation in the rainy season/2017 (205 mm) was three times higher than in the dry season/2016 (64 mm), a significant difference was not observed, unlike for the second year (Figure 3.2A). This can be explained by the fact that in the rainy season/2017 there were several days without rain (a similar number to the dry season/2016) and some days with high precipitation. Moreover, the rainy season showed air temperature and solar irradiation significantly higher than the dry season (Figure 3.2B-C). There was no significant difference in relative air humidity between seasons (Figure 3.2D).

### 3.2 Seasonal changes on extract yield

Leaf extracts showed higher yields than culm extracts. Such results were expected since the culms contain high levels of fibers. For both plant parts, the mean yield values of hydroethanol (**EE-L** 12.5%; **EE-C** 8.5%) and aqueous (**AE-L**: 12.0%; **AE-C**: 8.1%) extracts were higher than mean yield of hexane extracts (**HE-L**: 3.0%; **HE-C**: 0.93%), as can be seen in table 3.1. These results indicate the predominance of polar constituents instead of nonpolar. *Phyllostachys*

*pubescens* J. Houz. shoot skin was serially extracted using solvents of different polarities; methanol and aqueous extracts yielding 5 and 14 times more than hexane extract, respectively (Tanaka et al. 2011). Park and Jhon (2010) partitioned the methanol extract of *P. pubescens* and *P. nigra* (Lodd. ex Lindl.) Munro shoots and noted high yields of polar fractions, indicating that major soluble compounds were of high polarity. Studies about the chemical characterization of bamboos have reported mainly the presence of phenolic compounds in their leaves and culms (Keski-Saari et al. 2008; Van Hoyweghen et al. 2012; Tanaka et al. 2014; Gagliano et al. 2018).

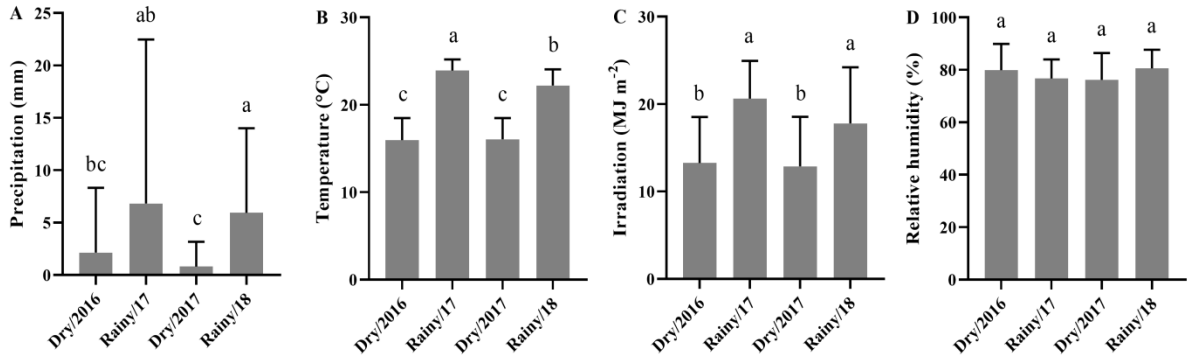


**Figure 3.2:** Meteorological data of 30 days before plant harvest. (A) Precipitation, (B) Temperature, (C) Solar irradiation, and (D) Relative air humidity. Data were obtained from the Meteorological Station of the Institute of Astronomy, Geophysics and Atmospheric Sciences of USP (IAG-USP). Different letters represent significantly different means ($P < 0.05$).

Hexane extracts from the second year of harvesting (dry season/2017 and rainy season/2018) showed higher yield than those obtained from plant material harvested in the first year. For culms, this increase was observed in both dry and rainy seasons, while for leaves a significant difference occurred only between rainy seasons. Apparently, there was a greater production of nonpolar compounds during the second year of harvesting (Table 3.1). These results may not be related only to seasonal changes but may be a result of plant growth and development.

## 3.3 Seasonal changes on bioactive compounds of *Merostachys neesii*

### 3.3.1 Estimation of bioactive compounds

Contents of bioactive constituents (flavonoids and terpenes) detected in extracts of leaves and culms from *M. neesii* are shown in table 3.2. Results of terpenes (lupenone and phytosterols) were expressed as mg $g^{-1}$, calculated by comparison with the area of nonadecane (internal standard). For flavonoids, results were expressed as mg equivalent to vitexin per gram of dry extract (mg VE $g^{-1}$).

Four major terpenes were detected in hexane extracts (**HE-L** and **HE-C**) being one triterpene and three phytosterols. Lupenone (**70**) was the only non-steroidal terpene detected in *M. neesii*. This triterpene was found only in culms. Campesterol (**63**), stigmasterol (**64**), and *β*-sitosterol (**68**) were the major phytosterols in both leaves and culms. Culms presented five, eight, and three times higher amounts of these phytosterols than leaves, respectively; hence, culms also showed significantly higher total terpene amounts than leaves.

**Table 3.1:** Extraction yield (%) of hexane (**HE**), hydroethanol (**EE**), and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) from *Merostachys neesii* harvested in the dry and rainy seasons (dry/2016, n=3; rainy/2017, n =3; dry/2017, n=4; and rainy/2018, n=4).

| Extracts | Seasons | | | | Averages* |
|---|---|---|---|---|---|
| | Dry/2016 | Rainy/2017 | Dry/2017 | Rainy/2018 | |
| **HE-L** | **1.92 ± 1.19ab** | **2.83 ± 0.05b** | **3.22 ± 0.25ab** | **3.72 ± 0.10a** | 3.00 ± 0.83 |
| **EE-L** | 12.24 ± 0.66 | 12.17 ± 1.29 | 11.83 ± 3.13 | 13.58 ± 1.19 | 12.49 ± 1.86 |
| **AE-L** | 11.23 ± 1.19 | 11.93 ± 1.35 | 12.49 ± 1.68 | 12.08 ± 1.54 | 11.98 ± 1.38 |
| **HE-C** | **0.53 ± 0.03b** | **0.71 ± 0.08b** | **1.12 ± 0.25a** | **1.22 ± 0.13a** | 0.93± 0.32 |
| **EE-C** $^{np}$ | 9.76 ± 1.91 | 7.79 ± 0.60 | 9.20 ± 1.37 | 7.24 ± 0.81 | 8.46 ± 1.52 |
| **AE-C** | 8.86 ± 1.03 | 7.89 ± 0.37 | 8.88 ± 1.51 | 6.75 ± 1.08 | 8.05 ± 1.37 |

Data are presented as mean ± standard deviation. Mean values in the same row followed by different letters indicate a statistically significant difference ($P < 0.05$). Extracts signalized with a superscript *np* ($^{np}$) indicate non-parametrical distributions. *Averages were calculated with the data obtained for all seasons (n=14).

Regarding flavonoids, ten flavones (five apigenin and five tricin derivatives) were found in aqueous and hydroethanol extracts. Flavonoids **79**, **82** and **83** (apigenin derivatives) were significantly more abundant in leaf extracts than in culm extracts. On the other hand, culm extracts showed higher levels of flavonoid **84** (tricin derivative) than leaves. Flavonoids **80** and **81** showed no significant difference between extracts. Flavonoid **85** (tricin derivative) was found only in **EE-L** and **EE-C** and culm extract showed significantly higher content of this compound than the leaf extract. Flavonoids **86**-**88** (tricin derivatives) were detected only in **EE-C**. All extracts showed a similar amount of total flavonoids; however, significant differences were observed for total apigenin and tricin contents. **EE-L** and **AE-L** presented higher total apigenin content than **EE-C** and **AE-C**. On the other hand, culm extracts had higher total tricin content than leaf extracts. **EE-C** also showed higher total tricin content than **AE-C**.

Gagliano et al. (2018) also quantified the flavonoid content of *M. pluriflora* by HPLC-DAD and found 5.9 and 1.8 mg $g^{-1}$ for EE-L and EE-C, respectively. Hydroethanol extract of *M. neesii* leaves had lower flavonoid content than *M. pluriflora*, but the opposite was observed for culms.

**Table 3.2:** Contents of compounds (mg $g^{-1}$) detected in of the hexane (**HE**), hydroethanol (**EE**), and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) from *Merostachys neesii*.

| **Compounds** | **Extracts** | | | | | |
|---|---|---|---|---|---|---|
| | **HE-L** | **HE-C** | **EE-L*** | **EE-C*** | **AE-L*** | **AE-C*** |
| Total Terpene | **50.25 ± 24.29** [b] | **218.96 ± 79.01** [a] | - | - | - | - |
| (**70**) Lupenone | - | 15.74 ± 11.74 | - | - | - | - |
| (**63**) Campesterol | **4.59 ± 1.96** [b] | **23.43 ± 9.81** [a] | - | - | - | - |
| (**64**) Stigmasterol | **7.17 ± 4.29** [b] | **55.75 ± 16.80** [a] | - | - | - | - |
| (**68**) *β*-Sitosterol | **38.49 ± 18.17** [b] | **124.04 ± 50.08** [a] | - | - | - | - |
| Total flavonoid | - | - | 4.28 ± 1.68 | 3.54 ± 1.13 | 4.48 ± 1.91 | 2.98 ± 0.84 |
| Total apigenin | - | - | **3.76 ± 1.52** [a] | **1.41 ± 0.65** [b] | **4.04 ± 1.83** [a] | **1.43 ± 0.47** [b] |
| (**79**) Apigenin *O*-hexoside *C*-hexoside | - | - | **1.41 ± 0.49** [a] | **0.33 ± 0.14** [b] | **1.57 ± 0.57** [a] | **0.34 ± 0.13** [b] |
| (**80**) Apigenin *O*-pentoside *C*-hexoside | - | - | 0.45 ± 0.15 | 0.46 ± 0.21 | 0.52 ± 0.20 | 0.47 ± 0.18 |
| (**81**) Isovitexin | - | - | 0.16 ± 0.11 | 0.18 ± 0.14 | 0.15 ± 0.10 | 0.18 ± 0.06 |
| (**82**) Apigenin *O*-hexoside *C*-pentoside | - | - | **0.42 ± 0.23** [a] | **0.10 ± 0.06** [b] | **0.47 ± 0.27** [a] | **0.11 ± 0.05** [b] |
| (**83**) Apigenin *O*-pentoside *C*-pentoside | - | - | **1.33 ± 0.72** [a] | **0.34 ± 0.24** [b] | **1.33 ± 0.78** [a] | **0.34 ± 0.18** [b] |
| Total tricin | - | - | **0.49 ± 0.20** [c] | **2.14 ± 0.59** [a] | **0.43 ± 0.11** [c] | **1.52 ± 0.50** [b] |
| (**84**) Tricin derivative 1 | - | - | **0.39 ± 0.16** [b] | **1.51 ± 0.46** [a] | **0.43 ± 0.11** [b] | **1.52 ± 0.50** [a] |
| (**85**) Tricin derivative 2 [np] | - | - | **0.10 ± 0.08** [b] | **0.24 ± 0.09** [a] | - | - |
| (**86**) Tricin derivative 3 | - | - | - | 0.13 ± 0.04 | - | - |
| (**87**) Tricin derivative 4 | - | - | - | 0.11 ± 0.07 | - | - |
| (**88**) Tricin derivative 5 | - | - | - | 0.15 ± 0.07 | - | - |

- : not detected.

Data are presented as mean ± standard deviation (n=14). Mean values in the same row followed by different letters indicate a statistically significant difference ($P < 0.05$). Compounds signalized with a superscript *np* ([np]) indicate non-parametrical distributions. * Values expressed in milligrams of compoud equivalent to vitexin per gram of dry extract. Numbers correspond to those shown in table 1.2 and 1.3 (see chapter 1).

### 3.3.2 Seasonal changes on bioactive compounds of *Merostachys neesii*

In order to verify a possible influence of seasonality on the content of bioactive compounds detected in *M. neesii*, levels of campesterol (**63**), stigmasterol (**64**), *β*-sitosterol (**68**), lupenone (**70**), and of the five apigenin-derived (**79-83**) and of the five tricin-derived flavones (**84-88**) were estimated in leaves and culms from *M. neesii* harvested in the dry and rainy seasons of two consecutive years. Chapter 1 integrated results and pointed out to a different chemical composition between hydroethanol and hexane extracts. Moreover, although all components found in aqueous extracts are also found in the hydroethanol extracts, the seasonal influence on the aqueous extracts was also evaluated due to the importance of infusions as a popular use of herbal preparation. Thus, all extracts were analyzed regarding seasonal influence on the content of the bioactive constituents. Tables 3.3 and 3.4 show the amounts of each constituent by date of plant harvesting.

Although the total terpene content in leaves of *M. neesii* did not vary significantly between seasons, campesterol showed a significant difference (Table 3.3). In the second year (dry season/2017 and rainy season/2018) there was higher campesterol production in the rainy season than in the dry season. The opposite was observed for culms where the total terpene content was significantly higher in the dry season/2016 compared to rainy seasons (2017 and 2018). This difference is mainly due to the campesterol and *β*-sitosterol levels that exhibited higher amounts in the dry season/2016. Additionally, campesterol content in the dry season/2016 was also higher than the dry season/2017.

Regarding flavonoids, seasonal variation was observed neither for total flavonoid, apigenin, and tricin nor for individual constituents in aqueous extracts of leaves and culms and hydroethanol extract of leaves. On the other hand, levels of apigenin *O*-hexoside *C*-pentoside (**82**), apigenin *O*-pentoside *C*-pentoside (**83**), and tricin derivative 4 (**87**) fluctuated among harvesting periods, especially for **EE-C**. Apigenin *O*-hexoside *C*-pentoside (**82**) content was higher in rainy season/2017 when compared to dry season/2016 and rainy season/2018. The amount of apigenin *O*-pentoside *C*-pentoside (**83**) was also higher in rainy season/2017 than in rainy season/2018. On the other hand, tricin derivative 4 content (**87**) varied between dry seasons, with greater content in 2017 (Table 3.4).

Studies evaluating seasonal changes in leaves from bamboo species observed that phenolic compounds had higher content mainly during autumn-winter compared to spring-summer (Ni et al. 2012; Ni et al. 2014; Ko et al. 2018). Ni et al. (2012) also observed that total terpene content in *Sasa argenteostriata* E.G. Camus leaves was also higher during autumn-winter. The bamboo species studied by these authors are from a temperate climate, where winter is the rainy season.

In the present work, phytosterols showed differences mainly between the dry and rainy seasons, while the few variations observed for flavonoids seem not to be related to seasonality.

Accumulated precipitation and its distribution 30 days before harvest were the meteorological factors that varied within the same seasons. Climate factors such as solar irradiation, precipitation, and temperature are known to influence secondary metabolites content; however other aspects, such as biotic factors and plant development, may also have influenced the observed changes (Gobbo-Neto and Lopes 2007).

**Table 3.3:** Contents of major bioactive compounds (mg $g^{-1}$) of the hexane (**HE**) extracts of leaves (**L**) and culms (**C**) of *Merostachys neesii* harvested in the dry (dry/2016, n=3; dry/2017, n=4) and rainy (rainy/2017, n =3; rainy/2018, n=4) seasons.

| **Compounds** | **HE-L** | | | |
|---|---|---|---|---|
| | **Dry/2016** | **Rainy/2017** | **Dry/2017** | **Rainy/2018** |
| Total terpene [np] | 76.42 ± 38.75 | 39.89 ± 6.24 | 31.67 ± 8.67 | 56.97 ± 11.93 |
| Campesterol | **6.35 ± 3.25** [ab] | **3.69 ± 0.56** [ab] | **3.00 ± 0.49** [b] | **5.54 ± 0.78** [a] |
| Stigmasterol [np] | 11.89 ± 6.70 | 5.60 ± 1.30 | 3.96 ± 0.97 | 8.02 ± 2.86 |
| *β*-sitosterol | 58.18 ± 28.85 | 30.60 ± 4.46 | 24.71 ± 7.33 | 43.41 ± 8.53 |
| Lupenone | - | - | - | - |
| **Compounds** | **HE-C** | | | |
| | **Dry/2016** | **Rainy/2017** | **Dry/2017** | **Rainy/2018** |
| Total terpene [np] | **321.08 ± 47.78** [a] | **176.45 ± 42.85** [b] | **227.51 ± 86.23** [ab] | **165.71 ± 27.45** [b] |
| Campesterol | **39.35 ± 4.07** [a] | **17.73 ± 3.77** [b] | **21.38 ± 7.80** [b] | **17.81 ± 1.54** [b] |
| Stigmasterol [np] | 74.09 ± 12.03 | 50.68 ± 11.24 | 55.77 ± 22.43 | 45.79 ± 6.74 |
| *β*-sitosterol | **196.70 ± 26.37** [a] | **91.64± 22.45** [b] | **127.64 ± 45.28** [ab] | **90.24 ± 12.15** [b] |
| Lupenone [np] | 10.95 ± 18.97 | 16.41 ± 5.62 | 22.71 ± 11.73 | 11.87 ± 9.54 |

Data are presented as mean ± standard deviation. Mean values in the same row followed by different letters indicate a statistically significant difference ($P < 0.05$). Compounds signalized with a superscript *np* ([np]) indicate non-parametrical distributions.

**Table 3.4:** Contents of major bioactive compounds (mg $g^{-1}$) of the hydroethanol (**EE**) and aqueous **(AE**) extracts of leaves (**L**) and culms (**C**) of *Merostachys neesii* harvested in the dry (dry/2016, n=3; dry/2017, n=4) and rainy (rainy/2017, n =3; rainy/2018, n=4) seasons.

| **Compounds** | **EE-L** | | | | **EE-C** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Dry/2016** | **Rainy/2017** | **Dry/2017** | **Rainy/2018** | **Dry/2016** | **Rainy/2017** | **Dry/2017** | **Rainy/2018** |
| Total apigenin | 4.19 ± 2.34 | 3.51 ± 1.59 | 3.52 ± 1.31 | 3.88 ± 1.61 | 0.85 ± 0.37 | 1.54 ± 0.37 | 1.92 ± 0.88 | 1.20 ± 0.35 |
| Apigenin *O*-hexoside *C*-hexoside | 1.65 ± 0.88 | 1.27 ± 0.34 | 1.31 ± 0.46 | 1.42 ± 0.37 | 0.19 ± 0.10 | 0.41 ± 0.15 | 0.35 ± 0.14 | 0.35 ± 0.12 |
| Apigenin *O*-pentoside *C*-hexoside | 0.40 ± 0.22 | 0.43 ± 0.17 | 0.50 ± 0.17 | 0.45 ± 0.10 | $^{np}$ 0.26 ± 0.12 | 0.51 ± 0.16 | 0.56 ± 0.25 | 0.48 ± 0.21 |
| Isovitexin | 0.19 ± 0.14 | 0.09 ± 0.05 | 0.17 ± 0.11 | 0.17 ± 0.13 | $^{np}$ 0.13 ± 0.07 | 0.13 ± 0.04 | 0.30 ± 0.21 | 0.12 ± 0.08 |
| Apigenin *O*-hexoside *C*-pentoside | 0.47 ± 0.30 | 0.43 ± 0.25 | 0.37 ± 0.20 | 0.44 ± 0.27 | **0.06 ± 0.01** $^{b}$ | **0.13 ± 0.01** $^{a}$ | **0.16 ± 0.08** $^{ab}$ | **0.07 ± 0.01** $^{b}$ |
| Apigenin *O*-pentoside *C*-pentoside | 1.48 ± 0.93 | 1.28 ± 0.78 | 1.17 ± 0.65 | 1.41 ± 0.87 | **0.21 ± 0.09** $^{ab}$ | **0.37 ± 0.04** $^{a}$ | **0.56 ± 0.36** $^{ab}$ | **0.18 ± 0.08** $^{b}$ |
| Total tricin | 0.37 ± 0.29 | 0.46 ± 0.19 | 0.52 ± 0.18 | 0.58 ± 0.18 | $^{np}$ 1.64 ± 0.51 | 2.22 ± 0.17 | 2.62 ± 0.50 | 1.96 ± 0.66 |
| Tricin derivative 1 | 0.28 ± 0.27 | 0.40 ± 0.16 | 0.43 ± 0.14 | 0.42 ± 0.10 | 1.25 ± 0.44 | 1.75 ± 0.28 | 1.78 ± 0.32 | 1.26 ± 0.59 |
| Tricin derivative 2 | 0.09 ± 0.03 | 0.06 ± 0.05 | 0.10 ± 0.06 | 0.16 ± 0.14 | 0.17 ± 0.06 | 0.22 ± 0.07 | 0.31 ± 0.12 | 0.24 ± 0.02 |
| Tricin derivative 3 | | | | | $^{np}$ 0.09 ± 0.05 | 0.13 ± 0.03 | 0.15 ± 0.04 | 0.13 ± 0.04 |
| Tricin derivative 4 | | | | | **0.04 ± 0.04** $^{b}$ | **0.05 ± 0.01** $^{ab}$ | **0.17 ± 0.06** $^{a}$ | **0.15 ± 0.07** $^{ab}$ |
| Tricin derivative 5 | | | | | 0.08 ± 0.02 | 0.07 ± 0.01 | 0.21 ± 0.08 | 0.18 ± 0.08 |
| Total flavonoid | 4.56 ± 2.63 | 3.97 ± 1.69 | 4.05 ± 1.46 | 4.53 ± 1.81 | 2.49 ± 0.80 | 3.76 ± 0.40 | 4.54 ± 1.21 | 3.17 ± 0.88 |
| **Compounds** | **AE-L** | | | | **AE-C** | | | |
| | **Dry/2016** | **Rainy/2017** | **Dry/2017** | **Rainy/2018** | **Dry/2016** | **Rainy/2017** | **Dry/2017** | **Rainy/2018** |
| Total apigenin | 4.56 ± 2.13 | 3.31 ± 2.29 | 3.66 ± 1.49 | 4.57 ± 2.07 | 1.03 ± 0.39 | 1.41 ± 0.04 | 1.76 ± 0.61 | 1.42 ± 0.40 |
| Apigenin *O*-hexoside *C*-hexoside | 1.80 ± 0.71 | 1.29 ± 0.67 | 1.41 ± 0.45 | 1.77 ± 0.61 | 0.25 ± 0.12 | 0.37 ± 0.06 | 0.39 ± 0.12 | 0.34 ± 0.18 |
| Apigenin *O*-pentoside *C*-hexoside | 0.48 ± 0.29 | 0.46 ± 0.24 | 0.56 ± 0.20 | 0.54 ± 0.18 | 0.32 ± 0.12 | 0.44 ± 0.06 | 0.54 ± 0.23 | 0.53 ± 0.18 |
| Isovitexin | $^{np}$ 0.23 ± 0.07 | 0.05 ± 0.06 | 0.16 ± 0.10 | 0.16 ± 0.12 | 0.15 ± 0.04 | 0.15 ± 0.06 | 0.22 ± 0.08 | 0.18 ± 0.03 |
| Apigenin *O*-hexoside *C*-pentoside | 0.52 ± 0.33 | 0.40 ± 0.34 | 0.41 ± 0.24 | 0.54 ± 0.31 | 0.07± 0.03 | 0.12 ± 0.01 | 0.15 ± 0.07 | 0.09 ± 0.03 |
| Apigenin *O*-pentoside *C*-pentoside | 1.53 ± 0.85 | 1.10 ± 1.00 | 1.12 ± 0.64 | 1.56 ± 0.91 | 0.25 ± 0.11 | 0.32 ± 0.01 | 0.47 ± 0.28 | 0.29 ± 0.14 |
| Total tricin | 0.44 ± 0.15 | 0.39 ± 0.12 | 0.45 ± 0.11 | 0.46 ± 0.10 | $^{np}$ 1.33 ± 0.56 | 1.62 ± 0.27 | 1.63 ± 0.31 | 1.47 ± 0.83 |
| Tricin derivative 1 | 0.44 ± 0.15 | 0.39 ± 0.12 | 0.45 ± 0.11 | 0.46 ± 0.10 | $^{np}$ 1.33 ± 0.56 | 1.62 ± 0.27 | 1.63 ± 0.31 | 1.47 ± 0.83 |
| Total flavonoid | 5.01 ± 2.21 | 3.71 ± 2.42 | 4.12 ± 1.56 | 5.03 ± 2.17 | 2.40 ± 0.87 | 3.04 ± 0.25 | 3.42 ± 0.65 | 2.92 ± 1.23 |

Data are presented as mean ± standard deviation. Data were compared among seasons for the same extract. Mean values in the same row followed by different letters indicate a statistically significant difference ($P < 0.05$). Compounds signalized with a superscript *np* ($^{np}$) indicate non-parametrical distributions.

## 3.4 Antioxidant potential of *Merostachys neesii*

### 3.4.1 Antioxidant capacity of *Merostachys neesii*

There are several *in vitro* assays that evaluate the antioxidant capacity of extracts and pure compounds. These assays may involve hydrogen transfer (*e.g*. ORAC) or electron transfer reactions (*e.g*. DPPH, ABTS, FRAP, and superoxide anion radical scavenging) (Chanda and Dave 2009). It is important to use more than one method to evaluate the antioxidant potential of extracts and other plant materials due to the complex nature of the chemical composition of these matrices, which may have compounds with different mechanisms of action. The use of different assays allows a better understanding of the antioxidant activity of the samples (Chu et al. 2000; Chanda and Dave 2009). Some of the most commonly used *in vitro* methods for determining antioxidant activity are DPPH, ABTS, FRAP, and ORAC.

The DPPH, ABTS, and ORAC methods evaluate the antiradicalar activity, while FRAP measures the iron-reducer power of a substance. DPPH assay is based on the reduction of DPPH (a purple stable synthetic radical) to hydrazine (a yellow compound) by electron transfer between compounds. This discoloration is measured spectrophotometrically at 515-528 nm. Similarly to the DPPH assay, the ABTS method also uses a synthetic radical. In this assay, the radical cation ABTS, which exhibits bluish-green color and maximum absorption at 734 nm, is generated in the presence of potassium persulfate. The method consists of measuring $ABTS^{\bullet+}$ discoloration due to electron transfer by antioxidants. Differently from the previous methods, in ORAC assay is used the peroxyl radical ($ROO^{\bullet}$), a biologically important radical. Besides, this method takes place at 37°C, simulating the human body temperature. ORAC assay uses a fluorescent probe (*e.g*. fluorescein) and AAPH, the peroxyl radical generator; this radical oxidizes fluorescein resulting in the loss of its fluorescence. This method is based on the ability of antioxidant compounds to donate a hydrogen atom to fluorescein, inhibiting or retarding oxidation by the peroxyl radical. Finally, FRAP method evaluates the capacity of antioxidants to transfer electrons by reducing the colorless $Fe^{3+}$-TPTZ (ferric tripyridyltriazine) complex to $Fe^{2+}$-TPTZ (ferrous tripyridyltriazine), a blue color complex with maximum absorption at 593 nm, in low pH (Magalhães et al. 2008; Singh and Singh 2008; Chanda and Dave 2009).

Antioxidant potential of *M. neesii* leaves and culms was expressed as $EC_{50}$, which is obtained from dose-response curves constructed using various sample concentrations; low $EC_{50}$ values indicate high activity (Table 3.5). Additionally, Trolox $EC_{50}$ values were calculated for all assays. Trolox is a water-soluble analogue to α-tocopherol with strong radical scavenging activity that is often used as a positive control in antioxidant assays (Oehlke et al. 2011).

**Table 3.5**: Effective concentration to achieve 50% of antioxidant activity ($EC_{50}$, μg $mL^{-1}$) of the hexane (**HE**), hydroethanol (**EE**), and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) of *Merostachys neesii*.

| Extracts | Assays | | | |
|---|---|---|---|---|
| | **DPPH** | **ABTS** [np] | **ORAC** [np] | **FRAP** |
| **HE-L** | NA | **444.62 ± 137.21** [ab] | **67.15 ± 23.17** [ab] | **216.92 ± 83.06** [a] |
| **HE-C** | NA | **536.75 ± 103.72** [a] | **84.49 ± 32.83** [a] | **258.83 ± 148.55** [a] |
| **EE-L** | **228.40 ± 96.36** [a] | **48.15 ± 23.00** [bc] | **10.33 ± 6.87** [bc] | **42.08 ± 20.29** [b] |
| **EE-C** | **142.49 ± 40.03** [b] | **30.76 ± 7.61** [cd] | **6.66 ± 2.27** [cd] | **24.84 ± 6.94** [c] |
| **AE-L** | **81.07 ± 23.96** [c] | **22.20 ± 2.90** [d] | **4.74 ± 1.22** [d] | **15.90 ± 4.44** [d] |
| **AE-C** | **107.91 ± 35.35** [bc] | **28.15 ± 7.31** [cd] | **5.87 ± 2.56** [cd] | **20.81 ± 7.47** [cd] |
| Trolox | 10.84 | 5.71 | 1.43 | 2.99 |

NA: no activity. Data are presented as mean ± standard deviation (n=14). Mean values in the same column followed by different letters indicate a statistically significant difference ($P < 0.05$). Assays signalized with a superscript *np* ([np]) indicate non-parametrical distributions.

In general, aqueous extracts were the most active, especially **AE-L**, followed by hydroethanol and hexane extracts for both leaves and culms. This result is interesting since infusions have been described as the main form for medicinal purposes (Šavikin et al. 2013).

In the DPPH assay, although **AE-L** showed a lower $EC_{50}$ value than **AE-C**, no significant difference was found; however, **AE-L** was significantly more active than leaf and culm hydroethanol extracts. Hexane extracts from leaves and culms did not exhibit antioxidant capacity by the DPPH assay.

Both for ABTS and ORAC assays, **AE-L**, **AE-C**, and **EE-C** showed similar activities and differed significantly from hexane extracts. Hexane extracts of leaves and culms were active but also presenting the highest $EC_{50}$ values.

Regarding FRAP assay, **AE-L** was again the most active extract, exhibiting activity comparable to **AE-C** and differing from all other extracts. **AE-C** and **EE-C** showed similar results. **EE-L** differed from all extracts, exhibiting intermediate activity amongst other samples. Once again, **HE-C** and **HE-L** were active but exhibited high $EC_{50}$ values.

All $EC_{50}$ values were higher than Trolox $EC_{50}$, although **AE-L**, the most active sample, showed $EC_{50}$ values 2.7 times higher than that found for Trolox in the ORAC assay. It should be highlighted that Trolox is a pure compound while the samples tested are crude extracts that have a complex composition.

The extracts showed better antioxidant activity in the ORAC assay. These results indicate that the antioxidant compounds present in *M. neesii* are good antiradicalar molecules acting by

hydrogen atom transfer. However, ABTS assay results indicated that leaf and culm extracts also have good antiradicalar capacity through electron transfer. In addition to antiradicalar activity, these extracts also showed promising iron-reducer activity. According to Cos et al. (2006), $EC_{50}$ values below 100 μg $mL^{-1}$ are considered promising for crude extracts. Thus, all aqueous and hydroethanol extracts showed promising antioxidant potential in all assays, except DPPH. Only **AE-L**, the most active extract in all assays, presented $EC_{50}$ below 100 μg $mL^{-1}$ in DPPH assay.

These results corroborate those reported for other bamboo species. Kim et al. (2012) evaluated the antioxidant potential by the DPPH method of leaf 80% methanol extract of *Sasa quelpaertensis* Nakai and found $EC_{50}$ values (862.5 μg $mL^{-1}$) higher than the ones found in the present study. On the other hand, Kim et al. (2009) observed a strong activity of leaf methanol extract of *Phyllostachys nigra* Munro using DPPH ($EC_{50}$: 5.5 μg $mL^{-1}$) and ABTS ($EC_{50}$: 4.3 μg $mL^{-1}$) assays. Aqueous methanolic extract of *Bambusa nutans* Wall. ex Munro leaves also showed high antioxidant activity for the DPPH assay ($EC_{50}$: 57.9 μg $mL^{-1}$) (Pande et al. 2018). Tripathi et al. (2015) evaluated the antioxidant capacity of leaf methanol extract of *B. nutans* (123.5 μg $mL^{-1}$) and *Bambusa vulgaris* Schrad. (262.9 μg $mL^{-1}$) using the DPPH assay. In this study, only *B. vulgaris* exhibited activity similar to the hydroethanol extract from *M. neesii* leaves.

Wróblewska et al. (2019) analyzed the antioxidant potential by the DPPH method of leaves and culms hydroethanol extracts from five Brazilian native bamboo species: *Aulonemia aristulata* (Döll) McClure, *Chusquea bambusoides* (Raddi) Hack., *Chusquea capituliflora* var. *pubescens* McClure & L.B.Sm., *Chusquea meyeriana* Rupr. ex Döll, and *Merostachys pluriflora*. *M. neesii* culms were more active than culm extracts of the five species while leaves exhibited activity similar to *A. aristulata C. bambusoides*, and *C. capituliflora* and lower activity than *C. meyeriana* and *M. pluriflora.*

Gagliano et al. (2018) observed that leaves and culms extracts of *M. pluriflora* showed high antioxidant activity. Hydroethanol extract of *M. pluriflora* leaves showed higher antiradicalar activity than *M. neesii* leaves. On the other hand, the hydroethanol extract of culms in the present study exhibited higher antiradicalar activity than *M. pluriflora*. Gagliano et al. (2018) correlated the antioxidant capacity of these extracts to the presence of phenolic compounds. Other bamboo studies have also associated antioxidant activity with phenolic compounds (Park and Jhon 2010; Goyal et al. 2011; Liu et al. 2016).

Phenolic compounds, such as phenolic acids, stilbenes, tannins, and flavonoids, are widespread in plants (Oroian and Escriche 2015). Flavonoids are an important group of phenolic compounds that have several biological activities, being the antioxidant capacity the best-studied for almost all phenolic subclasses (Kumar and Pandey 2013). They exhibit strong antioxidant activity and can avoid free radical damage through different mechanisms, such as ROS scavenging,

activation of antioxidant enzymes, metal-chelating activity, reduction of α-tocopheryl radicals, increase antioxidant properties of low molecular antioxidants, among others (Procházková et al. 2011).

Flavonoids, as mentioned above, were detected in aqueous and hydroethanol extracts of leaves and culms, which were the samples that showed the highest antioxidant activity. To verify if these compounds were the main responsible for the antioxidant activity in *M. neesii* extracts, Pearson's correlation analyses were performed for aqueous and hydroethanol extracts of both leaves and culms. For this, the quantification data (mg $g^{-1}$) of the individual flavonoids, total apigenin, tricin, and flavonoids (see table 3.4) and the $EC_{50}$ values (μg $mL^{-1}$) of the four antioxidant assays were used. It is noteworthy that since the antioxidant activity data used for the correlation analyses were the $EC_{50}$ values, the negative correlation data should be considered. Tables containing all correlation data are presented in supplementary material 3 (Table S3.1-S3.4). Overall, antioxidant capacity and flavonoid content were not strongly correlated. A moderate correlation was observed only for culm extracts. For **EE-C**, compound **84** (tricin derivative 1) showed moderate correlation to DPPH (*r* = **-0.73**) and FRAP (*r* = **-0.60**) assays; total tricin derivatives also presented moderate correlation to DPPH (*r* = **-0.65**). Concerning **AE-C**, total apigenin and tricin derivatives, and total flavonoids exhibited moderate correlation to DPPH (*r* = **-0.61**, **-0.67**, and **-0.74**, respectively); total tricin derivatives and flavonoid correlated also to ABTS (*r* = **-0.70** and **-0.68**, respectively). It seems that flavonoids influenced more effectively the antioxidant activity of culms than leaves. Furthermore, other classes of phenolic compounds may have been involved in the antioxidant activity of leaves, especially for **AE-L**. Although phenolic acids were not considered in this study, they were found in leaves and culms extracts of *M. neesii*. Van Hoyweghen et al. (2012) analyzed the antioxidant potential and phenolic compounds of leaves from 12 bamboo species and observed a strong correlation between phenolic acids and ORAC assay; as well as, the authors observed a moderate correlation between phenolic acids and DPPH and ABTS assays.

### 3.4.2 Seasonal changes on antioxidant capacity of *Merostachys neesii*

Results of the seasonal effect on the antioxidant potential of *M. neesii* extracts are shown in table 3.6. For the DPPH and ORAC assays, antioxidant activity was not influenced by the harvesting time; significant differences were observed neither for leaves nor for culms. For the ABTS assay, **EE-C** showed a significant difference between rainy seasons; **EE-C** antioxidant capacity was higher in the rainy season/2017 than in the rainy season/2018.

Regarding the iron-reducer power, almost all extracts showed significant differences between seasons. **HE-L** showed a significant difference between dry seasons, being the sample from dry season/2017 less active than the dry season/2016. On the other hand, **AE-C** exhibited a significant difference between rainy seasons; the highest antioxidant potential was observed in the

rainy season/2017. Significant changes between dry and rainy seasons were found for **HE-C** and **EE-C**. These extracts showed higher iron-reducer capacity in the rainy season/2017 than in the dry season/2017. These results indicate that the iron-reducer capacity was more influenced by seasonality than the antiradicalar capacity, mainly for *M. neesii* culms.

Ni et al. (2012) evaluated the antioxidant capacity of *Sasa argenteostriata* leaf extracts by DPPH and FRAP assays. They observed that the highest antioxidant activity for DPPH and FRAP was found during autumn/winter. Ni et al. (2014) also observed seasonal variations in the antioxidant potential of leaves of *Pleioblastus kongosanensis* f. *aureostriatus* Muroi & Yu. Tanaka and *Shibataea chinensis* Nakai. For *S. chinensis*, high antiradicalar activity (by DPPH assay) was observed during winter/spring while for *P. kongosanensis* f. *aureostriatus* it was high in the autumn/winter. Regarding the iron-reducer activity (FRAP assay), both species presented two peaks of activity; *S. chinensis* in February-April and September-October while *P. kongosanensis* f. *aureostriatus* had high activity in March and August. Antioxidant activity of *Sasa quelpaertensis* leaf extracts was also influenced by harvesting time; the greatest activity was found during autumn and winter (Ko et al. 2018).

These bamboo species belong to the Arundinarieae tribe, distributed mainly in temperate regions. In general, seasonality seems strongly influence the antioxidant activity in these species, showing more active extracts during autumn and winter (rainy season). For *M. neesii* leaves, the same pattern was not observed; leaves suffered few variations regarding the harvesting time, but not in a clear pattern. On the other hand, culms appear to have higher iron-reducer power in the rainy season than in the dry season. This was the first work evaluating seasonal changes in the antioxidant potential of an American bamboo species from the Bambuseae tribe.

**Table 3.6:** Seasonal changes on antioxidant potential ($EC_{50}$, µg $g^{-1}$) of the hexane (**HE**), hydroethanol (**EE**), and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) from *Merostachys neesii* harvested in the dry (dry/2016, n=3; dry/2017, n=4) and rainy (rainy/2017, n =3; rainy/2018, n=4) seasons.

| Assays | Seasons | Extracts | | | | | |
|---|---|---|---|---|---|---|---|
| | | **HE-L** | **EE-L** | **AE-L** | **HE-C** | **EE-C** | **AE-C** |
| **DPPH** | **Dry/2016** | NA | 150.85 ± 43.84 | 102.22 ± 37.99 | NA | 160.26 ± 75.10 | 136.91 ± 55.78 |
| | **Rainy/2017** | NA | 234.39 ± 175.10 | 72.05 ± 32.25 | NA | 128.87 ± 11.51 | 88.83 ± 18.30 |
| | **Dry/2017** | NA | 276.48 ± 95.64 | 74.99 ± 12.24 | NA | 116.24 ± 15.77 | 83.69 ± 12.25 |
| | **Rainy/2018** | NA | 233.99 ± 13.92 | 78.06 ± 9.59 | NA | 165.62 ± 26.74 | 124.70 ± 24.61 |
| **ABTS** | **Dry/2016** | 357.33 ± 108.00 | 32.38 ± 5.49 | 22.93 ± 3.42 | 521.77 ± 40.38 | **32.43 ± 14.05** $^{ab}$ | 30.01 ± 9.77 |
| | **Rainy/2017** | 454.20 ± 79.06 | 64.04 ± 50.92 | 20.03 ± 5.20 | 511.73 ± 27.61 | **25.08 ± 2.10** $^{b}$ | 22.64 ± 3.98 |
| | **Dry/2017** | 416.25 ± 130.05 | 48.16 ± 5.77 | 22.53 ± 1.05 | 490.46 ± 156.81 | **29.25 ± 6.90** $^{ab}$ | 24.32 ± 1.00 |
| | **Rainy/2018** | 531.28 ± 184.19 | 48.04 ± 3.17 | 22.95 ± 1.83 | 613.05 ± 94.68 | **35.27 ± 1.82** $^{a}$ | 34.73 ± 6.72 |
| **ORAC** | **Dry/2016** | 88.71 ± 45.02 | 6.81 ± 2.22 | 5.79 ± 1.93 | 120.54 ± 31.32 | 7.92 ± 4.35 | 7.69 ± 3.66 |
| | **Rainy/2017** | 64.15 ± 5.55 | 15.63 ± 15.37 | 4.69 ± 1.80 | 62.69 ± 4.64 | 5.99 ± 0.54 | 6.52 ± 3.74 |
| | **Dry/2017** | 57.40 ± 8.05 | 9.57 ± 0.94 | 4.15 ± 0.31 | 77.55 ± 21.93 | 6.11 ± 2.32 | 4.19 ± 0.10 |
| | **Rainy/2018** | 62.98 ± 16.34 | 9.74 ± 1.07 | 4.60 ± 0.39 | 80.73 ± 40.80 | 6.76 ± 1.30 | 5.70 ± 1.63 |
| **FRAP** | **Dry/2016** | **142.38 ± 53.88** $^{b}$ | 22.76 ± 8.10 | 15.55 ± 6.03 | **190.22 ± 52.95** $^{ab}$ | **25.58 ± 12.02** $^{ab}$ | **21.00 ± 10.07** $^{ab}$ |
| | **Rainy/2017** | **156.39 ± 16.82** $^{ab}$ | 45.21 ± 41.71 | 11.49 ± 4.54 | **155.23 ± 20.56** $^{b}$ | **18.71 ± 1.22** $^{b}$ | **13.81 ± 3.00** $^{b}$ |
| | **Dry/2017** | **290.41 ± 64.17** $^{a}$ | 47.99 ± 8.20 | 16.76 ± 2.84 | **408.63 ± 216.21** $^{a}$ | **23.52 ± 2.14** $^{a}$ | **18.50 ± 2.27** $^{ab}$ |
| | **Rainy/2018** | **244.72 ± 78.75** $^{ab}$ | 48.32 ± 5.60 | 18.61 ± 3.03 | **238.18 ± 28.12** $^{ab}$ | **30.20 ± 5.33** $^{ab}$ | **28.22 ± 5.78** $^{a}$ |

$EC_{50}$: effective concentration to achieve 50% of antioxidant activity. NA: no activity. Data are presented as mean ± standard deviation. Data were compared among seasons for the same assay and extract. Mean values in the same column followed by different letters indicate a statistically significant difference ($P < 0.05$). Compounds signalized with a superscript *np* ($^{np}$) indicate non-parametrical distributions.

## 3.5 Anticholinesterase potential and seasonal changes in *Merostachys neesii*

### 3.5.1 Anticholinesterase capacity of *Merostachys neesii*

For the inhibitory activity of cholinesterases (AChE and BChE), a colorimetric assay based on Ellman's method (1961) was used. This method is based on measuring the production rate of 5-thio-2-nitrobenzoate, a yellowish compound, at 412 nm. Acetylcholinesterase (AChE) hydrolyzes acetylthiocholine to produce thiocoline which reacts with DTNB leading to the formation of 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate. If the extracts contain AChE inhibitors, acetylthiocoline will not be hydrolyzed interrupting the other reactions (Rhee et al. 2001).

Table 3.7 shows the results of the antiacetylcholinesterase potential of *M. neesii*. Results were expressed as $IC_{50}$ and neostigmine was used as a positive control. Neostigmine is a synthetic AChE and BChE inhibitor developed from physostigmine, an alkaloid isolated from *Physostigma venenosum* Balf. (Fabaceae) that was the first AChE inhibitor discovered (Pinho et al. 2013).

**Table 3.7**: Concentration of the hexane (**HE**), hydroethanol (**EE**), and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) from *Merostachys neesii* to inhibit 50% of acetylcholinesterase activity ($IC_{50}$, µg $mL^{-1}$).

| Extracts | AChEI |
|---|---|
| **HE-L** | **1036.16 ± 578.34** $^{ab}$ |
| **HE-C** | **1036.70 ± 512.08** $^{a}$ |
| **EE-L** | **624.22 ± 189.14** $^{ac}$ |
| **EE-C** | **1008.95 ± 686.56** $^{ab}$ |
| **AE-L** | **446.79 ± 162.50** $^{c}$ |
| **AE-C** | **549.92 ± 231.37** $^{bc}$ |
| Neostigmine $^{*}$ | 15.37 |

Data are presented as mean ± standard deviation (n=14). Different letters indicate a statistically significant difference ($P < 0.05$). * $IC_{50}$ value in ng $mL^{-1}$. AChEI: acethylcholinesterase inhibition.

All extracts of *M. neesii* were able to inhibit AChE activity, but $IC_{50}$ values were high. Among the samples tested, aqueous extracts were the most active. Furthermore, no extract showed antibutyrylcholinesterase activity.

There are several therapeutic targets in the treatment of AD, including cholinergic neurotransmitters. Acetylcholine (ACh), a cholinergic neurotransmitter, is responsible for the conduction of electrical impulses. People with AD exhibit a reduction in the levels of this neurotransmitter. Increased levels of ACh can occur through the inhibition of cholinesterases,

enzymes that hydrolyze ACh. AChE and BChE are cholinesterases present in the human body (Anand and Singh 2013). There are a few AChE inhibitors available for the treatment of AD, making it necessary to search for new compounds that inhibit the activity of these enzymes.

Plants are a promising source of compounds with an inhibitory capacity of AChE and BChE. Several classes of plant secondary metabolites have been reported to inhibit such enzymes, such as alkaloids, coumarins, quinones, stilbenes, terpenes, flavonoids (especially flavones or isoflavones), among others (Pinho et al. 2013). Flavonoids, coumarins, and terpenes have been described in bamboos and some studies have reported neuroprotective activity of bamboo extracts. However, these studies did not report the class of responsible compounds for the neuroprotective activity (Eom et al. 2012; Liu et al. 2015; Sakagami et al. 2018).

Liu et al. (2015) evaluated the effects of a bamboo leaf extract on model rats with senile dementia (SD). They observed that SD-rats fed with bamboo leaf extract had better spatial memory capacity, increased levels of acetylcholine in the hippocampus, and reduced AChE activity in both the hippocampus and the cerebral cortex when compared to SD-rats not fed with this extract. Grombone-Guaratini et al. (2012) verified that the extract of *M. magellanica* inhibited AChE in a study using thin-layer chromatography (TLC). Although these results demonstrate the potential of bamboos in the search for cholinesterase inhibitors, *M. neesii* can be considered a non-promising species for this purpose. Furthermore, this is the first study evaluating the anticholinesterase activity using both AChE and BChE using an American bamboo species.

In a review by Uriarte-Pueyo and Calvo (2011) of AChE inhibitory activity of 128 natural and synthetic flavonoids, it was found that several flavones (41 compounds), among them isovitexin, were active. Isovitexin and other apigenin derivatives were found in leaves and culms of *M. neesii*. Thus, Pearson's correlation analyses were also performed between the identified compounds and the antiacetylcholinesterase activity of the extracts. Tables containing all correlation data are presented in supplementary material 3 (Table S3.5-3.10). No strong correlations were found between chemical compounds and antiacetylcholinesterase activity. These results show, once more, the low activity exhibited by the extracts, indicating the absence of compounds with high activity for this assay. The highest correlations were found for **AE-L**, the extract with greater AChE inhibition in the present study. Apigenin *O*-pentoside *C*-hexoside (**80**), isovitexin (**81**), apigenin *O*-hexoside *C*-pentoside (**82**), and apigenin *O*-pentoside *C*-pentoside (**83**) showed moderate to low correlation ($r$ = **-0.57**, **-0.58**, **-0.56**, and **-0.54**, respectively) for AChE inhibition activity.

### 3.5.2 Seasonal changes on anticholinesterase capacity of *Merostachys neesii*

Results of the seasonal effect on the antiacetylcholinesterase potential of *M. neesii* extracts are shown in table 3.8. Seasonal changes were found only for **EE-C**. As observed for this extract in

the FRAP assay, a significant difference was found between the harvestings performed in the rainy season/2017 and the dry season/2017. Nevertheless, this time the most active extract was obtained from the culms collected during the dry season/2017. Although no significant difference was observed, **AE-L** and **AE-C** also showed lower $IC_{50}$ values in this harvest.

**Table 3.8:** Seasonal changes on antiacetylcholinesterase potential ($IC_{50}$, µg $g^{-1}$) of the hexane (**HE**), hydroethanol (**EE**), and aqueous (**AE**) extracts of leaves (**L**) and culms (**C**) from *Merostachys neesii* harvested in the dry (dry/2016, n=3; dry/2017, n=4) and rainy (rainy/2017, n =3; rainy/2018, n=4) seasons.

| Extracts | Seasons | | | |
|---|---|---|---|---|
| | Dry/2016 | Rainy/2017 | Dry/2017 | Rainy/2018 |
| **HE-L** $^{np}$ | 579.44 ± 306.04 | 656.06 ± 197.28 | 1277.87 ± 558.08 | 1422.08 ± 648.10 |
| **EE-L** | 463.51 ± 181.25 | 571.30 ± 201.64 | 670.17 ± 172.42 | 757.38 ± 148.84 |
| **AE-L** | 609.83 ± 210.28 | 448.05 ± 58.65 | 361.88 ± 176.59 | 399.72 ± 114.12 |
| **HE-C** | 720.22 ± 110.25 | 1332.39 ± 610.17 | 1160.57 ± 763.16 | 928.41 ± 271.85 |
| **EE-C** $^{np}$ | **837.25 ± 59.05$^{ab}$** | **1512.73 ± 909.51$^{a}$** | **511.15 ± 121.75$^{b}$** | **1257.67 ± 881.93$^{ab}$** |
| **AE-C** | 650.03 ± 88.56 | 723.53 ± 309.50 | 308.76 ± 95.73 | 585.78 ± 185.20 |

$IC_{50}$: concentration to inhibit 50% of acetylcholinesterase activity. Data are presented as mean ± standard deviation. Mean values in the same row followed by different letters indicate a statistically significant difference ($P < 0.05$). Compounds signalized with a superscript *np* ($^{np}$) indicate non-parametrical distributions.

### 3.6 Principal component analysis (PCA)

To observe possible correlations between chemical composition and antioxidant potential, a principal component analysis (PCA) was carried out. For that, contents of lupenone (Lup), total phytosterols (Phyt), total flavonoid (Flav), total apigenin (Ap), total tricin (Tric), and antioxidant activity (DPPH, ABTS, ORAC, and FRAP - results expressed as mg equivalent to Trolox per g of dry extract) of leaves and culms were used.

PCA explained 79% of data variability within the first two axes (Figure 3.3). It was possible to observe the formation of four groups. Axis 1 separated hexane extracts (**groups 3 and 4**) from aqueous and hydroethanol extracts (**groups 1 and 2**), being the antioxidant activity and flavonoid content determinant for this result (Table 3.9). **Group 1**, placed on the positive side of axis 1, is formed by samples of the aqueous extract of leaves (**AE-L)**, while **group 2**, also placed on the positive side of axis 1 but on the negative side of axis 2, is formed by the samples of hydroethanol extracts (**EE-L** and **EE-C**). On the negative side of axis 1, samples of hexane extracts from leaves

(**HE-L**) and culms (**HE-C**) formed **group 3** and **group 4**, respectively. These two last groups showed low antioxidant activity and absence of flavonoids.

**Group 1** is composed by samples (**AE-L)** presenting the highest antioxidant activity, correlated with apigenin content. **Group 2** (**AE-C** and **EE-C**) showed correlation with tricin content.

Lupenone and total phytosterol contents were determinant to separate **HE-L** from **HE-C** (table 3.9) along axis 2. **Group 3** (**HE-C)**, placed on the positive side of axis 2, showed high phytosterol and lupenone contents, while **group 4** (**HE-L)** is placed on the negative side of both axes.

In summary, PCA corroborated previously discussed data of this work; seasonality was not determinant to change the chemical composition and/or antioxidant potential of *M. neesii*. In addition, leaves and culms have different chemical composition and antioxidant potential.

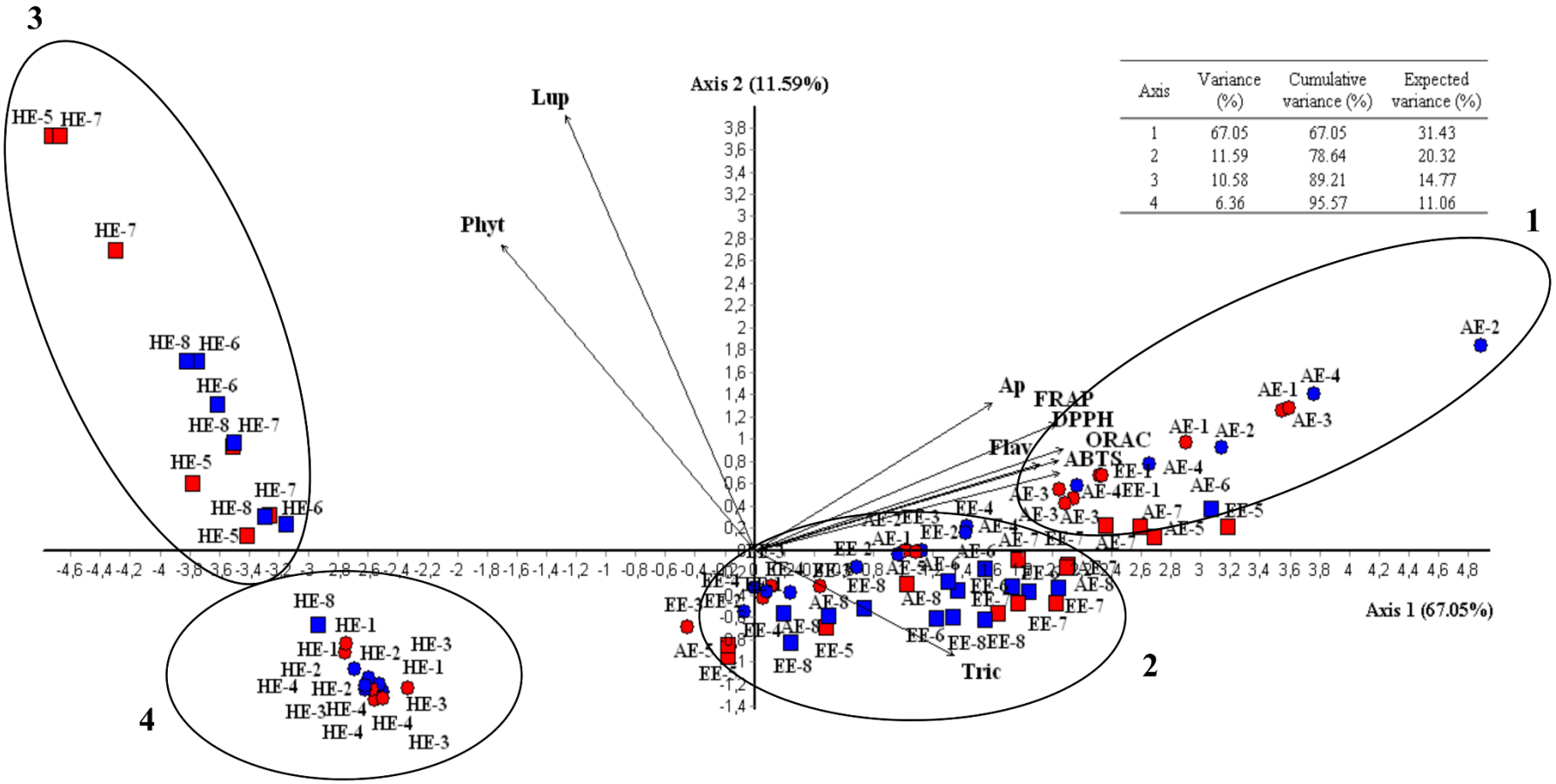


**Figure 3.3:** Principal component analysis (PCA) using nine variables (**Lup**: lupenone; **Phyt**: total phytosterol; **Flav**: total flavonoid; **Ap**: total apigenin; **Tric**: total tricin; **DPPH**: DPPH scavenging assay; **ABTS**: ABTS scavenging assay; **ORAC**: oxygen radical absorbance capacity assay; and **FRAP**: ferric-reducing antioxidant potential) evaluated in the hexane, hydroethanol, and aqueous extracts of leaves (**HE-L**, **EE-L**, and **AE-L**) (●) and culms (**HE-C**, **EE-C**, and **AE-C**) (■) of *Merostachys neesii* harvested in the dry (red symbols) and rainy seasons (blue symbols).

**Table 3.9:** Correlation coefficients between the variables used in the principal component analysis (PCA) and axes 1 and 2.

| Variables | Axis 1 | Axis 2 |
|---|---|---|
| DPPH | **0.38** | 0.17 |
| ABTS | **0.38** | 0.13 |
| ORAC | **0.38** | 0.15 |
| FRAP | **0.37** | 0.21 |
| Total apigenin | 030 | 0.24 |
| Total tricin | 0.25 | -0.17 |
| Total flavonoid | **0.35** | 0.14 |
| Total phytosterol | -0.31 | **0.51** |
| Lupenone | -0.23 | **0.72** |

## 4. Conclusions

Quantitative data on triterpenes and phytosterols were described for the first time for *Merostachys*. Culms presented phytosterol content 3 to 8 times higher than leaves. Total flavonoid content was similar for both plant parts and extracts (aqueous or hydroethanolic). However, leaf extracts exhibited higher amounts of apigenin derivatives and culm showed higher amounts of tricin derivatives. These results indicate that culm and leaf extracts, especially hydroethanol extracts, have different flavonoid composition, quantitatively and qualitatively.

Regarding seasonal changes, both phytosterols (campesterol and *β*-sitosterol) and flavonoids were affected. Phytosterol content varied between dry and rainy seasons; also, leaves exhibited higher content of campesterol in the rainy season while culms accumulate campesterol and *β*-sitosterol in the dry season.

Data in the present study corroborate those described for other bamboo species that report promising antioxidant activity. Aqueous and hydroethanol extracts of leaves and culms showed high antiradicalar and iron-reducer activity; aqueous extract of leaves (**AE-L**) was the most active sample in all assays. No strong correlations were observed between flavonoid content and antioxidant activity. Possibly, other compounds, mainly phenolic acids, also contributed to the antioxidant activity of these extracts.

Seasonal variations were found mainly for culm extracts in FRAP assay. In the harvestings performed in 2017, it was observed higher activity of **HE-C** and **EE-C** in the rainy season than the dry season. On the other hand, **AE-C** showed variations between harvestings during the rainy season, with higher activity in 2017. Precipitation seems to influence both the antioxidant activity

and the flavonoid content of the extracts; rainy season/2017 showed the highest accumulated precipitation 30 days before harvesting.

Although *M. neesii* extracts inhibited acetylcholinesterase activity, this species showed not promising results as a source of extracts or compounds with antiacetylcholinesterase activity.

In conclusion, the results obtained in this study suggest that *M. neesii* extracts, mainly leaf infusions, might have potential as a food antioxidant. Furthermore, since neither antioxidant activity nor flavonoid content was affected by seasonality, *M. neesii* leaves can be harvested at any time of the year.

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**Supplementary material 3**

**Table S3.1:** Pearson's simple correlation coefficients (*r*) for flavonoids (mg $g^{-1}$) and antioxidant capacity ($EC_{50}$, μg $mL^{-1}$) in hydroethanol extract of leaves (**EE-L**).

| | **DPPH** | **ABTS** | **ORAC** | **FRAP** | **Ap1** | **Ap2** | **Ap3** | **Ap4** | **Ap5** | **Tri1** | **Tri2** | **TAp** | **TTri** | **TF** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DPPH** | 1.00 | | | | | | | | | | | | | |
| **ABTS** | 0.81 | 1.00 | | | | | | | | | | | | |
| **ORAC** | 0.77 | 0.99 | 1.00 | | | | | | | | | | | |
| **FRAP** | 0.89 | 0.91 | 0.86 | 1.00 | | | | | | | | | | |
| **Ap1** | -0.21 | 0.02 | 0.00 | -0.04 | 1.00 | | | | | | | | | |
| **Ap2** | 0.06 | 0.30 | 0.27 | 0.30 | 0.55 | 1.00 | | | | | | | | |
| **Ap3** | -0.19 | -0.07 | -0.09 | -0.07 | 0.71 | 0.65 | 1.00 | | | | | | | |
| **Ap4** | -0.02 | 0.22 | 0.23 | 0.11 | 0.67 | 0.55 | 0.83 | 1.00 | | | | | | |
| **Ap5** | -0.02 | 0.21 | 0.22 | 0.12 | 0.70 | 0.56 | 0.86 | 1.00 | 1.00 | | | | | |
| **Tri1** | -0.20 | -0.09 | -0.13 | -0.05 | 0.37 | 0.61 | 0.42 | 0.53 | 0.49 | 1.00 | | | | |
| **Tri2** | -0.02 | 0.02 | 0.00 | 0.07 | 0.30 | 0.43 | 0.75 | 0.67 | 0.69 | 0.29 | 1.00 | | | |
| **TAp** | -0.09 | 0.16 | 0.16 | 0.08 | 0.86 | 0.67 | 0.89 | 0.95 | 0.96 | 0.52 | 0.62 | 1.00 | | |
| **TTri** | -0.17 | -0.07 | -0.10 | -0.01 | 0.42 | 0.67 | 0.65 | 0.70 | 0.68 | 0.91 | 0.65 | 0.67 | 1.00 | |
| **TF** | -0.10 | 0.14 | 0.13 | 0.07 | 0.83 | 0.69 | 0.90 | 0.95 | 0.96 | 0.58 | 0.66 | 0.99 | 0.74 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Ap1: apigenin *O*-hexoside *C*-hexoside; Ap2: apigenin *O*-pentoside *C*-hexoside; Ap3: isovitexin; Ap4: apigenin *O*-hexoside *C*-pentoside; Ap5: apigenin *O*-pentoside *C*-pentoside; Tri1: tricin derivative 1; Tri2: tricin derivative 2; TAp: total apigenin; TTri: total tricin; and TF: total flavonoid. $EC_{50}$: effective concentration to achieve 50% of antioxidant activity.

**Table S3.2:** Pearson's simple correlation coefficients (*r*) for flavonoids (mg $g^{-1}$) and antioxidant capacity ($EC_{50}$, μg $mL^{-1}$) in hydroethanol extract of culms (**EE-C)**.

| | **DPPH** | **ABTS** | **ORAC** | **FRAP** | **Ap1** | **Ap2** | **Ap3** | **Ap4** | **Ap5** | **Tri1** | **Tri2** | **Tri3** | **Tri4** | **Tri5** | **TAp** | **TTri** | **TF** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DPPH** | 1.00 | | | | | | | | | | | | | | | | |
| **ABTS** | 0.87 | 1.00 | | | | | | | | | | | | | | | |
| **ORAC** | 0.72 | 0.73 | 1.00 | | | | | | | | | | | | | | |
| **FRAP** | 0.86 | 0.86 | 0.62 | 1.00 | | | | | | | | | | | | | |
| **Ap1** | -0.36 | -0.23 | -0.28 | -0.29 | 1.00 | | | | | | | | | | | | |
| **Ap2** | -0.34 | -0.12 | -0.16 | -0.19 | 0.94 | 1.00 | | | | | | | | | | | |
| **Ap3** | -0.38 | -0.03 | 0.07 | -0.21 | 0.53 | 0.69 | 1.00 | | | | | | | | | | |
| **Ap4** | -0.33 | -0.06 | 0.01 | -0.26 | 0.32 | 0.44 | 0.76 | 1.00 | | | | | | | | | |
| **Ap5** | -0.28 | 0.00 | 0.09 | -0.24 | 0.26 | 0.37 | 0.80 | 0.96 | 1.00 | | | | | | | | |
| **Tri1** | **-0.73** | **-0.52** | -0.32 | **-0.60** | 0.26 | 0.34 | 0.42 | 0.53 | 0.41 | 1.00 | | | | | | | |
| **Tri2** | -0.23 | 0.09 | -0.12 | -0.04 | 0.46 | 0.52 | 0.77 | 0.80 | 0.79 | 0.30 | 1.00 | | | | | | |
| **Tri3** | -0.08 | 0.18 | -0.20 | 0.11 | 0.64 | 0.67 | 0.57 | 0.56 | 0.50 | 0.15 | 0.82 | 1.00 | | | | | |
| **Tri4** | -0.18 | 0.16 | -0.21 | 0.22 | 0.34 | 0.44 | 0.56 | 0.48 | 0.46 | 0.23 | 0.78 | 0.71 | 1.00 | | | | |
| **Tri5** | -0.11 | 0.25 | -0.09 | 0.30 | 0.27 | 0.39 | 0.58 | 0.47 | 0.48 | 0.18 | 0.77 | 0.70 | 0.98 | 1.00 | | | |
| **TAp** | -0.40 | -0.10 | -0.06 | -0.28 | 0.75 | 0.84 | 0.92 | 0.82 | 0.81 | 0.45 | 0.80 | 0.71 | 0.55 | 0.53 | 1.00 | | |
| **TTri** | **-0.65** | -0.33 | -0.33 | -0.40 | 0.40 | 0.50 | 0.64 | 0.70 | 0.60 | 0.90 | 0.64 | 0.50 | 0.61 | 0.57 | 0.67 | 1.00 | |
| **TF** | **-0.57** | -0.23 | -0.21 | -0.37 | 0.64 | 0.75 | 0.86 | 0.84 | 0.77 | 0.73 | 0.79 | 0.67 | 0.63 | 0.60 | 0.92 | 0.90 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Ap1: apigenin *O*-hexoside *C*-hexoside; Ap2: apigenin *O*-pentoside *C*-hexoside; Ap3: isovitexin; Ap4: apigenin *O*-hexoside *C*-pentoside; Ap5: apigenin *O*-pentoside *C*-pentoside; Tri1: tricin derivative 1; Tri2: tricin derivative 2; Tri3: tricin derivative 3; Tri4: tricin derivative 4; Tri5: tricin derivative 5; TAp: total apigenin; TTri: total tricin; and TF: total flavonoid. $EC_{50}$: effective concentration to achieve 50% of antioxidant activity.

**Table S3.3:** Pearson's simple correlation coefficients (*r*) for flavonoids (mg $g^{-1}$) and antioxidant capacity ($EC_{50}$, μg $mL^{-1}$) in aqueous extract of leaves (**AE-L**).

| | **DPPH** | **ABTS** | **ORAC** | **FRAP** | **Ap1** | **Ap2** | **Ap3** | **Ap4** | **Ap5** | **Tri1** | **TAp** | **TTri** | **TF** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DPPH** | 1.00 | | | | | | | | | | | | |
| **ABTS** | 0.82 | 1.00 | | | | | | | | | | | |
| **ORAC** | 0.89 | 0.77 | 1.00 | | | | | | | | | | |
| **FRAP** | 0.67 | 0.88 | 0.53 | 1.00 | | | | | | | | | |
| **Ap1** | -0.32 | -0.10 | -0.32 | -0.13 | 1.00 | | | | | | | | |
| **Ap2** | -0.29 | -0.01 | -0.33 | 0.00 | 0.84 | 1.00 | | | | | | | |
| **Ap3** | **-0.50** | -0.33 | **-0.50** | -0.19 | 0.67 | 0.65 | 1.00 | | | | | | |
| **Ap4** | **-0.59** | -0.31 | -0.41 | -0.27 | 0.32 | 0.20 | 0.60 | 1.00 | | | | | |
| **Ap5** | -0.40 | -0.13 | -0.24 | -0.09 | 0.30 | 0.25 | 0.65 | 0.92 | 1.00 | | | | |
| **Tri1** | -0.01 | 0.12 | -0.06 | 0.18 | 0.58 | 0.22 | 0.31 | 0.37 | 0.41 | 1.00 | | | |
| **TAp** | -0.29 | -0.40 | -0.34 | -0.27 | -0.10 | 0.15 | 0.35 | 0.07 | 0.28 | -0.10 | 1.00 | | |
| **TTri** | -0.06 | -0.28 | -0.32 | -0.05 | 0.06 | 0.19 | 0.41 | -0.09 | 0.04 | 0.13 | 0.69 | 1.00 | |
| **TF** | -0.28 | -0.40 | -0.34 | -0.27 | -0.09 | 0.15 | 0.36 | 0.06 | 0.27 | -0.09 | 1.00 | 0.72 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Ap1: apigenin *O*-hexoside *C*-hexoside; Ap2: apigenin *O*-pentoside *C*-hexoside; Ap3: isovitexin; Ap4: apigenin *O*-hexoside *C*-pentoside; Ap5: apigenin *O*-pentoside *C*-pentoside; Tri1: tricin derivative 1; TAp: total apigenin; TTri: total tricin; and TF: total flavonoid. $EC_{50}$: effective concentration to achieve 50% of antioxidant activity.

**Table S3.4:** Pearson's simple correlation coefficients (*r*) for flavonoids (mg $g^{-1}$) and antioxidant capacity ($EC_{50}$, μg $mL^{-1}$) in aqueous extract of culms (**AE-C**).

| | **DPPH** | **ABTS** | **ORAC** | **FRAP** | **Ap1** | **Ap2** | **Ap3** | **Ap4** | **Ap5** | **Tri1** | **TAp** | **TTri** | **TF** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DPPH** | 1.00 | | | | | | | | | | | | |
| **ABTS** | 0.86 | 1.00 | | | | | | | | | | | |
| **ORAC** | 0.61 | 0.33 | 1.00 | | | | | | | | | | |
| **FRAP** | 0.83 | 0.90 | 0.31 | 1.00 | | | | | | | | | |
| **Ap1** | 0.01 | 0.09 | -0.27 | -0.03 | 1.00 | | | | | | | | |
| **Ap2** | -0.32 | -0.16 | **-0.57** | -0.21 | 0.78 | 1.00 | | | | | | | |
| **Ap3** | 0.26 | 0.27 | 0.03 | 0.19 | 0.70 | 0.61 | 1.00 | | | | | | |
| **Ap4** | -0.02 | 0.04 | -0.36 | -0.03 | 0.89 | 0.79 | 0.65 | 1.00 | | | | | |
| **Ap5** | 0.03 | 0.08 | -0.29 | 0.01 | 0.92 | 0.77 | 0.68 | 0.99 | 1.00 | | | | |
| **Tri1** | -0.09 | -0.13 | -0.35 | 0.01 | 0.57 | 0.70 | 0.52 | 0.72 | 0.71 | 1.00 | | | |
| **TAp** | **-0.61** | -0.46 | -0.42 | -0.44 | 0.17 | 0.36 | 0.07 | 0.17 | 0.15 | 0.15 | 1.00 | | |
| **TTri** | **-0.67** | **-0.70** | -0.08 | **-0.56** | -0.07 | -0.03 | -0.22 | -0.12 | -0.11 | 0.13 | 0.48 | 1.00 | |
| **TF** | **-0.74** | **-0.68** | -0.28 | **-0.58** | 0.06 | 0.19 | -0.09 | 0.03 | 0.02 | 0.15 | 0.85 | 0.87 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Ap1: apigenin *O*-hexoside *C*-hexoside; Ap2: apigenin *O*-pentoside *C*-hexoside; Ap3: isovitexin; Ap4: apigenin *O*-hexoside *C*-pentoside; Ap5: apigenin *O*-pentoside *C*-pentoside; Tri1: tricin derivative 1; TAp: total apigenin; TTri: total tricin; and TF: total flavonoid. $EC_{50}$: effective concentration to achieve 50% of antioxidant activity.

**Table S3.5:** Pearson's simple correlation coefficients (*r*) for bioactive compounds (mg $g^{-1}$) and antiacetylcholinesterase capacity ($IC_{50}$, μg $mL^{-1}$) in hexane extract of leaves (**HE-L**).

| | **AChE** | **Camp** | **Stig** | ***β*-sit** | **TP** |
|---|---|---|---|---|---|
| **AChE** | 1.00 | | | | |
| **Camp** | 0.03 | 1.00 | | | |
| **Stig** | -0.12 | 0.95 | 1.00 | | |
| ***β*-sit** | -0.01 | 0.98 | 0.98 | 1.00 | |
| **TP** | -0.06 | 0.98 | 0.98 | 1.00 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Camp: campesterol; Stig: stigmasterol; *β*-sit: *β*-sitosterol; and TP: total phytosterol. AChE: acetylcholinesterase; $IC_{50}$: concentration to inhibit 50% of acetylcholinesterase activity.

**Table S3.6:** Pearson's simple correlation coefficients (*r*) for bioactive compounds (mg $g^{-1}$) and antiacetylcholinesterase capacity ($IC_{50}$, μg $mL^{-1}$) in hexane extract of culms (**HE-C**).

| | **AChE** | **Camp** | **Stig** | ***β*-sit** | **TP** | **Lup** |
|---|---|---|---|---|---|---|
| **AChE** | 1.00 | | | | | |
| **Camp** | -0.26 | 1.00 | | | | |
| **Stig** | -0.01 | 0.88 | 1.00 | | | |
| ***β*-sit** | -0.21 | 0.97 | 0.92 | 1.00 | | |
| **TP** | -0.17 | 0.97 | 0.94 | 0.99 | 1.00 | |
| **Lup** | 0.17 | 0.10 | 0.36 | 0.21 | 0.26 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Camp: campesterol; Stig: stigmasterol; *β*-sit: *β*-sitosterol; TP: total phytosterol; and Lup: lupenone. AChE: acetylcholinesterase; $IC_{50}$: concentration to inhibit 50% of acetylcholinesterase activity.

**Table S3.7:** Pearson's simple correlation coefficients (*r*) for bioactive compounds (mg $g^{-1}$) and antiacetylcholinesterase capacity ($IC_{50}$, μg $mL^{-1}$) in hydroethanol extract of leaves (**EE-L**).

| | **AChE** | **Ap1** | **Ap2** | **Ap3** | **Ap4** | **Ap5** | **Tri1** | **Tri2** | **TAp** | **TTri** | **TF** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **AChE** | 1.00 | | | | | | | | | | |
| **Ap1** | -0.02 | 1.00 | | | | | | | | | |
| **Ap2** | 0.33 | 0.55 | 1.00 | | | | | | | | |
| **Ap3** | -0.07 | 0.71 | 0.65 | 1.00 | | | | | | | |
| **Ap4** | -0.12 | 0.67 | 0.55 | 0.83 | 1.00 | | | | | | |
| **Ap5** | -0.12 | 0.70 | 0.56 | 0.86 | 1.00 | 1.00 | | | | | |
| **Tri1** | **0.51** | 0.37 | 0.61 | 0.42 | 0.53 | 0.49 | 1.00 | | | | |
| **Tri2** | 0.11 | 0.30 | 0.43 | 0.75 | 0.67 | 0.69 | 0.29 | 1.00 | | | |
| **TAp** | -0.05 | 0.86 | 0.67 | 0.89 | 0.95 | 0.96 | 0.52 | 0.62 | 1.00 | | |
| **TTri** | 0.45 | 0.42 | 0.67 | 0.65 | 0.70 | 0.68 | 0.91 | 0.65 | 0.67 | 1.00 | |
| **TF** | 0.01 | 0.83 | 0.69 | 0.90 | 0.95 | 0.96 | 0.58 | 0.66 | 0.99 | 0.74 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Ap1: apigenin *O*-hexoside *C*-hexoside; Ap2: apigenin *O*-pentoside *C*-hexoside; Ap3: isovitexin; Ap4: apigenin *O*-hexoside *C*-pentoside; Ap5: apigenin *O*-pentoside *C*-pentoside; Tri1: tricin derivative 1; Tri2: tricin derivative 2; TAp: total apigenin; TTri: total tricin; and TF: total flavonoid. AChE: acetylcholinesterase; $IC_{50}$: concentration to inhibit 50% of acetylcholinesterase activity.

**Table S3.8:** Pearson's simple correlation coefficients (*r*) for bioactive compounds (mg $g^{-1}$) and antiacetylcholinesterase capacity ($IC_{50}$, μg $mL^{-1}$) in hydroethanol extract of culms (**EE-C**).

| | **AChE** | **Ap1** | **Ap2** | **Ap3** | **Ap4** | **Ap5** | **Tri1** | **Tri2** | **Tri3** | **Tri4** | **Tri5** | **TAp** | **TTri** | **TF** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AChE** | 1.00 | | | | | | | | | | | | | |
| **Ap1** | 0.09 | 1.00 | | | | | | | | | | | | |
| **Ap2** | -0.16 | 0.94 | 1.00 | | | | | | | | | | | |
| **Ap3** | -0.36 | 0.53 | 0.69 | 1.00 | | | | | | | | | | |
| **Ap4** | -0.15 | 0.32 | 0.44 | 0.76 | 1.00 | | | | | | | | | |
| **Ap5** | -0.13 | 0.26 | 0.37 | 0.80 | 0.96 | 1.00 | | | | | | | | |
| **Tri1** | -0.37 | 0.26 | 0.34 | 0.42 | 0.53 | 0.41 | 1.00 | | | | | | | |
| **Tri2** | -0.04 | 0.46 | 0.52 | 0.77 | 0.80 | 0.79 | 0.30 | 1.00 | | | | | | |
| **Tri3** | -0.06 | 0.64 | 0.67 | 0.57 | 0.56 | 0.50 | 0.15 | 0.82 | 1.00 | | | | | |
| **Tri4** | -0.20 | 0.34 | 0.44 | 0.56 | 0.48 | 0.46 | 0.23 | 0.78 | 0.71 | 1.00 | | | | |
| **Tri5** | -0.24 | 0.27 | 0.39 | 0.58 | 0.47 | 0.48 | 0.18 | 0.77 | 0.70 | 0.98 | 1.00 | | | |
| **TAp** | -0.17 | 0.75 | 0.84 | 0.92 | 0.82 | 0.81 | 0.45 | 0.80 | 0.71 | 0.55 | 0.53 | 1.00 | | |
| **TTri** | -0.36 | 0.40 | 0.50 | 0.64 | 0.70 | 0.60 | 0.90 | 0.64 | 0.50 | 0.61 | 0.57 | 0.67 | 1.00 | |
| **TF** | -0.29 | 0.64 | 0.75 | 0.86 | 0.84 | 0.77 | 0.73 | 0.79 | 0.67 | 0.63 | 0.60 | 0.92 | 0.90 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Ap1: apigenin *O*-hexoside *C*-hexoside; Ap2: apigenin *O*-pentoside *C*-hexoside; Ap3: isovitexin; Ap4: apigenin *O*-hexoside *C*-pentoside; Ap5: apigenin *O*-pentoside *C*-pentoside; Tri1: tricin derivative 1; Tri2: tricin derivative 2; Tri3: tricin derivative 3.; Tri4: tricin derivative 4; Tri5: tricin derivative 5; TAp: total apigenin; TTri: total tricin; and TF: total flavonoid. AChE: acetylcholinesterase; $IC_{50}$: concentration to inhibit 50% of acetylcholinesterase activity.

**Table S3.9:** Pearson's simple correlation coefficients (*r*) for bioactive compounds (mg $g^{-1}$) and antiacetylcholinesterase capacity ($IC_{50}$, μg $mL^{-1}$) in aqueous extract of leaves (**AE-L**).

| | **AChE** | **Ap1** | **Ap2** | **Ap3** | **Ap4** | **Ap5** | **Tri1** | **TAp** | **TTri** | **TF** |
|---|---|---|---|---|---|---|---|---|---|---|
| **AChE** | 1.00 | | | | | | | | | |
| **Ap1** | -0.38 | 1.00 | | | | | | | | |
| **Ap2** | **-0.57** | 0.84 | 1.00 | | | | | | | |
| **Ap3** | **-0.58** | 0.67 | 0.65 | 1.00 | | | | | | |
| **Ap4** | **-0.56** | 0.32 | 0.20 | 0.60 | 1.00 | | | | | |
| **Ap5** | **-0.54** | 0.30 | 0.25 | 0.65 | 0.92 | 1.00 | | | | |
| **Tri1** | -0.03 | 0.58 | 0.22 | 0.31 | 0.37 | 0.41 | 1.00 | | | |
| **TAp** | -0.29 | -0.10 | 0.15 | 0.35 | 0.07 | 0.28 | -0.10 | 1.00 | | |
| **TTri** | -0.01 | 0.06 | 0.19 | 0.41 | -0.09 | 0.04 | 0.13 | 0.69 | 1.00 | |
| **TF** | -0.27 | -0.09 | 0.15 | 0.36 | 0.06 | 0.27 | -0.09 | 1.00 | 0.72 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Ap1: apigenin *O*-hexoside *C*-hexoside; Ap2: apigenin *O*-pentoside *C*-hexoside; Ap3: isovitexin; Ap4: apigenin *O*-hexoside *C*-pentoside; Ap5: apigenin *O*-pentoside *C*-pentoside; Tri1: tricin derivative 1; TAp: total apigenin; TTri: total tricin; and TF: total flavonoid. AChE: acetylcholinesterase; $IC_{50}$: concentration to inhibit 50% of acetylcholinesterase activity.

**Table S3.10:** Pearson's simple correlation coefficients (*r*) for bioactive compounds (mg $g^{-1}$) and antiacetylcholinesterase capacity ($IC_{50}$, μg $mL^{-1}$) in aqueous extract of culms (**AE-C**).

| | **AChE** | **Ap1** | **Ap2** | **Ap3** | **Ap4** | **Ap5** | **Tri1** | **TAp** | **TTri** | **TF** |
|---|---|---|---|---|---|---|---|---|---|---|
| **AChE** | 1.00 | | | | | | | | | |
| **Ap1** | 0.27 | 1.00 | | | | | | | | |
| **Ap2** | -0.10 | 0.78 | 1.00 | | | | | | | |
| **Ap3** | -0.05 | 0.70 | 0.61 | 1.00 | | | | | | |
| **Ap4** | 0.23 | 0.89 | 0.79 | 0.65 | 1.00 | | | | | |
| **Ap5** | 0.28 | 0.92 | 0.77 | 0.68 | 0.99 | 1.00 | | | | |
| **Tri1** | -0.04 | 0.57 | 0.70 | 0.52 | 0.72 | 0.71 | 1.00 | | | |
| **TAp** | -0.45 | 0.17 | 0.36 | 0.07 | 0.17 | 0.15 | 0.15 | 1.00 | | |
| **TTri** | -0.13 | -0.07 | -0.03 | -0.22 | -0.12 | -0.11 | 0.13 | 0.48 | 1.00 | |
| **TF** | -0.33 | 0.06 | 0.19 | -0.09 | 0.03 | 0.02 | 0.15 | 0.85 | 0.87 | 1.00 |

Interpretation of the *r* values (+ or -): very weak or no correlation, 0.00 to 0.25 or -0.25 to 0.00; weak correlation, 0.25 to 0.50 or -0.50 to -0.25; moderate correlation, 0.50 to 0.75 or -0.75 to -0.50; strong correlation, 0.75 to 1.00 or -1.00 to -0.75; and perfect correlation, 1.00 or -1.00 (Vieira 2008). Ap1: apigenin *O*-hexoside *C*-hexoside; Ap2: apigenin *O*-pentoside *C*-hexoside; Ap3: isovitexin; Ap4: apigenin *O*-hexoside *C*-pentoside; Ap5: apigenin *O*-pentoside *C*-pentoside; Tri1: tricin derivative 1; TAp: total apigenin; TTri: total tricin; and TF: total flavonoid. AChE: acetylcholinesterase; $IC_{50}$: concentration to inhibit 50% of acetylcholinesterase activity.

# Final considerations

Bamboos are an important socioeconomic resource, especially in Asian countries. Although Brazil also has a great diversity of bamboo species, this group is poorly explored and studied.

The aims of this study were to characterize the chemical composition of a Brazilian endemic bamboo species as well as to evaluate its biological potential, verifying its antioxidant and anticholinesterase activities. Plus, it was also studied the seasonal influence on the chemical composition and the nutritional and biological potential of *M. neesii*.

*M. neesii* showed similar chemical composition to other bamboo species. Among the nonpolar bioactive compounds were detected phytosterols, especially campesterol, stigmasterol, and *β*-sitosterol, and lupenone, a triterpene. These compounds have already been described for Bambusoideae. Regarding the phenolic composition, flavones *C*-glycosides derived from apigenin and flavones derived from tricin were found in leaves and culms. Flavones *C*-glycosides are the main class of secondary metabolites reported in bamboos. Moreover, this study described the presence of soluble carbohydrates, organic acids, catecholamines (reported for the first time in Bambusoideae), and fatty acids.

*M. neesii* also showed similar nutritional composition to other bamboo species. The results of nutritional composition suggest that this species might have potential as a supplementary forage and as a source of health beneficial compounds, such as fibers, polyunsaturated fatty acids, phytosterols, and phenolic compounds; thus, this species may also have potential as a food supplement and/or food additive.

In addition to nutritional potential, *M. neesii* also showed promising antioxidant activity, reinforcing its potential as a food additive; other bamboo species are already used for this purpose. On the other hand, this species showed not to be a promising source of compounds with acetylcholinesterase and butyrylcholinesterase inhibitory activity.

Regarding the seasonal changes, bioactive compound contents and nutritional and biological potential of *M. neesii* were not strongly influenced by climate factors.

The hypotheses of this study were: (i) Brazilian native bamboos, like the Asian bamboos, have medicinal and nutritional potential, and (ii) seasonality influences the phenolic contents, especially flavonoids, of *M. neesii*, which will result in differences in the bioactivity of its extracts. The results showed that this species has nutritional potential, as observed for other Asian bamboo species. However, differently from other studies on seasonal variations, especially with bamboo species, flavonoid contents and antioxidant and anticholinesterase activities were not affected by seasonal changes. This can be partly explained by the large climatic differences where other

bamboo species occur. Most of these species are from temperate regions where climate variations are much more intense than those observed for the subtropical regions where *M. neesii* is found.

In summary, the present work achieved the proposed objectives, contributing to the phytochemical knowledge of the Brazilian native flora and a better understanding of the effect of seasonality on the production of secondary metabolites, mainly flavonoids, and on the nutritional and biological potential of plant species.

# Resumo

Bambus têm sido usados como recurso alimentar e para o tratamento de doenças há séculos. Em regiões onde forragem é escassa em determinados períodos do ano, estas gramíneas podem ser usadas como suplemento alimentar. Além disso, os brotos de bambu constituem um alimento muito popular na culinária asiática e têm recebido crescente atenção devido ao seu valor nutricional e aos benefícios proporcionados à saúde. Os colmos e as folhas de bambu também apresentam importância nutricional. Os colmos têm sido usados como fonte de fibras para suplementos alimentares, enquanto um extrato de folhas de bambu (AOB, extrato antioxidante de bambu) foi aprovado pelo Ministério da Saúde da China como antioxidante alimentar. Os principais constituintes bioativos neste grupo de plantas são as substâncias fenólicas, especialmente ácidos fenólicos e flavonas *C*-glicosiladas. Sabe-se que vários fatores podem afetar a produção de metabólitos secundários, entre eles, a sazonalidade. Dessa forma, o conhecimento de como mudanças sazonais podem afetar o conteúdo de metabólitos secundários e, consequentemente, o potencial biológico de extratos vegetais é importante no estudo de plantas medicinais. O Brasil apresenta uma das maiores diversidade de espécies de bambu no mundo e constitui o centro de diversidade de vários gêneros, entre eles, *Merostachys* Spreng. Este gênero apresenta 53 espécies (47 nativas) e até o momento apenas três espécies apresentam algum estudo sobre composição química e atividade biológica. Dessa forma, *M. neesii*, uma espécie de bambu endêmico da Mata Atlântica, foi escolhida para a realização deste estudo. Os objetivos gerais deste trabalho foram contribuir para o conhecimento fitoquímico da flora brasileira, ajudar na busca de compostos bioativos e contribuir para o entendimento de como a sazonalidade pode afetar a produção de metabólitos secundários. Como principais resultados, observou-se que *M. neesii* apresentou composição química semelhante ao de outras espécies de bambu. Foram identificadas 64 substâncias, a maioria descrita pela primeira vez para o gênero. As principais classes de substâncias encontradas nesta espécie foram carboidratos solúveis, ácidos orgânicos, catecolaminas, ácidos graxos, fitosteróis, triterpenos (lupenona) e flavonoides, especialmente flavonas *C*-glicosiladas derivadas de apigenina e flavonas derivdas de tricina. A avaliação da composição nutricional revelou que *M. neesii* tem potencial como forragem suplementar bem como suplemento e aditivo alimentar. Além disso, os extratos, principalmente a infusão de folhas, apresentaram atividade antirradicalar e redutora de ferro. Entretanto, estes extratos apresentaram baixa atividade inibitória da acetilcolinesterase. Com relação às variações sazonais, os resultados revelaram que não houve diferença significante entre as estações no conteúdo dos nutrientes, exceto para o conteúdo de lipídeos nas folhas que apresentou maior concentração na estação chuvosa do que na estação seca.

Com relação aos constituintes bioativos e potencial biológico, foram observadas algumas variações entre as coletas para ao conteúdo de fitosteróis e flavonoides e também para a atividade antioxidante, porém não houve um padrão de variação claro. Dessa forma, fatores climáticos não foram determinantes nas mudanças observadas. Por fim, foi encontrada uma correlação moderada entre atividade antioxidante e flavonoides apenas para colmos. Outras classes de substâncias fenólicas, possivelmente ácidos fenólicos, podem estar envolvidas na atividade antioxidante, principalmente na infusão de folhas, que foi o extrato mais ativo. Embora ácidos fenólicos foram detectados nos extratos de folhas e colmos, eles não foram abordados no presente estudo. Dessa forma, estudos adicionais sobre a composição fenólica devem ser realizados a fim de obter uma melhor compreensão da atividade antioxidante destes extratos bem como de sua composição fenólica.

Palavras-chave: Bambusoideae; Sazonalidade; Flavonoide; Valor nutricional; Atividade antioxidante; Atividade anticolinesterásica.

# Abstract

Bamboos have been used as a food resource and for the treatment of diseases for centuries. In regions where fodder is scarce in some seasons, these grasses can be used as supplementary forage. Furthermore, bamboo shoots are a very popular food in Asian cuisine and have received increasing attention due to their nutritional value and health benefits. Bamboo culms and leaves are also nutritionally important. Culms have been used as a source of fiber for food supplements, while a bamboo leaf extract (AOB, antioxidant of bamboo leaves extract) has been certificated as a natural food antioxidant. The main bioactive constituents in these plants are phenolic compounds, mainly phenolic acids and flavones *C*-glycosides. Several factors are known to affect the production of secondary metabolites, including seasonality. Thus, the knowledge of how seasonal changes can affect secondary metabolite content and, consequently, the biological potential of plant extracts is important in the study of medicinal plants. Brazil has one of the largest diversity of bamboo species in the world and is the center of diversity of many genera, among them *Merostachys* Spreng. This genus has 53 species (47 natives) and so far, only three species have been studied regarding their chemical composition and biological activity. Thus, *M. neesii*, an endemic bamboo species from the Atlantic Rain Forest, was chosen for this study. The main objectives of this study were to contribute to the phytochemical knowledge of the Brazilian native flora, to help in the search for bioactive compounds and to contribute to the understanding of how seasonality can affect the production of secondary metabolites. As the main results, it was observed that *M. neesii* presented similar chemical composition to other bamboo species. Sixty-four compounds were identified in this work, most of them described for the first time for this genus. The main classes of compounds found were soluble carbohydrates, organic acids, catecholamines, fatty acids, phytosterols, triterpenes (lupenone), and flavonoids, especially flavones *C*-glycosides derived from apigenin and flavones derived from tricin. The evaluation of the nutritional composition of *M. neesii* revealed that this species has potential as supplementary forage as well as a food additive. Moreover, extracts of this species, especially leaf infusion, showed antiradicalar and iron-reducer activity. However, these extracts exhibited low acetylcholinesterase inhibitory activity. Regarding seasonal changes, there was no significant difference between seasons on the nutrient contents, except for the lipid content of leaves that was higher in the rainy season than in the dry season. There were some variations among harvestings for phytosterol, flavonoid contents, and antioxidant activity, but there was no clear variation pattern. Thus, climate factors were not relevant to determine these changes. Finally, there was a moderate correlation between antioxidant activity and flavonoids for culms. Other classes of phenolic compounds, probably phenolic acids, may be involved in the antioxidant

activity, especially in leaf infusion, which was the most active extract. Although phenolic acids were detected in leaf and culm extracts, they were not evaluated in the present study. Thus, further studies on phenolic composition should be performed for a better understanding of the antioxidant activity of these extracts, as well as their phenolic composition.

Keywords: Bambusoideae; Seasonality; Flavonoid; Nutritional value; Antioxidant activity; Anticholinesterase activity.