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### PHYTOCHEMICAL ANALYSIS

#### Phytochemical studies

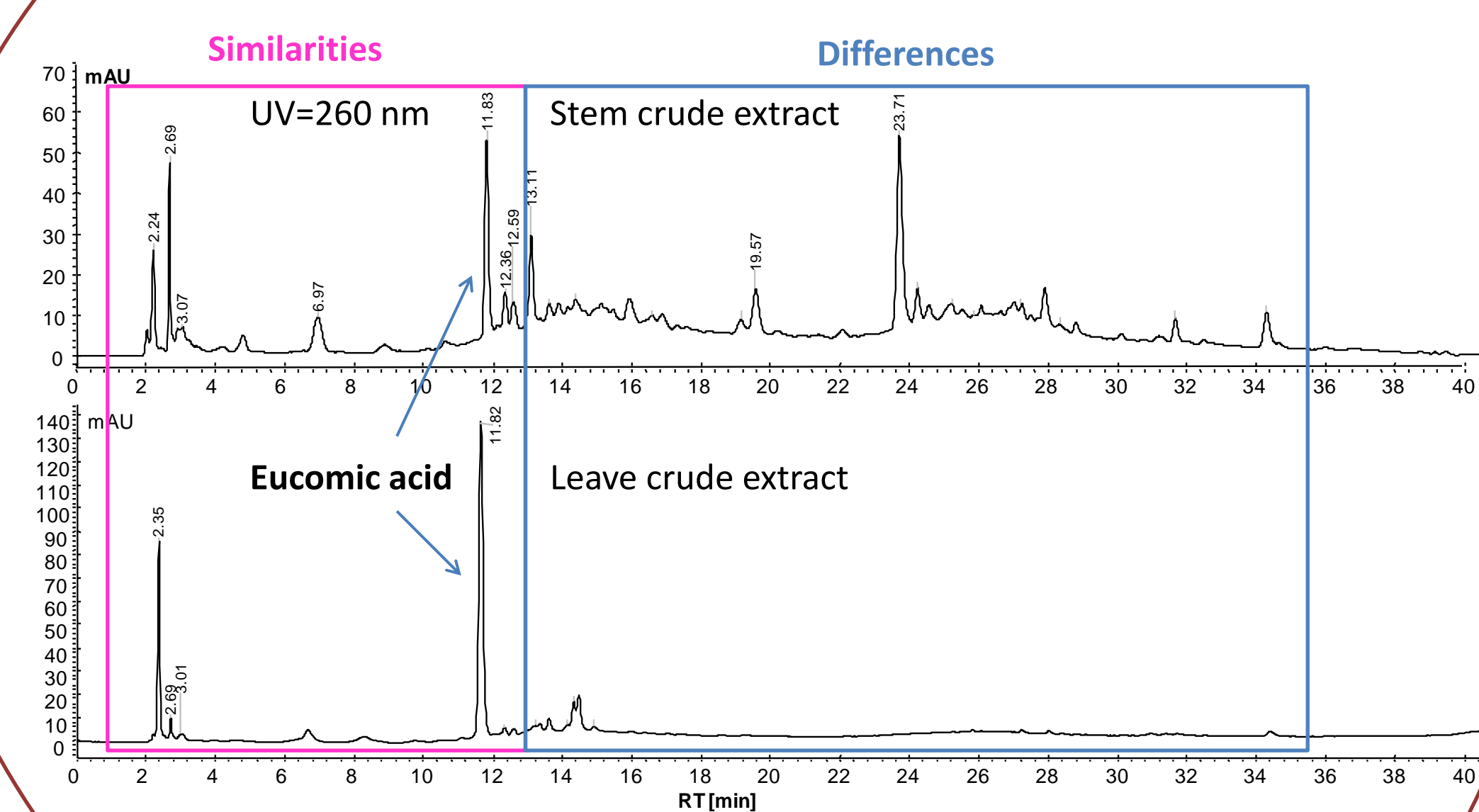
Leaves and stems of *Brassocattleya marcella* Koss were extracted with ethanol at reflux during 30 minutes.

The crude extracts were fractionated on C18 SPE with different ratio of water and methanol.

Two major compounds were isolated by semi-preparative RP-HPLC while one minor constituent was identified on-line thanks to hyphenated LC-MS-UV DAD-SPE-NMR technique.

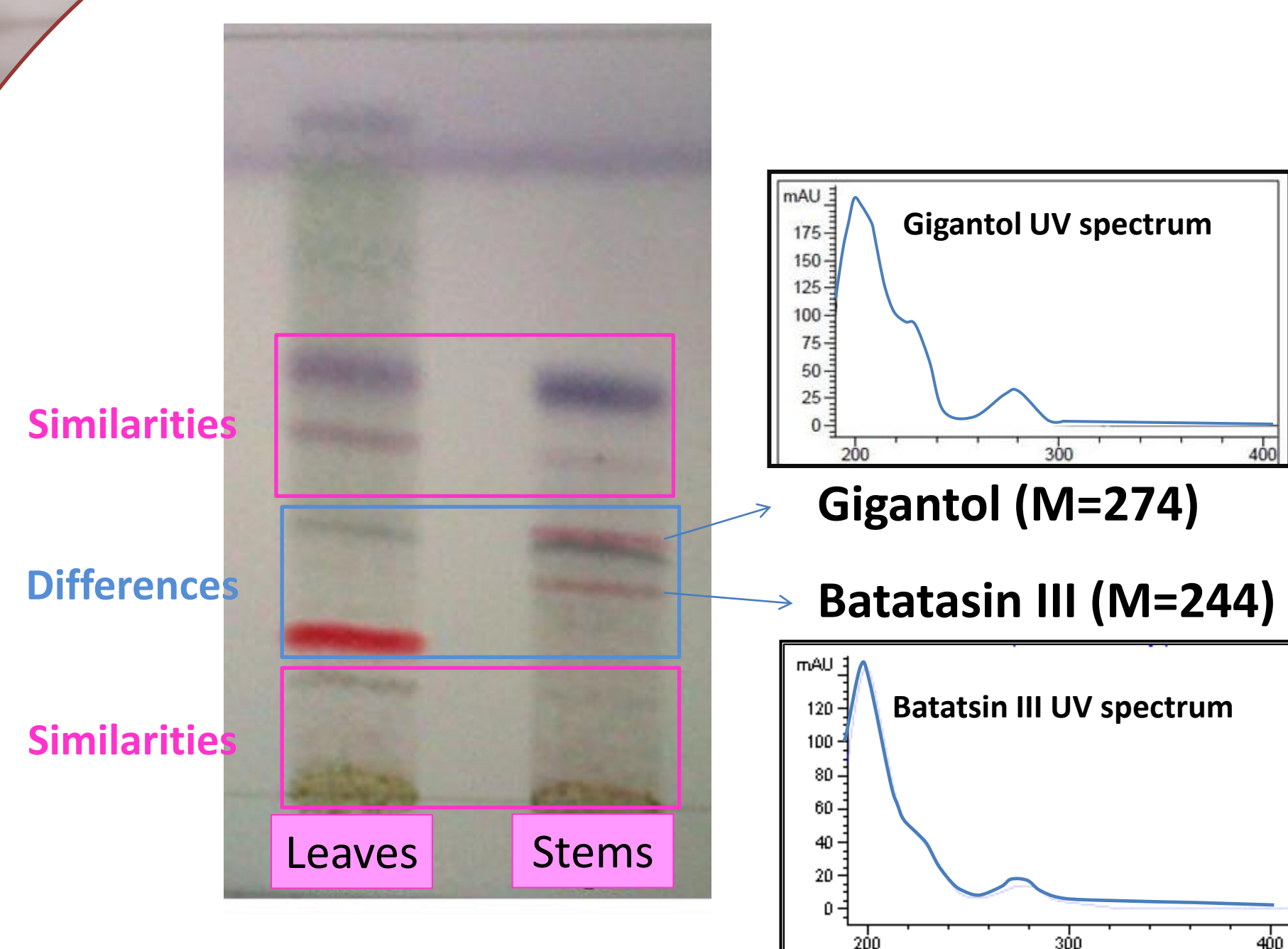
Their structures were confirmed by NMR and HR-MS (data not shown).

#### HPLC-DAD analysis of *B. Marcella* crude stem and leaf extracts



Column: Nucleodur C18ec 250x4,6 mm 5µm;  
Solvent A (Water+0,1% formic acid, B (MeOH+0,1% formic acid); Gradient-time min (%A; %B): 0-5 (75;25), 8 (55; 45), 10-13,5 (47; 53), 15-18 (45;55), 23-25 (20;80), 27,5-31 (15;85), 36-40 (0;100).

#### TLC profiles of *B. Marcella* crude stem and leave extracts



Stationary phase: TLC silica gel 60 Merck F254 Merck  
Mobile phase: Chloroform/Methanol 94/6 v/v  
Detection: Godin's reagent, UV-visible light after heating at 110°C

Disorders in pigmentation process and high sensibility to pro-inflammatory stress result in the appearance of age spots and a loss of radiance.

*Brassocattleya* extract appears to be suitable to use in global brightening cosmetic products, especially adapted to treat fragile and spot sensitive skin.

### BIOLOGICAL ACTIVITY

Specific genes are involved in melanin formation and in the control of epidermal architecture, which govern skin-light interactions.

- on **Melanocytes**  
Brassocattleya extract reduced activity on the gene encoding tyrosinase, MC1R, AP3B2, SILV, GPR143, VAMP2, STOML2 and SNAP23.
- on **Keratinocytes**  
It stimulated the gene encoding aquaporins 3, E cadherins, Ki67 but reduced the activity of the K16 gene and the activity of the gene encoding calgranulin.

PGE2 is synthesized by cyclo-oxygenase COX from the arachidonic acid, fatty-acid present at the level of lipids membrane. Following various stimuli such as UV irradiation, COX activity increases and will thus induce an increase of intracellular PGE2 leading to overstimulate the pigmentation.

- Brassocattleya* extract (2%) inhibits PGE2 release up to 88%, with and without UVB stimulation, in an equivalent manner to indomethacin (1µM)

### DISCUSSION

Phytochemical analysis of crude leaf and stem extracts led to the identification of two bibenzyl derivatives : gigantol (= 3',4-dihydroxy-3,5'-dimethoxybibenzyl) and batatasin III (= 3,3'-dihydroxy-5-methoxybibenzyl) together with their precursor eucomic acid (= 2-(4-Hydroxybenzyl) malic acid).

This metabolites are already described in other orchids and cannot be considered as identity markers of this species. Eucomic acid is common to *Brassocattleya* leaf and stem extracts while gigantol and batatasin III are only present in its stem extract.

Their implication to the bioactivity of the crude extract need to be demonstrated.

### Acknowledgements



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PARFUMS & COSMETIQUES

