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# Differentiating Two Species of ‘Mother-of-Thousands’: *Kalanchoe daigremontiana* and *Kalanchoe x houghtonii*

**Evelyn Assis de Andrade**<sup>1</sup>  
https://orcid.org/0000-0002-7542-3070

**Isadora Machinski**<sup>1</sup>  
https://orcid.org/0000-0002-2745-6687

**Valter Paes de Almeida**<sup>1</sup>  
https://orcid.org/0000-0003-0603-8184

**Wilmer Hervet Perera**<sup>2</sup>  
https://orcid.org/0000-0003-3607-8638

**Robert Thomas Williamson**<sup>3</sup>  
https://orcid.org/0000-0001-7450-3135

**Wendy Karen Strangman**<sup>3</sup>  
https://orcid.org/0000-0002-6911-4909

**Jane Manfron**<sup>1</sup>  
https://orcid.org/0000-0003-1873-2253

**Flávio Luís Beltrame**<sup>1\*</sup>  
https://orcid.org/0000-0001-7067-5802

<sup>1</sup>Universidade Estadual de Ponta Grossa, Programa de Pós-graduação em Ciências Farmacêuticas, Ponta Grossa, Paraná, Brasil; <sup>2</sup>CAMAG Scientific, Inc., Wilmington, North Carolina, USA; <sup>3</sup>University of North Carolina Wilmington, Department of Chemistry and Biochemistry, Wilmington, North Carolina, USA.

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\*Correspondence: flaviobeltra@gmail.com; Tel.: +55-42-999601108 (F.L.B.).

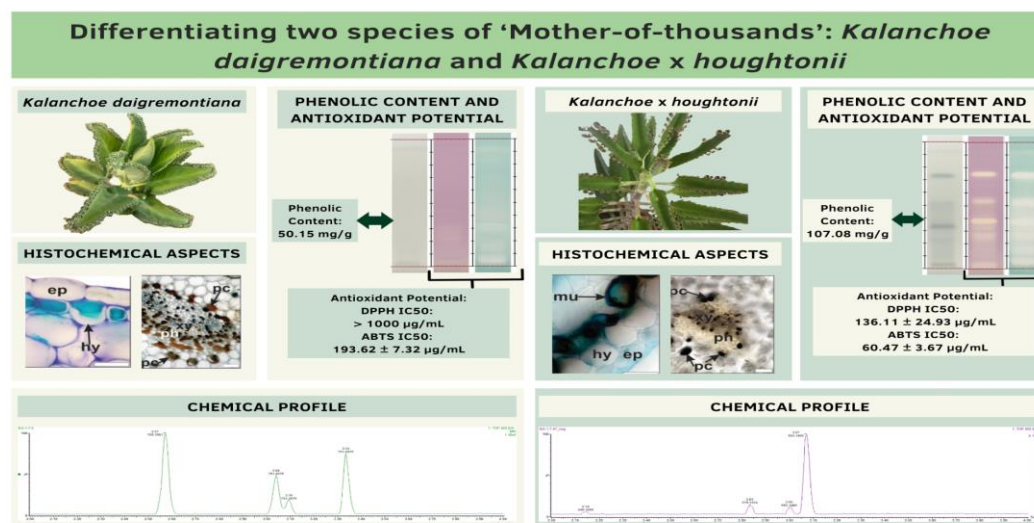
## HIGHLIGHTS

- Morphological and chemical comparison minimize confusion between these species.
- The HPTLC and UPLC-MS/MS assays help in the identification of chemical markers.
- These analyses provide comprehensive validation of species for medicinal use.

**Abstract:** The widespread medicinal use of *Kalanchoe daigremontiana* and *Kalanchoe x houghtonii*, both popularly known as “mother-of-thousands”, often leads to their interchangeable use in folk medicine despite their distinct species status. This research aims to delineate the histochemical, chemical, and biological characteristics of these two *Kalanchoe* species to aid in their accurate identification. Through a comprehensive evaluation of histochemical markers (reaction patterns in hypodermis), alongside *in vitro* assessments (correlation of antioxidant activities and phenolic content), and metabolomic profiles (using HPTLC and UPLC-MS/MS<sup>E</sup>), this study has identified significant differences between *K. daigremontiana* and *K. x houghtonii*. The findings reveal distinct variations in all evaluated parameters, underscoring the importance of proper species identification to ensure the safety and efficacy of their medicinal use. These results contribute valuable insights into the phytochemical and pharmacological bases for the traditional uses of these plants and highlight the need for distinguishing between species to avoid potential therapeutic inefficacies or adverse effects in folk medicine practices.

**Keywords:** Crassulaceae; traditional medicine; ethnopharmacology; histochemistry; antioxidant potential; flavonoids; metabolomics; UPLC-MS/MS; HPTLC.

## GRAPHICAL ABSTRACT



## INTRODUCTION

Plants have been indispensable sources of medicinal compounds for centuries, either directly as therapeutic agents or as inspiration for pharmaceutical development. Ethnopharmacology, which builds on traditional knowledge to scientifically investigate plants used in folk medicine, plays a pivotal role in guiding pharmacological, clinical, and chemical studies. This approach not only validates traditional uses but also uncovers new bioactive compounds. However, ethnopharmacological exploration is often complicated by the existence of multiple plant species sharing common names or morphological features, leading to confusion and potentially hazardous mistakes during preliminary research [1-2].

A prime example of such confusion is found within the *Kalanchoe* genus, renowned for its therapeutic applications across different cultures. Species like *K. daigremontiana* and *K. x houghtonii* are known as "miracle-leaf" for their pronounced healing properties [3-8]. However, their shared vernacular names and morphological similarities frequently result in their mistaken identification and use, raising concerns about treatment efficacy and safety.

With its broad distribution and recognized medicinal properties, *K. daigremontiana* has been the focus of extensive research. Studies have documented its antioxidant, cytotoxic, antimicrobial, anti-inflammatory, and antiviral activities, alongside its inhibitory effects on thrombin and plasmin enzymes [6-7, 9-13].

Conversely, *K. x houghtonii*, an artificial species from the hybridization of *K. daigremontiana* and *K. delagoensis*, has been less studied. Initially unrecognized and misnamed due to its close resemblance to its parent species, this hybrid was only officially described in 2006 [14]. Its capability for self-propagation through leaf propagules, a trait inherited from *K. daigremontiana*, has led to its popular name "mother-of-thousands". Unfortunately, this similarity has also fueled the interchangeability and confusion between these species in medicinal use, highlighted by the erroneous reporting in several studies [12,15].

Given this background of confusion and the significant medicinal potential of these species, the present study seeks to elucidate the histochemical, chemical, and biological distinctions between *K. daigremontiana* and *K. x houghtonii*. By evaluating their histochemical structures, identifying principal chemical constituents, and analyzing the biological effects of their secondary metabolites, this study aims to provide a scientific basis for the localization and identification of the metabolites in plant tissues, besides their biological activities, enhancing the safety and efficacy of their medicinal use.

## MATERIAL AND METHODS

### Plant Material

The plant materials were gathered during their winter-flowering season to ensure a stable phytochemical profile. The collection occurred in Ponta Grossa, Paraná, Brazil (25°5'38"S 50°12'34"W) in 2021. The species were identified by Dr. Gustavo Heiden from The Brazilian Agricultural Research Corporation (EMBRAPA), and Dr. Vijayasankar Raman, Botanist and National Taxonomist in the National Identification Services team in the APHIS-PPQ-PEIP division (USDA), and they were registered and deposited by Dr. Rosângela

Capuano Tardivo in the Herbarium of the State University of Ponta Grossa (HUPG) as *Kalanchoe daigremontiana* Raym.-Hamet & H.Perrier and *Kalanchoe x houghtonii* D.B.Ward, with registration numbers 22778 and 22808, respectively. Access to the botanical materials was documented in the Brazilian National System for Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under registration AFDD6B4. The extracts were prepared by milling fresh leaves and extracting them with distilled water (1:10, w/v) at room temperature in a high-speed blender for 5 minutes, a method designed to closely resemble traditional practices. To preserve the extracts' integrity, they were filtered, freeze-dried, and stored under refrigeration at 5 °C until carrying out the experiments.

## Histochemical Evaluation

The leaves and stems of *K. daigremontiana* and *K. x houghtonii* were fixed for 5 days in a solution of formalin:acetic acid:alcohol 70% (FAA 5:5:90, v/v/v) [16]. Afterwards, they were washed with purified water and stored in ethanol 70% (v/v) [17]. Histochemical analyses were performed on transverse sections of the stored material. Some *Kalanchoe* classes of secondary metabolites were investigated using the following reagents: phloroglucinol/HCl to identify lignified elements [18], Sudan III to observe the presence of lipophilic substances [19], ferric chloride 2% (v/v) [16] and potassium dichromate 10% (v/v) to detect phenolic compounds [20], methylene blue 1% (v/v) to evidence mucilages [18], and iodine 1% (v/v) to identify the presence of starch [16]. The slides were immediately photographed and analyzed under a light microscope coupled with a photographic camera (Olympus CX31 model with C 7070 control unit) for a detailed description of the features.

## HPTLC Analysis

Dried extracts of *K. daigremontiana* and *K. x houghtonii* were solubilized at a concentration of 10 mg/mL in water. Standard solutions of quercetin, chlorogenic acid and kaempferol were prepared at 0.2 mg/mL while rutin was at 0.4 mg/mL. For the System Suitability Test (SST), standard solutions were prepared at 0.5 mg/mL for guanosine and 1.0 mg/mL for thymidine and 9-hydroxyfluorene). 2 µL of the SST and standards and 10 µL of the samples were applied onto a 20 x 10 cm (length x height) silica gel plate (Merck) using an automatic TLC sampler 4 using the parameters described in the USP Chapter <203> [21] were used. The developing solvent used was composed of *n*-butyl acetate, methanol, water, formic acid (7.5:2:1:1, v/v/v/v) [22] and the plate was developed using an automatic developing chamber 2 (CAMAG®).

### HPTLC-Phenolic Compounds

For the detection of the phenolic content, the plate was immersed in Folin-Ciocalteu reagent, diluted 1:10 (v:v) in methanol, using the CAMAG® Immersion device, under conditions of time 0 and speed 5. The plate was set at room temperature for 30 minutes then images under transmission white light using a CAMAG® TLC Visualizer 2 were recorded.

### HPTLC-DPPH

For HPTLC-DPPH<sup>•</sup> antioxidant activity, 3 mL of 0.05% DPPH in methanol was sprayed on the plate using a CAMAG® derivatizer with a blue nozzle set at spraying level 3. After derivatization, the plate was kept in the dark for 30 minutes and then images under transmission white light using a CAMAG® TLC Visualizer 2 were recorded.

### HPTLC-ABTS

For the HPTLC-ABTS<sup>•+</sup> assay, 3 mL of 0.04% ABTS in water and subsequently diluted 1:1 in methanol was sprayed on the plate with the CAMAG® derivatizer with a yellow nozzle set at spraying level 3. After 30 minutes, the images under transmission white light were taken with CAMAG® TLC Visualizer 2.

## In vitro Analysis

### Total Phenolic Content

For *in vitro* quantification [23], extracts were prepared at a concentration of 1 mg/mL. For each assay, 0.5 mL of the extract was mixed with 6.5 mL distilled water and 0.5 mL of Folin-Ciocalteu reagent. After 30 seconds, 2.5 mL of 10.6% sodium carbonate was added, and the mixture was incubated at 50 °C for 5 minutes. Absorbance was measured at 715 nm. A gallic acid calibration curve (50 to 1000 µg/mL) was used for quantification. The results were expressed as milligrams of gallic acid equivalent per gram of extract. All procedures were performed in triplicate.

### DPPH<sup>•</sup> radical reduction

For the *in vitro* assay, antioxidant capacity was assessed using the DPPH<sup>•</sup> method [24], adding 300  $\mu$ L of sample extracts at varying concentrations (40, 80, 120, 160, 200, 240, 280, 320, 360, and 400  $\mu$ g/mL) in 300  $\mu$ L of DPPH<sup>•</sup> solution, mixing the test tubes and incubating for 15 minutes in the dark. Subsequently, 200  $\mu$ L from each reaction mixture was transferred to a 96-well plate, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Each sample was analyzed in triplicate, with quercetin as the reference standard.

### ABTS<sup>•+</sup> radical reduction

The ABTS<sup>•+</sup> cation radicals were produced by mixing 5 mL of ABTS<sup>•+</sup> stock solution with 88  $\mu$ L of potassium persulfate, allowing the mixture to stand in the dark at refrigerated temperature for 12 hours [25]. The solution was then diluted with phosphate-buffered saline (PBS, 10 mM) to the appropriate absorbance. In the *in vitro* assay, 300  $\mu$ L of the extracts of *K. daigremontiana* and *K. x houghtonii* (at concentrations of (40, 80, 120, 160, 200, 240, 280, 320, 360, and 400  $\mu$ g/mL) and 300  $\mu$ L of the ABTS<sup>•+</sup> solution was combined in each tube. After 30 minutes of incubation in the dark, 200  $\mu$ L from each mixture was transferred to a 96-well plate for absorbance reading at 734 nm using a UV-Vis spectrophotometer. Samples were tested in triplicate using quercetin as a reference standard.

### UPLC-MS/MS<sup>E</sup>

The extracts (100  $\mu$ g/mL) of *K. daigremontiana* and *K. x houghtonii* were analyzed using a Waters<sup>®</sup> I-Class UPLC system coupled with a Waters<sup>®</sup> G2-XS Q-TOF Mass Spectrometer. Chromatographic separation was achieved on an Acquity UPLC<sup>®</sup> BEH C18 (1.7  $\mu$ m, 2.1 x 100 mm) reversed-phase column, with 0.1% formic acid in water (v/v) (A) and 0.1% formic acid in acetonitrile (B) as a binary mobile phase. The gradient elution program began at 15% B, increasing to 30% B over 4 minutes, then to 100% B by 8.2 minutes, followed by a return to initial conditions in 0.1 minute and conditioned for 2 minutes prior to the next injection. The flow rate was 0.45 mL/min, with a 3  $\mu$ L injection volume and column temperature at 30°C. The mass spectrometer operated in both positive and negative ion modes across a *m/z* 50-1800 range, with collision energy for the MS<sup>E</sup> function ramped between 35-45 V. Triplicate samples were prepared from each extract and vials were randomized prior to sampling. The QC pooled sample was prepared by mixing 20  $\mu$ L of each sample. Lock-mass correction information was acquired for each sample with a continuous infusion of a 0.2 ng/mL solution of leucine enkephalin at a flow rate of 10  $\mu$ L/min, generating a reference ion for positive ion mode ([M+H]<sup>+</sup>: 556.2771) and negative ion mode ([M-H]<sup>-</sup>: 554.2615) to ensure accuracy during the MS analysis. Mass correction was applied post-acquisition in the Progenesis software.

### Data Processing and Statistical Analysis

The data obtained for *in vitro* tests were analyzed using GraphPad Prism<sup>®</sup> software (version 5.0) and expressed as mean  $\pm$  standard deviation. To determine the IC<sub>50</sub>, it was calculated from a nonlinear regression curve, where the X-axis consisted of concentrations in logarithmic scale and the Y-axis the average percentages of antioxidant radical inhibition, following the tutorial provided by GraphPad<sup>®</sup> software.

The LC-MS/MS<sup>E</sup> data were processed with MassLynxTM software (version 4.2) and further analyzed using Progenesis QI software (version 3.0.3), focusing on features with an Anova p-value > 0.05, maximum abundance > 1000, and retention time between 0.75 and 6.0 minutes. Data normalization was to the total ion intensity per chromatogram, with Principal Component Analysis (PCA) performed to elucidate patterns and differences between samples.

## RESULTS

### Histochemical analysis

The leaf cuticles of *K. daigremontiana* and *K. x houghtonii* reacted positively for lipophilic compounds (Figure 1a,c) through Sudan III, showing smooth and thin cuticles for both species. The hypodermis of the leaves of both species reacted with Sudan III (Figure 1a,c), ferric chloride (Figure 1m,o), potassium dichromate, and methylene blue (Figure 1e,g), indicating the presence of lipophilic, phenolic, and mucilaginous compounds, respectively. For *K. daigremontiana*, these reactions occur in the first layer below the epidermis (Figure 1a,e,m), while for *K. x houghtonii*, these reactions occur in the second layer below the epidermis (Figure 3c,g,o).

The presence of lignin is observed in the xylem vessel elements of the leaves of both studied species (Figure 1j,l). In the vascular system, positive reactions for phenolic compounds were observed in the phloem and parenchyma cells of the xylem in the leaves of *K. daigremontiana* (Figure 1b,n). On the other hand, for the leaves of *K. x houghtonii*, there was a positive reaction for phenolic (Figure 1p) and lipophilic (Figure 1d) compounds only in the parenchyma cells of the xylem.

Starch grains stored in the cells were found in the mesophyll and ground parenchyma of *K. daigremontiana* (Figure 1q,r). The same was observed for *K. x houghtonii*, but the reaction showed a much more significant amount of starch per cell (Figure 1s,t). A large number of idioblasts that react to phenolic compounds (Figure 1m-p), lipophilic compounds (Figure 1b-d), and mucilaginous compounds (Figure 1f,h,i,k) are observed in the mesophyll region, ground parenchyma, and near the vascular bundles in both species.

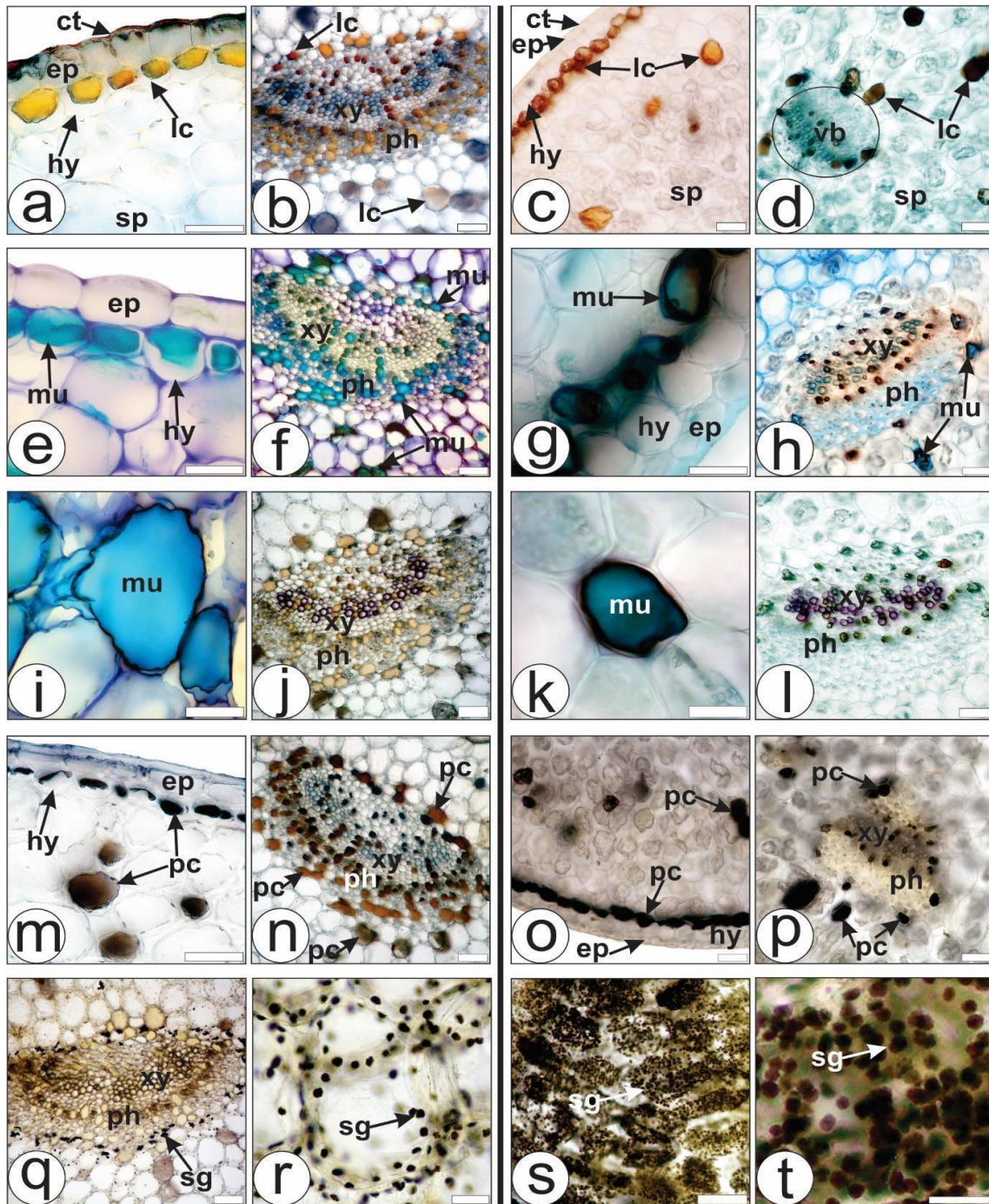


**Figure 1.** Histochemical aspects of *K. daigremontiana* and *K. x houghtonii* leaves. a,b,e,f,i,j,m,n,q,r: *K. daigremontiana*; c,d,g,h,k,l,o,p,s,t: *K. x houghtonii*; a-d: Sudan III; e-h, i, k: methylene blue; j,l: phloroglucinol/HCl; m,o,p: ferric chloride; n: potassium dichromate; q-t: iodine solution. ct: cuticle; ep: epidermis; hy: hypodermis; lc: lipophilic compounds; mu: mucilage; pc: phenolic compounds; ph: phloem; sg: starch grains; sp: spongy parenchyma; vb: vascular bundle; xy: xylem. Scale bar: 25  $\mu$ m (r,t); 50  $\mu$ m (a,e,g,i,k,m); 100  $\mu$ m (b,c,d,f,h,j,l,n,o,p,q,s).

Regarding the petiole features, the cuticle of *K. daigremontiana* and *K. x houghtonii* are smooth and thin (Figure 2a, c). Lipophilic compounds (Figure 2a, b), mucilaginous compounds (Figure 2e, f), and phenolic compounds (Figure 2m, n, q, r) are also observed in the petiole. They are present in the ground parenchyma, near the vascular bundles, in the phloem, and in the parenchyma cells of the xylem. On the other hand, in the petiole of *K. x houghtonii*, only phenolic compounds reactions are seen (Figure 2 o, p, s, t). Vessel elements in the xylem are present in both species (Figure 2 i, k). Starch grains surround the vascular bundles and are dispersed in the ground parenchyma of the petioles of both species (Figure 2 j, l).

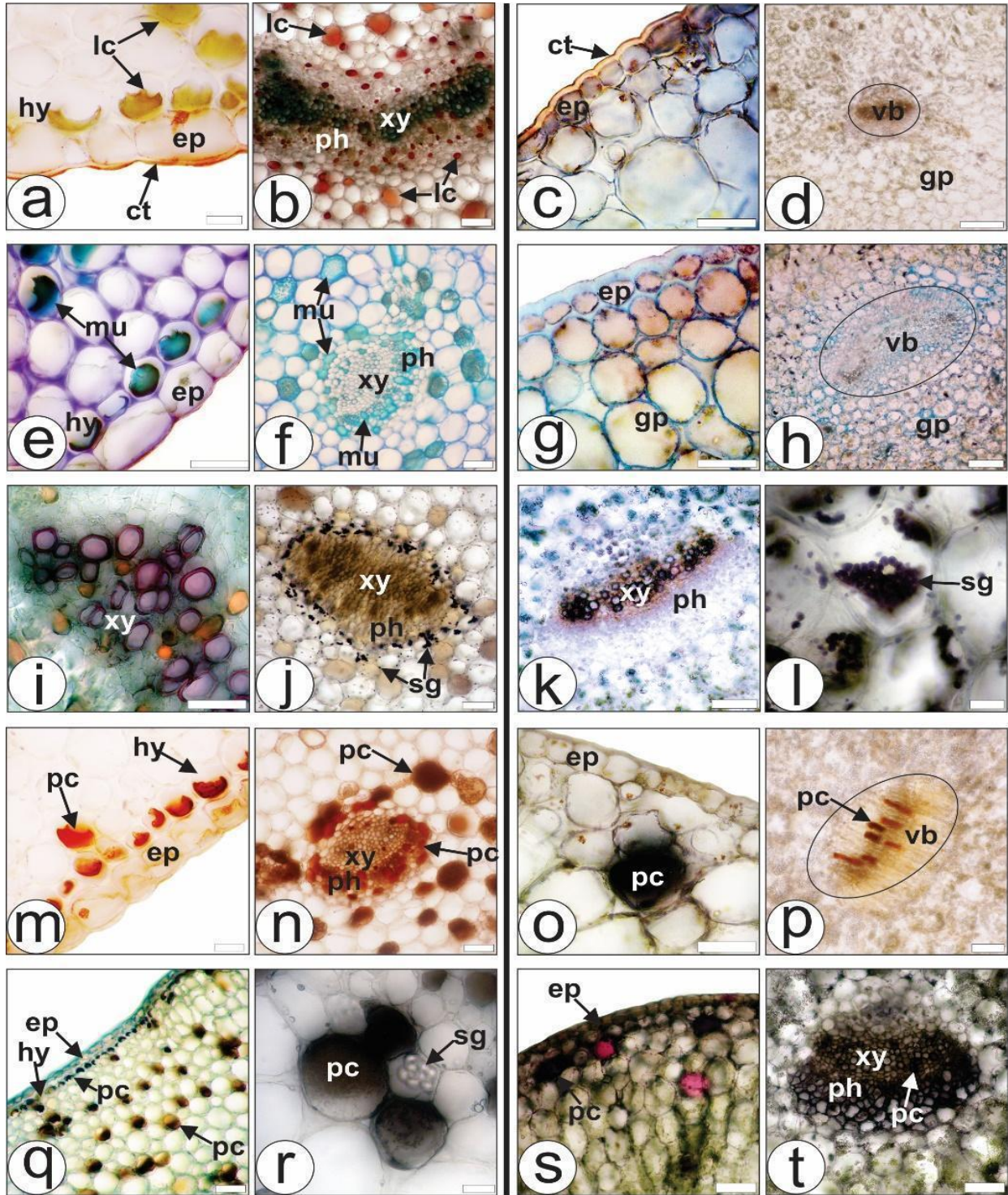


**Figure 2.** Histochemical aspects of *K. daigremontiana* and *K. x houghtonii* petioles. a,b,e,f,i,j,m,n,q,r: *K. daigremontiana*; c,d,g,h,k,l,o,p,s,t: *K. x houghtonii*; a-d: Sudan III; e-h: methylene blue; i,k: phloroglucinol/HCl; j,l: iodine solution; o,r-t: ferric chloride; m,n,p,q: potassium dichromate. ct: cuticle; ep: epidermis; gp: ground parenchyma; hy: hypodermis; lc: lipophilic compounds; mu: mucilage; pc: phenolic compounds; ph: phloem; sg: starch grains; vb: vascular bundle; xy: xylem. Scale bar: 25  $\mu$ m (l,r); 50  $\mu$ m (c,e,g,i,o); 100  $\mu$ m (a,b,f,h,j,k,m,n,p,q,s,t); 250  $\mu$ m (d).

The stems of *K. daigremontiana* and *K. x houghtonii* have a smooth and thin cuticle (Figure 3a,c,d). In the hypodermis, cortex, and pith of the stem, idioblasts are observed, which react to the presence of lipophilic compounds (Figure 3a,b), mucilaginous compounds (Figure 3e,f), and phenolic compounds (Figure 3m,n,q,r) in *K. daigremontiana*. While for *K. x houghtonii*, idioblasts are found in smaller quantities in the cortex and pith and only react to the presence of phenolic compounds (Figure 3o,p,s). Starch grains are found in the cortex and pith of *K. daigremontiana* (Figure 3i,j) and *K. x houghtonii* (Figure 3k,l). However, *K. x houghtonii* exhibits a higher amount of starch in the cells (Figure 3k,l).

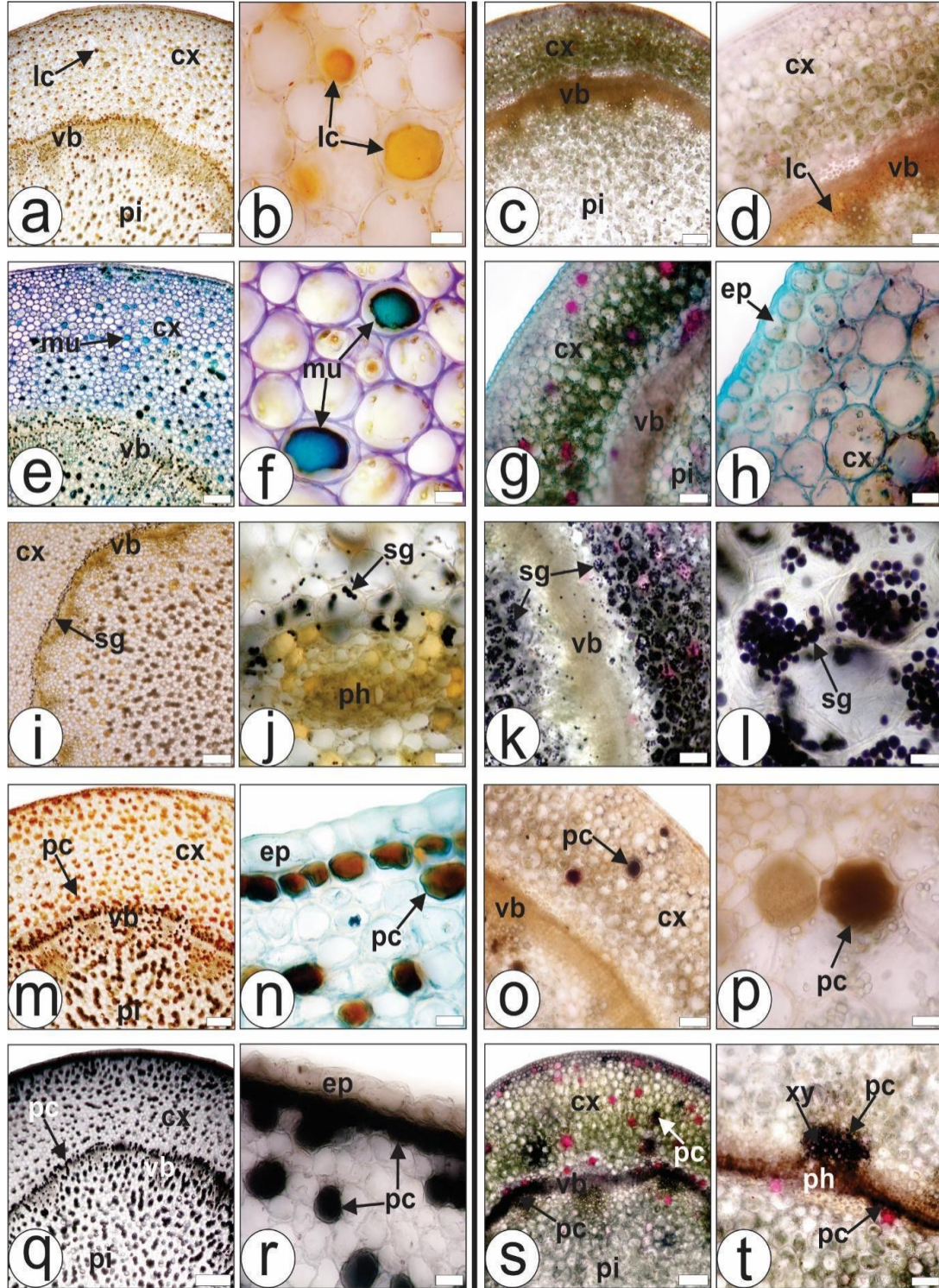


**Figure 3.** Histochemical aspects of *K. daigremontiana* and *K. x houghtonii* stems. a,b,e,f,i,j,m,n,q,r: *K. daigremontiana*; c,d,g,h,k,l,o,p,s,t: *K. x houghtonii*; a-d: Sudan III; e-h: methylene blue; i-l: iodine solution; m-p: potassium dichromate; q-t: ferric chloride. cx: cortex; ep: epidermis; pc: phenolic compounds; lc: lipophilic compounds; mu: mucilage; ph: phloem; pi: pith; sg: starch grains; vb: vascular bundle; xy: xylem. Scale bar: 25  $\mu$ m (b,f,h,l,n,p,r); 100  $\mu$ m (d,g,j,k,o,t); 250  $\mu$ m (a,c,e,i,m,q,s).

### HPTLC and UPLC-MS/MS analysis

The extracts of both species were screened to highlight the polyphenolic and radical scavenging compounds (Figure 4). Additionally, chemical profiles of the extracts of the two species were characterized by UPLC-MS/MS<sup>E</sup> and tentatively identified as kaempferol-glycosides derivatives based on MS<sup>E</sup> fragmentation data (Figure 5, Table 1).

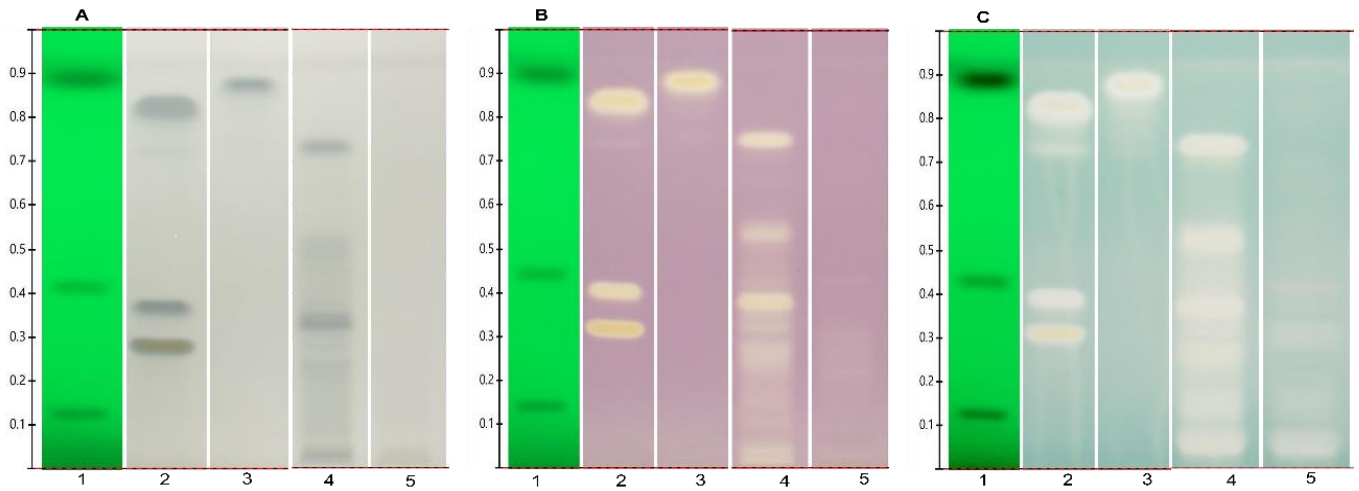


**Figure 4.** HPTLC fingerprint of *K. daigremontiana* and *K. x houghtonii* extracts post-derivatization with Folin-Ciocalteu (A), ABTS (B), and DPPH (C) reagents, under transmission white light. (1) SST (System Suitability Test). (2) Rutin, Chlorogenic Acid, Quercetin with increasing  $R_F$ . (3) Kaempferol. (4) *K. x houghtonii*. (5) *K. daigremontiana*.

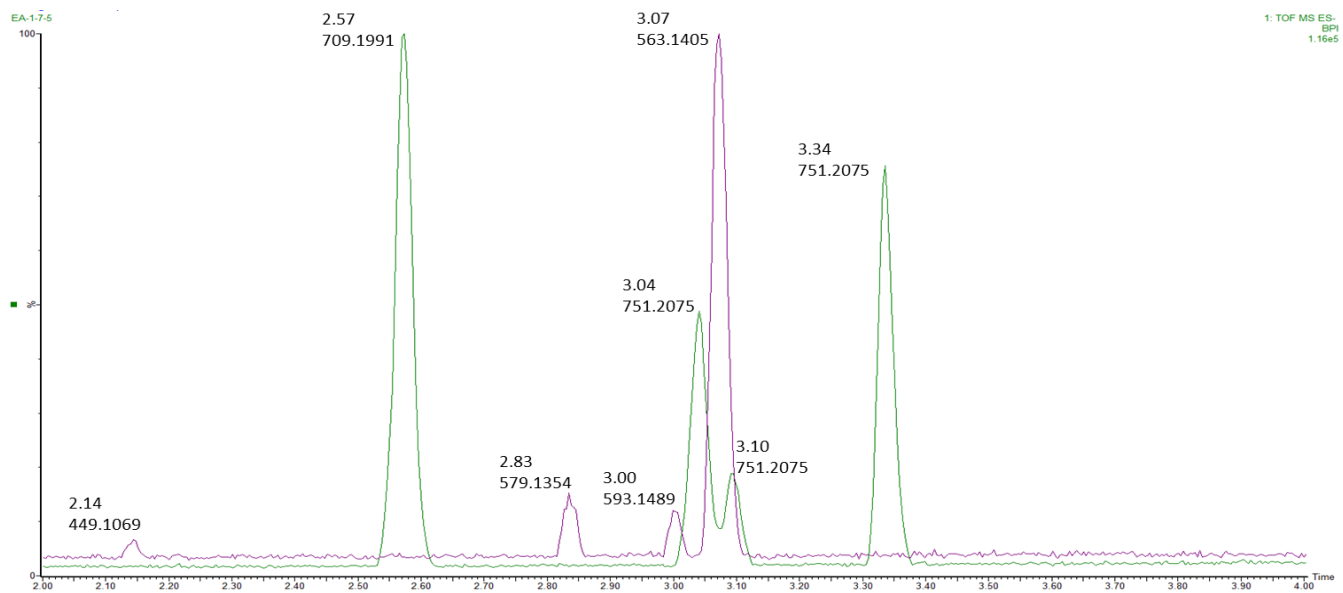


**Figure 5.** LC-MS chromatograms of *K. daigremontiana* (green) and *K. x houghtonii* (purple).

**Table 1.** Potential chemical markers for differentiation of *Kalanchoe daigremontiana* and *Kalanchoe x houghtonii* aqueous extracts

| Species                  | RT (min) | m/z                |                              |
|--------------------------|----------|--------------------|------------------------------|
|                          |          | [M-H] <sup>-</sup> | MS [-] MS/MS                 |
| <i>K. daigremontiana</i> | 2.57     | 709.2056           | 563.1481, 431.0993, 285.0423 |
|                          | 3.04     | 751.2075           | 563.1481, 473.1107, 285.0423 |
|                          | 3.34     | 751.2075           | 563.1404, 473.1024, 285.0362 |
| <i>K. x houghtonii</i>   | 2.83     | 579.1354           | 378.9161, 285.0397           |
|                          | 3.00     | 593.1489           | 520.9094, 378.9161, 285.0397 |
|                          | 3.07     | 563.1405           | 285.0362                     |

## Phenolic Content and *in vitro* antioxidant potential

The phenolic content of the extracts was quantified and can also be compared to the antioxidant capacity of each extract, based on the results that are shown in Table 2.

**Table 2.** Quantification of phenolic compounds and antioxidant activity of *Kalanchoe daigremontiana* and *Kalanchoe x houghtonii* extracts

| Species                         | Phenolic Content (mg/g) <sup>1</sup> | Antioxidant Potential <sup>2</sup> |                |
|---------------------------------|--------------------------------------|------------------------------------|----------------|
|                                 |                                      | ABTS (µg/mL)                       | DPPH (µg/mL)   |
| <i>Kalanchoe daigremontiana</i> | 50.15                                | 193.61 ± 7.32                      | > 1000         |
| <i>Kalanchoe x houghtonii</i>   | 107.08                               | 60.47 ± 3.67                       | 136.11 ± 24.93 |

<sup>1</sup>Expressed as gallic acid. <sup>2</sup>Data expressed in IC<sub>50</sub>.

## DISCUSSION

The application of histochemical tests has been pivotal in delineating the cellular constituents of the plant species, showcasing the presence of lipophilic and phenolic compounds, mucilage-storing cells, and other key features using various reagents [26]. In histochemical tests, Sudan III is a reagent that stains lipid compounds red or red-orange; phenolic compounds are visualized by ferric chloride solution, which produces a dark brown or black color, or by potassium dichromate solution, which turns a brown or reddish-brown color; and methylene blue is a reagent that reveals cells containing mucilage in blue [27]. The positive reactions observed for *K. daigremontiana* and *K. x houghtonii* resonate with literature observations [28], suggesting a widespread occurrence of these compounds within the genus.

The adoption of High-Performance Thin-Layer Chromatography (HPTLC) for qualitative analysis marks a great approach in the phytochemical investigation of *Kalanchoe* extracts, offering a unique visual fingerprint and facilitating the comparison of phenolic constituents across species. This methodology underscores a gap in the literature regarding the comprehensive profiling of secondary metabolites in *Kalanchoe* species, indicating an area of future research endeavors.

The subsequent chemical profiling through Ultra Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS) has provided deeper insights into the presence of specific phenolic compounds. During the exploration of chemical markers for distinguishing the two species within the *Kalanchoe* genus, the mass-to-charge ratios (*m/z*) for [M-H]<sup>-</sup> ions were correlated with compounds traditionally found in *Kalanchoe*, when available, as highlighted in existing research. Additionally, the proposed identifications for specific *m/z* values draw upon commonly observed phytochemicals in the plant species, including notable bioactive phenolic compounds. Analysis of MS<sup>E</sup> fragmentation data indicated a consistent presence of *m/z*: 285 [M-H]<sup>-</sup>, across major compounds in both extracts, pointing towards a kaempferol core [29].

Further examinations of the MS<sup>E</sup> data for both species suggest that these compounds are tentative identified as flavonol glycosides [30]. Specifically for *K. daigremontiana*, the presence of kaempferol glycoside derivatives is also associated in the literature with glycosylated flavonoids or extensive polyphenolic compounds [6-7,31-32].

These preliminary identifications require further verification through standard comparisons and detailed spectroscopic studies. Phenolic compounds are recognized as key bioactive components contributing to the medicinal benefits of the *Kalanchoe* genus. Specifically, flavonoids and kaempferol derivatives found in aqueous leaf extracts of *K. daigremontiana* have been documented [33]. These findings enhance our comprehension of the phenolic content in *Kalanchoe*, underscoring the genus's potential medical significance and the importance of further investigation for definitive compound identification and understanding of their biological effects.

Quantitative analysis using the Folin-Ciocalteu method revealed a higher concentration of total phenolics in *K. x houghtonii* extracts compared to *K. daigremontiana*, emphasizing the substantial phenolic diversity within the genus and its implications for medicinal potential [30, 34-38]. This integrated approach validates the presence of phenolic compounds and facilitates their precise quantification and analysis, laying a crucial foundation for future studies aiming at elucidating the phytochemical landscapes of these plants.

Variable amounts of phenolic compounds have been reported in *Kalanchoe* species in the literature. For example, the methanolic extract and fractions of *K. gracilis* stem ranged from 7.44-169.21 mg/g (catechin

equivalents) [39]. Furthermore, ethanolic extracts from different parts of *K. mortgagei* and *K. fedtschenkoi* varied from 331-1340 mg/g and 370-498 mg/g (gallic acid equivalent), respectively [37]. For *K. pinnata*, the total phenolic content was  $27.782 \pm 0.25$   $\mu\text{g}/\text{mg}$  (gallic acid equivalent) [40]; like the hydroalcoholic leaf extract, which had a content of  $28.4 \pm 2$   $\mu\text{g}/\text{mg}$  (quercetin equivalent) [41]. For the aqueous extracts (obtained by different methods), the concentrations ranged from 63.01-232.9  $\mu\text{g}/\text{mL}$  (gallic acid equivalent) [4], and for benzene, chloroform, acetone, and ethanolic extracts of leaves and stems, it was observed that leaves (0.49-1.17% (v/v)) had a significantly higher total phenolic content than stems (0.18-0.62% (v/v)) [42]. For the ethanolic extract of *K. laetivirens* obtained by maceration, a content of  $166.66 \pm 0.8$  mg/g (gallic acid equivalent) was obtained [43]. For the dichloromethane, ethyl acetate, and methanol extracts of *K. glaucescens* obtained by maceration, the contents were 0.15 mg/g, 0.30 mg/g, and 0.69 mg/g (gallic acid equivalent), respectively [44].

Significant variability in phenolic content across different studies highlights the influence of extraction methodologies, solvent choices, and plant collection times, underscoring the need for standardized procedures in phytochemical research [4,40-42,45-46].

An increasing amount of medicinal research has been dedicated to reactive oxygen species (ROS). Therefore, it is known that for proper physiological function, a balance between antioxidant substances and free radicals or reactive oxygen/nitrogen species (generated as byproducts in metabolic processes) is essential to play important roles in the prevention of diseases induced by reactive species [2,35].

The efficacy of plant extracts and natural compounds with high antioxidant activity is well documented. Most of the antioxidant potential in plants is attributed to the redox properties of phenolic compounds, which can exert such activity through various mechanisms. Many medicinal herbs with significant antioxidant activities have been employed as natural antioxidants. However, although the ability to prevent oxidative stress or minimize its harmful effects is one of the most frequently described biological activities of plant-derived substances, the antioxidant actions of extracts from different *Kalanchoe* species have not yet been fully described [9,35,47].

*Kalanchoe pinnata* is the most studied species in the genus regarding its antioxidant potential. The antioxidant activities of leaf and stem extracts from *K. pinnata* increase in a dose-dependent manner. By reducing the DPPH $^{\bullet}$  radical, benzene, ethanol, ethyl acetate, and acetone extracts exhibit  $\text{CI}_{50}$  values of 94-160  $\mu\text{g}/\text{mL}$  in leaves and 108-185  $\mu\text{g}/\text{mL}$  in stems. Meanwhile, by reducing the NO radical, the  $\text{CI}_{50}$  values range from 60-140  $\mu\text{g}/\text{mL}$  in leaves and 102-162  $\mu\text{g}/\text{mL}$  in stems [42]. The antioxidant activities of aqueous extracts obtained from *K. pinnata* leaves have been analyzed during plant growth [34], in organs of normal and hypertensive rats [48], and in renal oxidative damage caused by  $\text{CCl}_4$  in rats [49]. The aqueous extract improved the antioxidant potential in various organs (especially in the aorta), and this anti-hypertensive activity may be associated with an enhancement in antioxidant potential. Adverse changes resulting from  $\text{CCl}_4$  intoxication in rats were prevented by pre-treatment with the aqueous extract (25 and 50 mg/kg body weight), suggesting that this extract can protect the kidneys against  $\text{CCl}_4$ -induced oxidative damage. The methanolic extract of *K. pinnata* obtained by maceration exhibited an average inhibition of the DPPH $^{\bullet}$  radical ranging from 7.18-47.18% (concentrations of 50-1000  $\mu\text{g}/\text{mL}$ ) [48]. After, the same extract subjected to triple maceration showed 69.77% inhibition of the DPPH $^{\bullet}$  radical [38]. The ethanol extract of *K. pinnata* stem was evaluated using DPPH $^{\bullet}$  and demonstrated high antioxidant activity ( $\text{CI}_{50}$ : 37.28  $\mu\text{g}/\text{mL}$ ) [42] and compared to other leaf extracts (aqueous and petroleum ether), it exhibited the highest inhibitory effect ( $49.5 \pm 5.6\%$  (2000  $\mu\text{g}/\text{mL}$ )) [12].

Four major flavonoids obtained from hydroalcoholic leaf extracts of *K. pinnata* were evaluated for their antioxidant activities using DPPH $^{\bullet}$  and ABTS $^{•+}$  assays. It was found that only the quercetin derivatives exhibited radical scavenging activity, suggesting that quercetin 3-O- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranoside may be indicated as a specific marker for this species [50-51].

The antioxidant potential of four *Kalanchoe* species (*K. pinnata*, *K. daigremontiana*, *K. nyikae*, and *K. milloti*) was evaluated using the DPPH $^{\bullet}$  assay, and the obtained  $\text{CI}_{50}$  values ranged from 49.9-1410  $\mu\text{g}/\text{mL}$ , indicating a wide variation in the activity of the analyzed extracts [35]. Additionally, dichloromethane, ethyl acetate, and methanol extracts of *K. glaucescens* obtained by maceration showed average inhibition percentages of the DPPH $^{\bullet}$  radical ranging from 11.38-72.95% [44].

The antioxidant capacity of *K. daigremontiana* is also reported in the literature. The methanolic leaf extract was evaluated using DPPH $^{\bullet}$ , and a  $\text{CI}_{50}$  value of 19.2  $\mu\text{g}/\text{mL}$  was obtained, which was similar to results obtained for vitamin E [52]. No data were found in the literature regarding the antioxidant activity of the species *K. x houghtonii*.

Compared to other studies on the antioxidant activity of medicinal plants, it can be inferred that considerable antioxidant activities were determined in this study. Although the methods used have different

reaction mechanisms and may not necessarily correspond to the same activities, they clearly indicated that the studied plants have variable antioxidant activity. The present study obtained values that were similar and/or within the expected range considered satisfactory by other authors for the *Kalanchoe* genus, confirming that these species are natural sources of antioxidant agents. Moreover, it suggests that the antioxidant potential and efficacy of these species can be mainly attributed to polyphenolic compounds [39,44,53-55].

The exploration of antioxidant potentials further underscores the critical role of phenolic compounds in combating oxidative stress, with the methods employed in this study indicating variable antioxidant activity and reaffirming the species as valuable sources of natural antioxidants [9,18,47].

The literature discusses various groups of metabolites identified in plants, highlighting their functions and importance, as the secondary metabolites, which include phenolics, flavonoids, and terpenoids, playing significant roles in plant defense against pathogens and herbivores. These compounds are often antioxidants and can provide UV protection, contributing to the plant's ability to manage biotic and abiotic stresses; besides that, the cell wall components, such as lignin and polysaccharides, which helps in understanding plant structure and support. Lignin, for example, provides mechanical strength and is crucial for the plant's structural integrity [56].

Considering the intricate nature of secondary metabolites and their biological activities observed in *Kalanchoe daigremontiana* and *Kalanchoe x houghtonii*, future research should focus on expanding the phytochemical spectrum and bioactivity profiling of these species. There is a pressing need for standardized extraction and analytical methods to facilitate comparative studies and improve our understanding of their health benefits. Exploring the genetic basis of phytochemical variations could reveal new biosynthetic pathways for bioactive compounds, offering fresh perspectives in medicinal plant research. Additionally, clinical trials are warranted to substantiate the pharmacological claims of these species, effectively bridging traditional applications and scientific validation.

Considering the significant pharmacological potential of extracts from *Kalanchoe daigremontiana* and *Kalanchoe x houghtonii*, further studies aimed at the development of new drugs incorporating plant matrices or isolated substances are highly recommended. This would not only contribute to the advancement of herbal medicine but also provide a scientific foundation for the therapeutic use of these 'mother-of-thousands' species.

## CONCLUSION

The species *Kalanchoe daigremontiana* and *Kalanchoe x houghtonii* exhibit differences in histochemical, phenolic content, antioxidant potential, and chemical composition characteristics. These data, together, aid in the identification and, primarily, differentiation of the studied species, aiming to minimize and, if possible, eliminate doubts, exchanges, and confusion, especially regarding their medicinal use. Information related to the traditional use of medicinal plants contributes to the search for scientific evidence. Therefore, all this data, particularly the lack thereof, reinforces the presence of a gap between the therapeutic aspect and the applicability in experiments with an ethnopharmacological approach.

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## REFERENCES

1. Akentieva NP, Shushanov SS, Gizatullin AR, Prikhodchenko TR, Shkondina NI, D'agaro E. The effect of plant extracts *Kalanchoe daigremontiana* and *Aloe arborescens* on the metabolism of human multiple myeloma cells. *Biointerface Res Appl Chem*. 2021; 11:13171-86.
2. Chandana S, Morlock GE. Comprehensive bioanalytical multi-imaging by planar chromatography in situ combined with biological and biochemical assays highlights bioactive fatty acids in abelmosk. *Talanta*. 2021; 223 (Part 1):121701. ISSN 0039-9140.

3. Akulova-Barlow Z. *Kalanchoe*. Cactus Succul J. 2009; 81:268-76.
4. Bhavsar S, Bhavita D, Maitreyi Z, Divya C. A comparative pharmacognostical and phytochemical analysis of *Kalanchoe pinnata* (Lam.) Pers. leaf extracts. J Pharmacogn Phytochem. 2018; 7(5):1519-27.
5. Mawla F, Khatoon S, Rehana F, Jahan S, Shelley MR, Hossain S, et al. Ethnomedicinal plants of folk medicinal practitioners in four villages of Natore and Rajshahi districts, Bangladesh. Am Eurasian J Sustain Agric. 2012; 6:406-16.
6. Molina GA, Esparza R, Lopez-Miranda JL, Hernandez-Martinez AR, Espana-Sanchez B, Elizalde-Pena EA, et al. Green synthesis of Ag nanoflowers using *Kalanchoe daigremontiana* extract for enhanced photocatalytic and antibacterial activities. Colloids Surf B Biointerfaces. 2019; 180:141-9.
7. Stefanowicz-Hajduk J, Asztemborska M, Krauze-Baranowska M, Godlewska S, Gucwa M, Moniuszko-Szajwaj B, et al. Identification of Flavonoids and Bufadienolides and Cytotoxic Effects of *Kalanchoe daigremontiana* Extracts on Human Cancer Cell Lines. Planta Med. 2020; 86:239-46.
8. Zawirska-Wojtasiak R, Jankowska B, Piechowska P, Szkudlarczyk SM. Vitamin C and aroma composition of fresh leaves from *Kalanchoe pinnata* and *Kalanchoe daigremontiana*. Sci Rep. 2019; 9:19786.
9. Kolodziejczyk-Czepas J, Nowak P, Wachowicz B, Piechocka J, Glowacki R, Moniuszko-Szajwaj B, et al. Antioxidant efficacy of *Kalanchoe daigremontiana* bufadienolide-rich fraction in blood plasma in vitro. Pharm Biol. 2016; 54(12):3182-8.
10. Kolodziejczyk-Czepas J, Sieradzka M, Moniuszko-Szajwaj B, Pecio L, Ponczek MB, Nowak P, et al. Bufadienolides from *Kalanchoe daigremontiana* as thrombin inhibitors – in vitro and in silico study. Int J Biol Macromol. 2017; 99:141-50.
11. Kolodziejczyk-Czepas J, Pasinski B, Ponczek MB, Moniuszko-Szajwaj B, Kowalczyk M, Pecio L, et al. Bufadienolides from *Kalanchoe daigremontiana* modulate the enzymatic activity of plasmin - In vitro and in silico analyses. Int J Biol Macromol. 2018; 120:1591-600.
12. Quintero EJ, Leon EG, Moran-Pinzon J, Mero A, Leon E, Cano LPP. Evaluation of the Leaf Extracts of *Kalanchoe Pinnata* and *Kalanchoe Daigremontiana* Chemistry, Antioxidant and Anti-inflammatory Activity. Eur J Med Plants. 2021; 32(5):45-54.
13. Ürményi FGG, Saraiva GN, Casanova LM, Matos AS, Camargo LMM, Romanos MTV, et al. Anti-HSV-1 and HSV-2 flavonoids and a new kaempferol triglycoside from the medicinal plant *Kalanchoe daigremontiana*. Chem Biodivers. 2016; 13(12):1707-14.
14. Herrando-Moraira S, Vitales D, Nualart N, Gomez-Bellver C, Ibanez N, Masso S, et al. Global distribution patterns and niche modeling of the invasive *Kalanchoe × houghtonii* (Crassulaceae). Sci Rep. 2020;10(1):1-18.
15. Chernetskyy M. Structure of leaves and phenolic acids in *Kalanchoe daigremontiana* Raym.-Hamet & H. Perrier. Acta Sci Pol Hortorum Cultus. 2018; 17(4):137-55.
16. Johansen DA. Plant Microtechnique. New York: McGraw Hill Book; 1940. p. 41-193.
17. Berlyn GP, Miksche JP. Botanical microtechnique and cytochemistry. Ames: Iowa State University; 1976. p. 121.
18. Oliveira F, Akisue G, Akisue MK. Farmacognosia: Identificação de Drogas Vegetais. {Pharmacognosy: Identification of Plant Drugs.} Atheneu; 2014.
19. Pearse AGE. Histochemistry: Theoretical and Applied. The Williams & Wilkins Company; 1972.
20. Gabe M. Techniques histologiques. Paris: Masson et Cie; 1968.
21. USP (2017) High-Performance Thin Layer Chromatography procedure for identification of articles of botanical origin. In: United States Pharmacopeia and National Formulary USP40–NF35. United States Pharmacopoeial Convention, Rockville, MD, USA, Chapter 203, pp. 258–60.
22. Perera WH, Frommenwiler DA, Sharaf MHM, Reich E. 2021. An improved high-performance thin-layer chromatographic method to unambiguously assess *Ginkgo biloba* leaf finished products. J Planar Chromat Mod TLC.34 (6):559–60.
23. Swain T, Hills WE. The phenolic constituents of *Prunus domestica*. I quantitative analysis of phenolics constituents. J Sci Food Agric. 1959;19(1):63-8.
24. Soares JR, Dinis TC, Cunha AO, Almeida LM. Antioxidant activity of some extracts of *Thymus zygis*. Free Radic Res. 1997; 26:469.
25. Pellegrini N, Ke R, Yang M, Rice-evans C. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2'-azinobis(3-ethylenebenzothiazoline-6 sulfonic acid radical cation decolorization assay. Methods Enzymol. 1999; 299:379-89.
26. Manfron J. [Pharmacobotany: an important tool for detecting adulterations in plant raw materials.]. In: Baratto LC, editor. A farmacognosia no Brasil. Petrópolis, RJ: Ed. do autor; 2021.
27. Almeida VP, Monchak IT, da Costa Batista JV, Grazi M, Ramm H, Raman V, et al. Investigations on the morphology and histochemistry of the European mistletoe: *Viscum album* L. subsp. album. Sci Rep. 2023; 13:4604.
28. Abdel-Raouf HS. Anatomical traits of some species of *Kalanchoe* (Crassulaceae) and their taxonomic value. Ann Agric Sci. 2012; 57(1).
29. Kouri G, Tsimogiannis D, Bardouki H, Oreopoulou V. Extraction and analysis of antioxidant components from *Origanum dictamnus*. Innov Food Sci Emerg Technol. 2007;8(2):155-62. ISSN 1466-8564.

30. Hegazy MM, Metwaly AM, Mostafa AE, Radwan MM, Mehany AB, Ahmed E, et al. Biological and chemical evaluation of some African plants belonging to *Kalanchoe* species: Antitrypanosomal, cytotoxic, anti-topoisomerase I activities and chemical profiling using ultra-performance liquid chromatography/quadrupole-time-of-flight mass spectrometer. *Pharmacogn Mag.* 2021; 17:6-15.
31. De Araujo ERD, Félix-Silva J, Xavier-Santos JB, Fernandes JM, Guerra GCB, de Araújo AA, et al. Local anti-inflammatory activity: Topical formulation containing *Kalanchoe brasiliensis* and *Kalanchoe pinnata* leaf aqueous extract. *Biomed. Pharmacother.* 2019;113,108721.
32. Beszterda M, Frański R. Electrospray ionization mass spectrometric behavior of flavonoid 5-O-glucosides and their positional isomers detected in the extracts from the bark of *Prunus cerasus* L. and *Prunus avium* L. *Phytochem Anal.* 2021 May;32(3):433-9.
33. Andrade EA, Machinski I, Ventura ACT, Barr SA, Pereira AV, Beltrame FL, et al. A review of the popular uses, anatomical, chemical, and biological aspects of *Kalanchoe* (Crassulaceae): A genus of plants known as “miracle leaf”. *Molecules.* 2023; 28:5574.
34. Nascimento LBS, Leal-Costa MV, Coutinho MAS, Moreira NS, Lage CLS, Barbi NS, et al. Increased antioxidant activity and changes in phenolic profile of *Kalanchoe pinnata* (Lamarck) Persoon (Crassulaceae) specimens grown under supplemental blue light. *Photochem Photobiol.* 2013; 89:391-9.
35. Bogucka-Kocka A, Zidorn C, Kasprzycka M, Szymczak G, Szewczyk K. Phenolic acid content, antioxidant, and cytotoxic activities of four *Kalanchoe* species. *Saudi J Biol Sci.* 2018; 25(4):622-30.
36. Elansary HO, Abdelgaleil SAM, Mahmoud EA. Effective antioxidant, antimicrobial and anticancer activities of essential oils of horticultural aromatic crops in northern Egypt. *BMC Complement Altern Med.* 2018; 18:214.
37. Richwagen N, Lyles JT, Dale BLF, Quave CL. Antibacterial activity of *Kalanchoe mortagei* and *K. fedtschenkoi* against ESKAPE pathogens. *Front Pharmacol.* 2019; 10:67.
38. Palumbo A, Casanova LM, Correa MFP, Costa NM, Nasciutti LE, Costa SS. Potential therapeutic effects of underground parts of *Kalanchoe gastonis-bonnierei* on benign prostatic hyperplasia. *Evid Based Complement Alternat Med.* 2019.
39. Lai ZR, Ho YL, Huang SC, Huang TH, Lai SC, Tsai JC, et al. Antioxidant, Anti-Inflammatory, and Antiproliferative Activities of *Kalanchoe gracilis* (L.) DC Stem. *Am J Chin Med.* 2011; 39(6):1275-90.
40. Bhandari R, Sabina G, Nisha A, Devika G, Kalpana P, Abaru P, et al. Evaluation of phytochemical, antioxidant, and memory-enhancing activity of *Garuga pinnata* Roxb. Bark and *Bryophyllum pinnatum* (Lam) Oken. leaves. *Sci World J.* 2021;2021(5):1-7.
41. Jaiswal S, Chawla R, Sawhney S. *Kalanchoe pinnata* - a promising source of natural antioxidants. *Eur J Med Plants.* 2014; 4(10):1210-22.
42. Bhatti M, Kamboj A, Saluja AK, Jain U. In vitro evaluation and comparison of antioxidant activities of various extracts of leaves and stems of *Kalanchoe pinnatum*. *Int J Green Pharm.* 2012; 6(4):340-7.
43. Pinheiro NAP, Alves AMB, Campos AEQR, Furtado ML, Lima AS, Siqueira SMC. [In vitro evaluation of the photoprotective activity of *Bryophyllum laetivirens* (desc.) V.v. byalt.]. *Rev Coleta Científica.* 2020, 4(7):11-16.
44. Adam M, Elhassan GOM, Yagi S, Senol FS, Orhan IE, Ahmed AA, et al. In Vitro Antioxidant and Cytotoxic Activities of 18 Plants from the Erkowit Region, Eastern Sudan. *Nat Prod Bioprospecting.* 2018; 8:97-105.
45. Simões CMO, Schenkel EP, Mello JCP, Mentz LA, Petrovick PR. [Pharmacognosy: From Natural Product to Medicine]. *Artmed;* 2017.
46. Mishra R, Pounikar Y, Gangwar M. Extraction, Phytochemical screening and quantitative determination of phenols and flavonoids in extract of *Kalanchoe pinnata* and *Pongamia pinnata*. *J Drug Deliv Ther.* 2019; 9(4):192-6.
47. Casanova JM, Nascimento LBS, Casanova LM, Leal-Costa MV, Costa SS, Tavares ES. Differential Distribution of Flavonoids and Phenolic Acids in Leaves of *Kalanchoe delagoensis* Ecklon & Zeyher (Crassulaceae). *Microsc.* 2020; 26(5):1061-8.
48. Bopda OSM, Longo F, Bella TN, Edzah PMO, Taiwe GS, Bilanda DC, et al. Antihypertensive activities of the aqueous extract of *Kalanchoe pinnata* (Crassulaceae) in high salt-loaded rats. *J Ethnopharmacol.* 2014; 153(2):400-7.
49. Anadozie SO, Akinyemi JA, Agunbiade S, Ajiboye BO, Adewale OB. *Bryophyllum pinnatum* inhibits arginase II activity and prevents oxidative damage occasioned by carbon tetrachloride (CCl<sub>4</sub>) in rats. *Biomed Pharmacother.* 2018; 101:8-13.
50. Kenderson CA, Kagoro ML, Adelakun EA. Phytochemical and Pharmacological Evaluation of Nigerian *Kalanchoe pinnata* (Lam.) Stem-Bark. *J Chem Soc Nigeria.* 2021; 46(4):751-6.
51. Fernandes JM, Ortiz S, Tavares RPM, Mandova T, Araujo ER, Andrade AWL, et al. *Bryophyllum pinnatum* markers: CPC isolation, simultaneous quantification by a validated UPLC-DAD method and biological evaluations. *J Pharm Biomed Anal.* 2021;193.
52. Elizondo-Luévano JH, Perez-Narvaez OA, Sanchez-Garcia E, Castro-Rios R, Hernandez-Garcia ME, Chavez-Montes, A. In Vitro Effect of *Kalanchoe daigremontiana* and Its Main Component, Quercetin against *Entamoeba histolytica* and *Trichomonas vaginalis*. *Iran J Parasitol.* 2021; 16(3):394-401.
53. Fonseca AG, Dantas LLSFR, Fernandes JM, Zucolotto SM, Lima AAN, Soares LAL, et al. In Vivo and In Vitro Toxicity Evaluation of Hydroethanolic Extract of *Kalanchoe brasiliensis* (Crassulaceae) Leaves. *J Toxicol.* 2018.
54. Lai ZR, Peng WH, Ho YL, Huang SC, Huang TH, Lai SC, et al. Analgesic and anti-inflammatory activities of the methanol extract of *Kalanchoe gracilis* (L.) DC stem in mice. *Am J Chin Med.* 2010; 38(3):529-46.

55. Mayorga OAS, Costa YFG, Silva JB, Scio E, Ferreira ALP, Sousa OV, et al. *Kalanchoe brasiliensis* Cambess., a promising natural source of antioxidant and antibiotic agents against multidrug-resistant pathogens for the treatment of *Salmonella gastroenteritis*. *Oxid Med Cell Longev*. 2019.
56. Yadav V, Arif N, Singh VP, Guerriero G, Berni R, Shinde S, et al. Histochemical Techniques in Plant Science: More Than Meets the Eye. *Plant Cell Physiol*. 2021 Oct;62(10):1509-27.



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