

High-Performance Thin-Layer Chromatography and Morpho-anatomy of *Monteverdia ilicifolia* “Espinheira-santa”.

Collaborative project

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Introduction

Monteverdia ilicifolia (Mart. ex Reissek) Biral (Celastraceae) syn. *Maytenus ilicifolia* Mart. ex Reissek

✓ Plant species from the genus *Maytenus* were recently studied by molecular biology and reorganized in two genera as follows:

- **Maytenus (7)**: *Maytenus boaria*; *M. magellanica*; *M. chubutensis*; *M. disticha*; *M. conferta*; *M. verticillata* and *M. woodsonni*.

- **Monteverdia (45)**: *Monteverdia ilicifolia* and *Monteverdia aquifolia*.

Biral, L.; Simmons, M. P.; Smidt, E. C, et al. Systematics of New World *Maytenus* (Celastraceae) and a New Delimitation of the Genus. *Syst. Bot.* **2017**, *42*, 1–14.

Introduction

- ✓ Leaves are widely used in Brazil as a tea to treat gastritis and gastric ulcers, a treatment that has been scientifically proven.
- ✓ Flavonoids, triterpenes and tannins have been reported, although some studies suggest epigallocatechin and epicatechin gallate as the compounds responsible for the biological activity.
- ✓ *M. ilicifolia* is very popular in Brazil and one of the most commercialized medicinal plants in Paraná state.
- ✓ Due to the high demand of *M. ilicifolia*, it is commonly adulterated, some examples of adulterants are *Monteverdia aquifolia* (Mart.) Biral, *Sorocea bonplandii* (Baill.) W.C. Burger, *Jodina rhombifolia* (Hook. & Arn.) Reissek, *Citronella gongonha* (Mart.) R.A. Howard and *Zollernia ilicifolia* (Brongn.) Vogel. A.

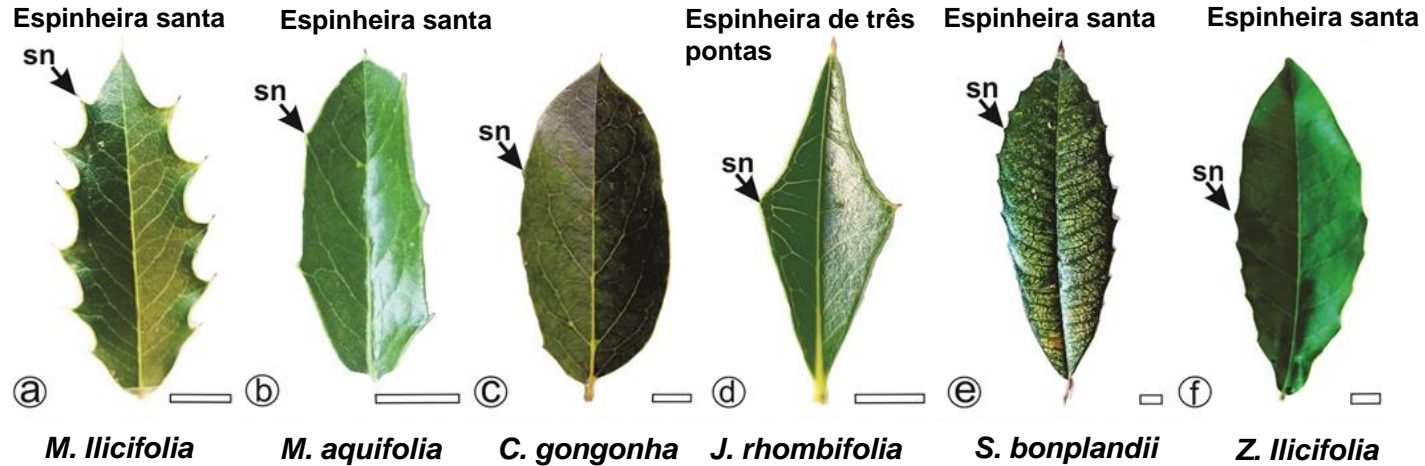
Carlini, E. A. (Coord.). **Estudo da ação antiúlcera gástrica de plantas brasileiras: *Maytenus ilicifolia* (Espinheira-santa) e outras.** Brasília: CEME/AFIP. 87p., 1988.

Mariot, M. P.; Barbieri, R. L. Metabólitos secundários e propriedades medicinais da espinheira-santa (*Maytenus ilicifolia* Mart. Ex Reiss. e *M. aquifolium* Mart.). **Revista Brasileira de Plantas Medicinai**s, v.9, n.3, p.89-99, 2007.

Feitosa Filho, J. L.; Modesto, K. R. Alcaçuz e espinheira-santa no tratamento de gastrite. **Revista de Iniciação Científica e Extensão**, v.2, n.2, p.269-273, 2019.

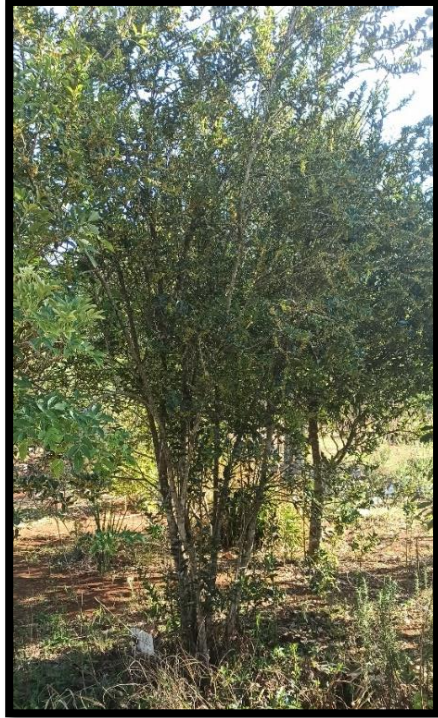
Paula, M.N.; Kelm, M.; Symma, N. *et al.* Anti-adhesive Activity of *Maytenus ilicifolia* Against *Helicobacter pylori*. **Rev. Bras. Farmacogn.** 31, 726–731, 2021.

Morphology of *espinheira-santa* leaf and its adulterants



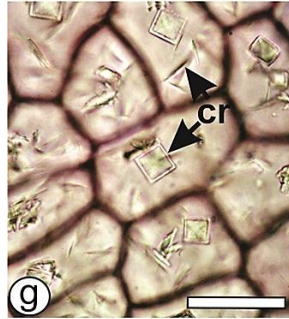
- Leaves with similar morphology motivate adulterations/substitutions
- The adulterations of herbal drugs can be defined as:
 - 1- Intentional: economically motivated, by adding plant species of low or no commercial value
 - 2- Unintentional: missidentification of the species, collecting species with similar common name, etc

Monteverdia ilicifolia (Mart. ex Reissek) Biral (Celastraceae)

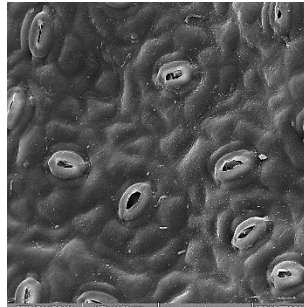


Plant in its habitat (By Kevin Antunes)

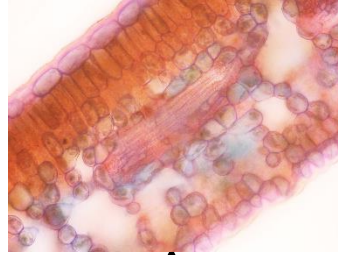
Crystals in the adaxial Epidermis (frontal view)



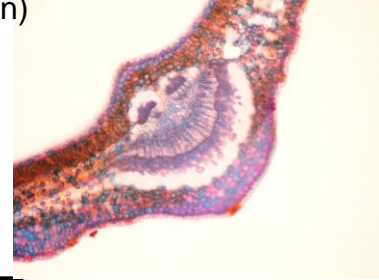
Stomata on abaxial side (frontal view)



Dorsiventral mesophyll (cross-section)

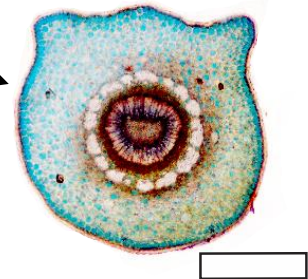


Biconvex shape of midrib (cross-section)



By Leonardo Biral

Biconvex petiole (cross-section)



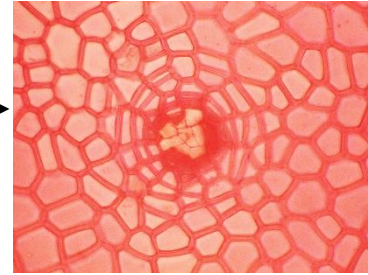
Monteverdia aquifolia (Mart.) Biral (Celastraceae)



Plant in inhabit (By Kevin Antunes)



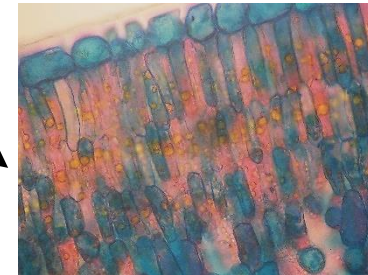
(By Kevin Antunes)



Crystals on adaxial side (frontal view)



Biconvex shape of midrib (cross-section)



Dorsiventral mesophyll (cross-section)

Citronella gongonha (Mart.) R.A.Howard (Cardiopteridaceae)

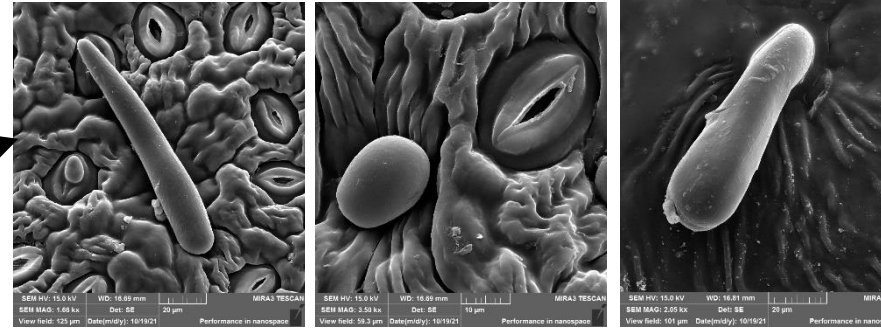


By Martin Molz

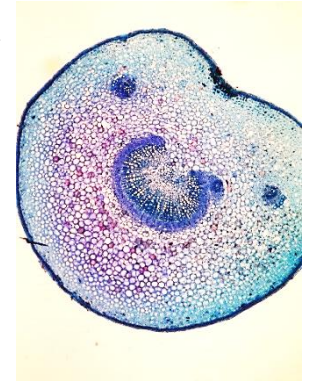


By Rafael Barbizan Sühs

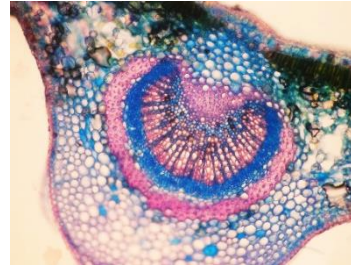
Non-glandular and glandular trichomes (frontal view)



Concave-convex petiole shape (cross-section)



Flat-convex midrib shape (cross-section)



Jodina rhombifolia (Hook. & Arn.) Reissek (Santalaceae)



<http://uruguay1.blogspot.com/2011/07/sombra-de-toro-jodina-rhombifolia.html>

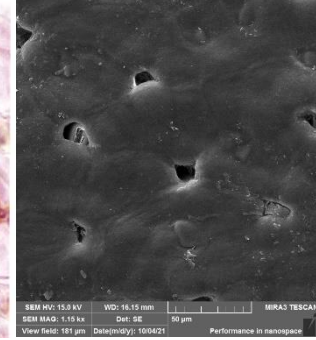
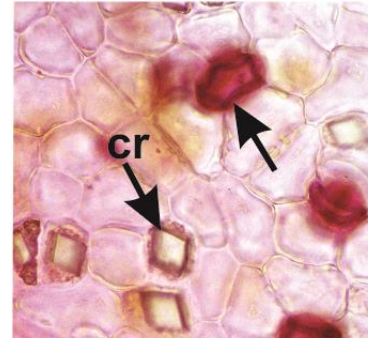


<https://hortodidatico.ufrpe.br/cancorosa-de-tres-pontas/>

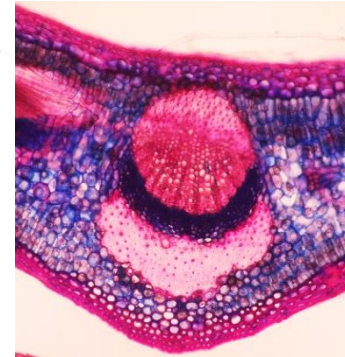


Flat-convex shape of petiole (cross-section)

Crystals and stomata on both epidermis (frontal view)



Flat-convex shape of midrib (cross-section)



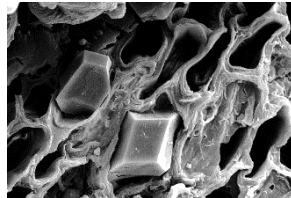
Sorocea bonplandii (Baill.) W.C. Burger (Moraceae)



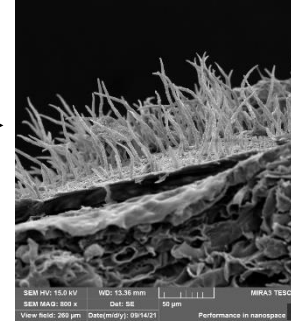
<https://www.arvores.brasil.nom.br/new/folhaserra/index.htm>



Crystals of calcium oxalate
(rhomboidal, diamond)



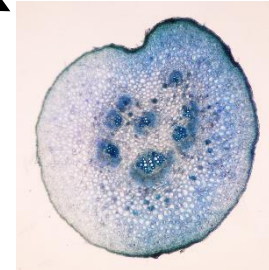
Non-glandular and glandular trichomes



Prominent convexity on abaxial side of midrib in cross-section



Concave-convex shape of petiole



Zollernia ilicifolia (Brongn.) Vogel (Fabaceae)

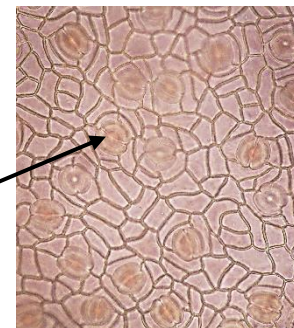
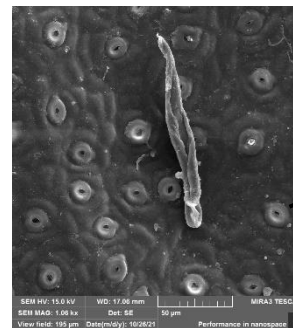


<https://www.colecionandofrutas.com.br/zollerniaolicifolia.htm>

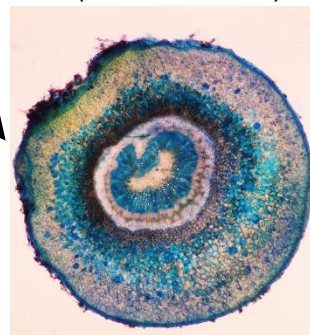


By Marcio Verdi

Stomata and non-glandular trichome (frontal view)



Flat-convex shape of petiole (cross-section)



Flat-convex shape of midrib (cross-section)



Thin-Layer Chromatography of *Monteverdia ilicifolia*



- ✓ The herbal drug consists of the dried leaves of *Monteverdia ilicifolia* with a content 2.0 % of total tannins and a minimum content of epicatechin of 0.28%
- ✓ Thin-layer Chromatography method (non-standardized methodology)
 - **Sample preparation:** reflux 5 g in 50 mL of water for 5 min then filter
 - **Reference solution:** epicatechin 1 mg/mL in methanol
 - **Stationary phase:** Silica gel F₂₅₄ (0.250 mm)
 - **Developing solvent:** ethyl acetate, formic acid, water 90:5:5 (V/V)
 - **Band application:** 10 µL for samples and 3 µL for reference solution
 - **Detection:** under UV 254 nm
 - **Derivatization:** vanillin then heat 110 °C for 10 min

Extraction methods

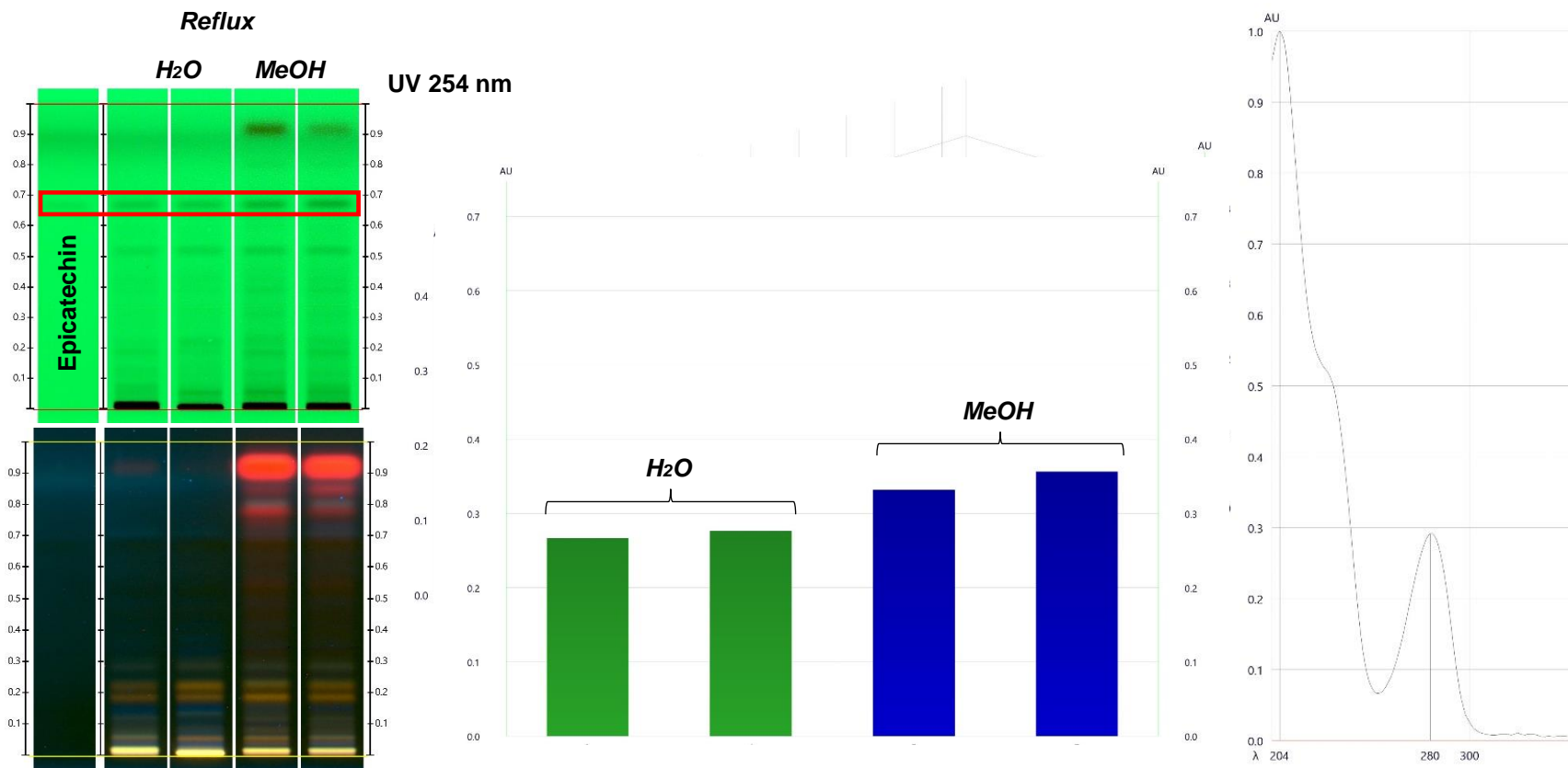
This work is registered at SISGEN (National Genetic Heritage Management System) under the access authorization number AE6D3C6.

5 g of *M. ilicifolia* previously milled were extracted with 50 mL for 15 min using the following conditions:

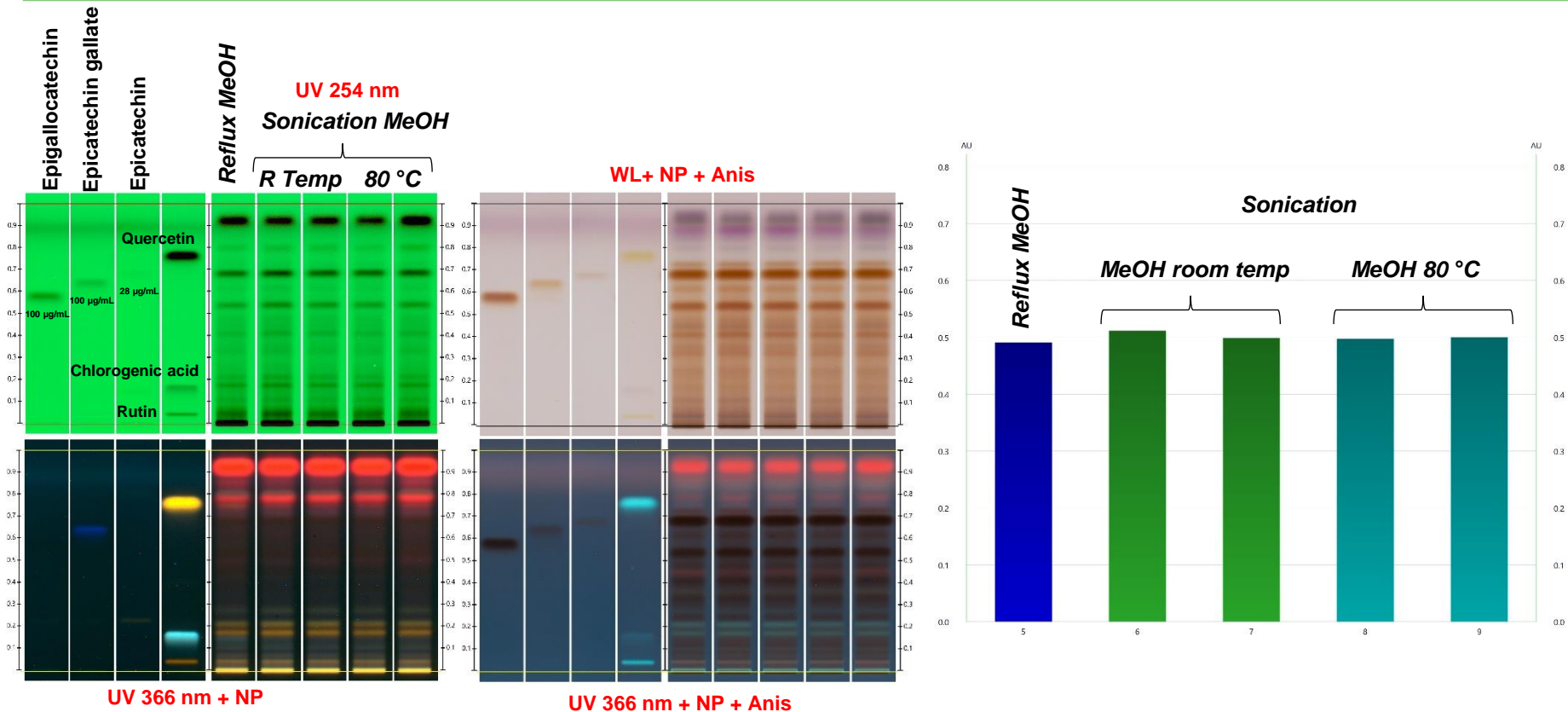
1. Refluxed with distilled water.
2. Refluxed with methanol.
3. Sonicated with methanol at 80 °C.
4. Sonicated with methanol at room temperature.

The solution was filtered and dried using a rotovap.

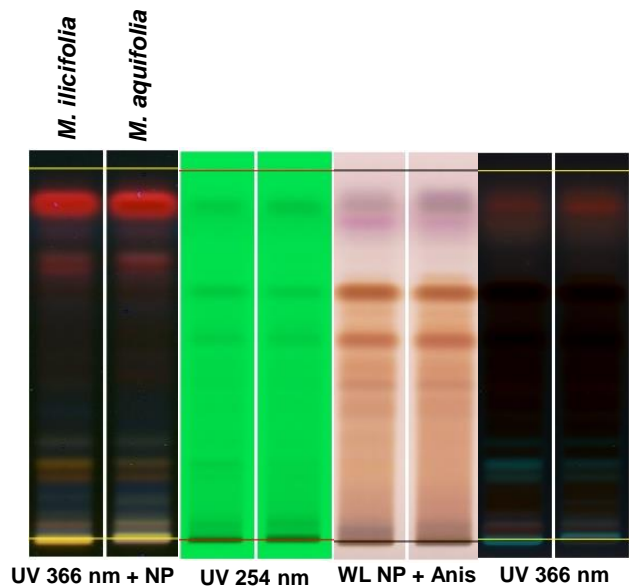
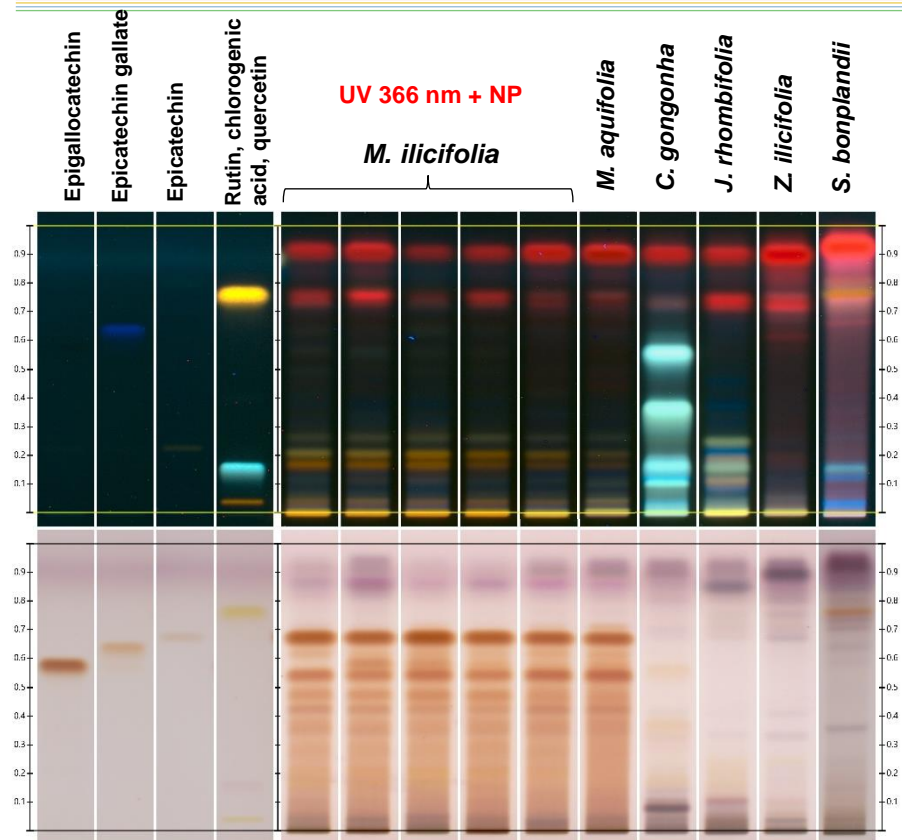
Transferring *M. ilicifolia* TLC method into HPTLC (2 μ L application)



Reflux vs. sonication (5 μ L application volume)

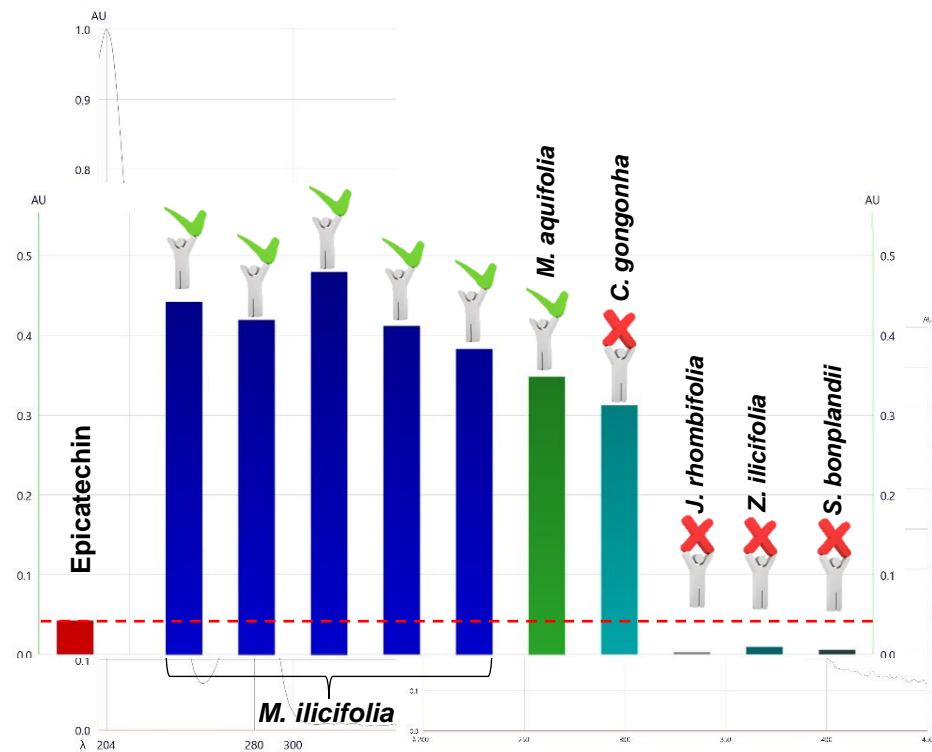
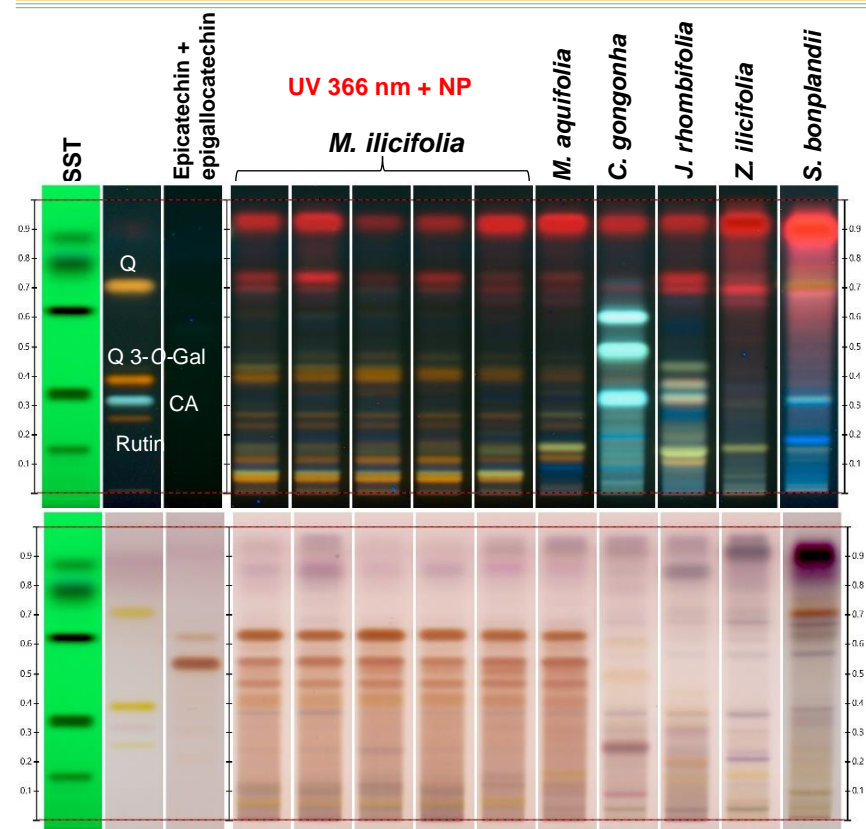


HPTLC fingerprint of *M. ilicifolia* and its adulterants



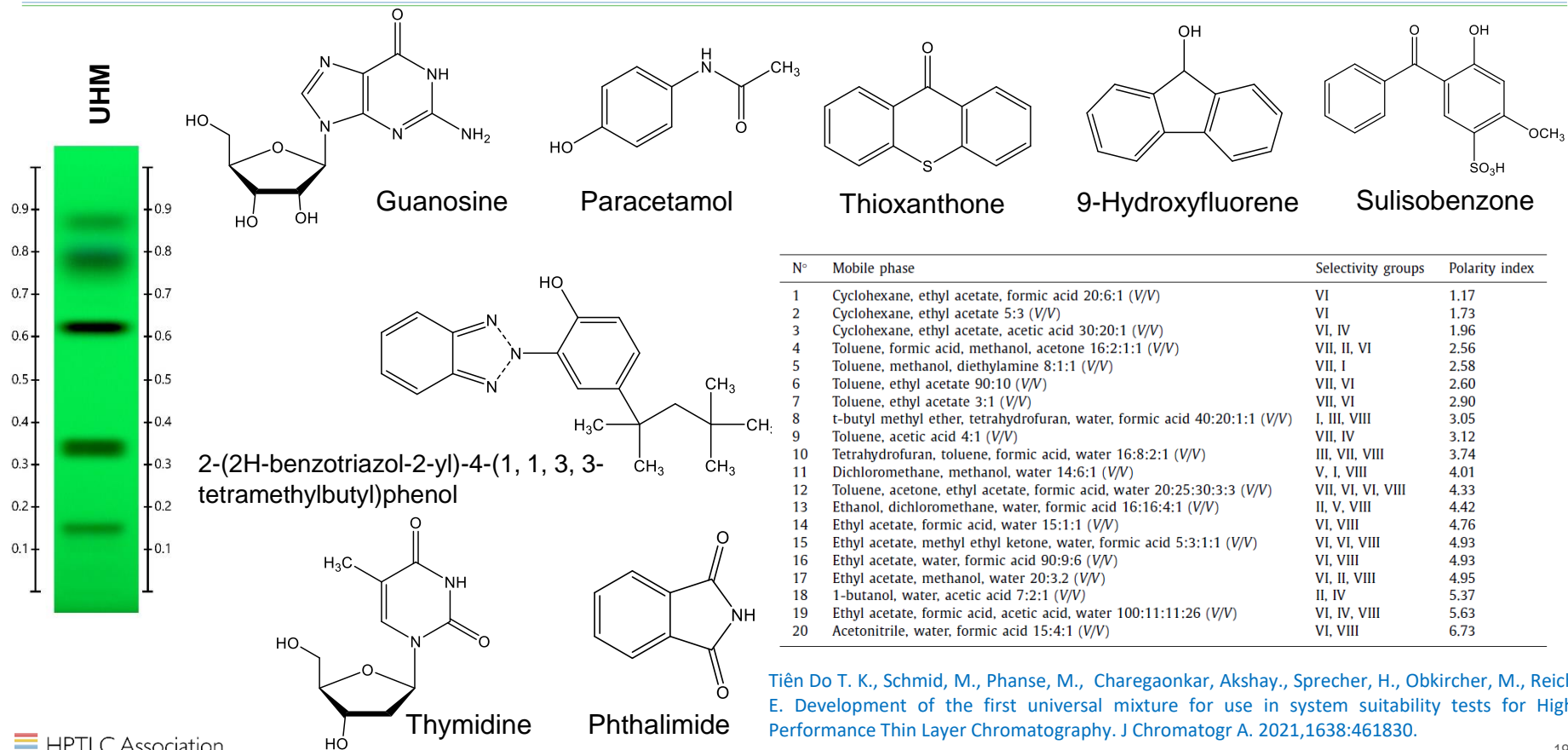
No differences in fingerprint

Comprehensive HPTLC: *n*-butyl acetate, methanol, water, formic acid (7.5:2:1:1, V/V)

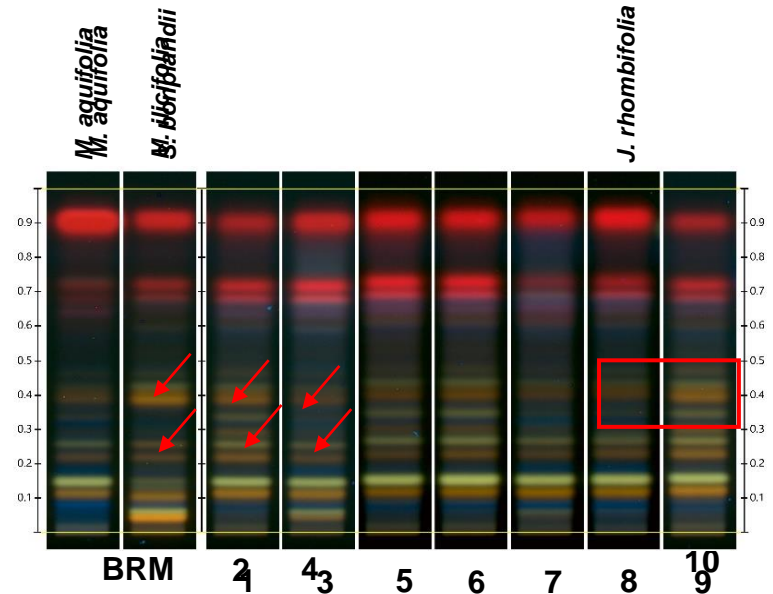
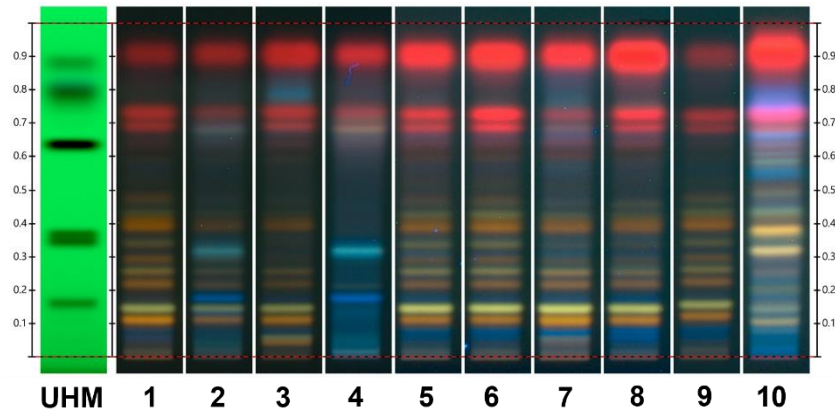


Perera, WH.; Frommenwiler, DA.; Sharaf, M. H. M. and Reich, E. An improved high-performance thin-layer chromatographic method to unambiguously assess *Ginkgo biloba* leaf finished products. JPC – Journal of Planar Chromatography – Modern TLC. 2021, 34, 559-560.

Universal HPTLC Mixture Concept



Feasibility study with commercial samples



Sample # 2: *M. aquifolia* + *S. bonplandii*
 Sample # 10: *J. rhombifolia* + ?
 Sample # 1, 5, 6, 8 and 9: *M. aquifolia*; sample # 3 and 7: *M. ilicifolia*
 Sample # 4: *S. bonplandii*, also observed in anatomic studies

Conclusions

- ✓ Anatomic study discriminates *Monteverdia ilicifolia* leaf from most of the adulterants except *M. aquifolia*.
- ✓ The comprehensive HPTLC method based on the flavonoid fingerprint, assesses the minimum content of epicatechin in *Monteverdia ilicifolia* leaf as described in the Brazilian Pharmacopeia and differentiates the species from all common adulterants.
- ✓ HPTLC is the only approach studied herein that discriminates *M. ilicifolia* from *M. aquifolia* leaf in commercial samples.

Thank you!