HPTLC/AMD: a valuable method for rapid analysis of rare orchid plant extracts

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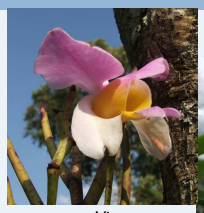
INTRODUCTION

LVMH Recherche investigates plant extracts with potential cosmetic activity.

In order to characterize primary and secondary metabolites, LVMH Recherche has developed a multi-step phytochemical screening using HPTLC with isocratic or gradient elution.

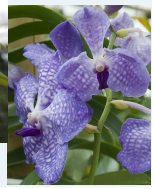
Our investigations led us to be interested in rare Orchids from Yunnan, South China. Some are used in traditional medicine and we focused on epiphyte *Vanda* sp. stems known to possess antioxidant and anti-inflammatory activities.

The idea is to determine specific chemical features of each five *Vanda* species, *Vb*, *Vc*, *Vd*, *Vs* and *Vt*, in order to differenciate them. Global molecular characterization was first realised by phytochemical screening. Then, an elution based on AMD system allows the identification of some specific compounds in *Vanda*'s ethanolic extracts and especially targeting phenolics, terpenoïds and fatty acids. This migration will be followed by a densitometric study (densitogram, UV spectra) before staining.



Vt

Vd



Vc

METHODS

ELUTION BY HPTLC/AMD

Introduction

The 'phytochemical screening', with its specific elutions and stainings allowed us to underline some sugars, especially monosaccharids, in every *Vanda* species: *Vb*, *Vc*, *Vd*, *Vs* and *Vt*.

Amino acids are mostly detected in *Vd* specy and in *Vs* . Chorophylls are particularly present in *Vt*, *Vb* and *Vc* species. Tanins are more suspected in *Vd*, *Vb* and *Vc* species.

The phenolic and lipidic areas are studied thanks to AMD system in order to detect specificity in these families.

Principle of Automated Multiple Development elution

It's a migration by gradient: polarity of mobile phase is decreased at each step of elution, with a preconditionning and a drying steps between each, allowing a 'focalisation' of compounds: they are more concentrated before each new step.

The plate is developed in the same way, several times, increasing the migration distance at each step.

We aim at having in one plate compounds with large polarity, to compare different phytochemicals families in one run.

Gradient

Step	1	2	5	11	16	21
Bottle	1	2	3	4	5	6
Methanol	40	30	20			
Diisopropyl ether	60	70	80	50	30	
Heptan				50	70	100
Drying	5	5	5	4	3	3
Preconditionning	Acidic					

This gradient includes twenty five steps, with three solvents chosen in function of Snyder and Hildebrand parameters: these are methanol, diisopropyl ether and heptan. The basis solvent is essentially a mixture of methanol and diisopropyl ether.

Diol, the stationary phase used, is less polar permitting a decrease of the retention of the polar compounds such as sugar derivatives.

The final drying step lastes ten minutes.

CONCLUSION

HPTLC/AMD proved to be a valuable method for rapid analysis of orchid extracts.

We revealed that every *Vanda* species' stems contain sugars, phenolic compounds, terpenoïds (especially sterols), fatty acids, triglycerids and sterol ester: *Vt*, but also *Vb* and *Vc* stand out from others with their chlorophylls content.

By containing flavonoids, *Vb* and *Vt* have atypical profile in comparison with *Vc*, *Vd* and *Vs*.

***Vt* has a few terpenoïds molecules. *Vc* and *Vs* have nearly the same molecular outline. *Vb* has its specific profile with a more apolar phenolic compound. *Vd* also shows a new marker.**

In all species, terpenoïds family shows common molecules, phytosterols like stigmasterol, β sitosterol (as seen in bibliography) and a phenanthren derivative.

This phytochemical study allows a better understanding of these *Vanda* extracts related to anti-ageing properties.

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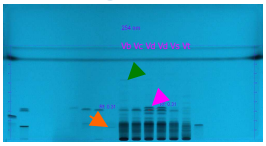
RESULTS

Approach of ethanolic *Vanda* extracts at the native step

254 nm



Organs studied: stems

366 nm

Caption - 1: resveratrol/oleanolic acid – 2: luteolin 7-O glucosid/p-coumaric acid – 3: β sitosterol – 4: stigmasterol/cholesteryl oleate – 5: aucubin – 6: gigantol – 7: methoxycoelonin – 8: imbricatin – 9: Vt1 – 10: *Vb* – 11: *Vc* – 12: *Vd* – 13: *Vd* – 14: *Vs* – 15: *Vt* – 16: chlorogenic acid – 17: linoleic acid - 18: trielaïdin -19: sclareol/geraniol - 20: α hédérin/ursolic acid

Under 254 nm, we can observe some common compounds with black spots (as tanins in the low Rf) and in particular some specific for *Vd* .

Under 366 nm, we can observe a lot of blue fluorescent components, especially two for *Vd* and *Vs* species. Chlorophylls may hide them in *Vt*. In fact, these 'markers' (Rf about 0.3 and 0.6) might be present in all these extracts. Thus, their 'concentration' could be a differenciation marker. Indeed, the intensity of coloration of spots enables to anticipate a 'semi-quantitative' analysis, which will be in concordance with densitometric study (peak area).

In comparison with standards, these black and fluorescent 'spots' may belong to stilbenoïds and/or phenolic acids components.

Focus in phenolic area

Stilbenoïds/Flavonoïds

Neu + Peg 366 nm

Caption - 1: resveratrol/oleanolic acid – 2: luteolin 7-O glucosid/p-coumaric acid – 3: β sitosterol – 4: stigmasterol/cholesteryl oleate – 5: aucubin – 6: gigantol – 7: methoxycoelonin – 8: imbricatin – 9: Vt1 – 10: *Vb* – 11: *Vc* – 12: *Vd* – 13: *Vd* – 14: *Vs* – 15: *Vt* – 16: chlorogenic acid – 17: linoleic acid - 18: trielaïdin -19: sclareol/geraniol - 20: α hédérin/ursolic acid

In comparison with the standards, we can assert that:

- Neu + Peg shows that the flavonoïds are not detected in *Vanda* species, except for the *Vt* and for the *Vb*.

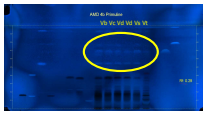
- There are a lot of similar compounds, 'markers' of *Vanda* species, but with a strong difference between their intensity.

- The hypothesis of stilbenoïds in every species and may be phenolic or organic acids derivatives, is confirmed.

Focus in 'lipidic' area

Terpenoïds/Fatty acids

Primulin 366 nm



Sulfuric Vanillin visible

Caption - 1: fructose – 2: myricitrin – 3: α hédérin/ursolic acid – 4: stigmasterol/cholesteryl oleate – 5: rhapontin – 6: gigantol – 7: methoxycoelonin – 8: imbricatin – 9: Vt1 – 10: *Vb* – 11: *Vc* – 12: *Vd* – 13: *Vd* – 14: *Vs* – 15: *Vt* – 16: aucubin – 17: linoleic acid - 18: trielaidin -19: sclareol - 20: geraniol

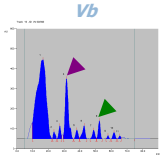
These two plates give rise again to a lot of similar compounds in these *Vanda* extracts. We can see the presence of a group of sterol, fatty acids and triglycerids especially (first staining reagent). The second one shows unsaturated fatty acids but probably saturated triglycerids. Some iridoïds and triterpens may be present. Also sterol ester, except in *Vs*.

We observe a graduate color for the phenolics specific to each specy. The quantification could be a mean to differenciate them.

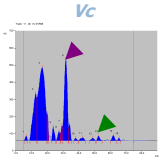
It 's interesting to notice that in one elution, we can see sugars up to sterol esters.

Densitograms at 254 nm - Specificity in *Vanda* species

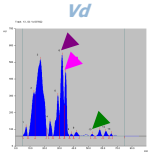
Vb



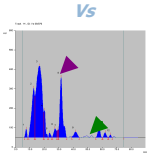
Vc



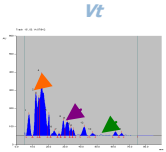
Vd



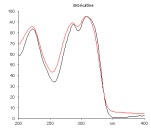
Vs



Vt



UV spectra from *Vc*



Molecular qualitative profil by plate seems to be nearly the same because of a lot of similar compounds. Their concentration could make the difference.

Even with a common marker in every extracts (*Vd*) densitometric study at several wavelengths, and especially at 254 nm, shows different outlines, in relation with specific compound due to specy as an other marker with a concentration decreasing.

For example, we can see that the green peak is decreasing from *Vb* to *Vt* specy.

Some UV spectra give structural information (dihydro-phenanthrens for *Vc* and hydroxybenzyl derivatives for *Vt*). The study of other markers' spectra is in progress.

UV spectra from *Vt*