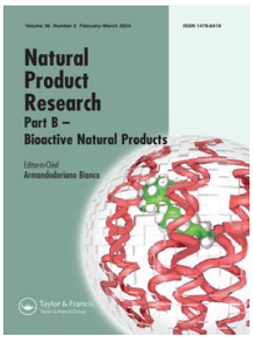

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Comprehensive high-performance thin-layer chromatography analysis of *Monteverdia ilicifolia* leaf and its adulterants

Kevin Alves Antunes[a], Luciane Mendes Monteiro[a], Christopher Howard[b], Eike Reich[c], Gustavo Heiden[d], Ernestino de Souza Gomes Guarino[d], Vera Lúcia Pereira dos Santos[e], Jane Manfron[a] and Wilmer H. Perera[b]

[a]Postgraduate Program in Pharmaceutical Sciences, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil; [b]CAMAG Scientific, Inc., Wilmington, NC, USA; [c]HPTLC Association, Rheinfelden, Switzerland; [d]Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil; [e]Escola Superior de Educação, Humanidades e Línguas (ESEHL), Centro Universitário Internacional Uninter, Curitiba, Paraná, Brazil

ABSTRACT

The leaves of *Monteverdia ilicifolia* (syn. *Maytenus ilicifolia*) are widely used in traditional South American medicine to treat gastrointestinal problems such as gastritis and ulcers. Several herbal products containing the leaves of *M. ilicifolia* can be found in the market. However, other species with similar leaf morphology are confounding materials, e.g. *Monteverdia aquifolia* (Celastraceae), *Citronella gongonha* (Cardiopteridaceae), *Jodina rhombifolia* (Santalaceae), *Sorocea bonplandii* (Moraceae) and *Zollernia ilicifolia* (Fabaceae). This study aimed to identify *M. ilicifolia* and distinguish it from its potential adulterants using high-performance thin-layer chromatography (HPTLC) technique. Comprehensive HPTLC analysis revealed specific fingerprints that can be used to assess the minimum content of epicatechin and the quality of commercial espinheira-santa samples. The results of the study demonstrated that the HPTLC method is capable of detecting adulterations and distinguishing *M. ilicifolia* from all confounding materials in commercial products available on the market, showing that most of the products are of poor quality due to adulterations.

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CONTACT Jane Manfron janemanfron@hotmail.com; Wilmer H. Perera wilmer.perera@camag.com
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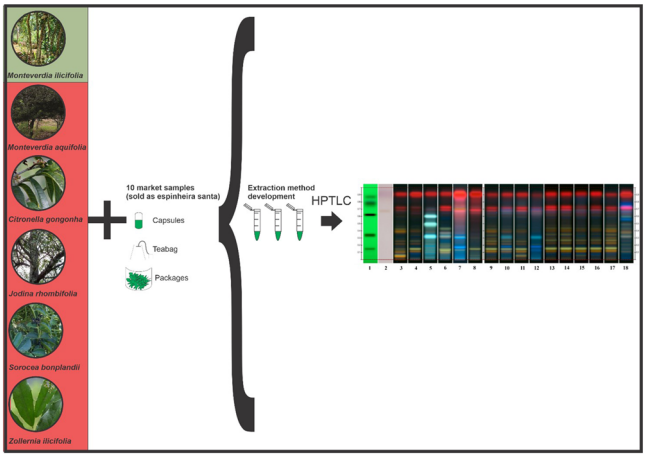

1. Introduction

Monteverdia ilicifolia (Mart. ex Reissek) Biral (Celastraceae), commonly known as espinheira-santa is a popular medicinal plant in Brazil and one of the most commercialised in Paraná State, to treat gastritis and gastric ulcers (Carlini 1988; Feitosa Filho and Modesto 2019). Flavonoids, triterpenes, and tannins have been reported, although some studies suggest epigallocatechin and epicatechin gallate as the compounds responsible for the biological activity (Mariot and Barbieri 2007; de Paula et al. 2021; Martins et al. 2023).

Several herbal products are available in the market and it is well known that the low quality of multiple marketed medicinal plants and their derivatives is a global issue (Nicoletti et al. 2013; Chiara et al. 2014; Frommenwiler et al. 2019). Frequently, commercial materials of espinheira-santa contain mixed, misidentified, or unidentified parts and species, mainly due to the use of plant species with the same common name, similar morphology, or similar therapeutic uses (Manfron 2021).

Despite the prevailing uncertainty and widespread substitution of the true espinheira-santa with several other species, only a few studies on the authentication of commercial products containing espinheira-santa based on morphoanatomy are available in the literature. The most common adulterants of *Monteverdia ilicifolia* are *Monteverdia aquifolia* (Mart.) Biral (Celastraceae), *Citronella gongonha* (Mart.) R.A. Howard (Cardiopteridaceae), *Jodina rhombifolia* (Hook. & Arn.) Reissek (Santalaceae), *Sorocea bonplandii* (Baill.) W.C. Burger et al. (Moraceae), and *Zollernia ilicifolia* (Brongn.) Vogel (Fabaceae) (Jacomassi and Machado 2003; Machado and Santos 2004; Duarte and Debur 2005). Recently morphoanatomy and microscopy approaches were developed to differentiate *M. ilicifolia* from its adulterants. Although the method performs well, *M. ilicifolia* cannot be distinguished from its related species *M. aquifolia* in powdered form (Antunes et al. 2023).

High-performance thin-layer chromatography (HPTLC) is considered the gold standard for the identification of botanicals in many countries and multiple examples have been published in the scientific literature (Reich and Schibli 2007; Booker et al. 2018; Khokhlova and Zdoryk 2020). In order to differentiate *M. ilicifolia* from its known

adulterants, an HPTLC method that generates fingerprints of flavonoids and phenolic acids was developed herein with further analysis of the minimum content of epicatechin for *M. ilicifolia*. The suitability of the method for quality control was assessed using commercial samples from Brazil and the USA.

2. Results and discussion

2.1. HPTLC analysis

2.1.1. Method development

The TLC method of the Brazilian Pharmacopeia monograph on *M. ilicifolia* leaf uses ethyl acetate, formic acid, and water 90:5:5 (*v/v*) as the developing solvent (BRASIL 2019). The parameters of the TLC method are not standardised. Therefore, they were converted into HPTLC parameters following the United States Pharmacopoeia General Chapter 203 (USP 2017). The derivatisation reagent vanillin in sulphuric acid was replaced by natural product (NP) reagent. A first analysis compared the sample preparation by reflux using water or methanol (Supplementary Figure S1).

The HPTLC method was used to compare the concentration of epicatechin after performing densitometric analysis at UV 280 nm prior to derivatisation because the herbal drug *M. ilicifolia* is defined in the Brazilian Pharmacopoeia (BRASIL 2019) as the dried leaves containing no less than 0.28% of epicatechin and 2.0% of total tannins expressed as pyrogallol. The application of 3 µL provides a weak fingerprint, and epicatechin was not detected after derivatisation with NP. The application volume was increased to 5 µL and extraction by reflux with methanol was compared with sonication at room temperature and 80 °C. At the same time, a two-step detection with anisaldehyde sulphuric acid (AS) reagent after NP reagent was explored (Supplementary Figure S2).

5 µL application showed a fingerprint in all detection modes, and catechin standards can be easily detected after derivatisation with NP + AS. There is no significant difference between reflux with methanol and sonication at room temperature and 80 °C when peak areas of epicatechin are compared. Hence, sonication with methanol was selected as the most efficient extraction method for further analysis of *M. ilicifolia* and its adulterants (Supplementary Figure S3).

The HPTLC fingerprint of *M. ilicifolia* is clearly different from those of *C. gongonha*, *J. rhombifolia* and *S. bonplandii*. However, it is very similar to that of *M. aquifolia*, and no characteristic bands are observed in the fingerprint of *Z. ilicifolia* that can be used to identify it when mixed with *M. ilicifolia*. Collection sides of plant species are shown in supplementary, Table S1)

Therefore, another developing solvent, *n*-butyl acetate, methanol, water, and formic acid (7.5:2:1:1, *v/v*) was evaluated (Supplementary Figure S4). It keeps the upper part of the chromatogram very similar to that obtained with the method of the Brazilian Pharmacopoeia but moves the more polar flavonoids further from the application position and separates them well (Perera et al., 2021).

The fingerprint of *M. ilicifolia* is very different from those of the related species. Considering both detection modes, there are characteristic zones for each of the species. Observation in longwave UV (350 nm broadband) after derivatisation with NP

reagent is the most suitable detection mode for identification. The fingerprint of *M. ilicifolia* is described as follows: three reddish zones at R_F 0.91, 0.72, and 0.69, a faint zone at R_F 0.46, greenish and yellowish zones at R_F 0.42 and 0.39, a couple of yellowish zones at R_F 0.26 and 0.22, a faint greenish zone at R_F 0.14 and a yellowish zone at R_F 0.11 and a greenish and yellowish zone at R_F 0.06 and 0.04, respectively.

The UHM, a ready-to-use mixture of guanosine, sulisobenzone, thymidine, paracetamol, phthalimide, 9-hydroxyfluorene, thioxanthen-9-one, and 2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol is proposed as system suitability test (SST) for routine analytical work (Do et al., 2021). It generates quenching zones in shortwave UV (254 nm) at R_F 0.87 ± 0.03, 0.79 ± 0.02, 0.62 ± 0.03, 0.34 ± 0.02 and 0.15 ± 0.02.

With the HPTLC method developed herein, the minimum content for epicatechin can also be verified with no extra chromatographic work (Supplementary Figure S5). The only requirement is to apply epicatechin on the plate at an amount that is equivalent to its minimum content in sample. Both *M. ilicifolia* and *M. aquifolia* pass the test for minimum content. However, based on our findings, the minimum content specified by the Brazilian Pharmacopoeia seems to be too low.

The last part of the work focused on the application of the proposed method to the analysis of 10 market samples. The fingerprints of the samples on tracks 8, 12, 13, 15, and 16 match that of the *M. aquifolia* botanical reference material on track 3. The sample on track 9 also shows a fingerprint of *M. aquifolia*, but a couple of extra blueish zones are observed at R_F 0.18 and 0.30, indicating the presence of *S. bonplandii* (track 6).

The sample on track 11 shows a fingerprint matching that of *S. bonplandii*. The sample on track 10 reveals a clear fingerprint similar to that of the *M. ilicifolia* botanical reference material. At the same time, the sample on track 14 also shows similarities to *M. ilicifolia* with a fainter zone of the flavonoid's oligo glycosides at R_F ~0.05. The sample on track 17 exhibits a few zones characteristic of *J. rhombifolia*, although other zones not associated with any adulterants also appear. Only two of the 10 analysed samples match the fingerprint of *M. ilicifolia*.

3. Conclusions

The HPTLC method developed herein distinguishes *M. ilicifolia* from its common adulterants, *C. gongonha*, *J. rhombifolia*, *S. bonplandii*, and *Z. ilicifolia*. Although *M. ilicifolia* and *M. aquifolia* have different habitats, the substitution of *M. ilicifolia* for *M. aquifolia* in commercial samples is the most common. This fact confirms that the popular name and the morphological similarity of the leaves favour the misidentification of *M. ilicifolia*, leading to low-quality raw materials. *S. bonplandii* was the second most common adulterant found in commercial materials. HPTLC is a good approach to quickly discriminate *M. ilicifolia* from its adulterants in commercialised samples using the flavonoid fingerprint and efficiently assesses the minimum content of epicatechin.

Author contributions

KAA purchased samples from the market and prepared the extracts. LMM prepared the standards. WHP, CH, and ER performed the HPTLC and densitometric analysis and reviewed the manuscript. GH, VLPS, and ESGG collected the plants. WHP and KAA wrote the manuscript. JM

created the project and supervised the laboratory work along with WHP. All the authors have read the final version and approved the submission.

Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Kevin Alves Antunes http://orcid.org/0000-0002-3209-1125
Luciane Mendes Monteiro http://orcid.org/0000-0003-2896-8147
Eike Reich http://orcid.org/0000-0003-4320-4946
Gustavo Heiden http://orcid.org/0000-0002-0046-6500
Ernestino de Souza Gomes Guarino http://orcid.org/0000-0001-8286-4889
Vera Lúcia Pereira dos Santos http://orcid.org/0000-0002-9250-2248
Jane Manfron http://orcid.org/0000-0003-1873-2253
Wilmer H. Perera http://orcid.org/0000-0003-3607-8638

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