INTRODUCTION

Species from Malvaceae family are widely used in Brazil as remedy, food and as forage. This family consists of 250 genera and 4,230 species spread worldwide. In Brazil it is represented by 68 genera and 735 species found throughout the territory. An amount of 431 species are endemic to Brazil and found in all regions of the country.

The actual Malvaceae circumscription comprises nine subfamilies: Bombacoideae Brownlowioideae, Byttnerioideae, Dombeyoideae, Grewioideae, Helicteroideae, Malvoideae, Sterculioideae, and Tiliioideae. The subfamily Malvoideae Burnett (former Malvaceae family) is the largest of the nine subfamilies of Malvaceae, and has emerged as a monophyletic group with 111 genera and 1,040 species. The group’s taxonomy is quite complex, and in its most recent treatment Malvoideae are divided into three tribes: Gossypioideae Alefeld, Hibisceae Reichenbach, and Malveae J. Presl, the latter being classified under ten different genera and 1,040 species in 70 genera.

The comparative phytochemistry can contribute greatly to the chemotaxonomic analysis of the family, especially due their ability in preventing inflammation, thrombotic events, hypertension, diabetes, renal diseases, rheumatoid arthritis, and cancer.

Phytochemical investigations have reported the fatty acids content of ten Malvoideae species was analyzed and its chemotaxonomic significance has been investigated. The aerial parts of the species were collected in the Northeast of Brazil and their fatty acid methyl esters were analyzed by gas chromatography with flame ionization detector. The chemometric analysis consisted of principal component analysis (PCA) and hierarchical clustering analysis (HCA) with the euclidean distance between the samples given by the Ward.D2 algorithm. This is the first report of fatty acids from Wissadula periplocifolia, Herissantia crispa, Bakridesia pickelli, Sidastrum micranthum, Pavonia cancellata and Pavonia malacophylla. The results showed the predominance of palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids in the studied species. By the PCA and HCA analysis, the fatty acid composition was able to distinguish the species Herissantia crispa and Pavonia malacophylla. Our findings showed a chemotaxonomic proximity among species from different genera reflecting the taxonomic and phylogenetic closeness previously demonstrated by molecular investigations on Malvoideae species. Furthermore, our results demonstrated that the fatty acid analysis may be an interesting tool to support the taxonomic investigations on Malvoideae species.

Keywords: Malvoideae; fixed oils; gas chromatography; PCA; HCA.
registered at National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen - A568B8A).

**Extraction procedures and preparation of fatty acids methyl esters**

The plant material (aerial parts) of each species was separately dried (oven at 40 °C for 72 h), grounded and macerated with absolute ethanol (EtOH) for 72 h. The ethanolic solution was concentrated with a rotary evaporator to obtain the crude ethanolic extract (CEE). CCE was solubilized using EtOH:H2O (9:1), and the solution was solvent extracted in a separation funnel using hexane to obtain the lipid-rich fractions (LF).

Fatty acids methyl esters (FAME) were prepared according to the method described by Maia. The obtained LF (30 mg) of each extract was saponified, in a test tube with a screw cap with 5 mL methanolic solution of sodium hydroxide (0.5 mol L−1) at 90 °C for 2 min; 170 °C-173 °C (1.5 °C min−1); 173 °C-180 °C for 7 min; 180 °C-230 °C (6 °C min−1); 230 °C for 20 min. Then, a saturated solution of sodium chloride (4 mL) was added to the test tube under agitation (30 s) followed by addition of hexane (5 mL) (Tedia, chromatographic grade). After separation of phases, the FAME samples were stored in vials (2 mL) in a freezer (−20 °C).

**Gas Chromatography conditions**

The separation and quantification (area normalization method) of the FAME were performed by gas chromatography with flame ionization detector (GC-FID, Shimadzu, GC-2010) using a fused silica capillary column OV-5 (Ohio Valley Specialty Chemical, Inc. 30.0 m x 0.25 mm x 0.25 μm), gas Helium (1.7 mL min−1), injector temperature of 220 °C, detector at 230 °C and split ratio 1:30. An aliquot of the FAME (200 μL) was diluted using 1 mL of hexane and 1 μL of the diluted solution was analyzed in the following temperature program: 110 °C for 1 min, 110 °C-170 °C (10 °C min−1); 170 °C for 2 min; 170 °C-173 °C (1.5 °C min−1); 173 °C-180 °C (1 °C min−1); 180 °C for 7 min; 180 °C-230 °C (6 °C min−1); 230 °C for 20 min.

The identification of the FAME were carried out by comparing the observed retention times with methyl esters of the following standard fatty acids: oleic acid (Sigma, L-126H3446, ≥ 99%), palmitic acid (Sigma, L-26H8491, 99%), arachidic acid (Sigma, 56H0479, 99%) and behenic acid (Sigma, 051M1395V). All FAME standards were analyzed under the same operating conditions of the samples, with the following retention times (RT): of methyl esters from lauric acid (7.869 min), myristic acid (11.442 min), palmitic acid (17.773 min), linoleic acid (26.734 min), oleic acid (26.782 min), stearic acid (28.018 min), arachidic acid (33.632 min) and behenic acid (39.173 min) (Supplementary material, Figures 1S-10S).

**RESULTS AND DISCUSSION**

The results of the chemical composition of fatty acids (relative percentage) were submitted to hierarchical clustering analysis (HCA) and Principal Component Analysis (PCA), using the R project. The chemical composition data were standardized with the decostand function and centralized using the scale function. The Principal Component Analysis (PCA) was performed using the factoMine1R and factoextra packages. The hierarchical clustering analysis (HCA) using Euclidian Distance and Ward.D2 method was carried out with the Vegan package.

### RESULTS AND DISCUSSION

The following fatty acids were identified from all studied species: lauric acid (C12:0), myristic acid (C14:0), stearic acid (C16:0), arachidic acid (C20:0), behenic acid (C22:0), palmitic acid (C16:0), linoleic acid (C18:2), and oleic acid (C18:1). The last three substances were found as the major constituents, showing different percentages among the species (Table 1). This result is in agreement with previous studies on fatty acids composition of Malvoideae and Malvaceae species.

The hierarchical clustering analysis (HCA) divided the species into two clusters (Figure 1). Cluster I was formed by Herissantia crispa and Pavonia malacophylla, species with a higher relative proportion of oleic acid, 30.8% and 32.6%, respectively. Sida rhombifolia, Sidastrum paniculatum, Sidastrum micranthum, Sida galheirensis, Bakeridesia pickelii, Herissantia tiubae, Wissadula periplocifolia and Pavonia cancelata, species with a higher relative proportion of palmitic acid and linoleic acid, composed the cluster II.

According to the PCA (Figure 2) the oleic acid content in H. crispa (30.8%) and P. malacophylla (32.6%) contributed to

### Table 1. Fatty acid composition (%) of (Malvoideae) Malvaceae species

| Species                        | C12:0 | C14:0 | C16:0 | C18:0 | C18:1 | C18:2 | C20:0 | C22:0 |
|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Sidastrum paniculatum          | 1.0   | 1.7   | 27.5  | 2.6   | 23.7  | 24.0  | 0.6   | 1.5   |
| Sidastrum micranthum           | 0.4   | 1.9   | 27.9  | 2.9   | 22.8  | 26.4  | 0.9   | 0.9   |
| Sida rhombifolia               | 0.4   | 1.0   | 28.0  | 3.0   | 25.1  | 26.8  | 0.7   | 2.3   |
| Sida galheirensis              | 1.1   | 2.0   | 22.5  | 3.8   | 22.3  | 21.8  | 1.1   | 5.0   |
| Pavonia malacophylla           | 0.3   | 0.5   | 29.1  | 4.7   | 32.6  | 11.7  | 1.2   | 1.0   |
| Pavonia cancellata             | 1.4   | 3.1   | 29.3  | 3.5   | 19.6  | 15.5  | 0.7   | 1.5   |
| Herissantia tiubae             | 0.6   | 3.2   | 28.8  | 5.4   | 19.7  | 22.4  | 3.4   | 3.4   |
| Herissantia crispa             | 0.8   | 1.0   | 17.9  | 2.9   | 30.8  | 16.6  | 1.7   | 3.0   |
| Wissadula periplocifolia       | 0.5   | 2.0   | 27.5  | 3.4   | 20.3  | 18.4  | 0.8   | 2.2   |
| Bakeridesia pickelii           | 0.4   | 1.4   | 25.4  | 2.6   | 16.6  | 22.0  | 1.8   | 4.5   |

C12:0 = lauric acid, C14:0 = myristic acid, C16:0 = palmitic acid, C18:0 = stearic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C20:0 = arachidic acid, C22:0 = behenic acid.
differentiate them from the other species, as observed in the cluster analysis (Figure 1). *P. malacophylla* presented greater abundance of palmitic acid (29.1%) than *H. crispa* (17.9%).

The other species of the genera *Sida*, *Sidastrum*, *Pavonia* and *Wissadula* were subdivided into species rich in linoleic acid (*S. galheiensis*, *S. rhombifolia*, *S. paniculatum* and *S. micranthum*), and species rich in palmitic acid (*P. cancellata* and *W. periplocifolia*). The species *B. pickelli* and *H. tiubae* were found to be great producers of both linoleic and palmitic acids.

The fatty acid composition in aerial parts of *H. tiubae*, *S. paniculatum* and *S. galheiensis* was different from the fatty acid composition of seeds collected in the same bioma reported by Silva et al (2010).\(^8\) For the seeds, the palmitic acid represented 47% of total fatty acids, while for aerial parts it was around 28%. The relative proportion of linoleic acid in seeds of *H. tiubae* was 5.3%, in seed of *S. galheiensis* was 63.7%, and this acid was not detected in seeds of *S. paniculatum*.\(^8\) For aerial parts, however, the linoleic acid content was similar for those three species, varying from 22.4% to 24.0%
demonstrated that the fatty acid analysis may be an interesting tool to demonstrate by molecular investigations. Furthermore, our results reflect the taxonomic and phylogenetic closeness previously demonstrated by molecular investigations on the Abutilon Alliance by Aguilar et al. (2003), and is currently included in the Abutilon alliance. However, the taxon of the “generic Sida alliance” are still poorly understood and, in phylogenetic studies, do not form a monophyletic group.

The great amount of palmitic acid found in W. periplocifolia and P. cancellata, placed these species in the same cluster with Sida and Sidastrum species. Historically, some Wissadula species used to be classified as Sida, indicating a taxonomic closeness between the two genera.

The mentioned taxonomic proximity of many species from Malvoideae subfamily has raised the need for molecular investigations to better understand their phylogenetic position. These investigations include citogenetics, sequencing and analysis based on molecular markers (especially plastid markers). Many of these studies have suggested to reconstruct phylogenetic relationships within Malvoidae subfamily. As showed in cluster II (Figure 1), our findings showed a chemotaxonomic proximity among species from different genera reflecting the taxonomic and phylogenetic closeness previously demonstrated by molecular investigations. Furthermore, our results demonstrated that the fatty acid analysis may be an interesting tool to support the taxonomic investigations on Malvoideae species.

CONCLUSIONS

The present study is the first report of fatty acids from Wissadula periplocifolia, Herissantia crispa, Bakederiea pickelti, Sidastrum micranthum, Pavonia cancellata and Pavonia malacophylla. Palmitic, oleic and linoleic acids were the major compounds identified. Our findings showed a chemotaxonomic proximity among species from different genera reflecting the taxonomic and phylogenetic closeness previously demonstrated by molecular investigations on Malvoideae species. Furthermore, our results demonstrated that the fatty acid analysis may be an interesting tool to support the taxonomic investigations on Malvoideae species.

SUPPLEMENTARY MATERIAL

Table 1S and the chromatograms are freely available at http://quimicanova.sbq.org.br in PDF format.

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