

Une identification rapide de composés naturels de plantes par HPTLC-MS

Expériences en pédagogie
Expériences en recherche

Présenté par

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Yuka MUNAKATA, PhD

Une identification rapide de composés naturels de plantes par CCMHP

Expériences en pédagogie

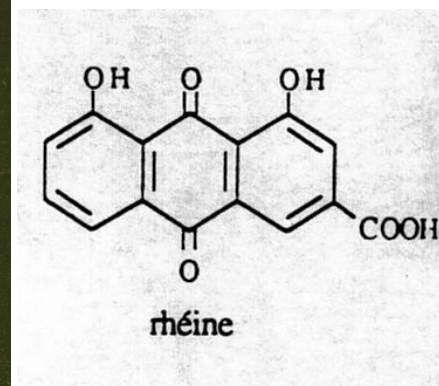
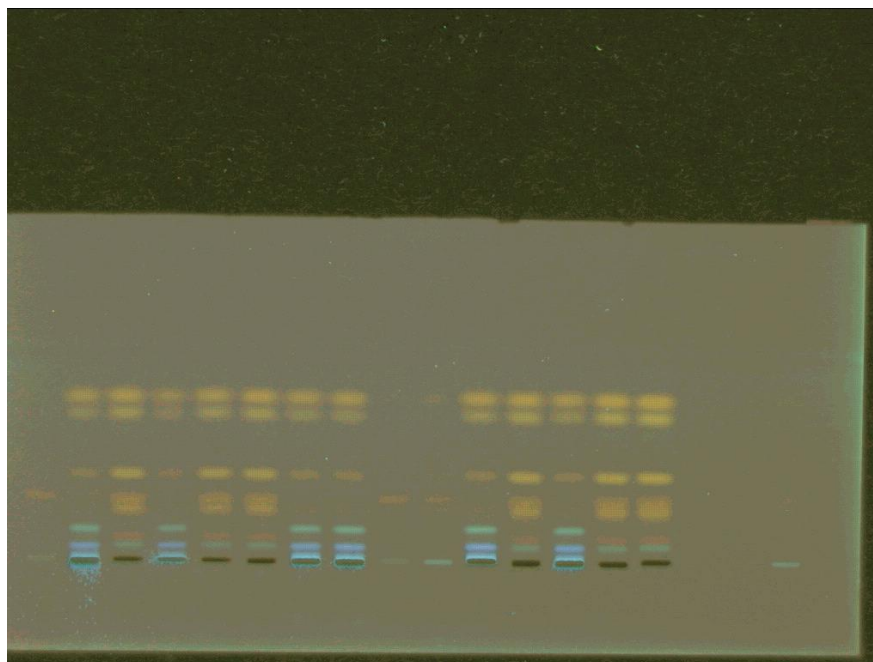


Une identification rapide de composés naturels de plantes par CCMHP

Expériences en pédagogie

Dans le cadre des TP de Pharmacognosie en 3^{ème} année de Pharmacie:
Extraction et caractérisation de dérivés anthraquinoniques

- Rhéine chez Rhubarbe officinale
- Rhaponticoside chez Rhubarbe des jardins



Une identification rapide de composés naturels de plantes par HPTLC-MS

Expériences en recherche

I) Identification de polyphénols dans des plantes de Djibouti:

fruits d' *Indigofera caerulea*

II) Identification d'anthocyanes dans des plantes du Burkina Faso:

fleurs de *Tapinanthus dodoneifolius* et *Eichhornia crassipes*

I) Identification de polyphénols dans des plantes de Djibouti: fruits d' *Indigofera caerulea*

Screening
Phytochimique &
pharmacologique



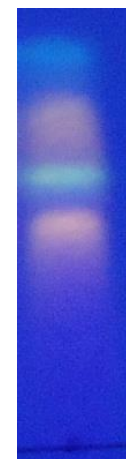
Extraction



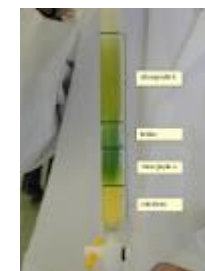
Screening
des activités
biologiques



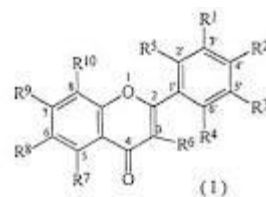
Screening
phytochimique



purification



Caractérisation structurale



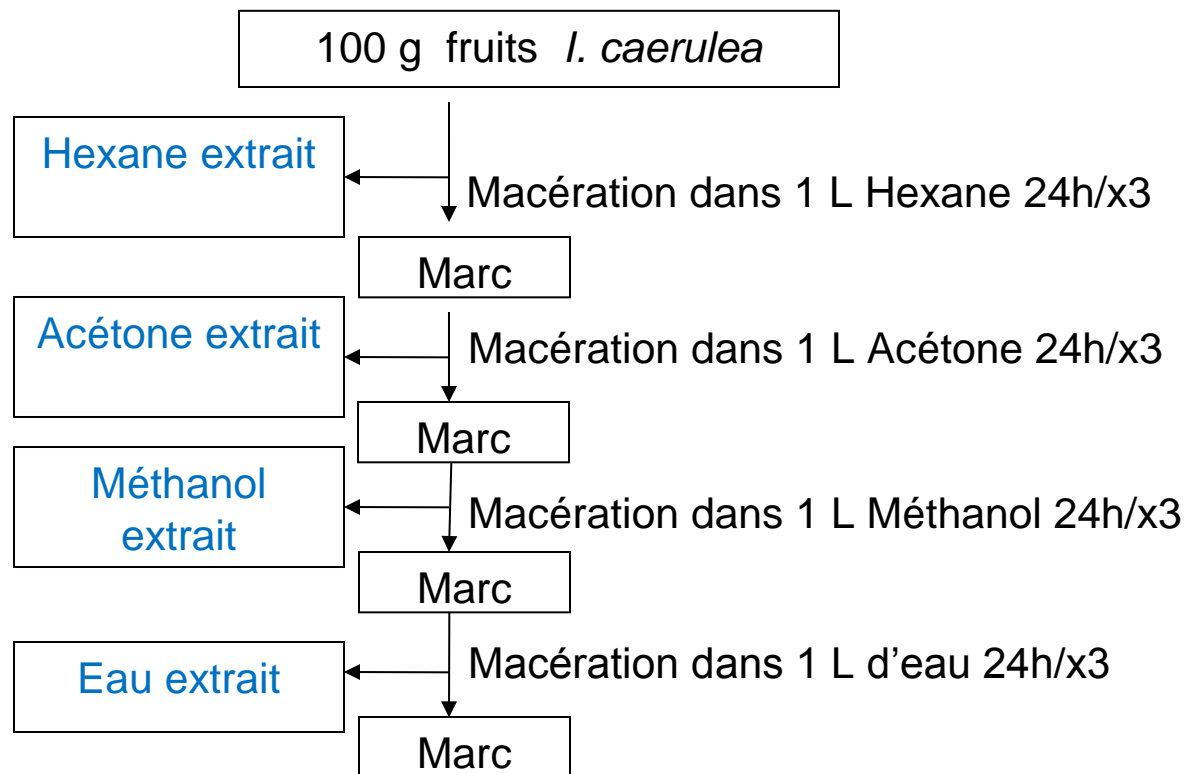
Composés purs

Caractérisation pharmacologique

Indigofera caerulea

Lieu de récolte: Abaitou, Dikhil (Djibouti)

Preparation des extraits avec des solvants de polarité croissante



Indigofera caerulea



fruits secs

Activité antimicrobienne des extraits de fruits d'*I. caerulea*

Pathogènes	Concentration Minimale Inhibitrice CMI (µg/mL) des extraits			
	Hexane	Acétone	Méthanol	eau
<i>Acinetobacter baumannii</i> ATCC19606	256	>256	>256	>256
<i>Escherichia coli</i> ATCC 25922	>256	>256	>256	>256
<i>Enterobacter cloacae</i> ATCC 13047	>256	>256	>256	>256
<i>Enterococcus faecalis</i> ATCC 29212	128	>256	128	>256
<i>Klebsiella pneumoniae</i> ATCC 700603	>256	>256	>256	>256
<i>Pseudomonas aeruginosa</i> ATCC 27853	>256	>256	>256	>256
<i>Staphylococcus aureus</i> ATCC 29213	256	>256	128	>256
<i>Candida albicans</i> ATCC 10231	>256	>256	>256	>256
<i>Aspergillus niger</i> ATCC 16888	>256	>256	>256	>256
<i>Aspergillus fumigatus</i> ATCC 1022	>256	>256	>256	>256

❖ Les extraits hexanique et méthanolique montrent des activités antibactériennes significatives contre *S. aureus* et *E. faecalis*.

❖ Seul l'extrait hexanique montre une activité antibactérienne contre *Acinetobacter baumannii*.

Activités antioxydantes des extraits de fruits d'*I. caerulea*

**Determination: des activités antioxydantes selon les méthodes DPPH, ABTS et FRAP
et FRAP
de la teneur en polyphénols totaux (TPC) et flavonoïdes (TFC)**

	DPPH IC50 ($\mu\text{g/mL}$)	ABTS IC50 ($\mu\text{g/mL}$)	FRAP $\mu\text{mol Evc/g}$ d'extrait sec	TPC (mg EAG/g d'extrait sec)	TFC (mg EQ/g d'extrait sec)
ME	97 \pm 2,2	25 \pm 1,3	625 \pm 3,1	185,1 \pm 3,1	23,1 \pm 2,3
AC	150 \pm 3,4	20 \pm 1	828 \pm 3,6	32,46 \pm 3,0	8 \pm 1,4
Vc*	70 \pm 7	70 \pm 1,5	-	-	-
Tx*	110 \pm 2,5	18 \pm 3,3	-	-	-

Standards: Vc: vitamine C Tx: Trolox
ME: Méthanol AC: Acétone

Caractérisation des polyphénols présents dans l'extrait méthanolique par HPTLC-MS

Rf= 0.53



Rf= 0.31

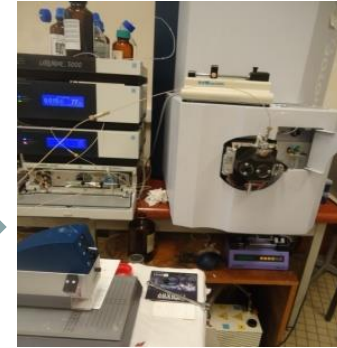
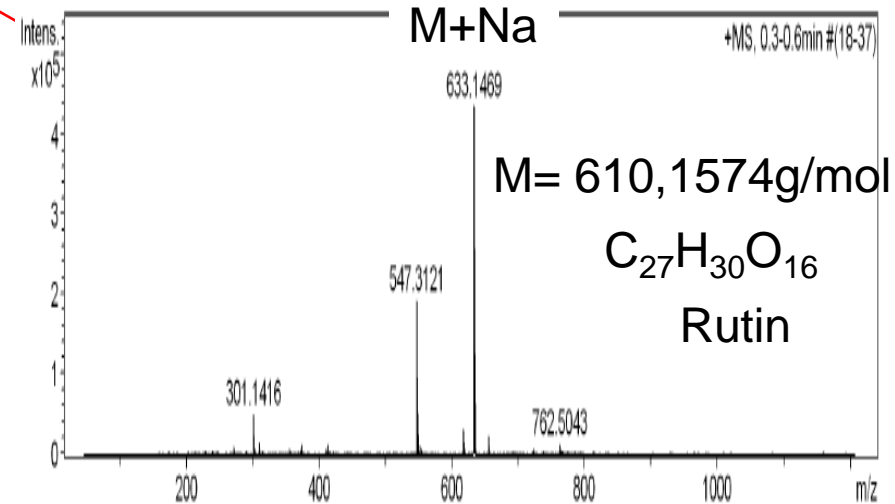
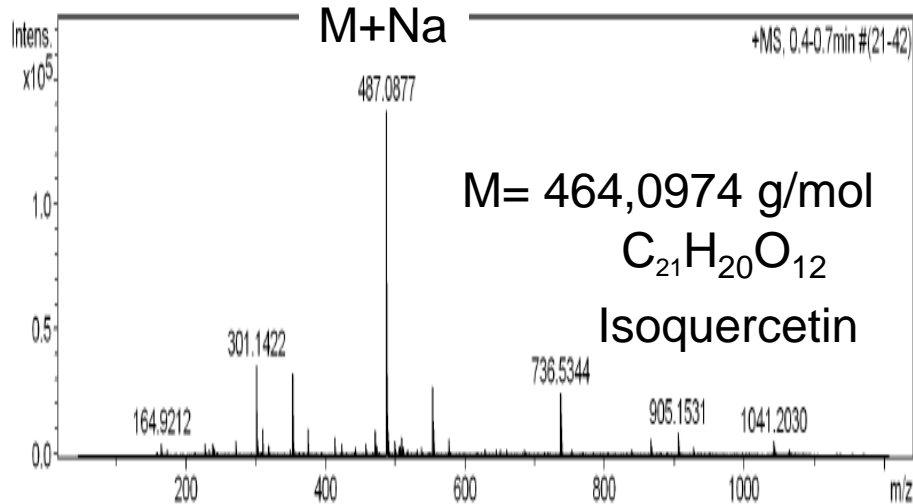


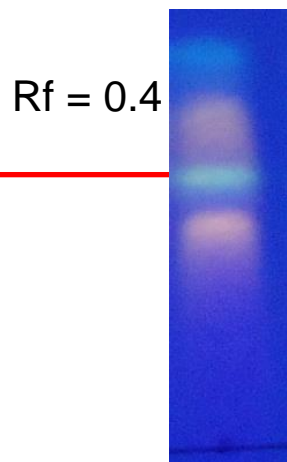
Plate HPTLC F254, R. Neu

TLC-MS interface

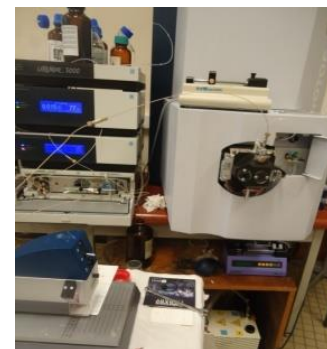
Mass spectrometry



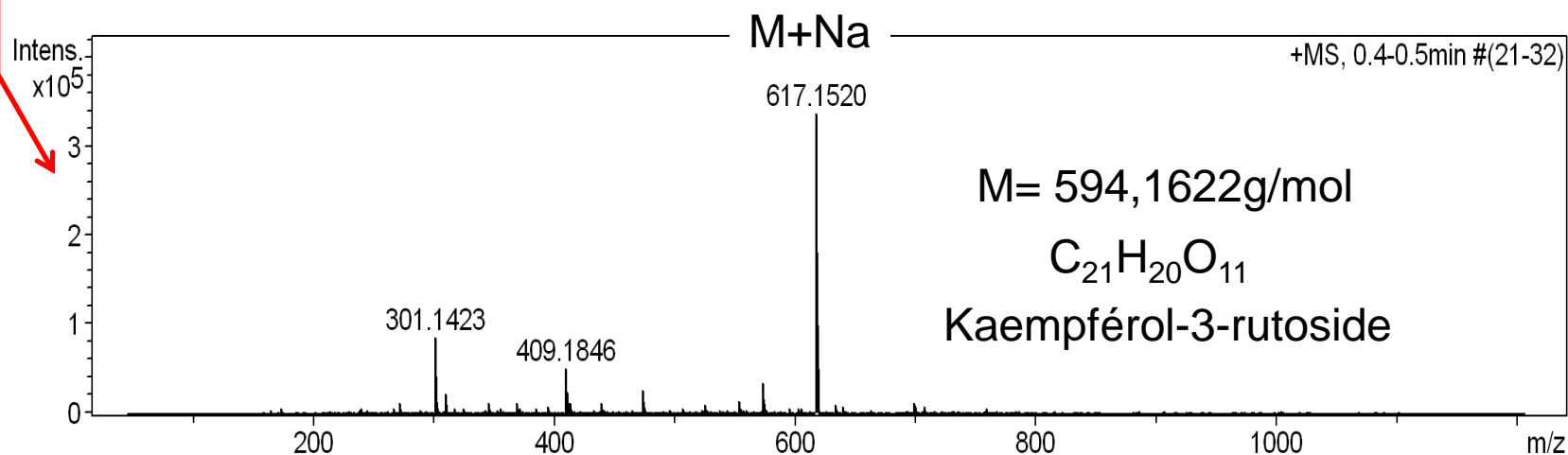
Caractérisation des polyphénols présents dans l'extrait méthanolique par HPTLC-MS



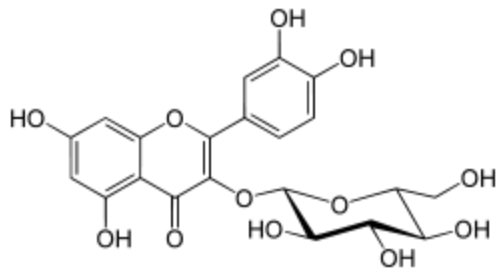
TLC-MS interface



Mass spectrometry

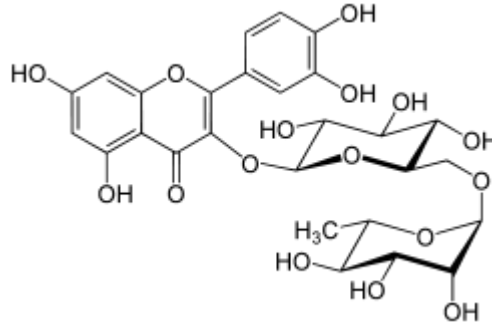


Screening phytochimique des fruits d' *Indigofera caerulea*



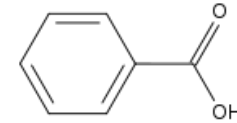
Isoquercétine

2.1 mg/g DW

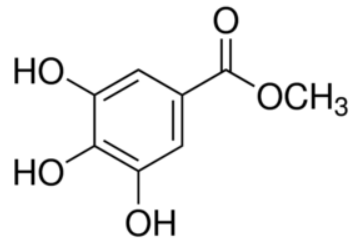


Rutine

1 mg/g DW

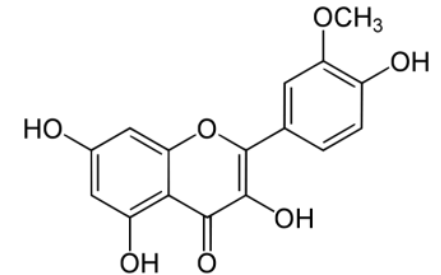
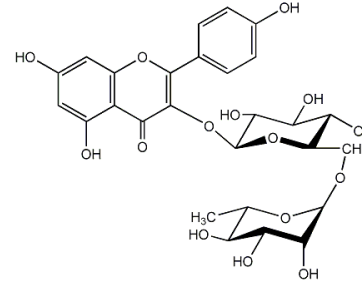


A. benzoïque

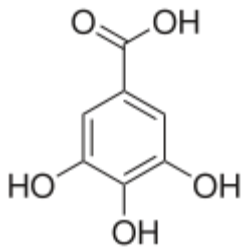


Methyl gallate

11.2 mg/g DW

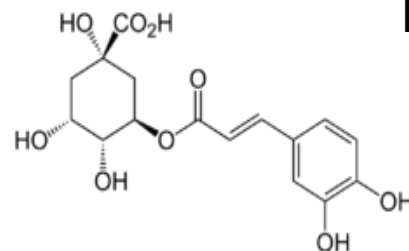


Isorhamnétine



A. gallique

1 mg/g DW



A. chlorogénique

Kaempférol-3-O- rutoside

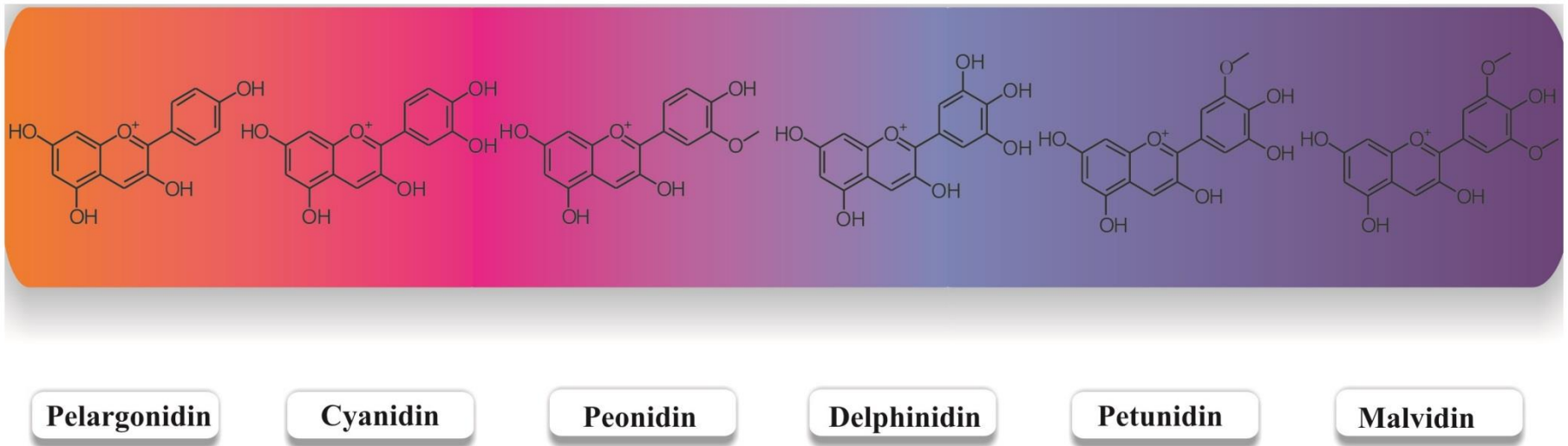
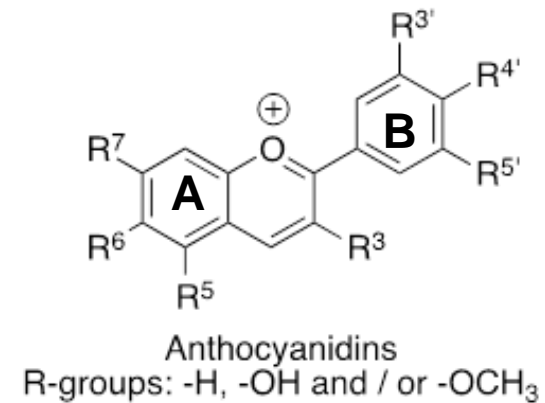
1 mg/g DW

A.Elmi, R. Spina, F. Abdoul-Latif, S. Yagi, S. Fontanay, A. Risler, R.E Duval, D. Laurain-Mattar (2018).

Industrial crops and products, 125, 123-130.

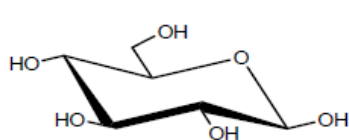
II) Identification of anthocyanins in various plants from Burkina Faso by HPTLC/MS

Background- Anthocyanidins chemistry

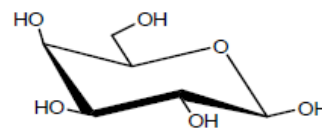


> 300 structurally different anthocyanins

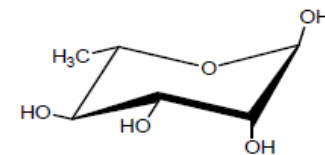
Sugars



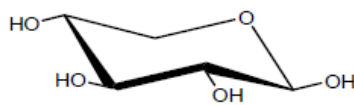
β-D-glucopyranose



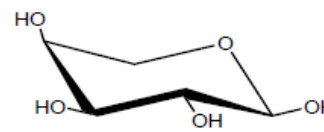
β-D-galactopyranose



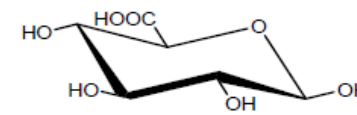
α-L-rhamnopyranose



β-D-xylopyranose

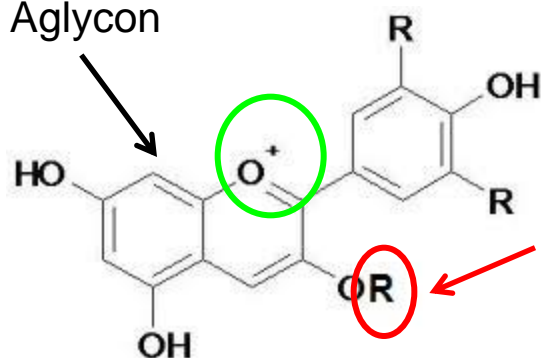


α-L-arabinopyranose

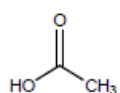


β-D-glucuronic acid

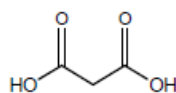
Aglycon



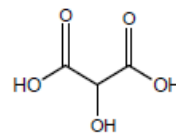
2-Phenylbenzopyrylium cation



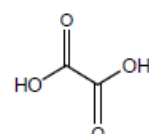
acetic acid



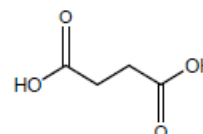
malonic acid



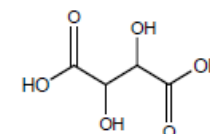
malic acid



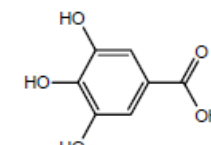
oxalic acid



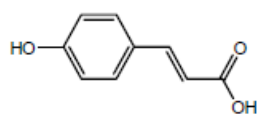
succinic acid



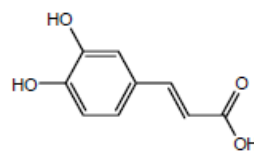
tartaric acid



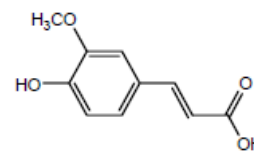
gallic acid



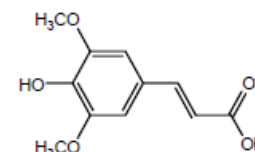
p-coumaric acid



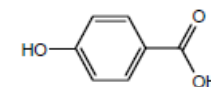
caffeic acid



ferulic acid



sinapic acid



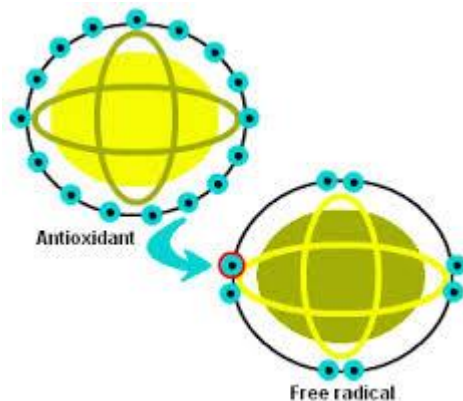
p-hydroxybenzoic acid

Acyl moiety

Are used as a food-colorant

E163

Have antioxidant properties



Potential therapeutic role to:

-Cardiovascular diseases

-Cancer

-Inhibition of several virus

- Improvement of visual acuity

Ref. Kong et al. Phytochemistry 64 (2003) 923-933

Burkina Faso's plants studied

HPTLC separation conditions

for qualitative and quantitative analysis of anthocyanins



Tapinanthus dodoneifolius
Loranthaceae
Flowers



Burkina Faso



Eichhornia crassipes
Pontederiaceae
Flowers



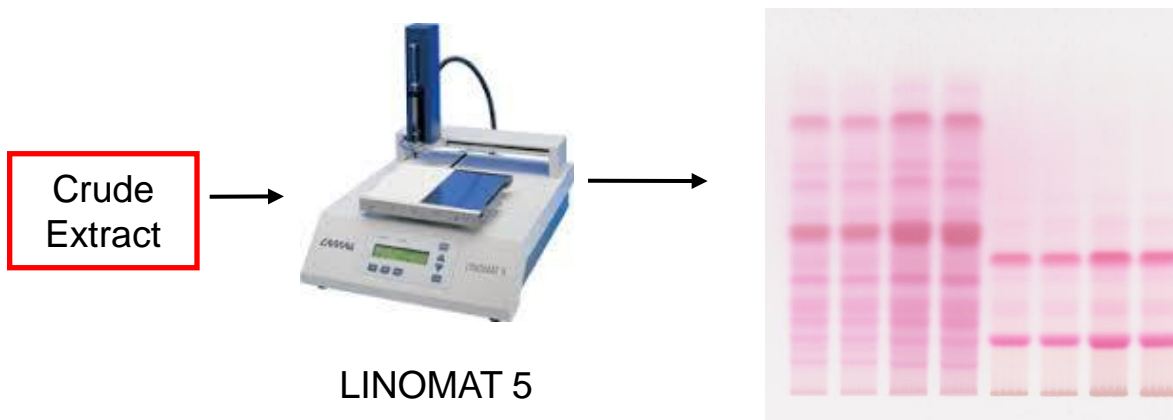
TLC/MS
INTERFACE
CAMAG

SCANNER TLC 4
CAMAG





Screening stationary and mobil phase



HPTLC plate silica gel 60 F₂₅₄

Different solvents systems

HPTLC plates RP-18 WF_{254S}

Different solvents systems





Results by HPTLC/MS from crude extract of *Tapinanthus dodoneifolius*

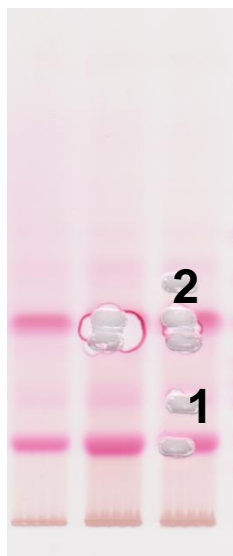
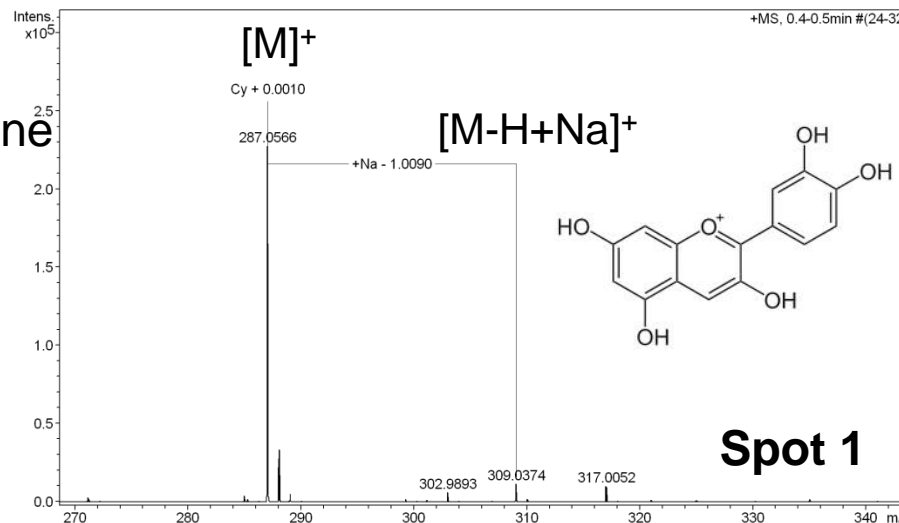


Tapinanthus dodoneifolius
Loranthaceae
Flowers

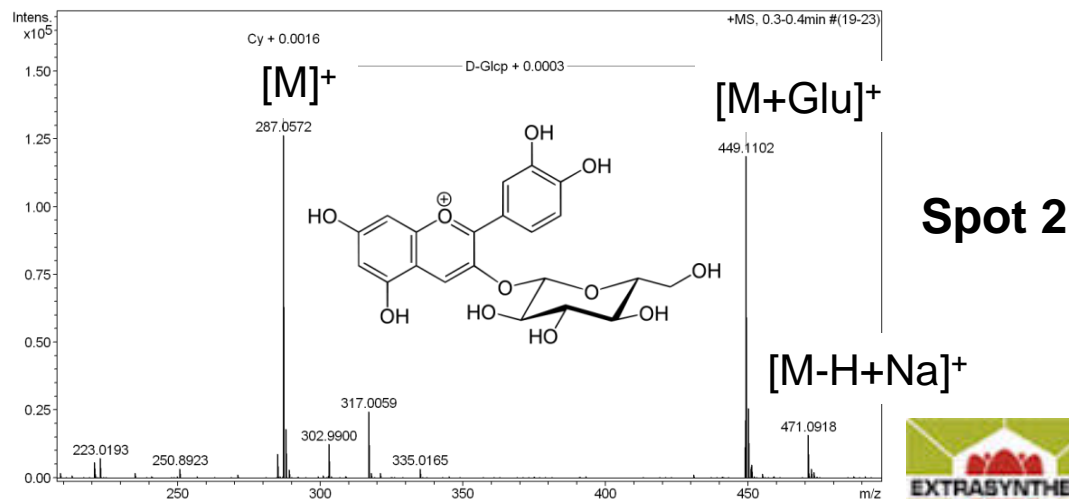


MicroTofQ
Bruker

Cyanidine



TLC/MS interface



Cyanidine glucoside



Results by HPTLC/MS from crude extract of *Eichhornia crassipes*



Eichhornia crassipes
Pontederiaceae
Flowers

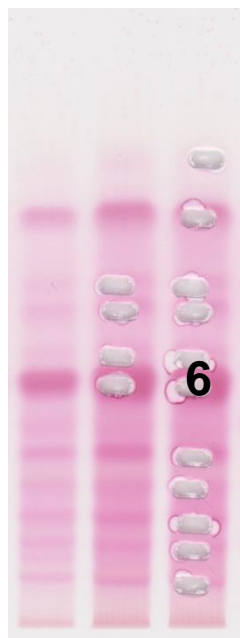
		[M] ⁺	MS/MS
Spot 11	No detectable	-	-
Spot 10	Delphinidin-3,5-diglucoside	627.1586	465.1057/ 303.0414
Spot 9	Delphinidin diglucoside + malic acid	713.1622	551.1121/465.1029/ 303.0514
Spot 8	Delphinidin diglucoside+succinic acid	727.1793	565.1376/ 303.0514
Spot 7	Delphinidin derivatives 2	611.1648	303.0414
Spot 6	Delphinidin diglucoside+succinic acid	727.1736	565.1172/465.1032/ 303.0492
Spot 5	Delphinin- derivatives 1	551.1075	303.0509
Spot 4	Delphinidin-3-glucoside	465.1062	303.0509
Spot 3	Pelargonidin-3-glucoside	433.1016	271.0530
Spot 2	Pelargonidin 3-(6''coumaroyl) glucoside	579.1379	271.0530
Spot 2	Delphinidin	303.0483	
Spot 1	Cyanidin	287.0470	

Results from HRMS

Example of HPTLC/MS from *Eichhornia crassipes*



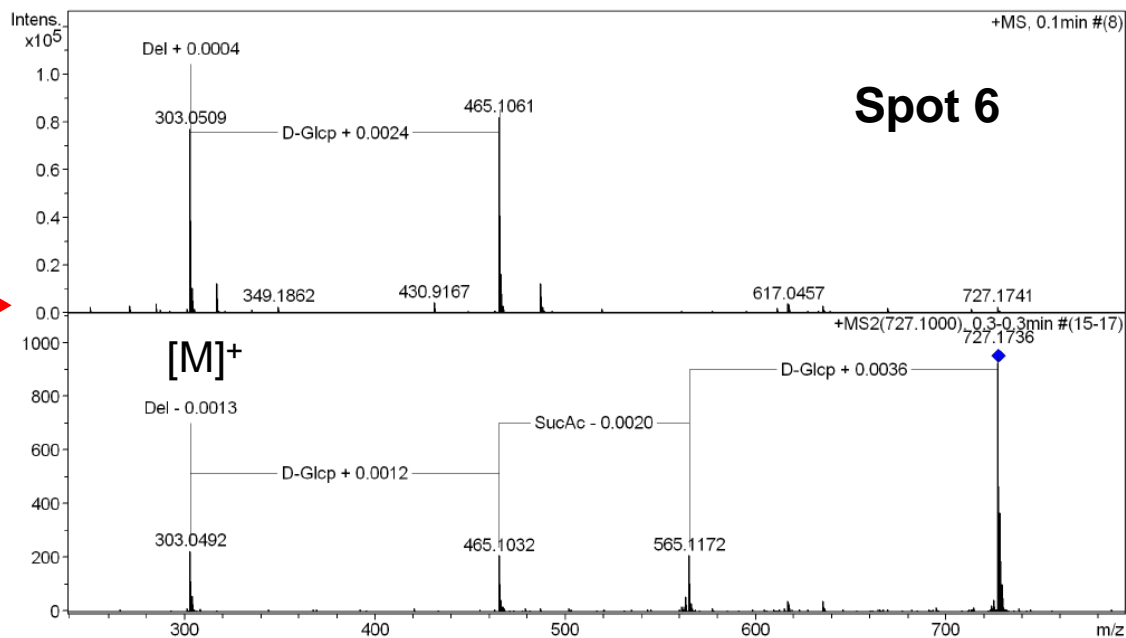
Eichhornia crassipes
Pontederiaceae
Flowers



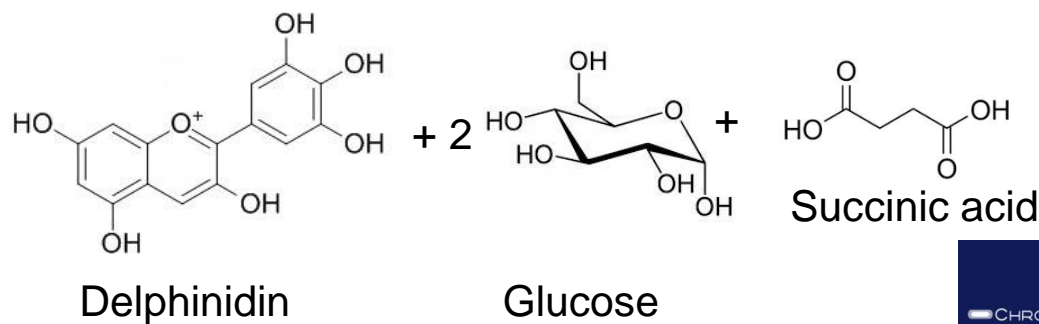
MicroTofQ
Bruker



TLC/MS interface



MS and MS/MS spectra





**SCANNER TCL 4
CAMAG**

Sample	Compound	nm	ng/spot	r	sdv %
<i>Tapinanthus dodoneifolius</i>	Cyanidin	530	1918	0.99782	2.06
<i>Eichhornia crassipes</i>	Cyanidin	530	736.25	0.99782	2.06
<i>Tapinanthus dodoneifolius</i>	Cyanidin-3-glucoside	530	1896	0.99741	2.94
<i>Eichhornia crassipes</i>	Pelargonidin	510	605.25	0.99739	1.33
<i>Eichhornia crassipes</i>	Pelargonidin-3-glucoside	520	840.87	0.99853	1.57
<i>Eichhornia crassipes</i>	Delphinidin	555	1109	0.99292	6.33
<i>Eichhornia crassipes</i>	Delphinidin-3-glucoside	555	1712	0.99800	3.79
<i>Eichhornia crassipes</i>	Delphinidin-3,5-diglucoside	530	1816	0.99917	1.08



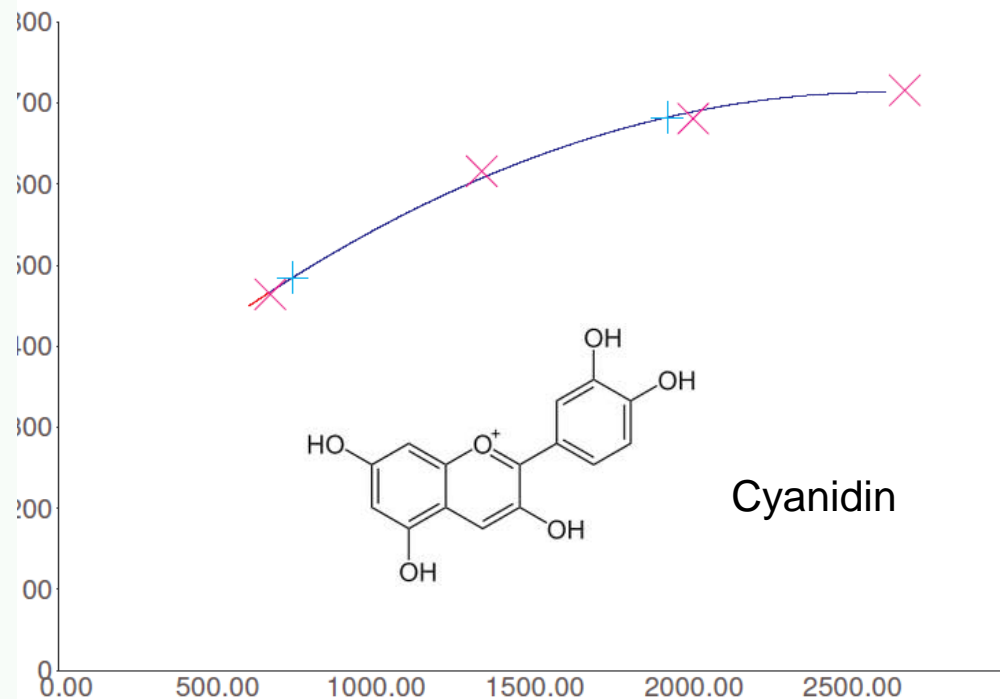
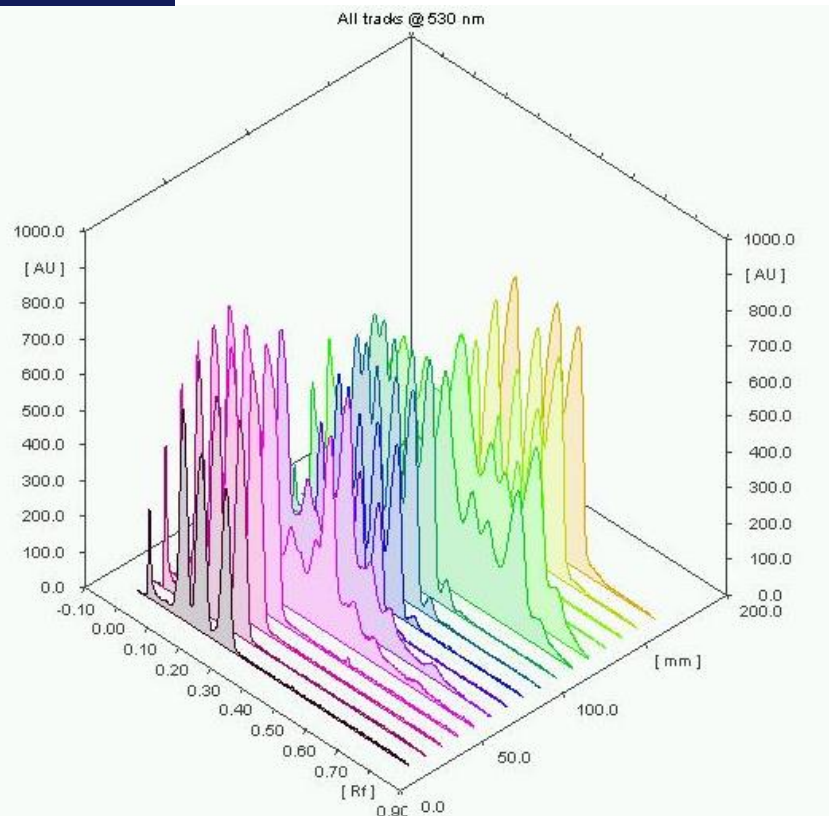
Correlation coefficient > 0.99
Regression via height: polynomial;
Volume applied of samples 10 μ L;

Concentration of solutions of crude extract: 5 mg/0,5 mL in MeOH

Substance: cyan @ 530 nm

Regression via height: Polynomial $Y = 268.3 + 0.3419 * X + -6.569e-005 * X^2$
 $r = 0.99782$ $sdv = 2.06$

CHROMACIM



HPTLC 3D-densitogram of absorbance measurement at 530nm for all tracks

Polynomial calibration of cyanidin via peak height by absorption at 530 nm



Conclusion



Methanolic extract



Linomat 5 CAMAG

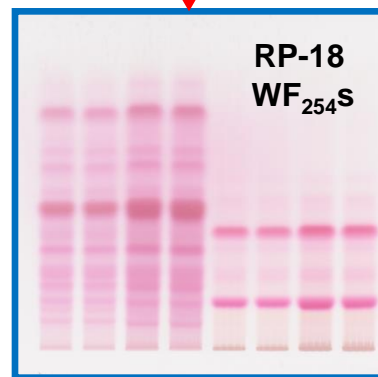


Methanolic extract



Scanner TLC 4
CAMAG

Quantification of
7 anthocyanins

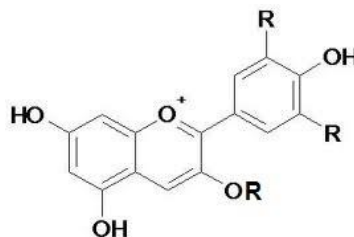


TLC-MS interface
CAMAG



MicroTofQ
BRUKER

Determination of
14 anthocyanins



Merci pour votre attention
Merci au club de CCM

