



IPB University
Bogor Indonesia

- Departemen Kimia FMIPA
- Pusat Studi Biofarmaka Tropika LPPM
- AR-Lab LPPM

Peran Kemometrik dan Metabolomik dalam Kendali Mutu Produk Bahan Alam

Mohamad Rafi & Rudi Heryanto

Kuliah Tamu Fakultas Farmasi dan Sains, UHMAKA
10 Desember 2021



Biosketch



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Dosen dan peneliti di Departemen Kimia FMIPA dan Pusat Studi Biofarmaka Tropika LPPM IPB. Saat ini diberi amanah sebagai Kaprodi S2/S3 Kimia, PIC Lab Metabolomik AR-Lab, dan ketua editor Jurnal Jamu Indonesia, IPB. Selain itu juga sebagai Ketua Himpunan Kimia Indonesia 2020-2021.

Penelitian yang dilakukan banyak di bidang metabolomik dan kemometrik untuk pengembangan metode kendali mutu, identifikasi senyawa penciri dan standardisasi tumbuhan untuk bahan baku obat herbal/pangan. Email: mra@apps.ipb.ac.id

Himpunan profesi yang diikuti:

- Himpunan Kimia Indonesia/Indonesian Chemical Society
- Metabolomics Society
- The Society of Chromatographic Science, Japan
- HPTLC Association
- Perhimpunan Peneliti Bahan Obat Alami/The Indonesian Association of Natural Drugs Researcher



<http://mra.staff.ipb.ac.id/>

Education

- B.Sc - IPB University, Indonesia
- M.Sc – IPB University, Indonesia
- Ph.D – Gifu University, Japan

PUBLIKASI

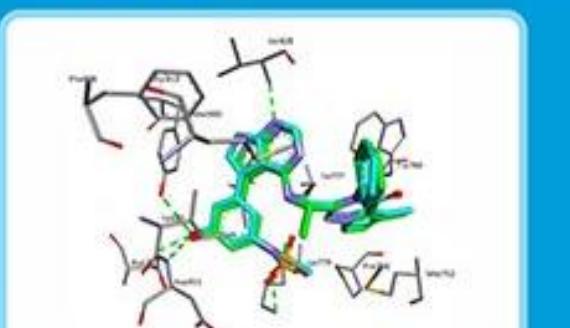
01

Feasibility of UV-Vis spectral fingerprinting combined with chemometrics for rapid detection of *Phyllanthus niruri* adulteration with *Leucaena leucocephala* (accepted in *Sains Malaysiana*. 2021)



Indonesian Journal of Chemistry

Vol. 20, No. 5, October 2020

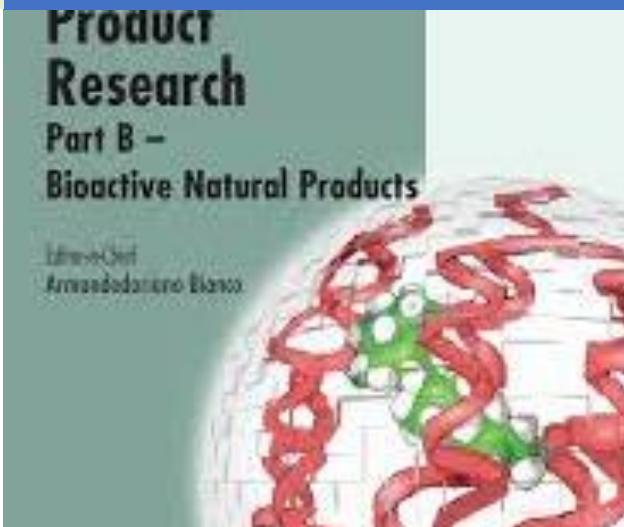


02

FTIR-based fingerprinting and chemometrics for rapid investigation of antioxidant activity from *Syzygium polyanthum* extracts (*Indones. J. Chem.* 2021)

03

Ripe Pulp Metabolite Profiling of Ten Indonesian Dessert Banana Cultivars Using UHPLC-Q-Orbitrap HRMS (*Eur Food Res Technol.* 2021)



04

Metabolite Profiling of *Andrographis paniculata* Leaves and Stem Extract Using UHPLC-Orbitrap-MS/MS (*Nat. Prod. Res.* 2020)



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Materi presentasi



Kemometrik dan metabolomik:
definisi dan ruang lingkup

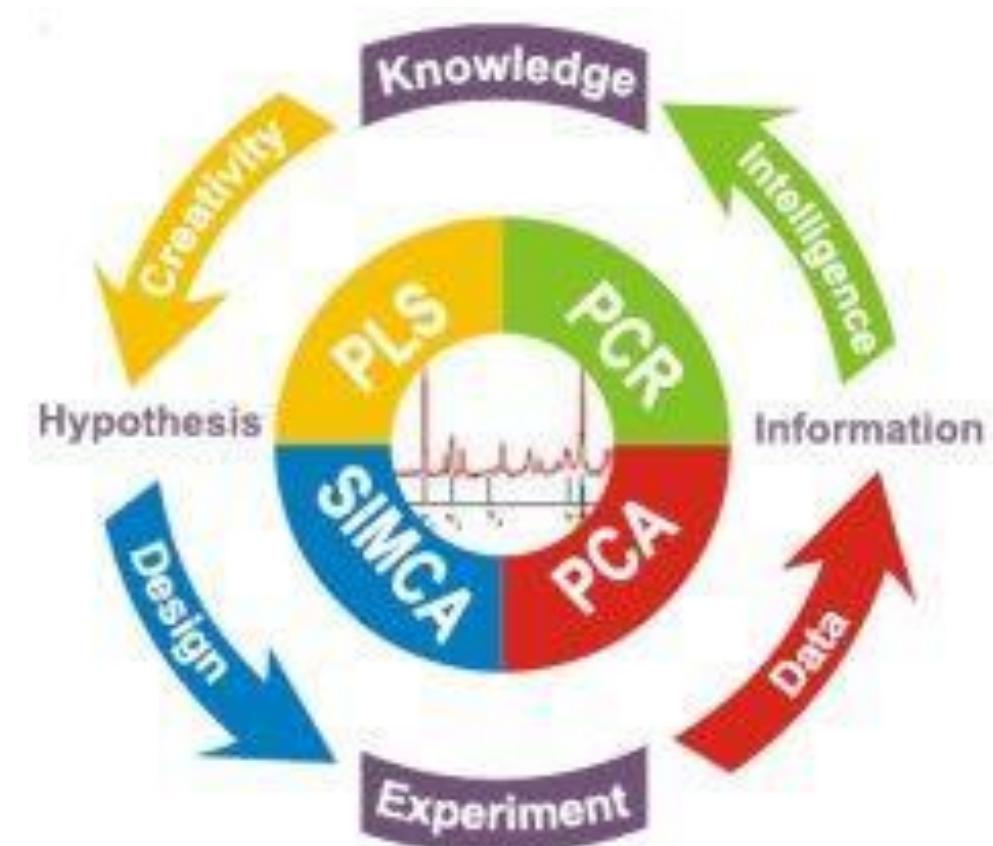
Karakteristik tumbuhan obat dan obat herbal

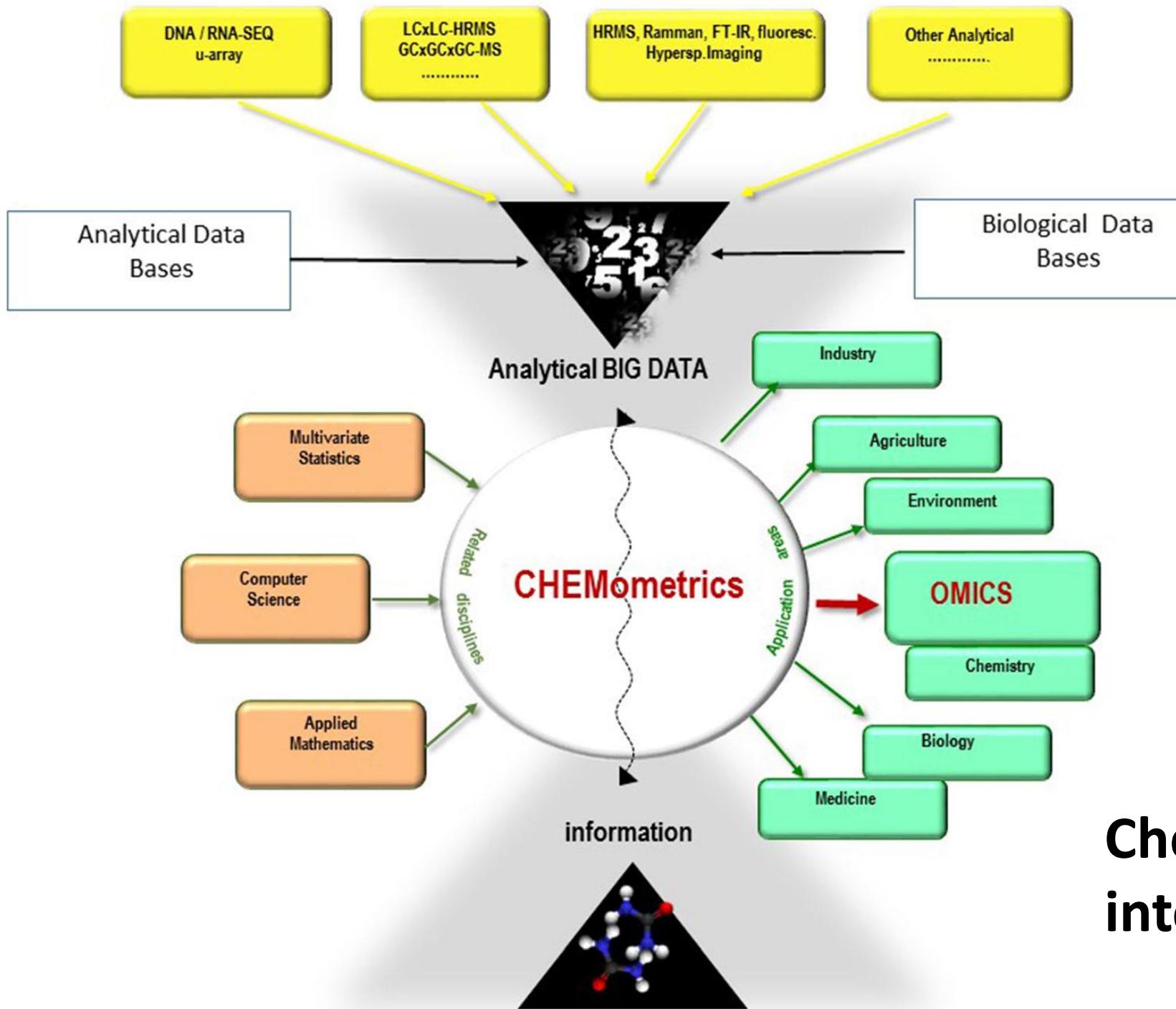
Kendali mutu produk herbal menggunakan kemometrik dan metabolomik

Kemometrik --- ilmu yang terkait dengan pengukuran yang dilakukan dalam sistem kimia atau prosesnya via aplikasi metode matematika atau statistika (International Chemometrics Society)*

Komponen dasarnya:

- Metode
 - Desain eksperimen
 - *Pattern recognition*
 - Kalibrasi
- Peranti lunak
- Instrumentasi
- Aplikasi



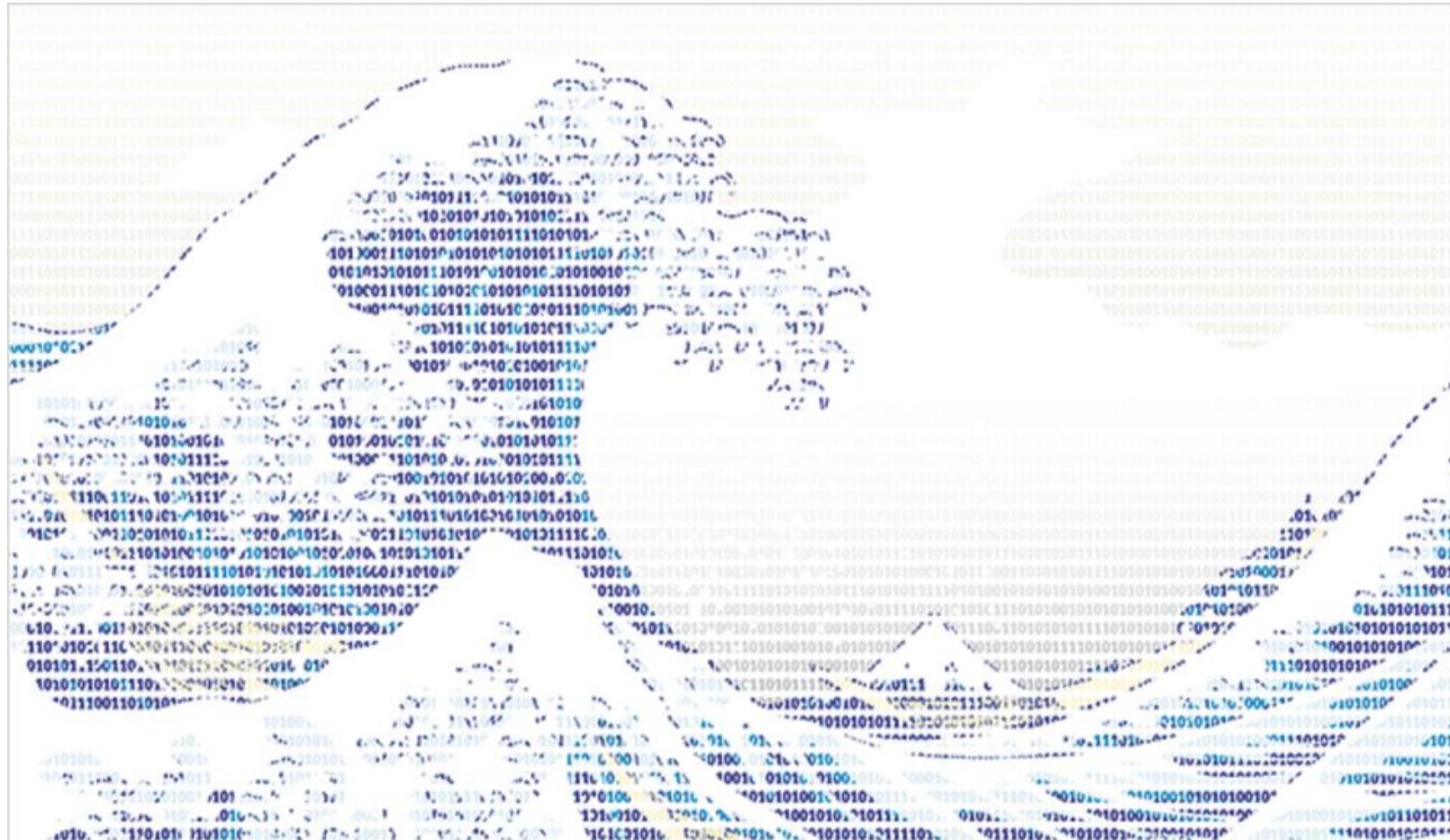


**Chemometrics as an
interdisciplinary field**

Kemometrik



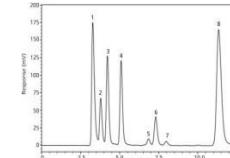
Adanya banjir data yang dihasilkan oleh instrumen analitik modern, memberikan angka-angka dalam jumlah besar untuk memahami dan mengkuantifikasi fenomena disekeliling kita



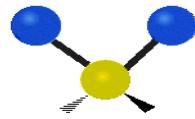
Kemometrik



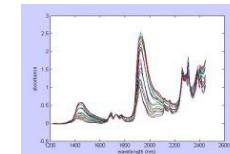
HPLC (or GC)



10¹ - 10² peaks



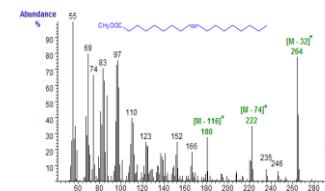
IR (FT/IR)/ UV



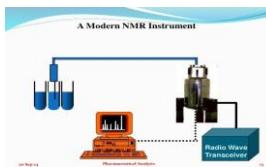
10³ DATA POINTS



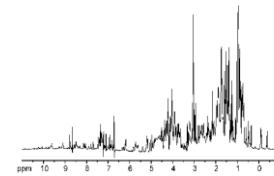
HPLC/GC-MS



10⁵ – 10⁶ DATA POINTS



NMR

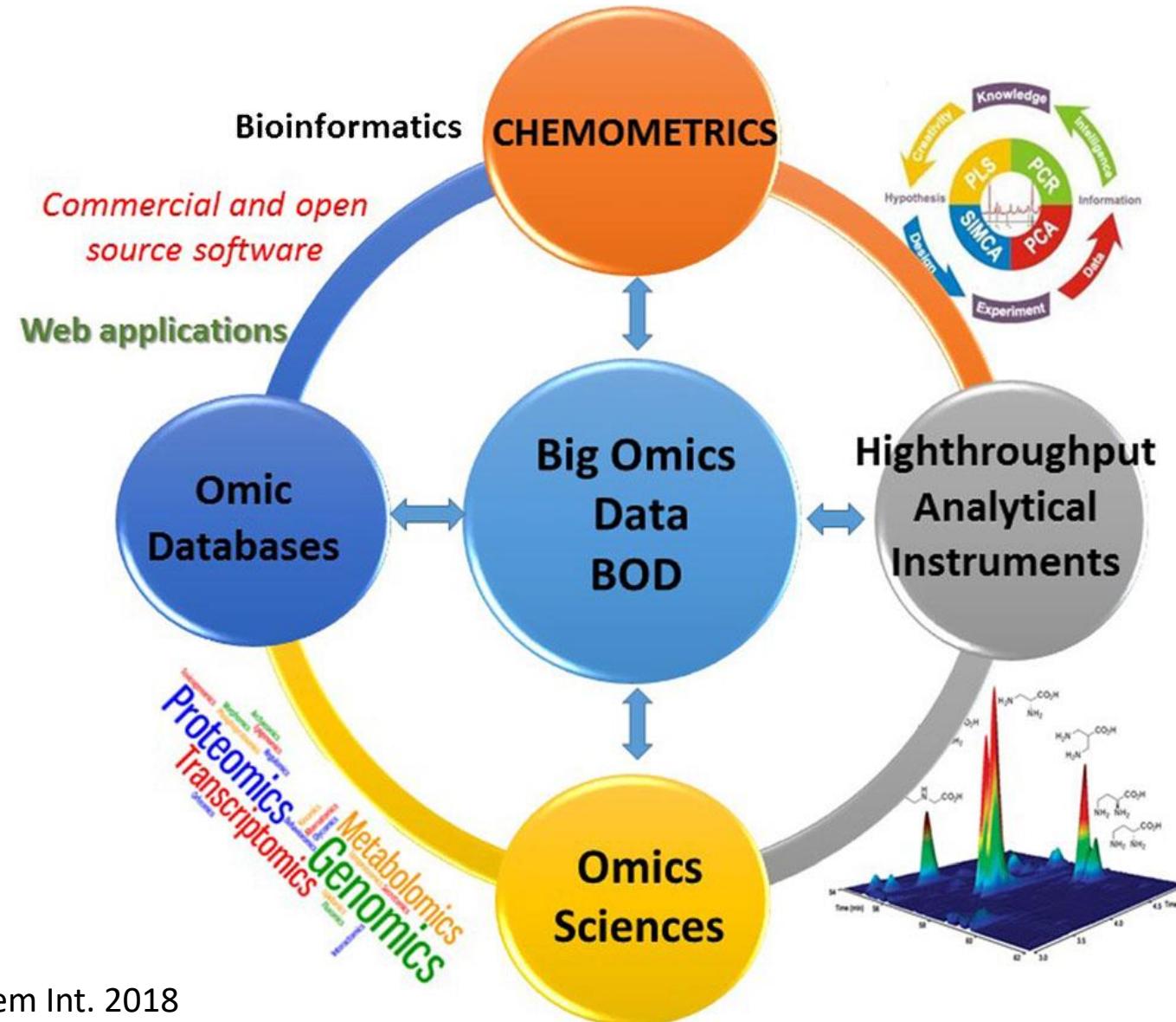


10⁴ - 10⁵ DATA POINTS

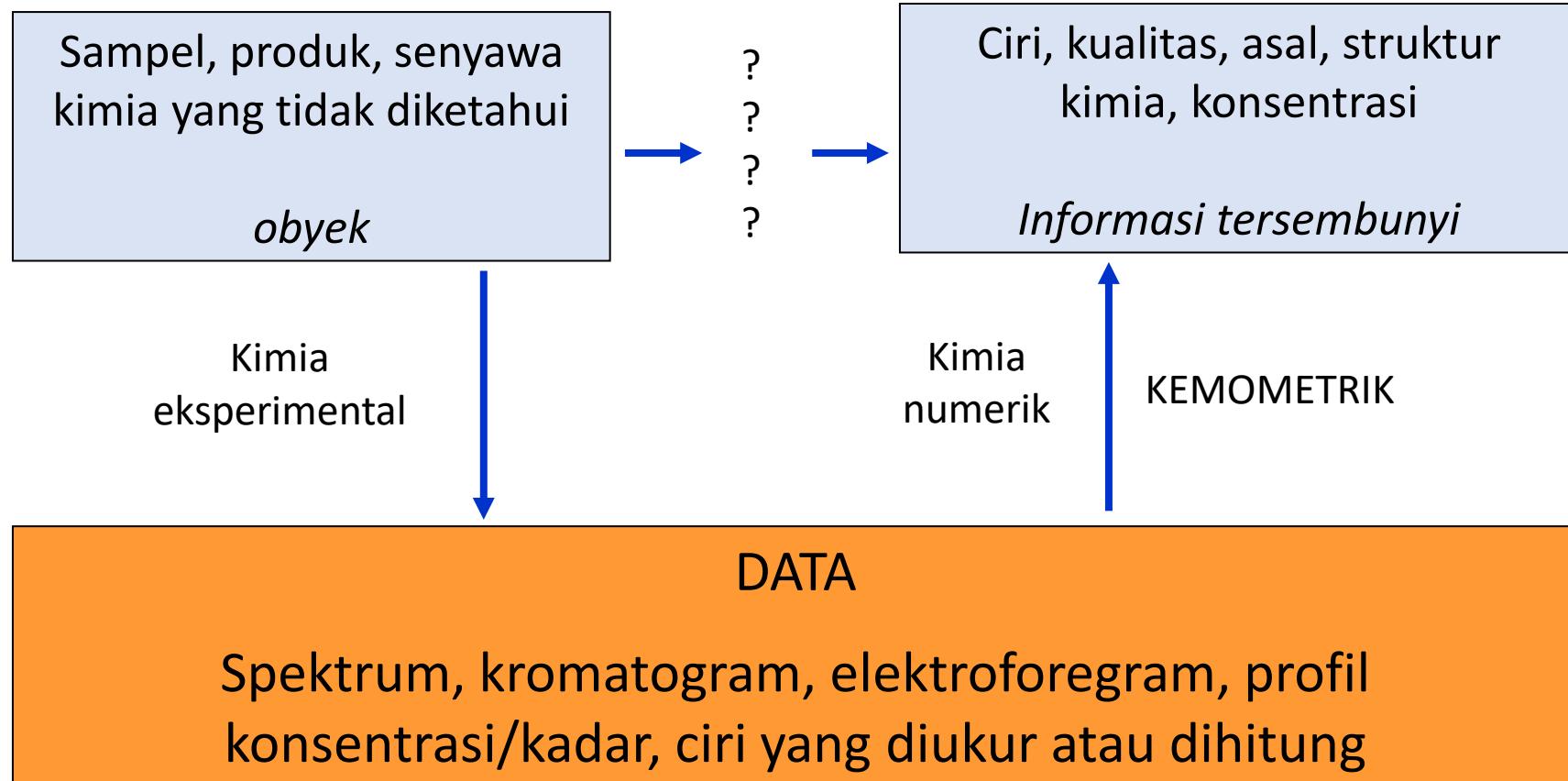
Kemometrik



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Tantangan Data - Informasi



Multivariate data analysis



Exploratory data analysis: to describe how the data is distributed automatically (unsupervised), looking for groups of similar objects, looking for outliers ---
Principal component analysis, Cluster Analysis



Classification: for pattern recognition based on a predefined class (supervised) ---
Discriminant analysis, Support Vector Machines



Multivariate calibration: build a mathematical model that describes the relationship between independent variable data and response variables --- Multiple Linear Regression, Principal Component Regression, Partial Least Square Regression

Permasalahan yang muncul dari produk obat herbal



Sesuatu yang sangat
penting dalam
produk obat herbal

- Substitusi bahan baku
- Penambahan bahan pemalsu dengan kadar tertentu
- Pencampuran dengan bahan kimia berbahaya maupun obat (jamu)

Permasalahan yang muncul dari produk obat herbal



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Perlunya Kendali Mutu Tumbuhan --- Pangan dan Obat Herbal



Kunyit (*C. longa*)



Temulawak
(*C. xanthorrhiza*)



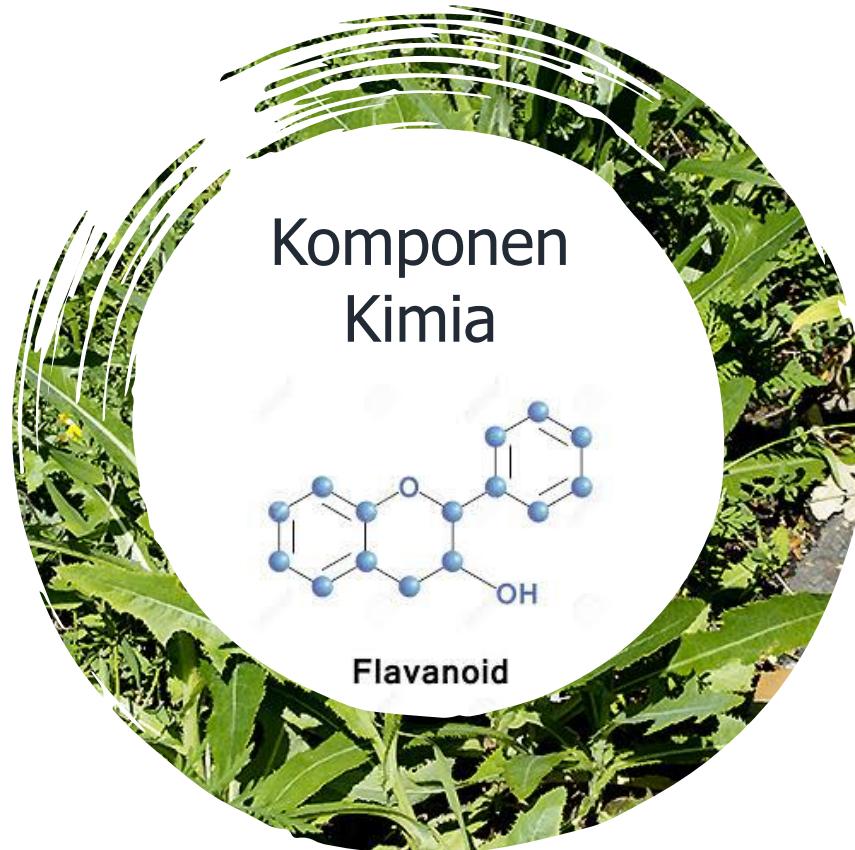
Sudan S Orange



Serbuk dari:

- Kunyit??
- Temulawak??
- Campuran kunyit dan temulawak??
- Atau bubuk kunyit plus *sudan s orange*?
- Atau.....

Tumbuhan obat

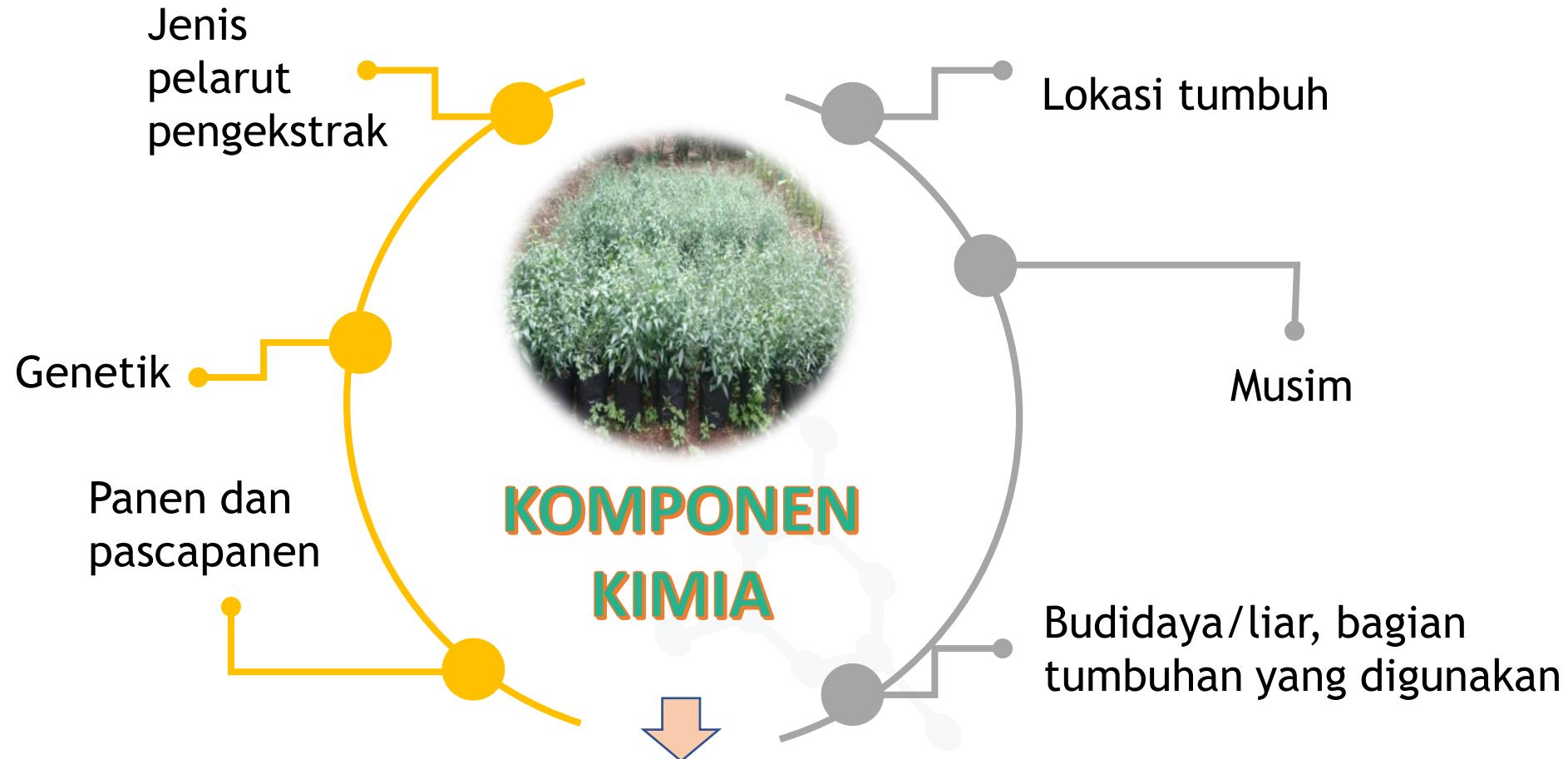


Aktivitas
Biologis

Karakteristik tumbuhan obat

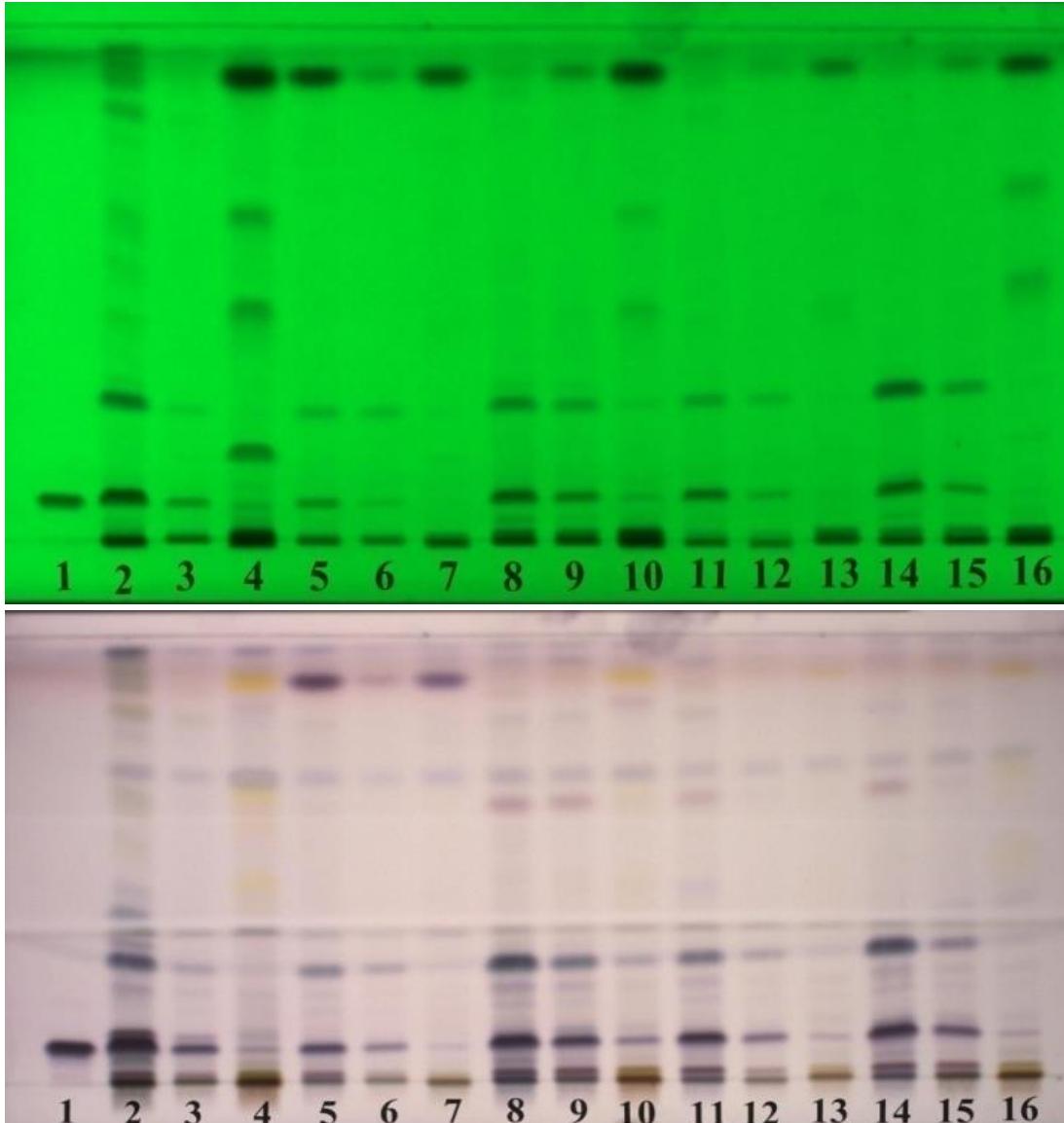


Faktor yang mempengaruhi komposisi dan konsentrasi metabolit



Mempengaruhi level aktivitas biologis yang ditimbulkan

Karakteristik obat herbal



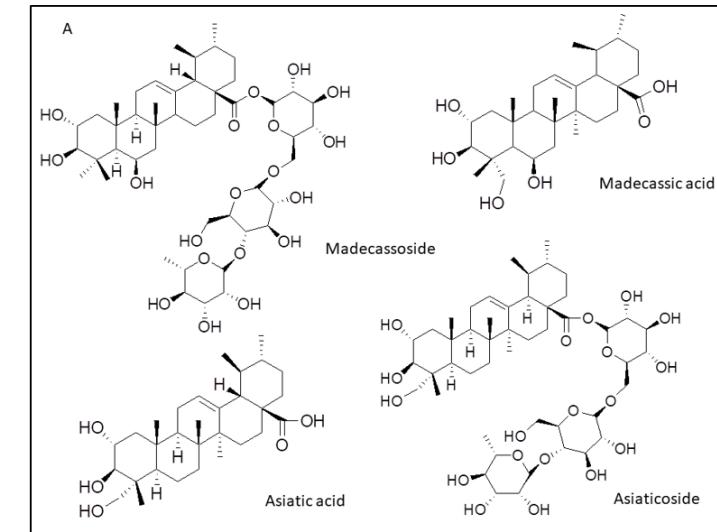
Track	Plant parts and sample origin
1	Andrographolide
2	AP leaves (Bogor, West Java)
3	AP stem sambiloto (Bogor, West Java)
4	AP roots (Bogor, West Java)
5	AP leaves (Sleman, Special Region of Yogyakarta)
6	AP stem (Sleman, Special Region of Yogyakarta)
7	AP roots (Sleman, Special Region of Yogyakarta)
8	AP leaves (Solo, Central Java)
9	AP stem (Solo, Central Java)
10	AP roots (Solo, Central Java)
11	AP leaves (Sukoharjo, Central Java)
12	AP stem (Sukoharjo, Central Java)
13	AP roots (Sukoharjo, Central Java)
14	AP leaves (Boyolali, Central Java)
15	AP stem (Boyolali, Central Java)
16	AP roots (Boyolali, Central Java)

Karakteristik obat herbal



CA samples	Analyte content (mg/g), n = 3			
	MS	AS	MA	AA
Bogor	7.62	7.71	2.8	2.6
Sleman	2.39	2.47	1.28	1.51
Boyolali	0.56	1.6	0.65	0.93
Kuningan	1.87	2.59	0.52	0.59
CA-1	4.22	2.99	2.1	0.69
CA-2	1.29	1.1	2.86	2.24
CA-3A1	8.09	8.23	2.09	1.52
CA-3A2	7.55	7.51	2.43	2.06
CA-3A3	6.02	6.03	1.98	1.49
CA-3A4	5.73	5.85	1.98	1.33
CA-3A5	6.18	6.25	1.8	1.32
CA-3A6	8.04	7.52	1.73	1.48
CA-3A7	5.55	5.96	2.3	2.44
CA-4A1	2.59	2.45	6.39	4.76
CA-4A2	8.44	8	5.22	3.68
CA-4A3	6.81	6.6	5.48	4.46
CA-4A4	9.92	9.77	4.14	3.1
CA-4A5	15.12	13.55	1.27	1.16
CA-4A6	7.45	7.82	4.1	3.36
CA-4A7	7.1	6.59	4.49	3.18
CA-5A1	8.22	8.21	1.25	1.11
CA-5A2	7.52	7.45	0.9	0.43
CA-5A3	7.56	7.16	0.66	0.08
CA-5A4	8.93	9.05	0.8	0.31
CA-5A5	6.78	6.85	0.61	0.04
CA-5A6	7.53	7.41	0.6	0.11
CA-5A7	7.61	7.14	0.65	0.04

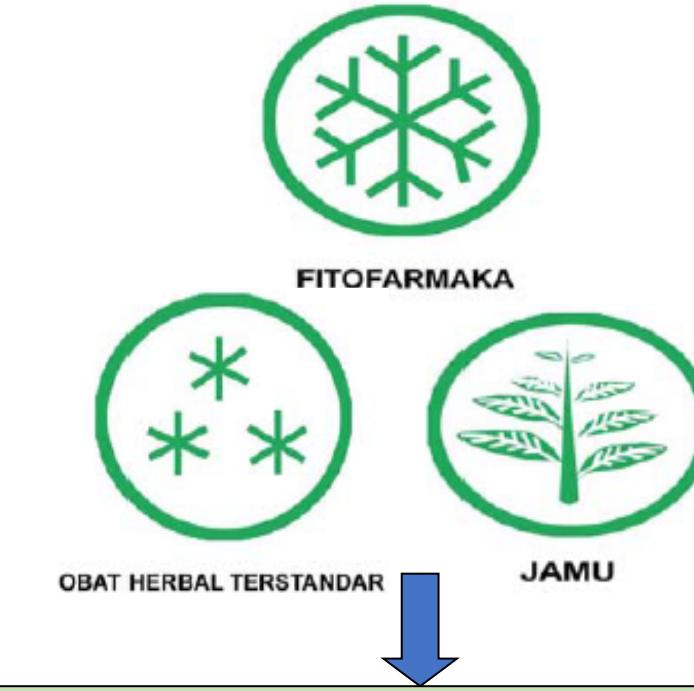
Kadar madekasosida, asiatikosida, asam madekasat dan asam asiatat pada pegagan dengan waktu panen berbeda



Karakteristik obat herbal



Multikomponen

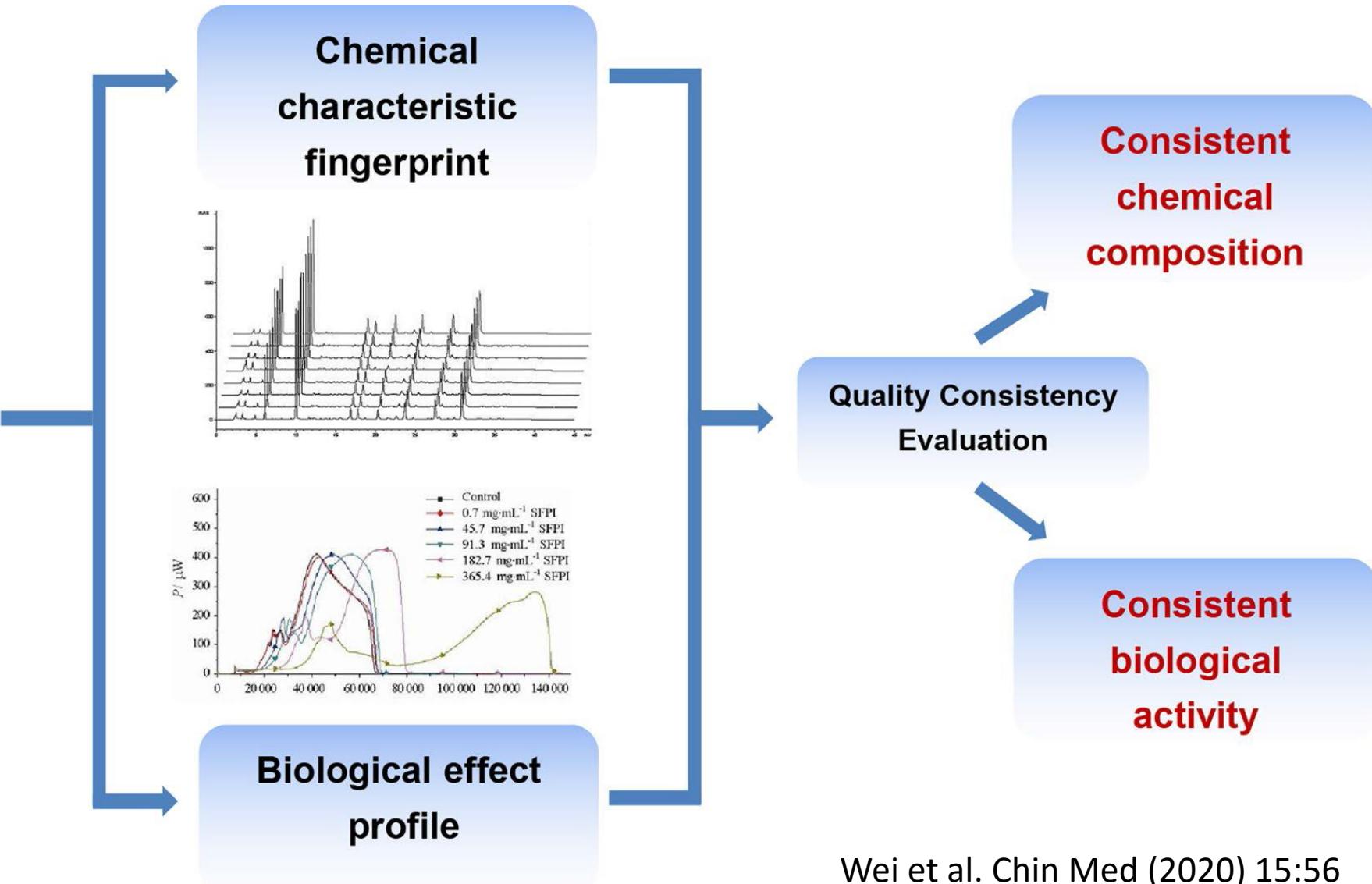


Dapat lebih dari satu bahan --
Multikomponen

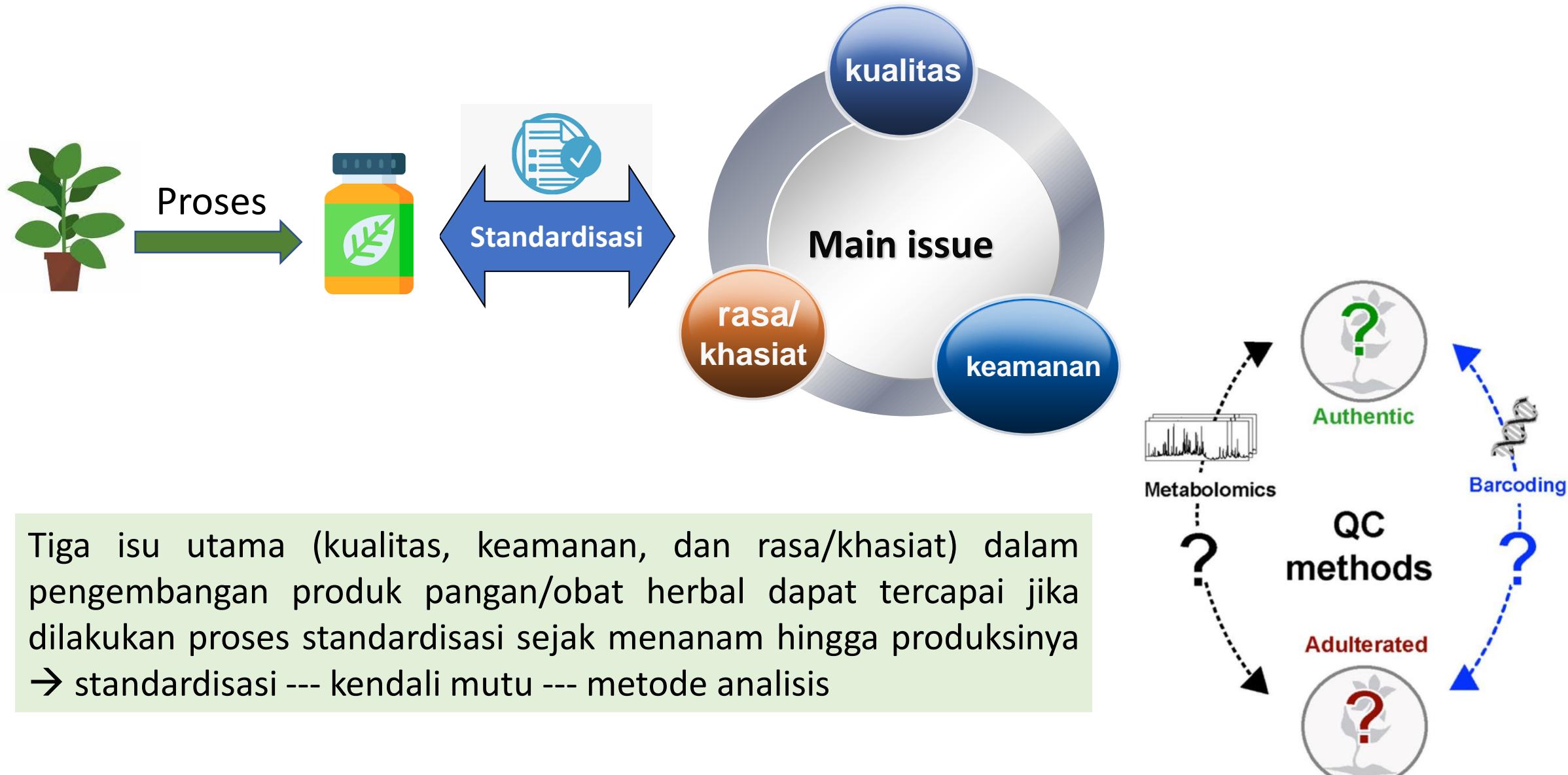


Sinergi atau antagonis?

Standardisasi produk obat herbal



Standardisasi produk obat herbal



Metabolomik



.....identifikasi dan kuantifikasi sistematis molekul kecil/metabolit* hasil metabolisme (metabolom)^{\$} dari suatu sistem biologis (sel, jaringan, organ, cairan biologis, atau organisme) pada titik waktu tertentu

(<https://www.nature.com/subjects/metabolomics>)

* Metabolit: setiap molekul organik yang terdeteksi dalam tubuh dengan MW <1500 Da

\$ Metabolom: koleksi lengkap metabolit/molekul kecil di sel, organ, jaringan atau organisme

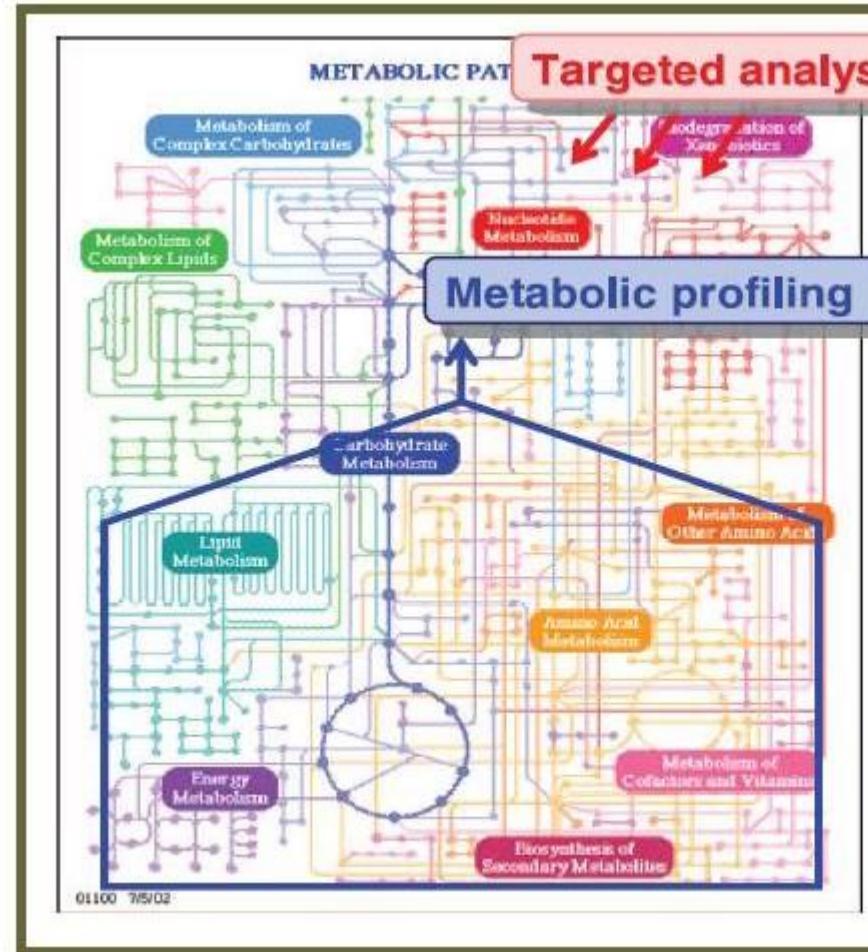
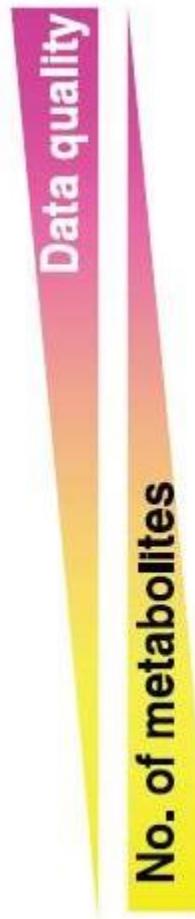


Nothing new in metabolomics!

Just:

- Different direction
- Combination of present technology

Strategi metabolomik



Detection and precise quantification of target compounds

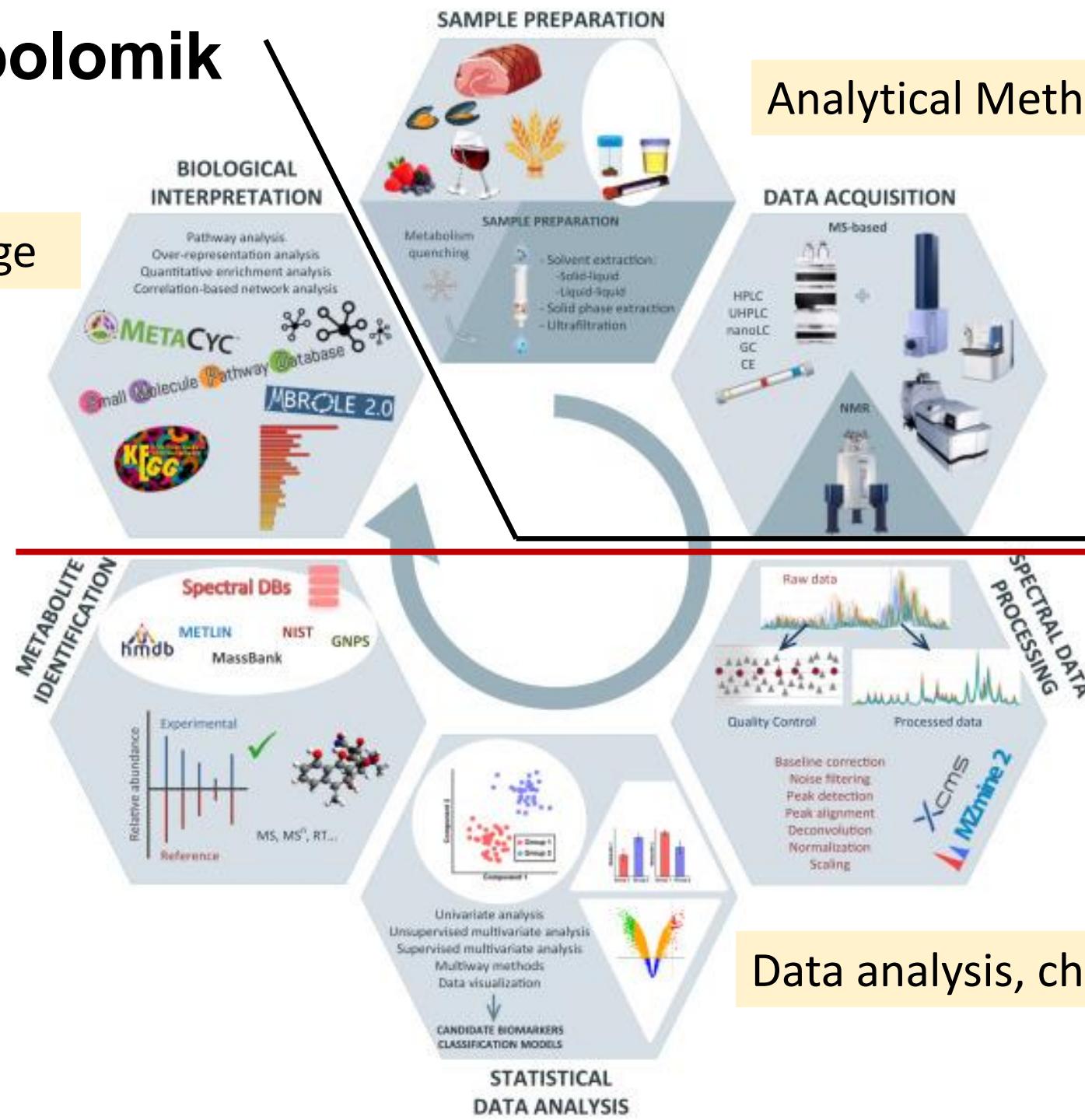
Detection, identification, and approximate quantification of a large set of target compounds

Metabolic fingerprinting

Spectral analysis of total compositions without knowledge of compound classes

Strategi metabolomik

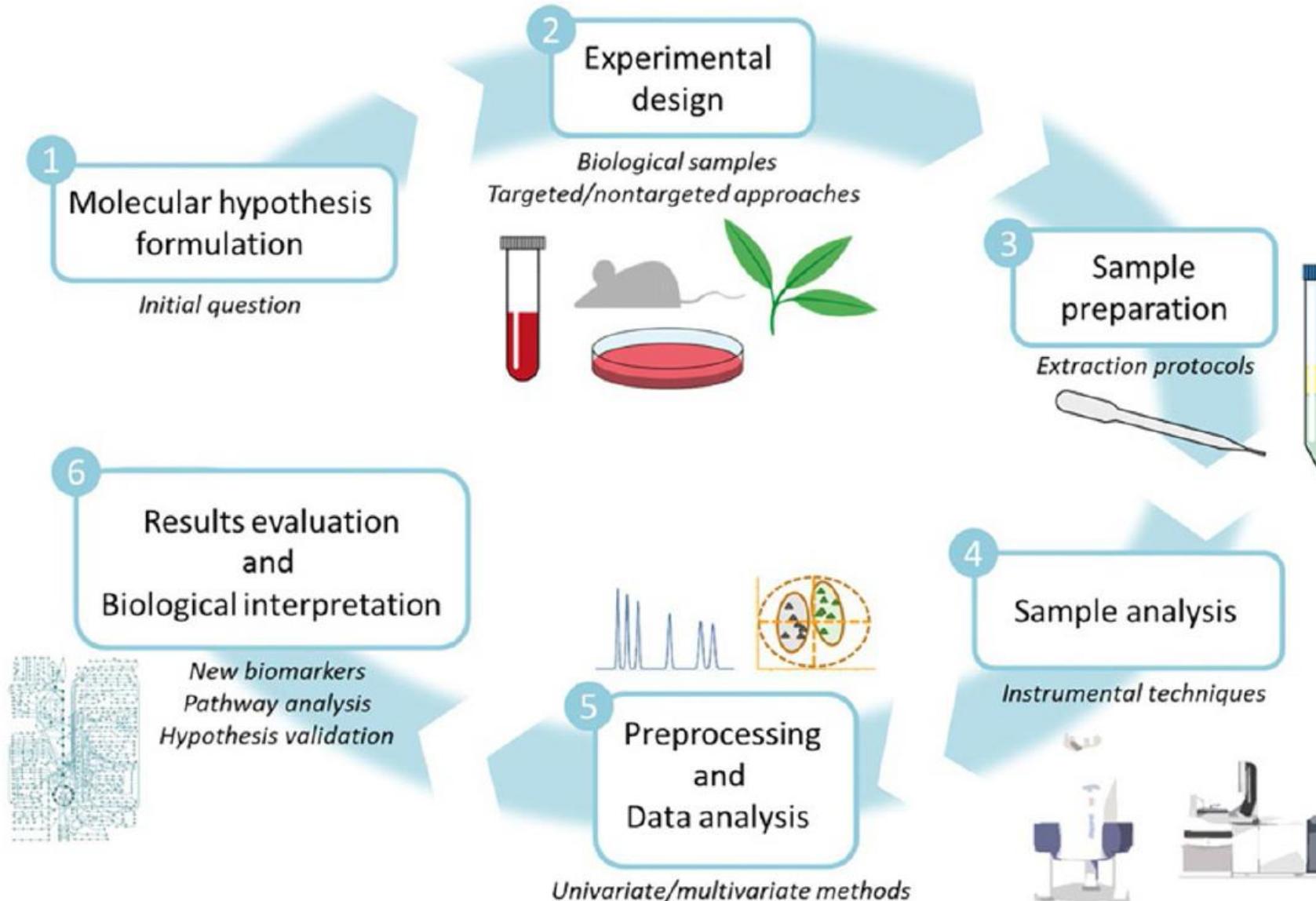
Biological knowledge



Analytical Methods

Data analysis, chemometrics

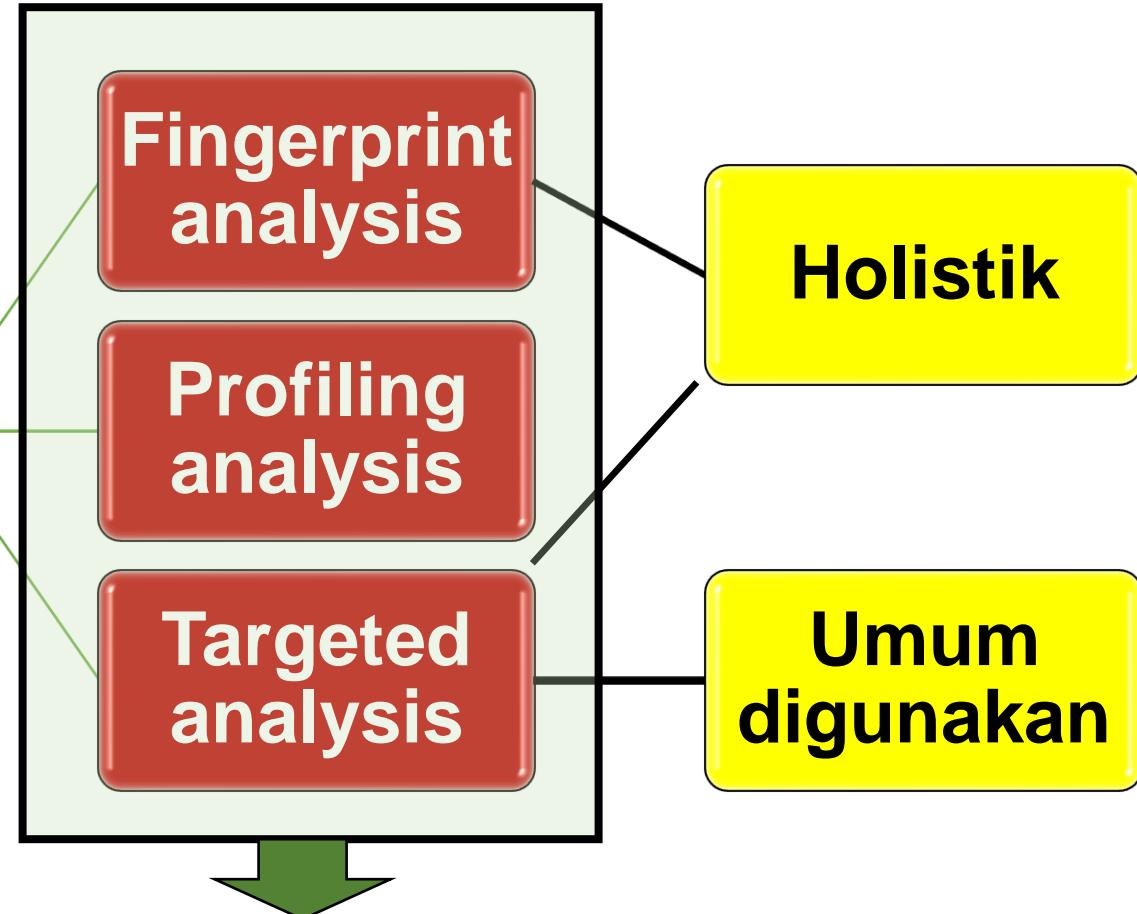
Metabolomics workflow



Kendali mutu tumbuhan obat

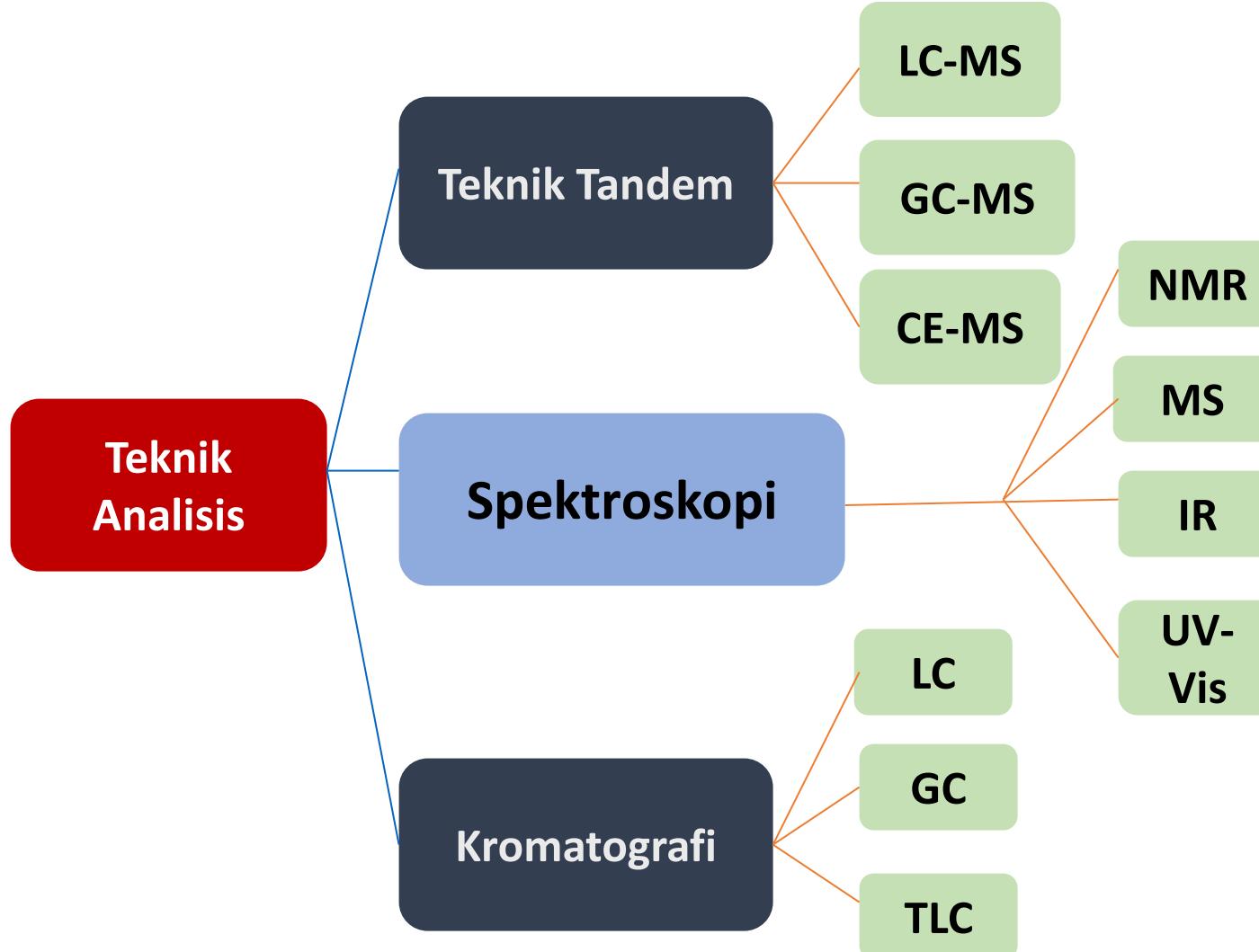


Metode QC
dengan
pendekatan
metabolomik

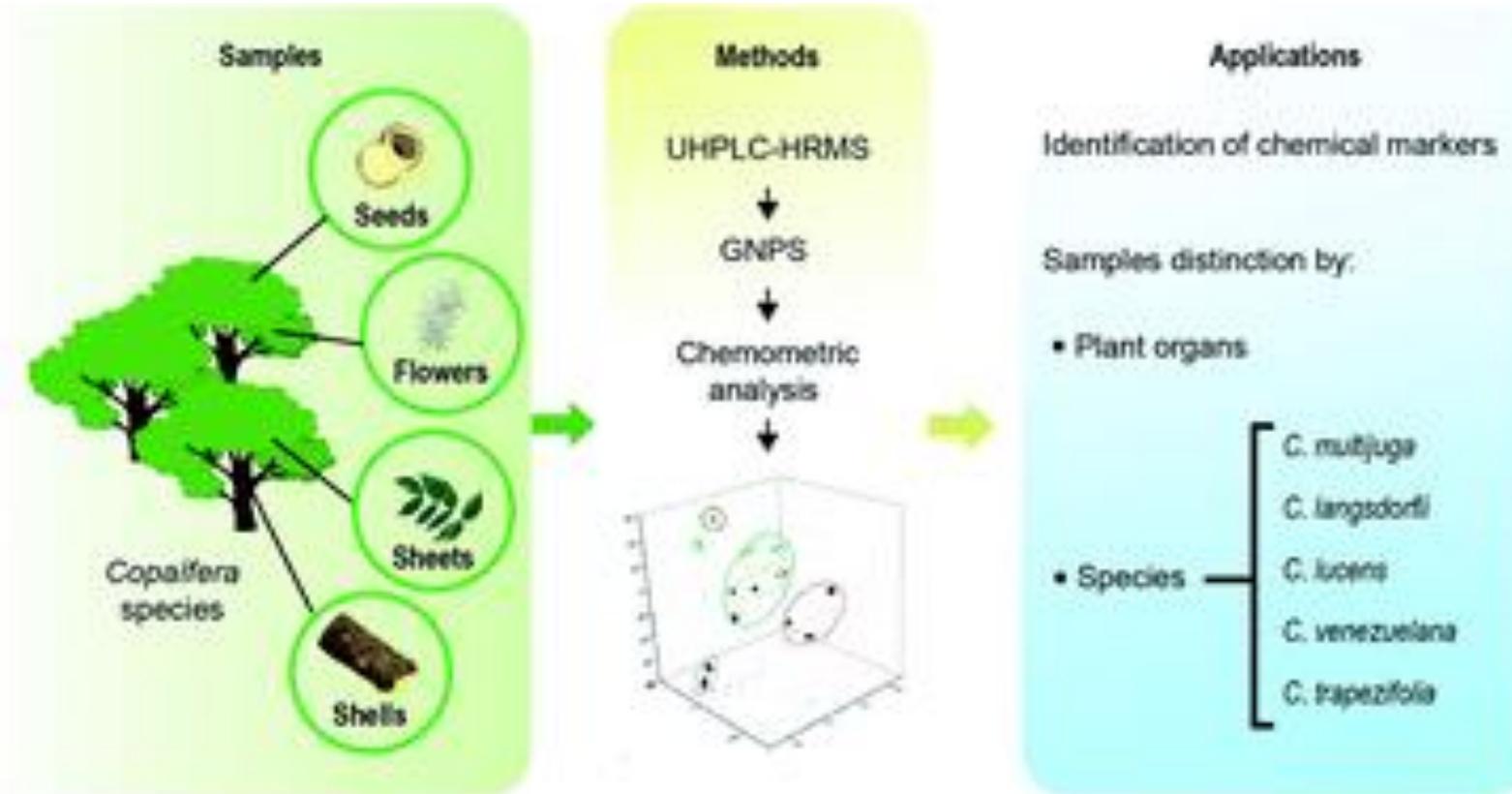


Identifikasi, diskriminasi, dan autentikasi (IDA)
tumbuhan obat

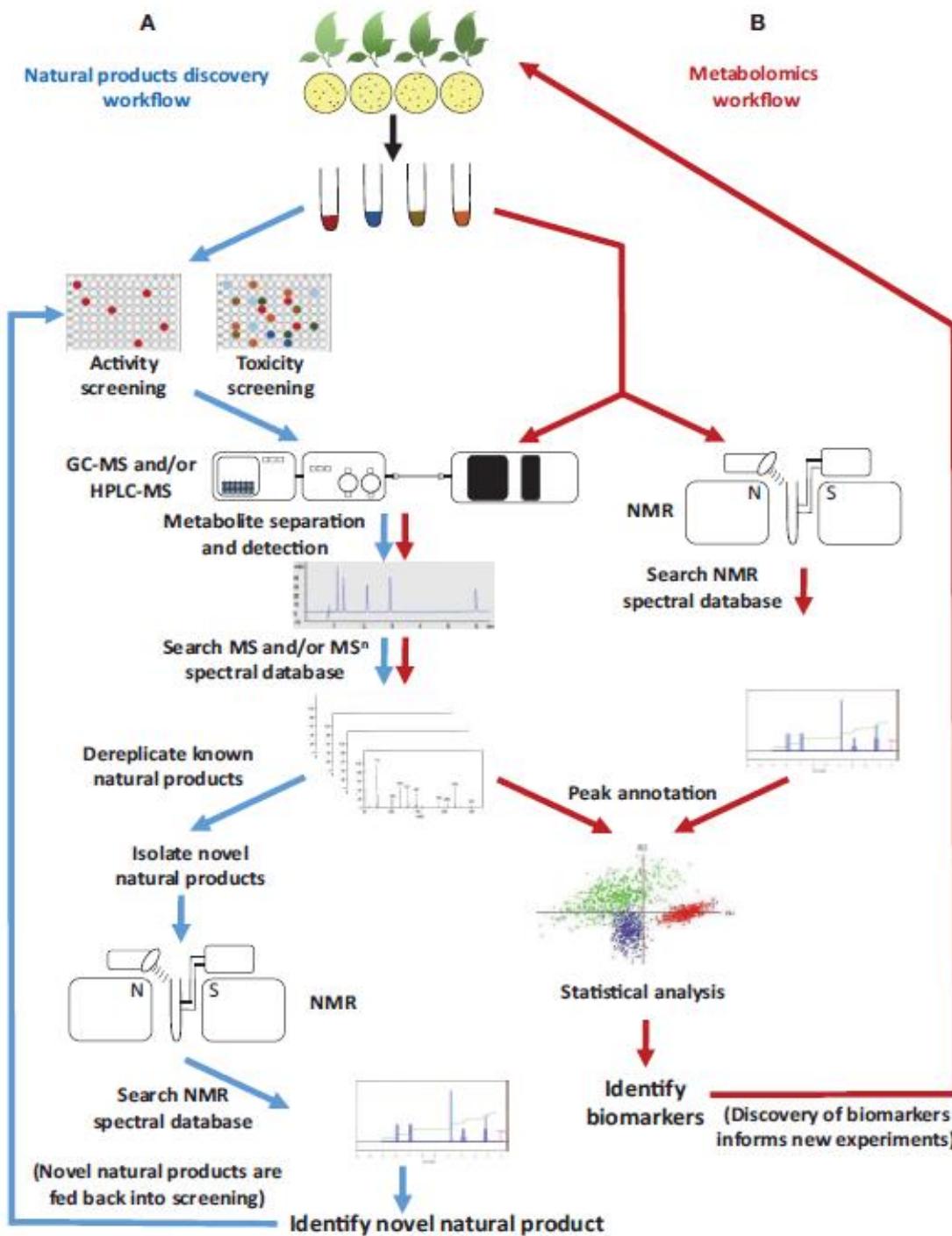
Instrumentasi analitik



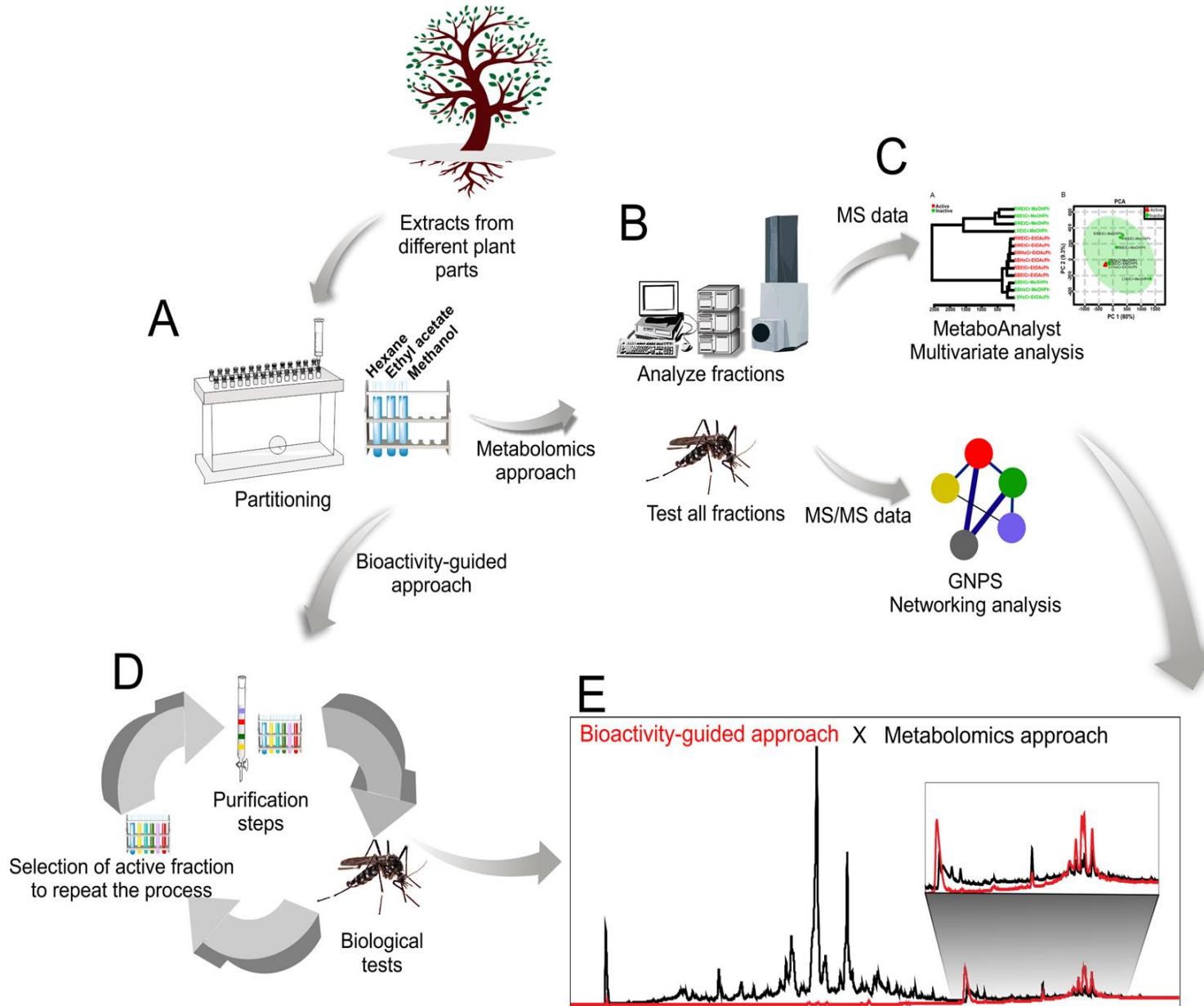
Bahan Alam



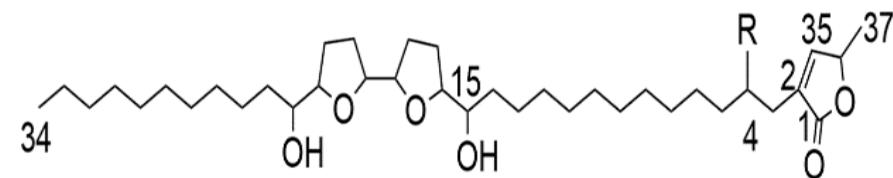
Bahan alam



Pendekatan metabolomik dalam riset bahan alam

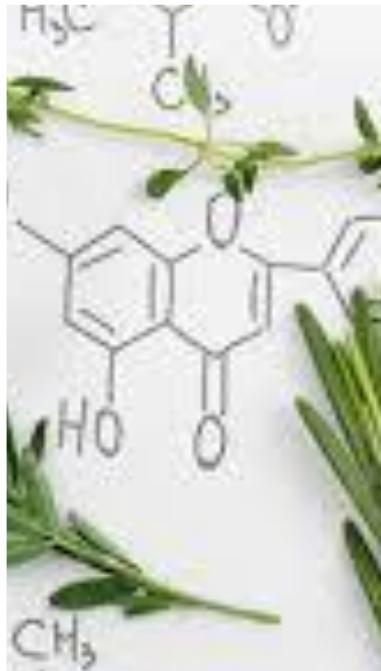


Identification of active compound from *Annona crassiflora* extracts as anti-larvicidal for *Aedes aegypti*



Asetogenin, R = H, 1a
annonacin; R = OH, 1b
squamocin

Demarque, D.P., Dusi, R.G., de Sousa, F.D.M. et al. Mass spectrometry-based metabolomics approach in the isolation of bioactive natural products. *Sci Rep* **10**, 1051 (2020).



Aplikasi kemometrik-metabolomik dalam metode kendali mutu tumbuhan obat

Identifikasi dan autentikasi meniran



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Sains Malaysiana 50(4)(2021): 997-1006
<http://doi.org/10.17576/jsm-2021-5004-10>

Feasibility of UV-Vis Spectral Fingerprinting Combined with Chemometrics for Rapid Detection of *Phyllanthus niruri* Adulteration with *Leucaena leucocephala*
(Kebolehlaksanaan Gabungan Spektrum Cap Jari UV-Vis dengan Kemometri untuk Pengesahan Pantas *Phyllanthus niruri* Dicemarkan dengan *Leucaena leucocephala*)

MOHAMAD RAFI*, BAYU NURCAHYO, WULAN TRI WAHYUNI, ZULHAN ARIF, DEWI ANGGRAINI SEPTANINGSIH,
SASTIA PRAMA PUTRI & EIICHIRO FUKUSAKI



Phyllanthus niruri



Leucaena leucocephala

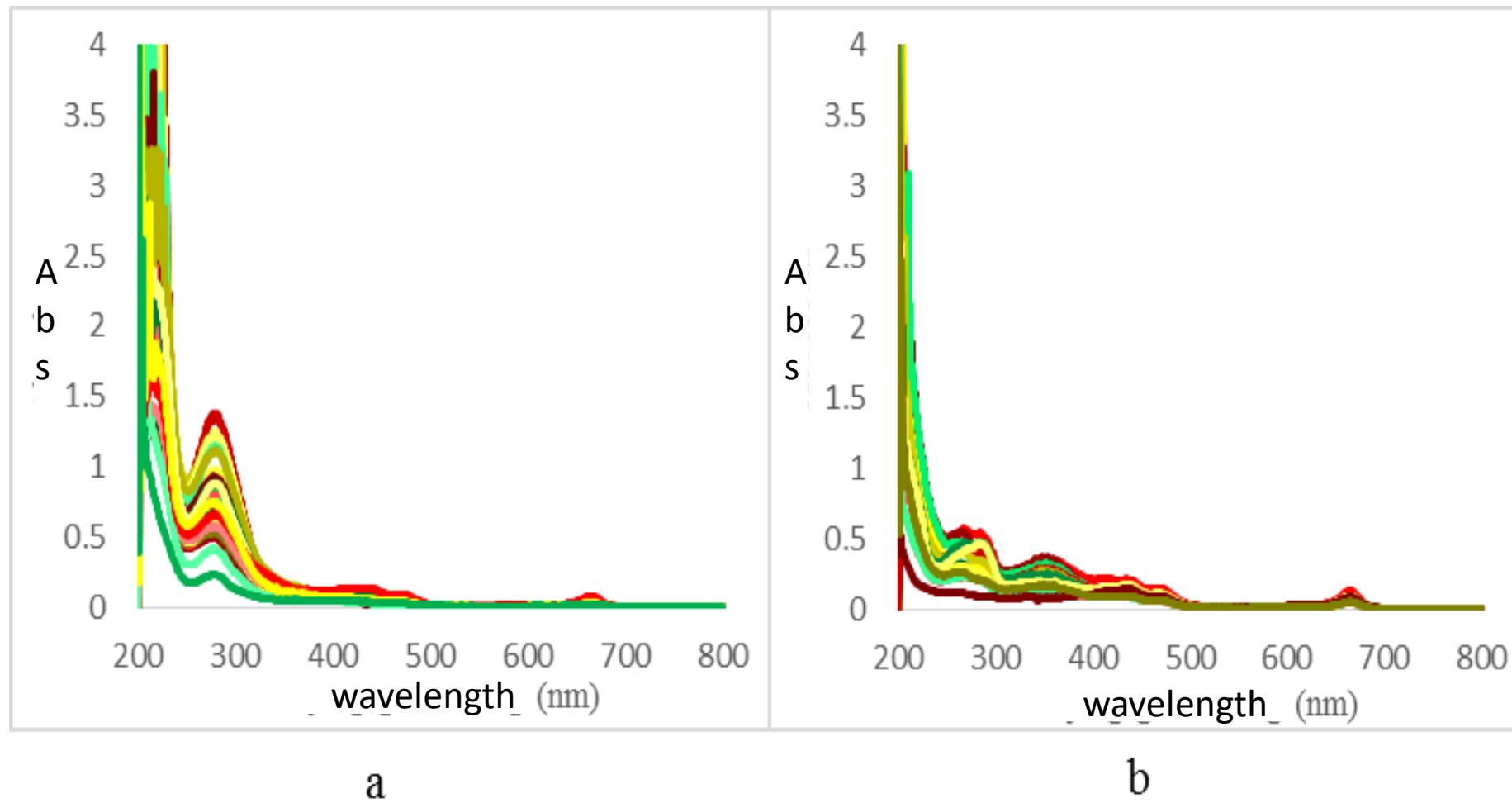


Serbuk daun.....?

Identifikasi dan autentikasi meniran



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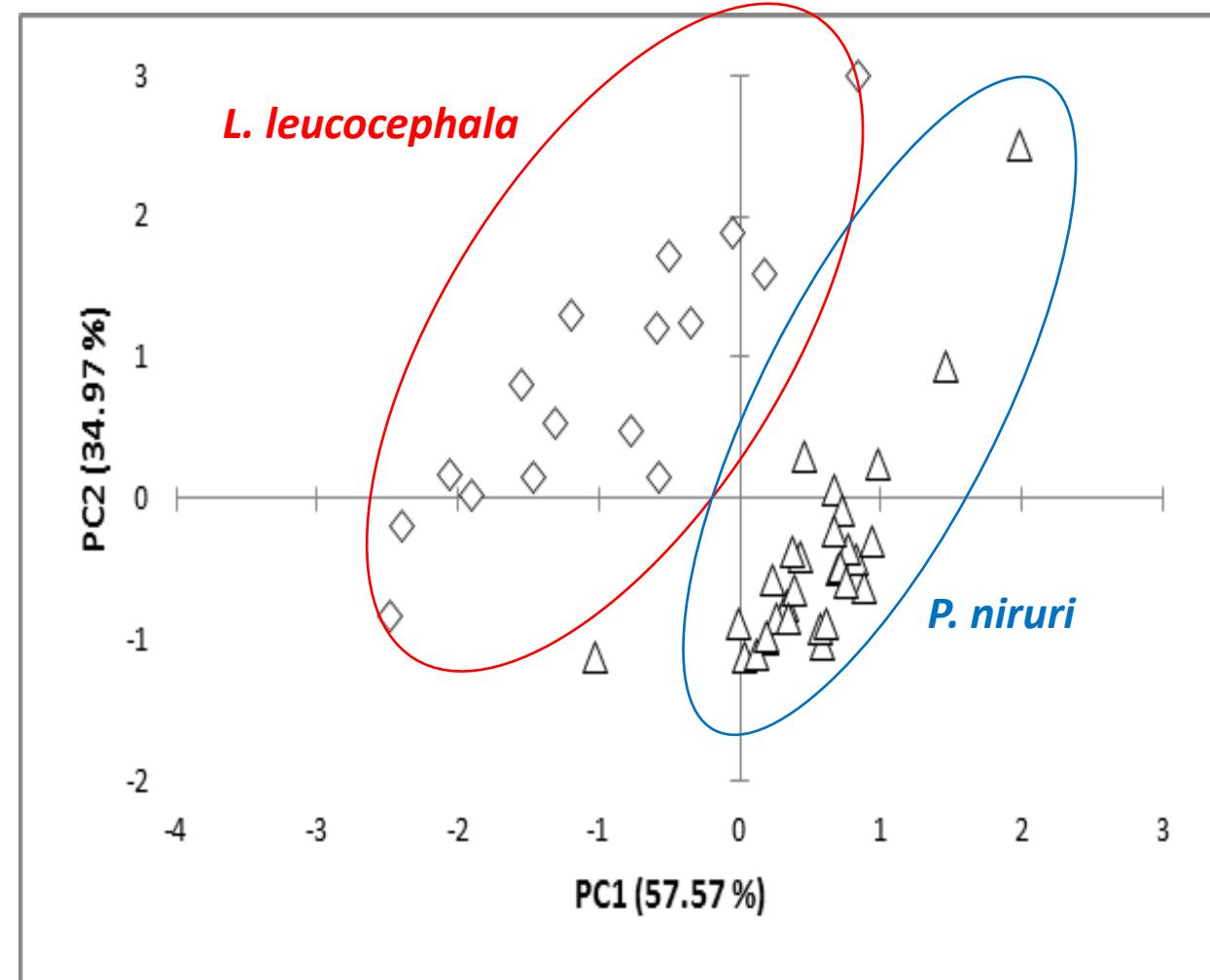


Spektrum UV-Vis *P. niruri* (a) dan *L. Leucocephala* (b)

Identifikasi dan autentikasi meniran

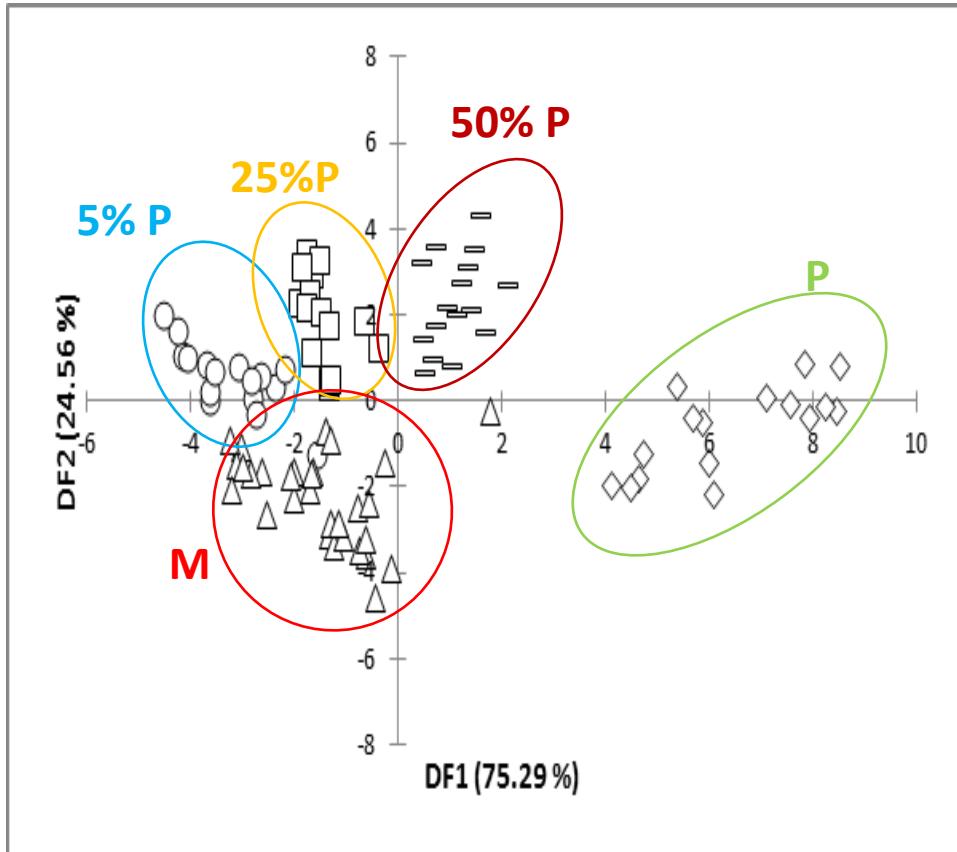


- Instrumentasi:
Spektrofotometer UV-Vis
- Variabel: absorbans (250-700 nm)
- Prapemprosesan sinyal:
standard normal variate
- Metode kemometrik:
principal component analysis (PCA)



PCA Plot

Identifikasi dan autentikasi meniran



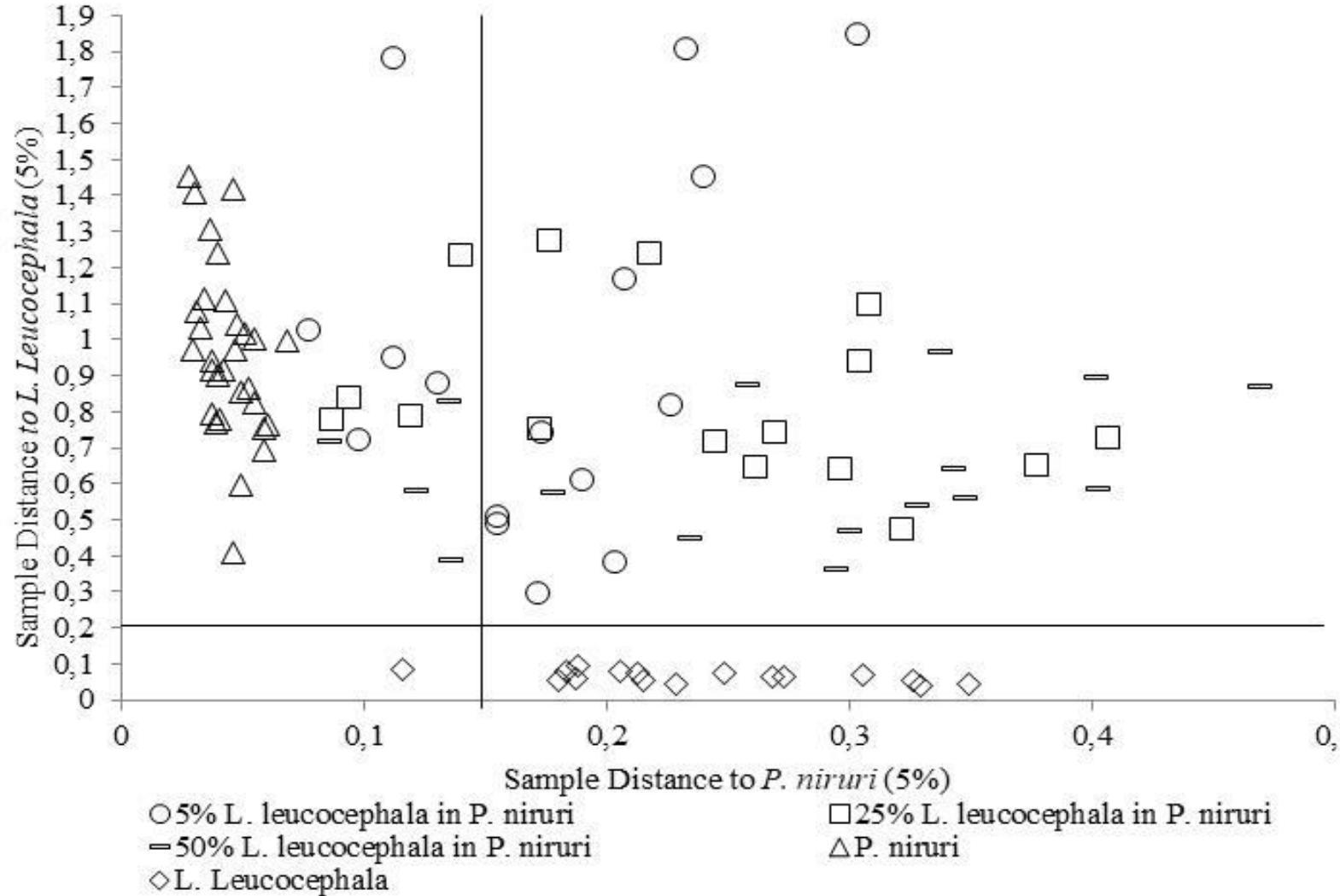
CVA plot of PN (Δ), 5% LL in PN (\circ), 25% LL in PN (\square),
50% LL in PN (-), dan LL (\diamond)

- Instrumentasi:
Spektrofotometer UV-Vis
- Variabel: absorbans (250-700 nm)
- Prapemprosesan sinyal:
standard normal variate
- Metode kemometrik:
canonical variate analysis (CVA)

Identifikasi dan autentikasi meniran



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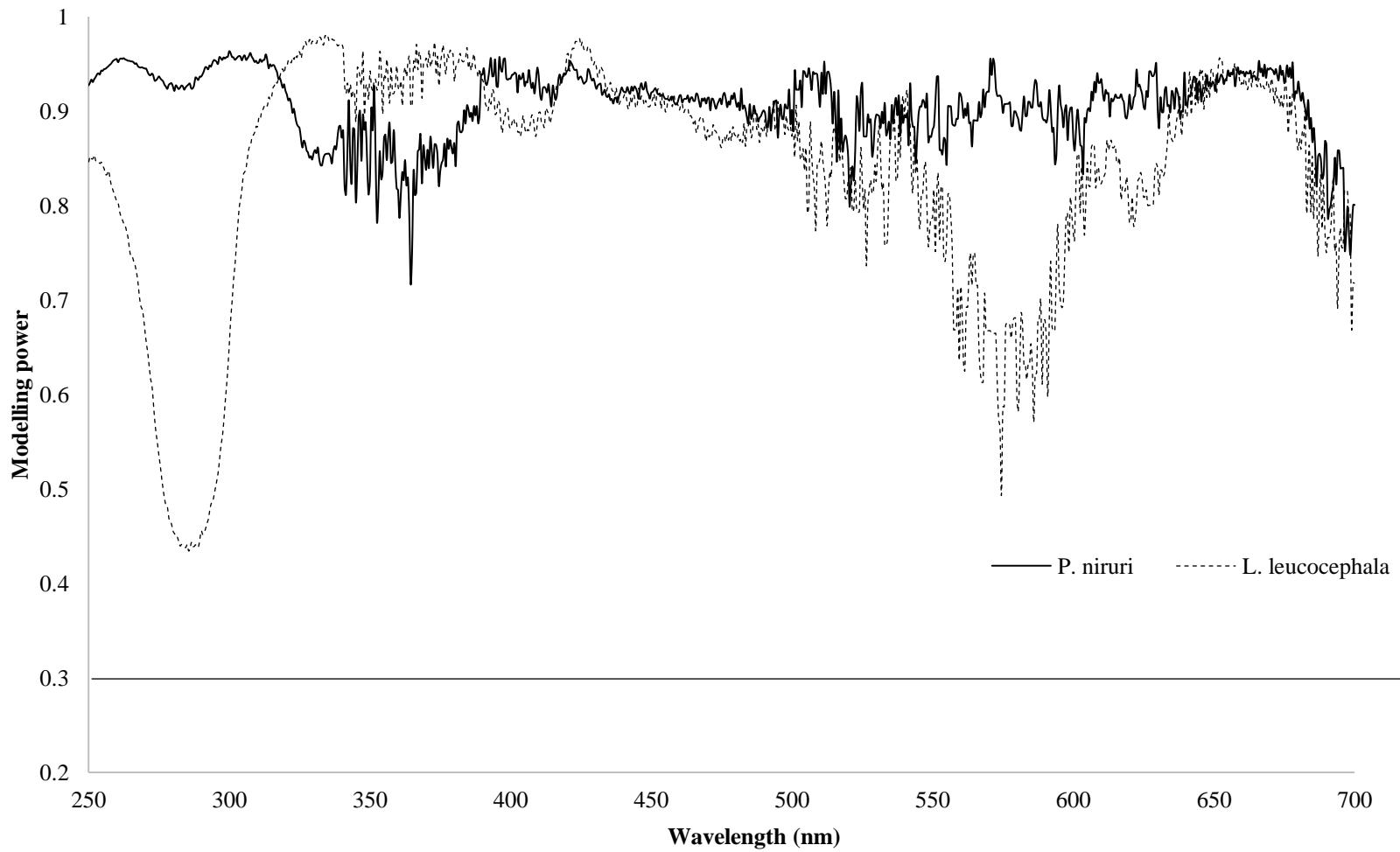
Plot Cooman SIMCA
(Soft Independent
Modelling of Class
Analogy)

Identifikasi dan autentikasi meniran



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Plot of modeling power in SIMCA model of *P. niruri* and *L. leucocephala* in the range of 250-700 nm



Identifikasi meniran hijau



1



2



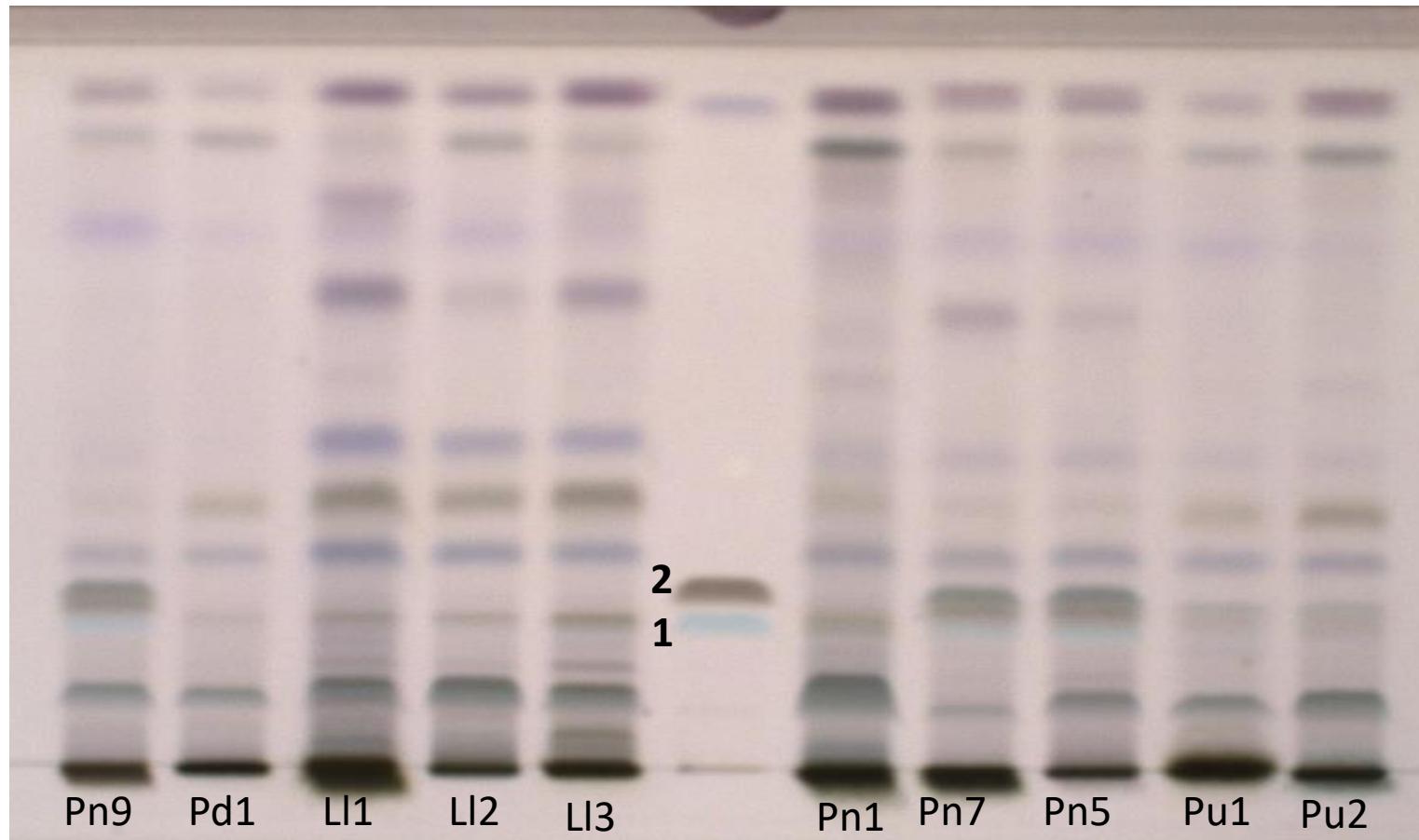
3

Manakah yang merupakan *meniran hijau* (*Phyllanthus niruri*)?

Identifikasi dan autentikasi meniran hijau



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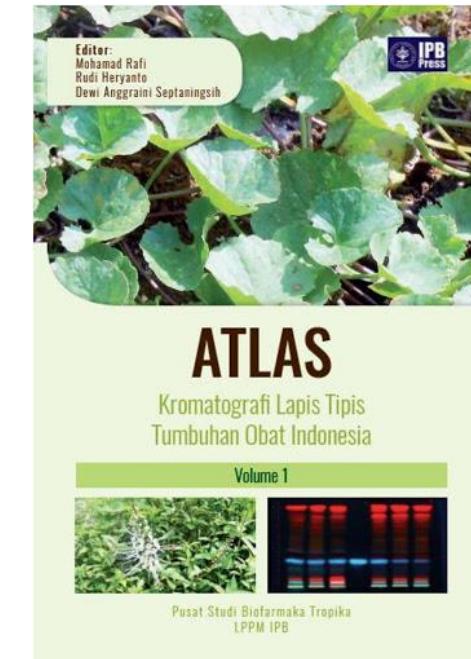
Pn: *Phyllanthus niruri*

Pd: *Phyllanthus debilis*

Pu: *Phyllanthus urinaria*

LI: *Leucaena leucocephala*

1. Filantin
2. Hipofilantin



[http://biofarmaka.ipb.ac.id/
news/2334-download-atlas-
kromatografi-lapis-tipis-
tumbuhan-obat-indonesia-
volume-1-2019](http://biofarmaka.ipb.ac.id/news/2334-download-atlas-kromatografi-lapis-tipis-tumbuhan-obat-indonesia-volume-1-2019)

Identifikasi meniran hijau



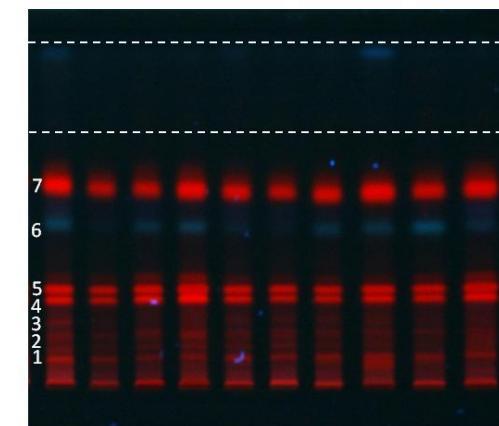
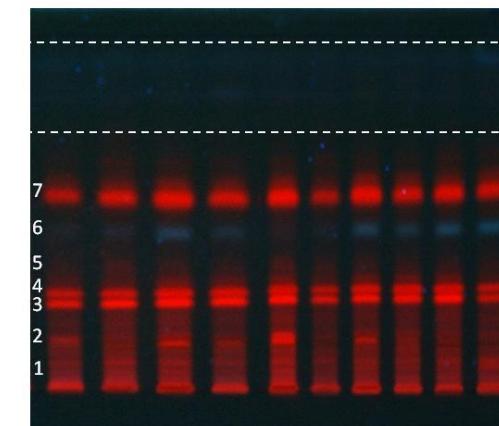
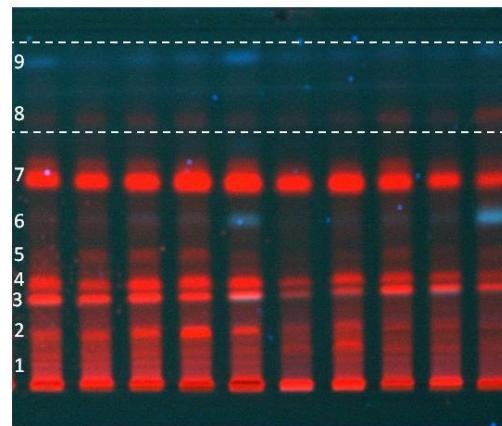
IPB University
Bogor Indonesia

P. niruri

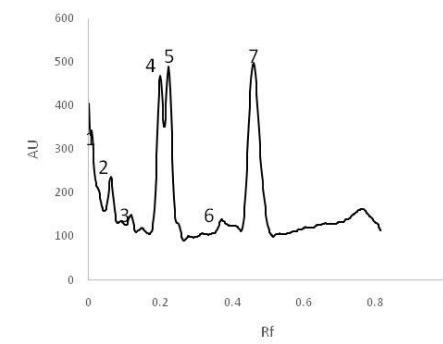
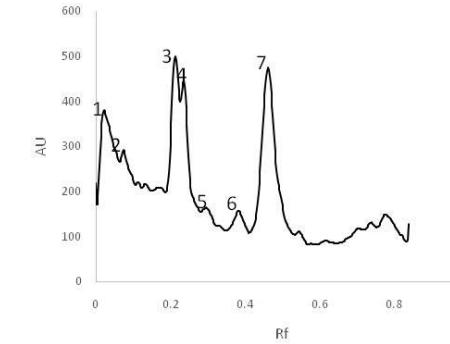
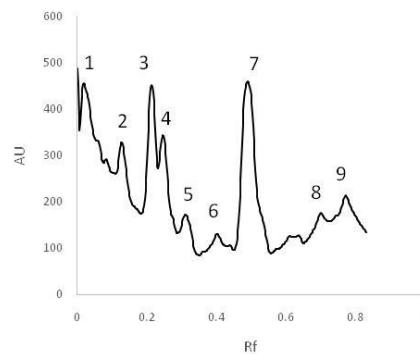
P. debilis

P. urinaria

Pola sidik jari KLT



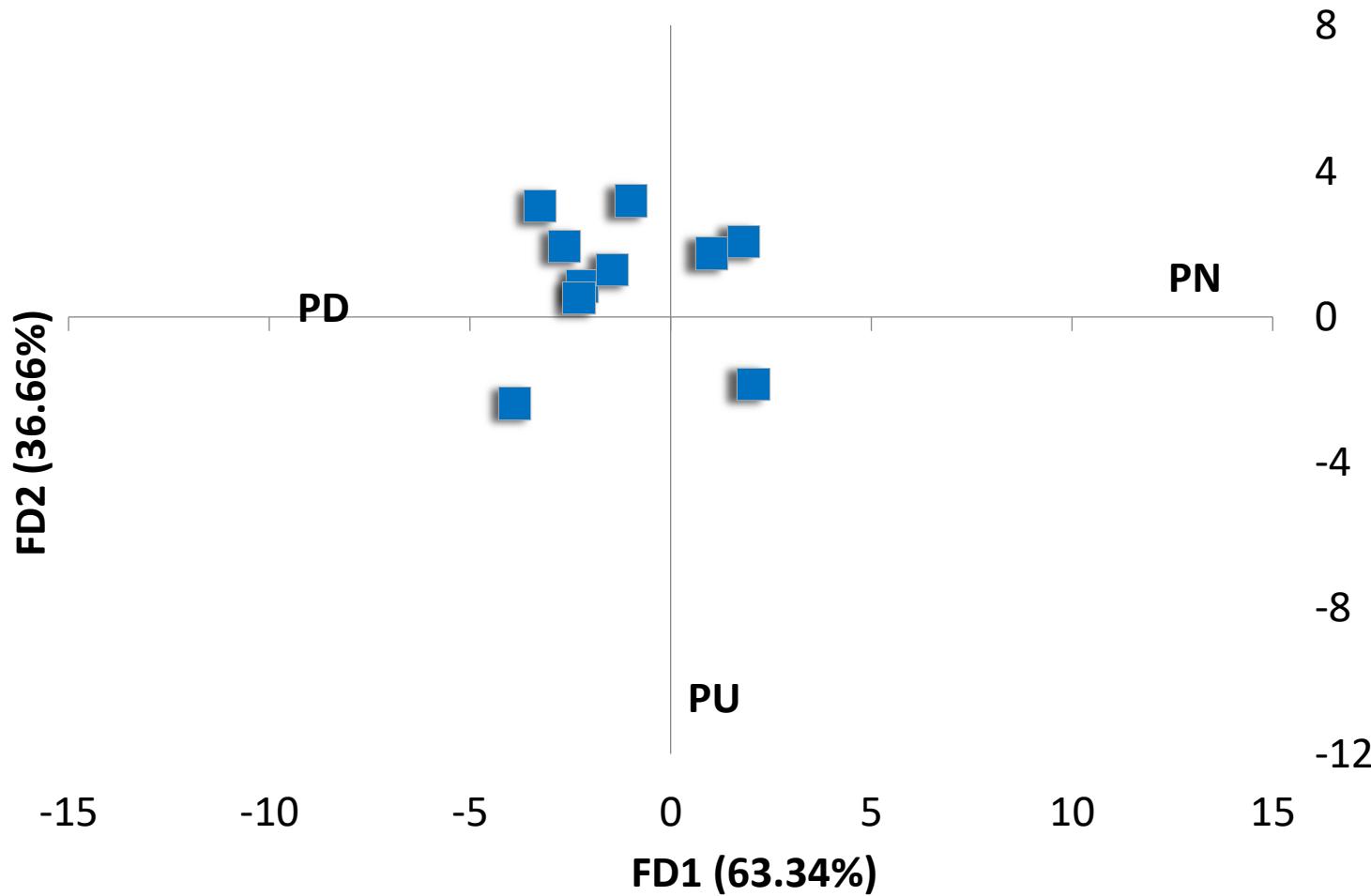
Densitogram



Identifikasi meniran hijau



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Plot AD

Identifikasi dan autentikasi meniran hijau



IPB University
Bogor Indonesia

Revista Brasileira de Farmacognosia 28 (2018) 527–532



Original Article

HPLC fingerprint and simultaneous quantitative analysis of phyllanthin and hypophyllanthin for identification and authentication of *Phyllanthus niruri* from related species



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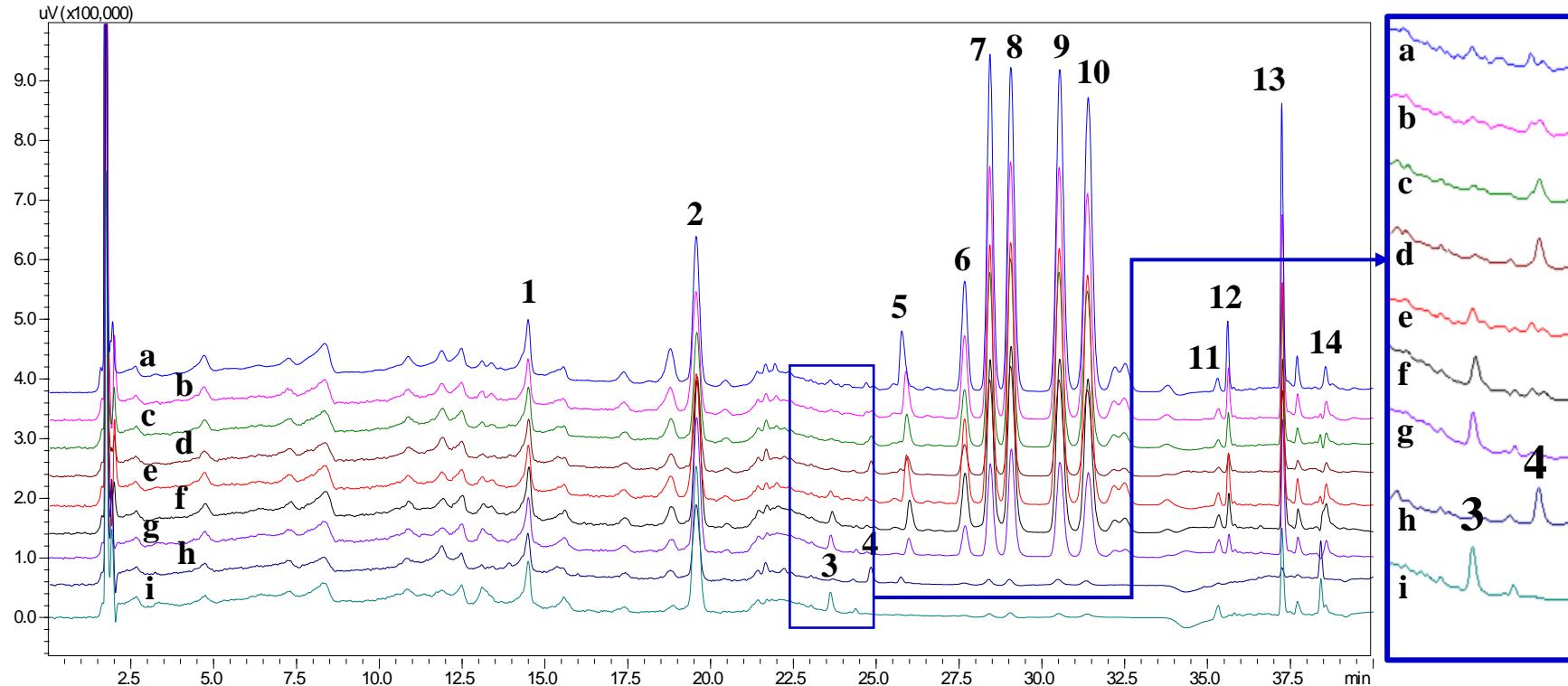
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Identifikasi dan autentikasi meniran hijau



IPB University
Bogor Indonesia



(a) PN-5 100 %

(b) PN-5:PU-5 (75:25 %)

(c) PN-5:PU-5 (50:50 %)

(d) PN-5:PU-5 (25:75 %)

(e) PN-5:PD-5 (75:25 %)

(f) PN-5:PD-5 (50:50 %)

(g) PN-5:PD-5 (25:75 %)

(h) PU-5 100 %

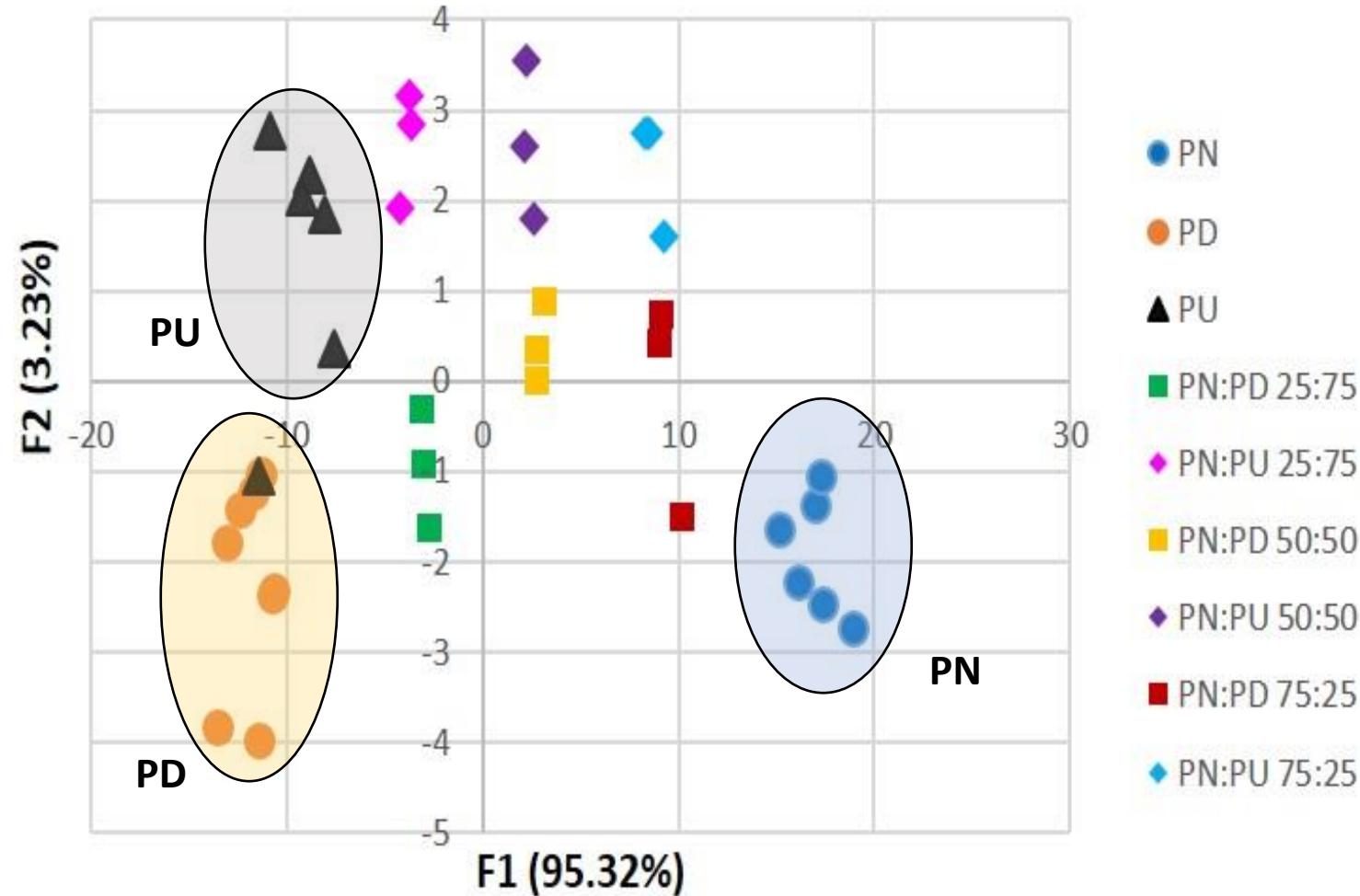
(i) PD-5 100 %

Identifikasi dan autentikasi meniran hijau



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Plot analisis
diskriminan



Diskriminasi umur tanam pegagan



Cultivation age could affect the concentration of metabolite in plant

Differences in the concentration of metabolite will give fluctuation in biological activity

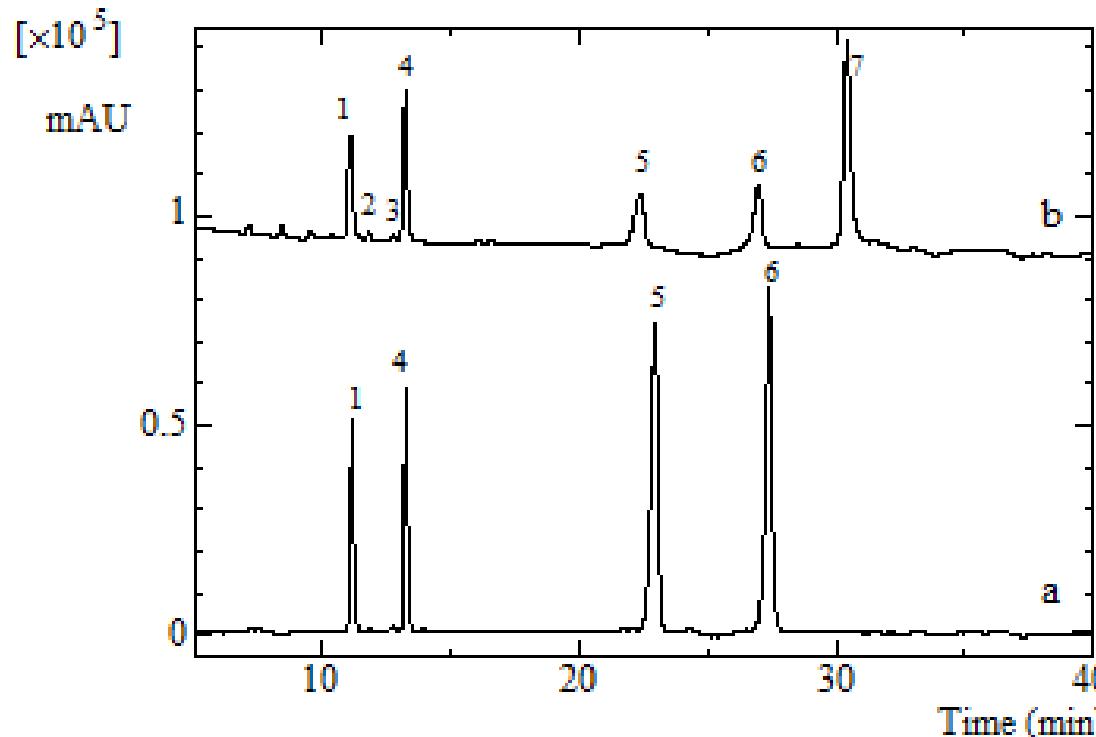
Evaluation using metabolomics approach

Diskriminasi umur tanam pegagan



Indian pennywort, pegagan/pegaga, gotu kola; mempunyai beberapa aktivitas farmakologi yang telah dilaporkan

Separasi 4 senyawa triterpenoid pada *C. asiatica*



- Kolom: Shim-pack VP-ODS C18 (150 mm x 4.6 mm)
- Suhu oven kolom: 40°C
- Fase gerak: 0-20': 20-45%, 20-40': 45-65% ACN dalam air
- Laju alir: 1 mL/min
- Deteksi: 206 nm
- Sampel: (a) standar 4 senyawa triterpenoid (madekasosida 100 µg/mL (1), asiatikosida 100 µg/ml (4), asam madekasat 100 µg/ml (5) dan asam asiatat 100 µg/ml (6))

Diskriminasi umur tanam pegagan



Kadar senyawa bioaktif penciri pada pegagan dengan umur tanam berbeda

Analit	Kadar (mg/g)				
	2 BST	3 BST	4 BST	5 BST	6 BST
Madecasosside	0.38	1.18	2.92	1.94	1.59
Asiaticoside	0.59	0.86	2.79	1.17	0.80
Madecassic acid	0.16	1.09	1.98	1.29	0.63
Asiatic acid	0.57	1.83	3.33	2.40	1.74

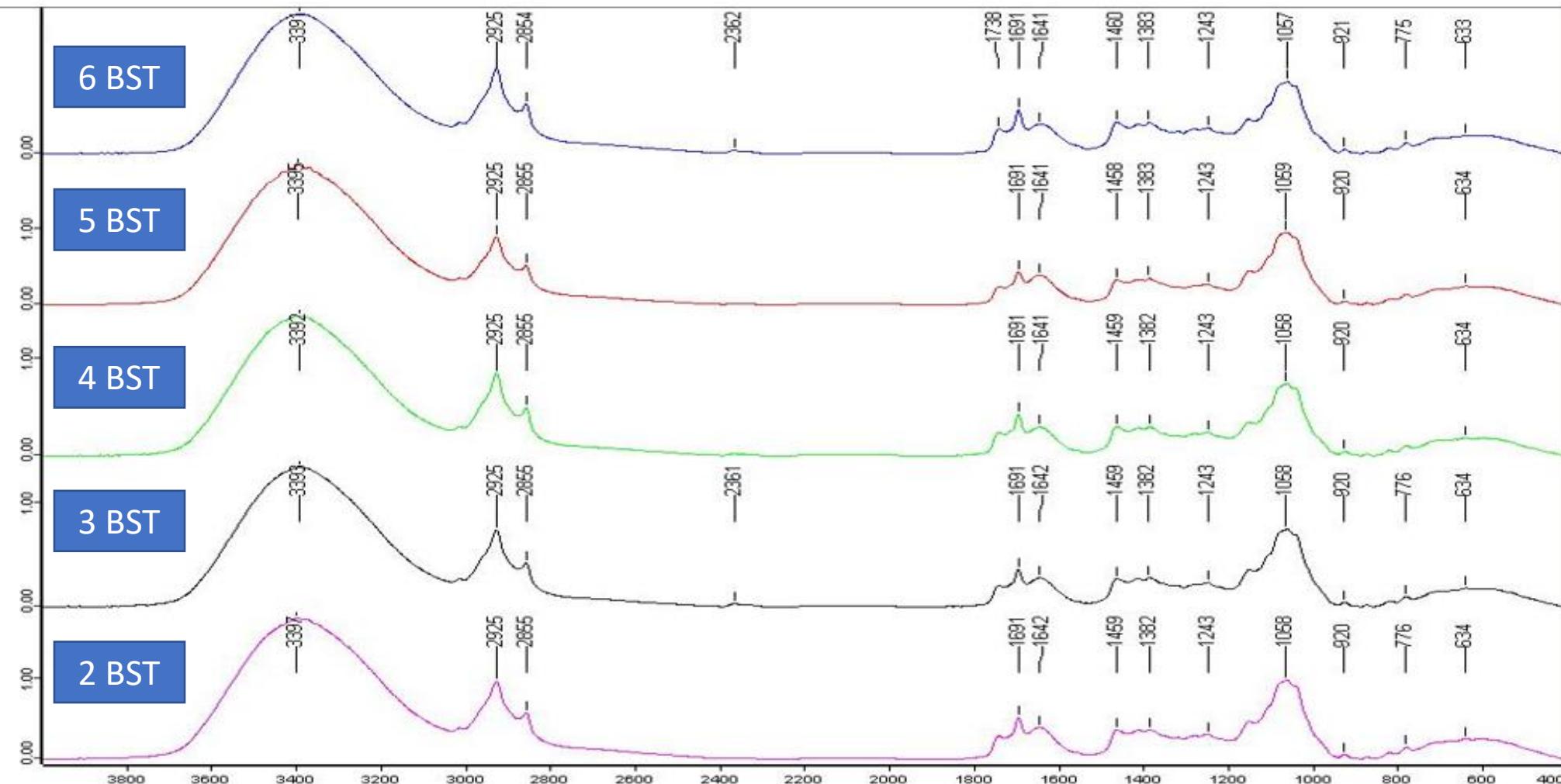
Diskriminasi umur tanam pegagan



Persentase inhibisi DPPH (kapasitas antioksidan) pegagan berbagai konsentrasi

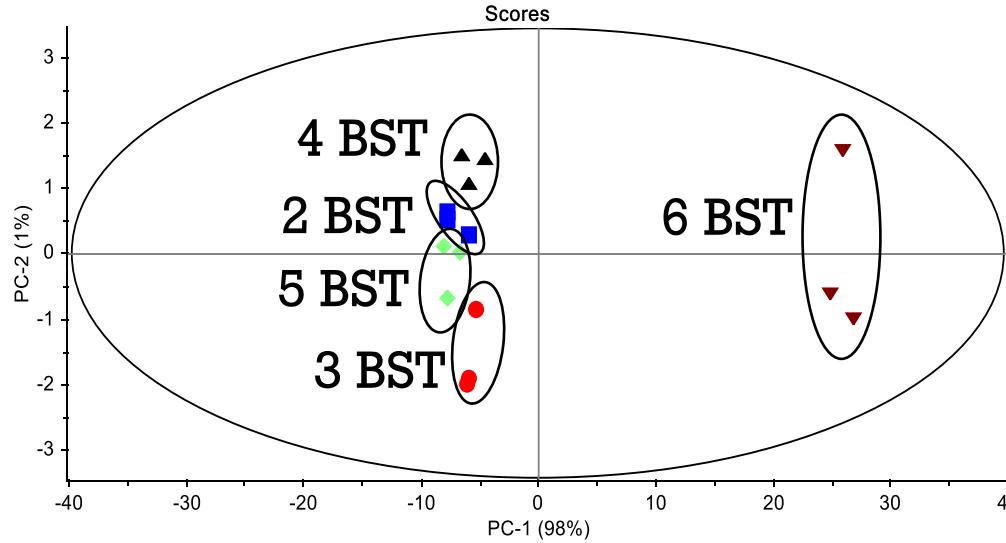
Konsentrasi ($\mu\text{g/mL}$)	% Inhibisi				
	2BST	3BST	4BST	5BST	6BST
500	80.2 \pm 1.3	84.59 \pm 1.30	95.78 \pm 0.60	81.07 \pm 1.17	80.53 \pm 2.45
400	68.46 \pm 0.9	77.37 \pm 1.30	89.55 \pm 0.92	73.65 \pm 0.67	70.17 \pm 2.03
300	57.80 \pm 1.65	69.43 \pm 0.36	77.71 \pm 1.20	65.06 \pm 0.67	63.12 \pm 1.49
200	47.93 \pm 1.60	57.76 \pm 0.90	64.45 \pm 2.17	57.64 \pm 1.17	53.11 \pm 0.97
100	39.13 \pm 1.66	48.37 \pm 0.55	53.81 \pm 0.92	52.56 \pm 1.79	42.69 \pm 2.25

Diskriminasi umur tanam pegagan



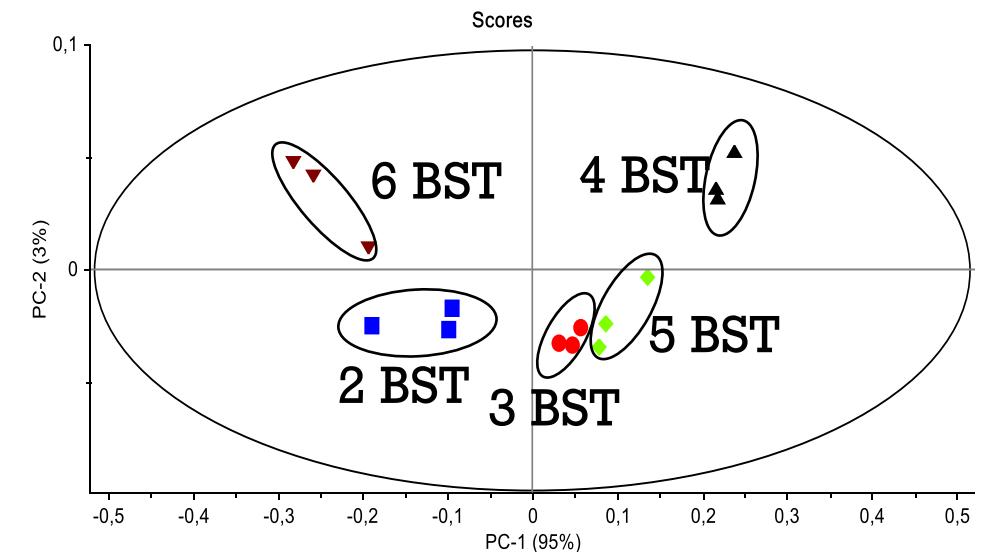
Spektra FTIR pegagan yang berbeda umur tanam

Diskriminasi umur tanam pegagan



Plot PCA
(spektra FTIR)

Plot PCA (variabel: kadar senyawa penciri)



Sambiloto



- Sambiloto merupakan salah satu tumbuhan obat yang banyak digunakan sebagai antidiabetes.
- Salah satu tumbuhan obat yang masuk dalam program saintifikasi jamu Kemenkes RI.



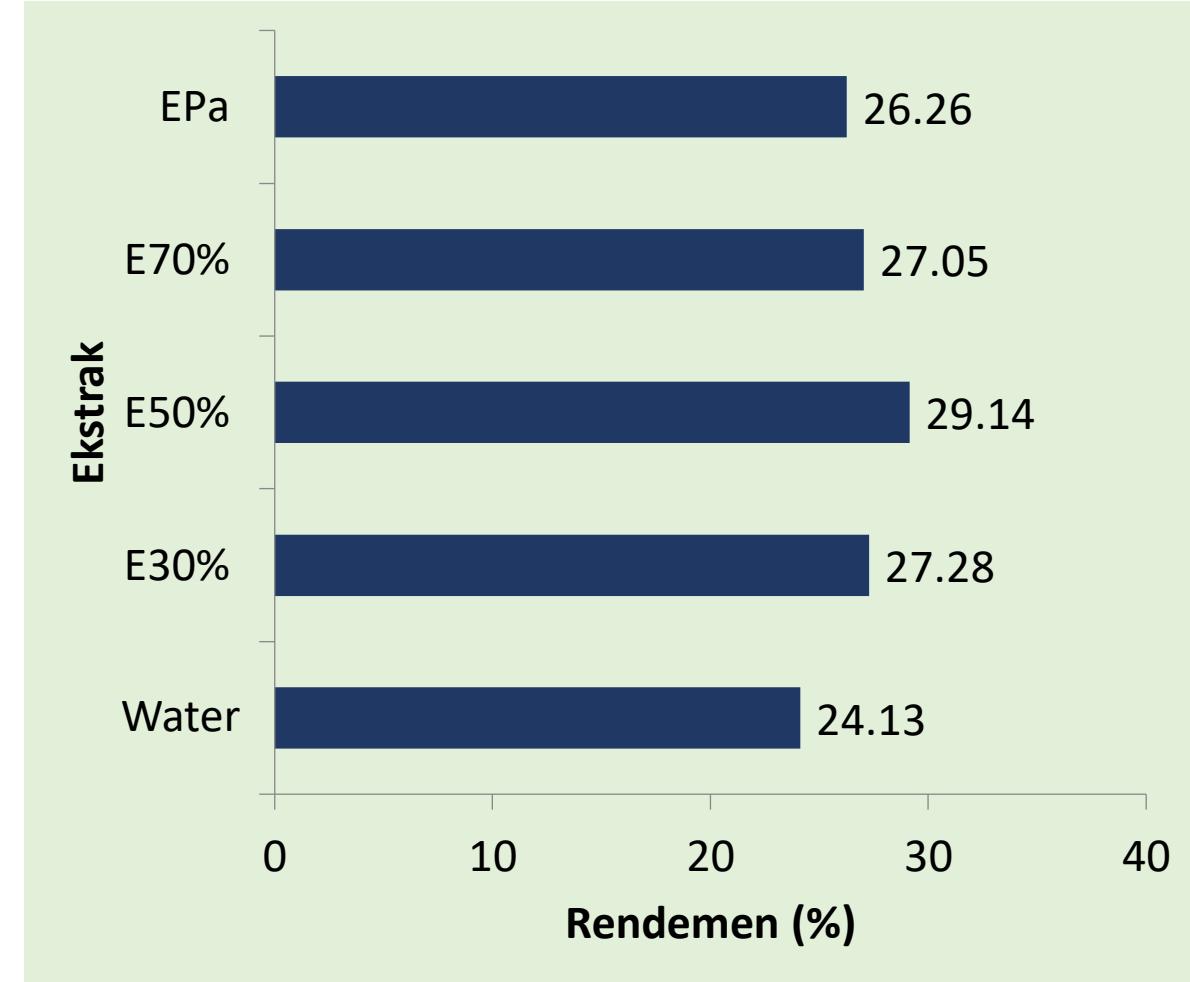
Klasifikasi ekstrak sambiloto



Extraksi sambiloto (3 BST) dari kebun percobaan Trop-BRC, IPB menggunakan konsentrasi pelarut pengekstrak berbeda



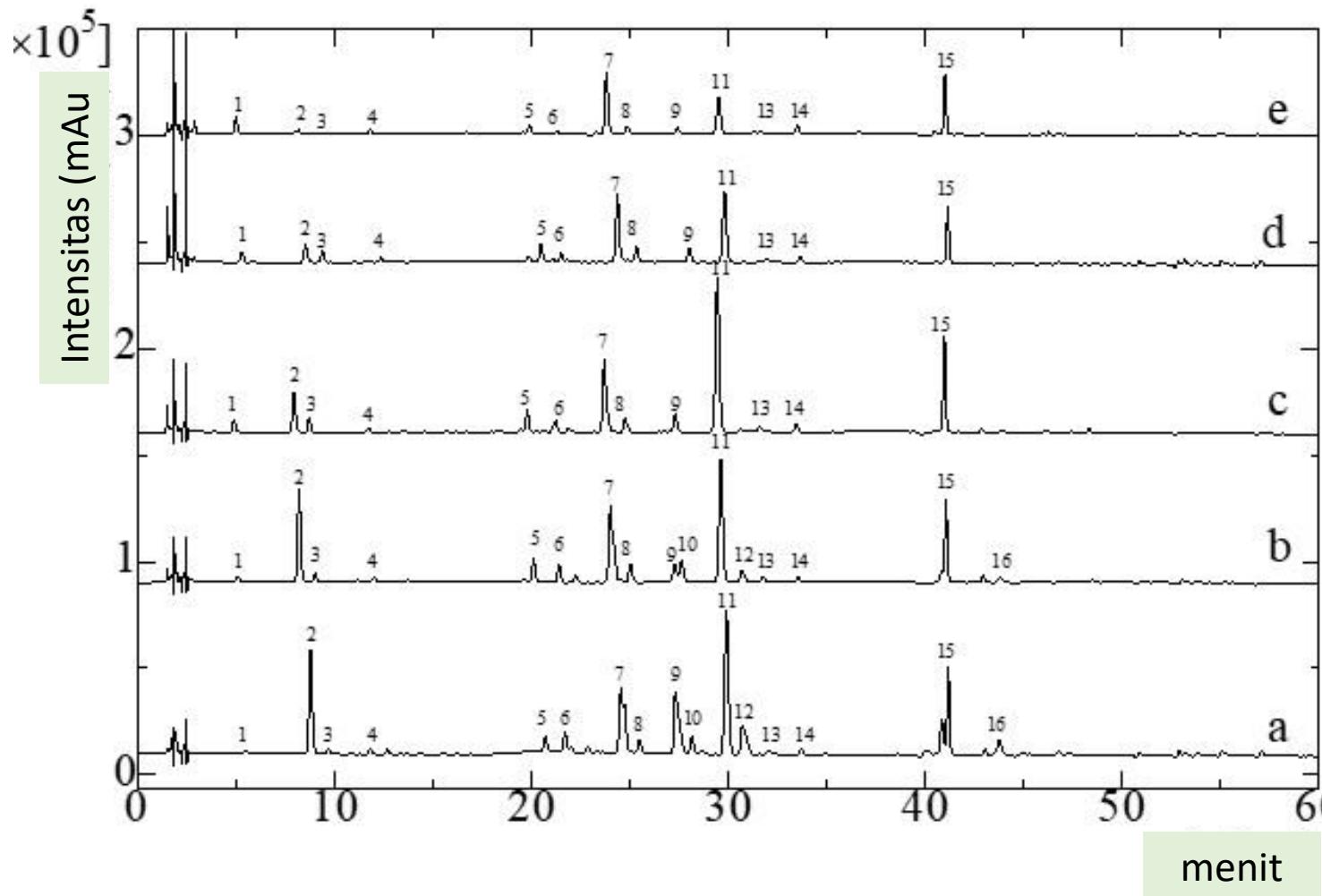
Rendemen teertinggi pada ekstrak EtOH 50%



Klasifikasi ekstrak sambiloto



Analisis sidik jari KCKT

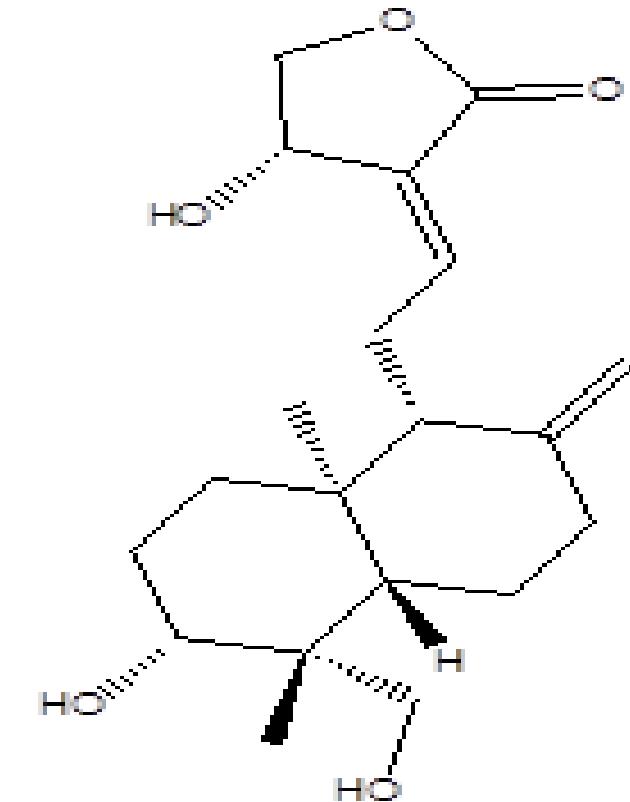


- Kromatogram KCKT:
- EtOH p.a.
 - EtOH 70%
 - EtOH 50%
 - EtOH 30%
 - Air

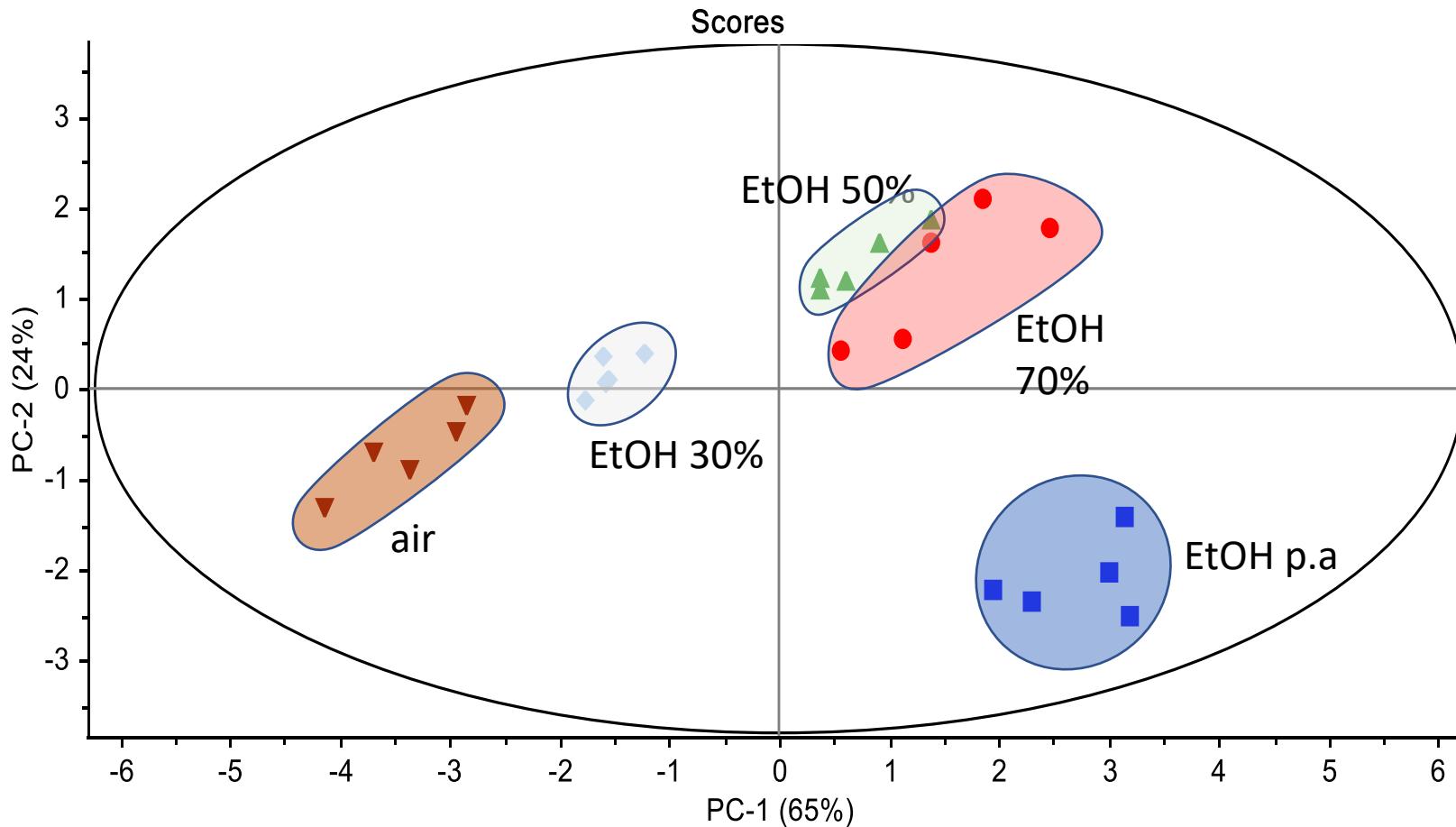
Klasifikasi ekstrak sambiloto



Pelarut pengekstrak	Persentase inhibisi enzim α -glukosidase (% \pm SD)	Kadar andrografolida (mg/g \pm SD)
Air	54.80 \pm 4.05	25.18 \pm 1.49
Etanol 30%	58.42 \pm 2.41	50.29 \pm 1.43
Etanol 50%	79.66 \pm 6.45	114.56 \pm 2.30
Etanol 70%	60.02 \pm 0.32	96.48 \pm 0.89
Etanol p.a.	49.58 \pm 0.97	102.08 \pm 2.73

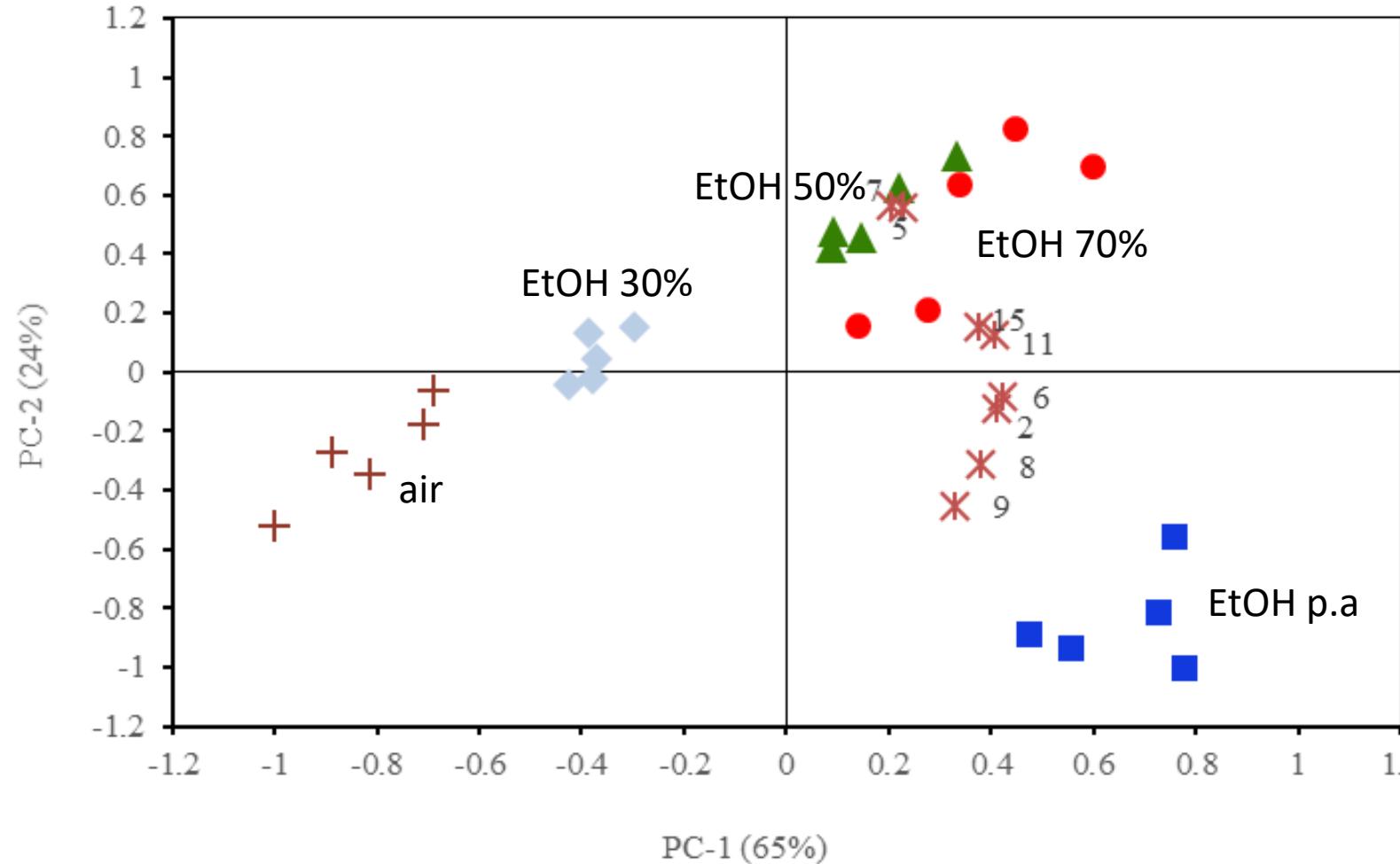


Klasifikasi ekstrak sambiloto



Plot PCA ekstrak sambiloto
Variabel: luas pita 2, 5, 6, 7, 8, 9, 11,15

Klasifikasi ekstrak sambiloto



Biplot PCA (Plot skor dan loading) ekstrak sambiloto

Pemrofilan metabolit sambiloto



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LC-MS/MS analysis

UHPLC tandem Q Exactive Plus Orbitrap-HRMS
Ionization source ESI(+), mass analyzer: Q-orbitrap
Column: Accucore™ Phenyl Hexyl (100×2.1 mm, 2.6 µm)
Ionization energy: 18, 35, 53 eV
Mobile phase: 0.1% FA in MeOH)

Sample extraction

Powdered sample of leaves and stem were extracted with 70% EtOH (1:10) using sonication method in room temperature for 30 minutes

Sample preparation

3-month-old of AP was used and separate between leaves and stem

1

2

3

4

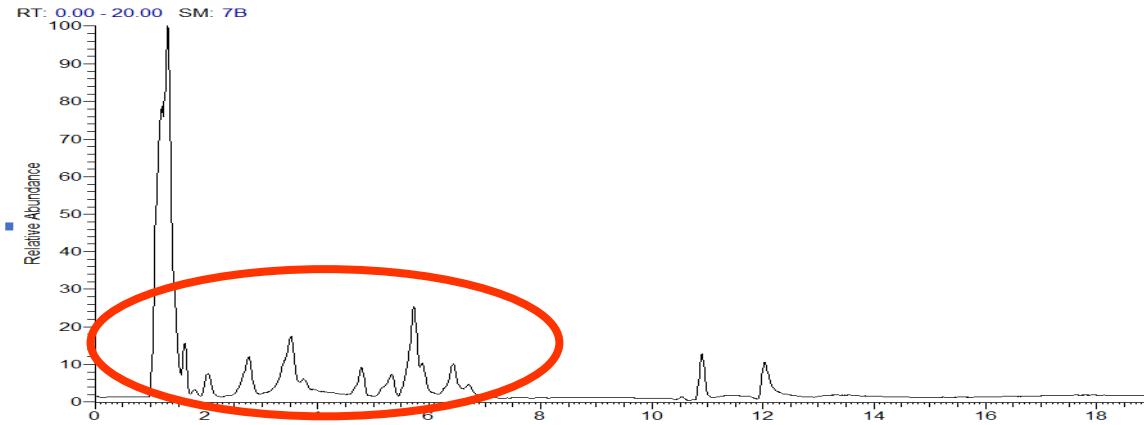
5

Identification of metabolite

Software Compound Discoverer 2.1 with local database

Classification using PCA

Software The Unscrambler X 10.4.



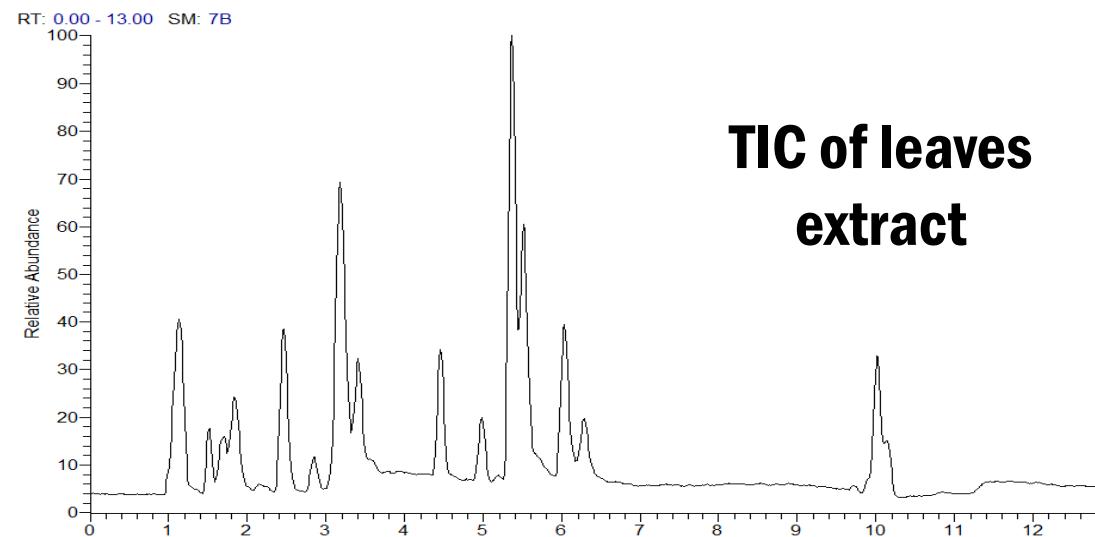
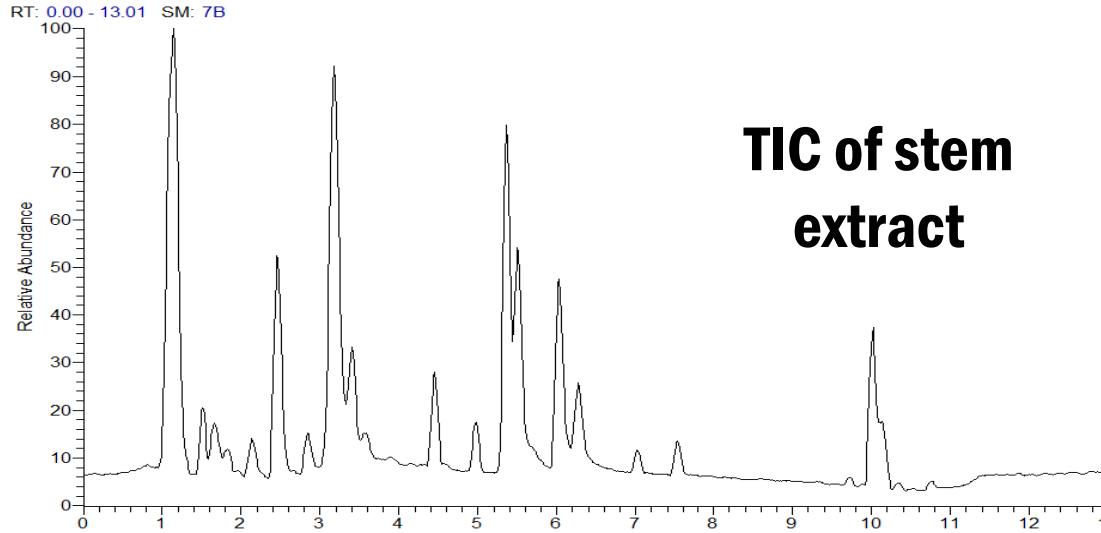
- a. 0.1% FA in water
- b. 0.1% FA in ACN
- c. 0.1% FA in MeOH

Modification from the developed method of Song *et al.* 2013

Pemrofilan metabolit sambiloto



IPB University
Bogor Indonesia



Pemrofilan metabolit sambiloto



Ditepene lactone

1. Paniculide C
2. Paniculide B
3. 14-deoxyandrographiside
4. 14-deoxy-11-oxoandrographolide
5. 12S-hydroxyandrographolide
6. 14-deoxy-17-hydroxyandrographolide
7. Andrographic acid
8. Andrographolide
9. Neoandrographolide
10. Paniculide A
11. Andrographanin
12. 14-deoxyandrographolide
13. Andrographolactone
14. Dehydroandrographolide
15. Bis-andrographolide A
16. 14-acetylandrographolide

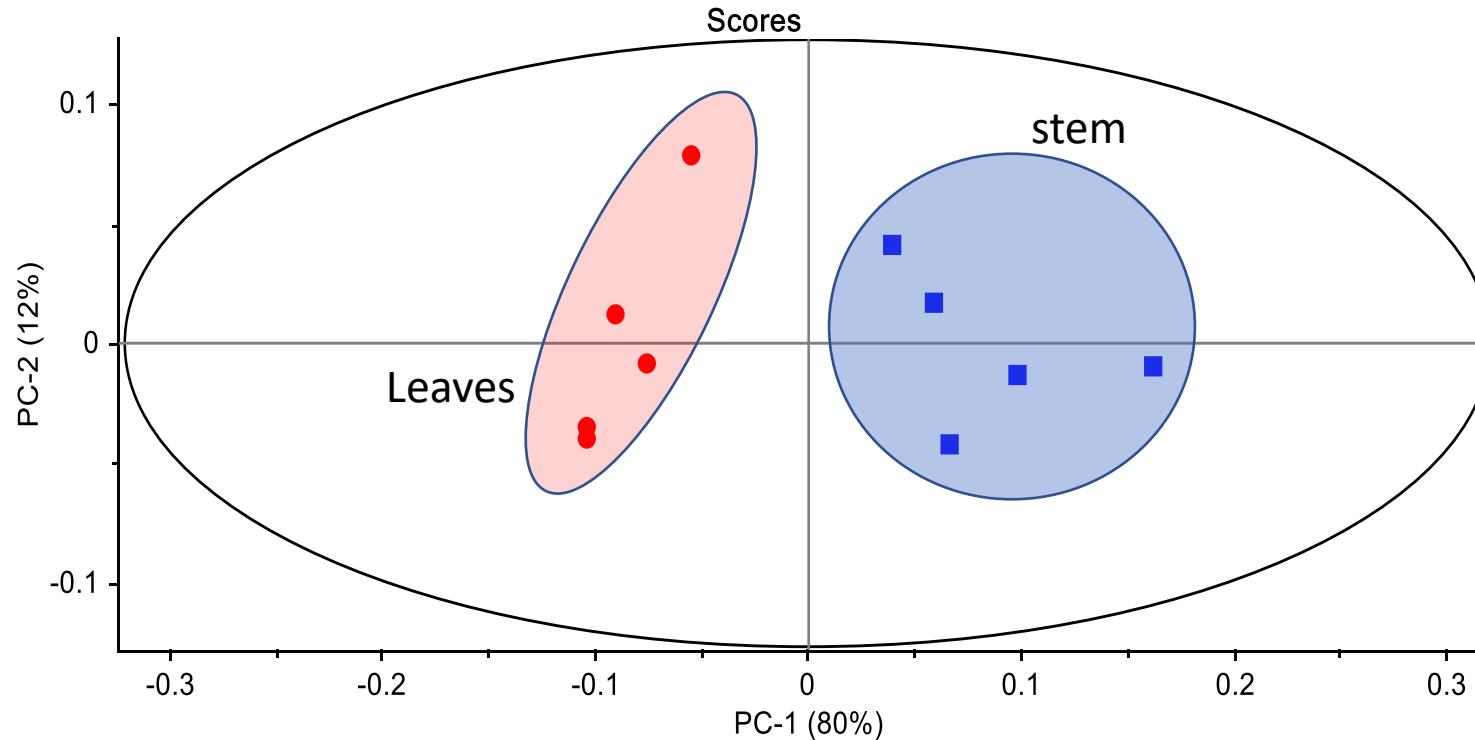
Flavonoid

1. Luteolin
2. Apigenin
3. 5,7-dihydroxy-8-methoxyflavone
4. Apigenin-7-O- β -D-glucoronide
5. Dihydroskullcapflavone I
6. 5,4'dihydroxy-7,8-dimethoxy flavone
7. 5-hydroxy-7,8,2'-trimethoxy flavone 5-glucoside
8. 5-hydroxy-3,7,8,2'-tetramethoxy flavone
9. Apigenin-7,4'-dimethylether
10. 5-hydroxy-7,8-dimethoxy flavanone
11. 5-hydroxy-7,2',6'-trimethoxy flavone
12. Andrographidin A

Phenolic acid

1. Cinamic acid
2. Caffeic acid
3. 3-O-Caffeoylcuinic acid

Pemrofilan metabolit sambiloto



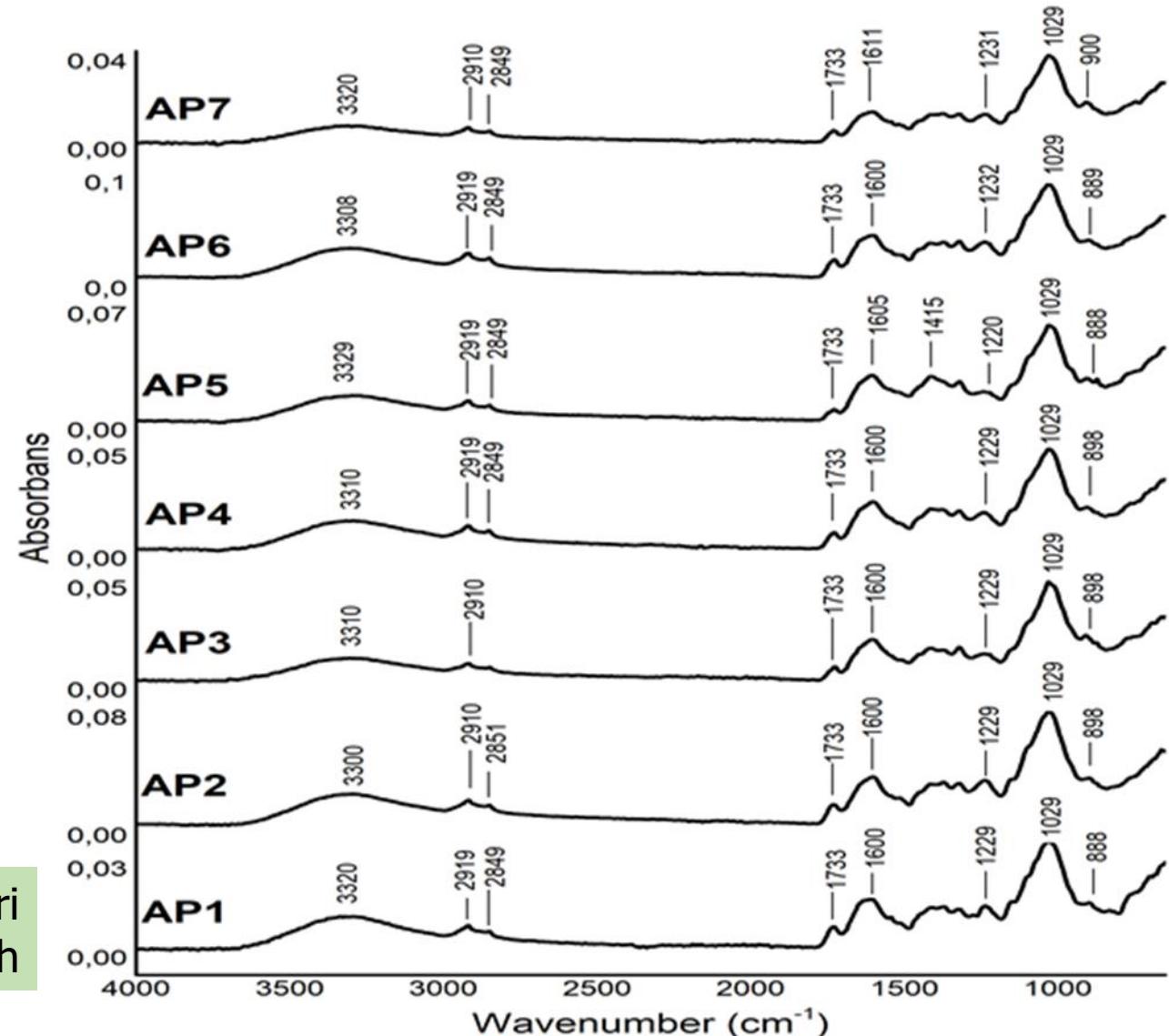
Variable: peak area from 18 major components

Sambiloto dari berbagai lokasi tumbuh



Kode sampel	Lokasi pengambilan sampel
AP1	Dramaga, Bogor, Jawa Barat
AP2	Nagrak, Sukabumi, Jawa Barat
AP3	Bayumanik, Semarang, Jawa Tengah
AP4	Karangpandan, Karanganyar, Jawa Tengah
AP5	Kejayan, Pasuruan, Jawa Timur
AP6	Batu, Jawa Timur
AP7	Poncol, Magetan, Jawa Timur

Spektrum FTIR sambiloto dari berbagai lokasi tumbuh



Sambiloto dari berbagai lokasi tumbuh



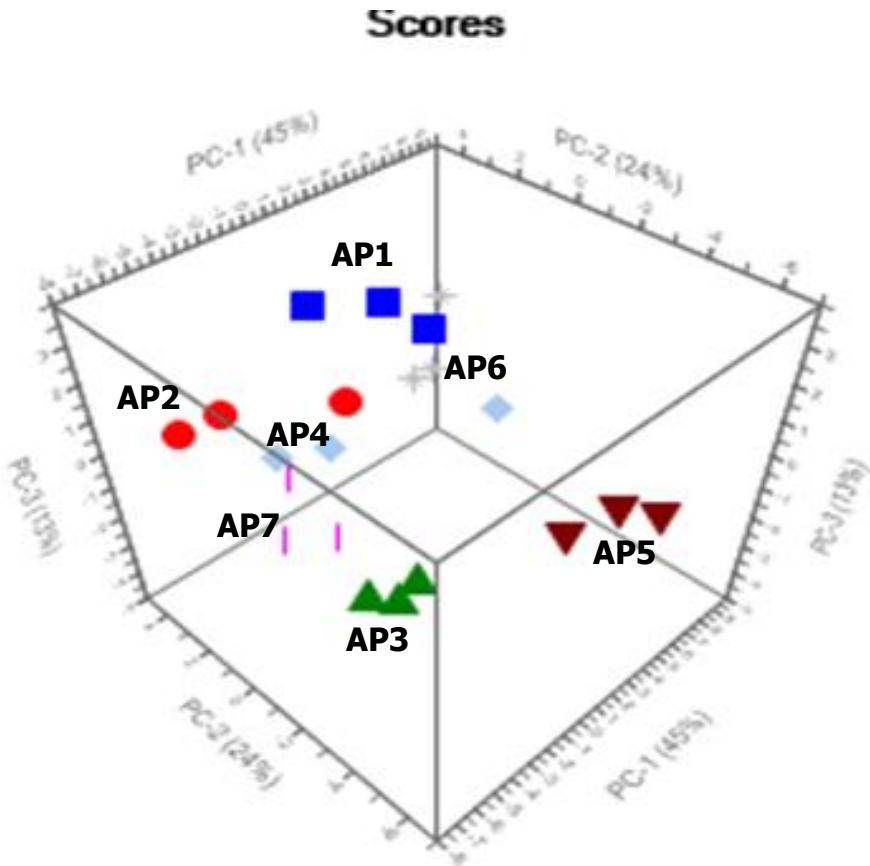
Rendemen ekstrak dan IC₅₀ inhibisi enzim α-glucosidase ekstrak etanol sambiloto

Code of Sampel	Yield (%)		IC ₅₀ (mg/mL) (n = 3)	
	EtOH	Water	EtOH	Water
AP1	14,97	12,20	6.56±0.22 ^a	13.86 ± 0.23 ^b
AP2	13,84	11,47	8.58±0.22 ^a	12.65 ± 0.46 ^b
AP3	14,21	11,58	8.24±0.44 ^a	11.82 ± 0.72 ^b
AP4	14,63	12,15	8.77±0.23 ^a	11.55 ± 0.17 ^b
AP5	14,02	12,01	9.11±0.34 ^a	25.39 ± 3.03 ^b
AP6	13,77	11,23	9.21±0.27 ^a	12.09 ± 0.07 ^b
AP7	13,91	11,68	6.56±0.24 ^a	13.17 ± 0.64 ^b
Acarbose				3.89 ± 0.20 ^a
Andrographolide				7.22 ± 0.33 ^a

No	Senyawa	Mw	MS dan MS/MS	Formula	AP1	AP2	AP3	AP4	AP5	AP6	AP7
1	3-O-Caffeoylquinic acid	354.09502	355, 163	C ₁₆ H ₁₈ O ₉	✓	✓	✓	✓	✓	✓	✓
2	Cinnamic acid	148.05241	149, 131, 105	C ₉ H ₈ O ₂	✓	✓	✓	✓	✓	✓	✓
3	Caffeic acid	180.04243	181, 163, 136	C ₉ H ₈ O ₄	✓	✓	✓	✓	✓	✓	✓
4	3-O-β-D-Glucopyranosyl andrographolide	512.26133	513, 351, 333, 315, 297, 285, 257	C ₂₆ H ₄₀ O ₁₀	✓	✓	✓	✓	✓	✓	✓
5	Apigenin	270.05214	271, 153, 119	C ₁₅ H ₁₀ O ₅	✓	✓	✓	✓	✓	✓	✓
6	12S-Hydroxy andrographolide	368.2197	369, 351, 333, 315, 297, 285, 257	C ₂₀ H ₃₂ O ₆	✓	✓	✓	✓	✓	✓	✓
7	Paniculide B	280.13095	281, 263, 245, 217	C ₁₅ H ₂₀ O ₅	✓	✓	✓	✓	✓	✓	✓
8	5,7-Dihydroxy-8-methoxyflavone	284.06819	285, 119	C ₁₆ H ₁₂ O ₅	✓	✓	✓	✓	✓	✓	✓
9	Apigenin-7-O-beta-D-glucuronide	446.12082	447, 271	C ₂₂ H ₂₂ O ₁₀	✓	✓	✓	✓	✓	✓	✓
10	5,4'-Dihidroxy-7,8,2',3'-tetramethoxyflavone	374.09998	375, 360, 342	C ₁₉ H ₁₈ O ₈	-	✓	✓	✓	✓	✓	✓
11	Luteolin	286.04656	287	C ₁₅ H ₁₀ O ₆	✓	✓	✓	✓	✓	✓	✓
12	14-Deoxy-17-hydroxy-andrographolide	352.22466	353, 335, 317, 299, 287, 259	C ₂₀ H ₃₂ O ₅	✓	✓	✓	✓	✓	✓	✓
13	5-Hydroxy-7,8,2'-trimethoxyflavone 5-glucoside	490.14757	491, 329	C ₂₄ H ₂₆ O ₁₁	✓	✓	✓	✓	✓	✓	✓
14	Paniculide C	278.11549	279, 261	C ₁₅ H ₁₈ O ₅	-	✓	✓	✓	-	✓	✓
15	5,4'-dihydroxy-7,8-dimethoxyflavone	314.07876	315, 197, 119	C ₁₇ H ₁₄ O ₆	✓	✓	✓	✓	✓	✓	✓
16	Andrographic acid	364.18871	365	C ₂₀ H ₂₈ O ₆	-	✓	✓	✓	✓	✓	-
17	14-deoxy-andrographiside	496.26592	497, 317, 299, 287, 259	C ₂₆ H ₄₀ O ₉	✓	✓	✓	✓	✓	✓	✓
18	Andrographolide	350.20816	351, 297, 285, 257	C ₂₀ H ₃₀ O ₅	✓	✓	✓	✓	✓	✓	✓
19	Apigenin 7,4'-dimethyl ether	298.08378	299, 133	C ₁₇ H ₁₄ O ₅	✓	✓	✓	✓	✓	✓	✓
20	5-Hydroxy-7,8-dimethoxyflavanone	300.09918	301, 197, 105	C ₁₇ H ₁₆ O ₅	✓	✓	✓	✓	✓	✓	✓
21	Andrographidin A	462.15198	463, 301, 197	C ₂₃ H ₂₆ O ₁₀	✓	✓	✓	✓	✓	✓	✓
22	5-Hydroxy-7,2',6'-trimethoxyflavone	328.09435	329, 314, 299	C ₁₈ H ₁₆ O ₆	✓	✓	✓	✓	✓	✓	✓
23	5-Hydroxy-3,7,8,2'-tetramethoxyflavone	358.10486	359, 329, 197	C ₁₉ H ₁₈ O ₇	✓	✓	✓	-	-	✓	✓
24	Neoandrographolide	480.27237	481	C ₂₆ H ₄₀ O ₈	✓	✓	✓	-	-	-	-
25	14-Acetyl-andrographolide	392.22	393, 315, 297, 285, 257	C ₂₂ H ₃₂ O ₆	✓	✓	✓	✓	✓	✓	✓
26	Andrograpanin	318.2187	319, 301, 289, 261	C ₂₀ H ₃₀ O ₃	✓	✓	✓	✓	✓	✓	✓
27	14-Deoxy-11-oxoandrographolide	348.1936	349, 331, 313, 285	C ₂₀ H ₂₈ O ₅	-	-	✓	-	-	-	-
28	14-Deoxy-andrographolide	334.2134	335, 317, 299, 287	C ₂₀ H ₃₀ O ₄	✓	✓	✓	✓	✓	✓	✓
29	Andrographolactone	296.1769	297, 269	C ₂₀ H ₂₄ O ₂	✓	✓	✓	✓	✓	✓	✓
30	Dehydro-andrographolide	332.1977	333, 315, 297, 285, 257	C ₂₀ H ₂₈ O ₄	✓	✓	✓	✓	✓	✓	✓
31	5,7,2',3'-Tetramethoxy-flavanone	344.1259	345	C ₁₉ H ₂₀ O ₆	✓	✓	✓	✓	✓	✓	✓
32	Dihydroskullcap-flavone I	316.0945	317, 299, 197, 121	C ₁₇ H ₁₆ O ₆	✓	✓	✓	✓	✓	✓	✓

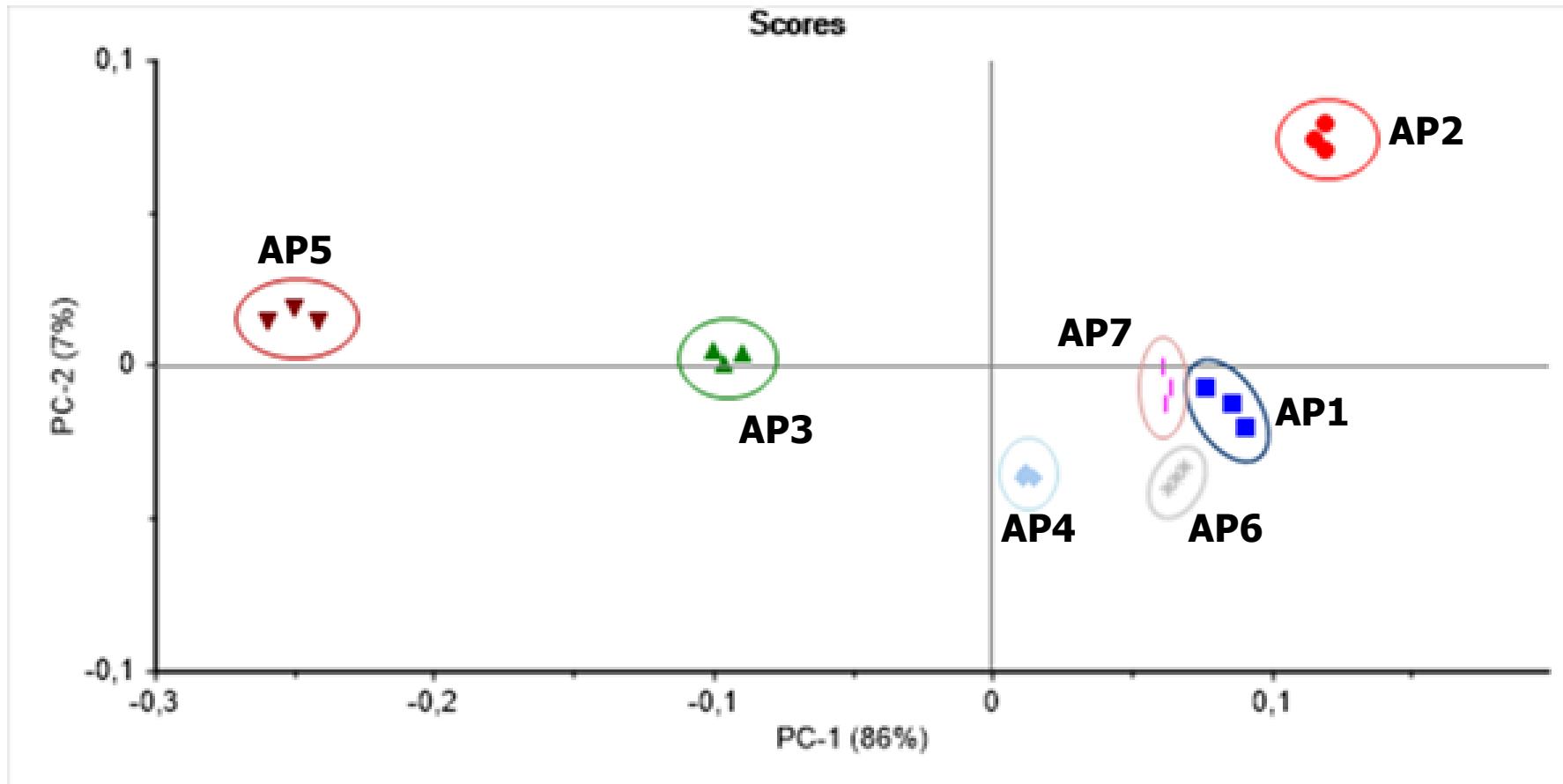
Komposisi
senyawa kimia
sambiloto yang
diambil dari
berbagai lokasi

Sambiloto dari berbagai lokasi tumbuh

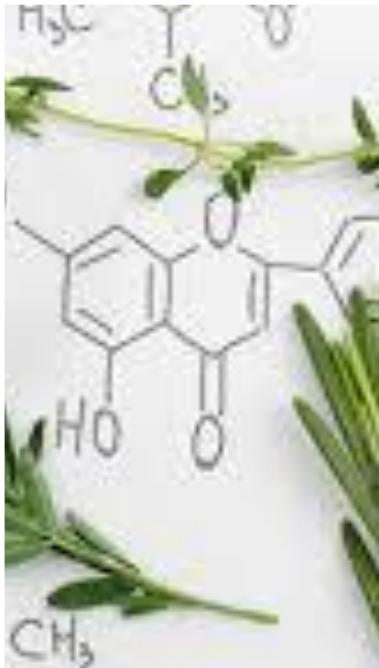


PCA score plot of FTIR Spectra in 3D

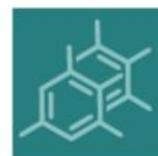
Sambiloto dari berbagai lokasi tumbuh



PCA score plot of 32 identified metabolites (UHPLC-Q-Orbitrap HRMS) in 2D



Contoh aplikasi kemometrik-metabolomik dalam studi farmakologi tumbuhan obat



Article

Anti-Diabetic Activity and Metabolic Changes Induced by *Andrographis paniculata* Plant Extract in Obese Diabetic Rats

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† These authors contributed equally to this work.

Abstrak



Abstract: *Andrographis paniculata* is an annual herb and widely cultivated in Southeast Asian countries for its medicinal use. In recent investigations, *A. paniculata* was found to be effective against Type 1 diabetes mellitus (Type 1 DM). Here, we used a non-genetic out-bred Sprague-Dawley rat model to test the antidiabetic activity of *A. paniculata* against Type 2 diabetes mellitus (Type 2 DM). Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy in combination with multivariate data analyses was used to evaluate the *A. paniculata* and metformin induced metabolic effects on the obese and obese-diabetic (obdb) rat models. Compared to the normal rats, high levels of creatinine, lactate, and allantoin were found in the urine of obese rats, whereas, obese-diabetic rats were marked by high glucose, choline and taurine levels, and low lactate, formate, creatinine, citrate, 2-oxoglutarate, succinate, dimethylamine, acetoacetate, acetate, allantoin and hippurate levels. Treatment of *A. paniculata* leaf water extract was found to be quite effective in restoring the disturbed metabolic profile of obdb rats back towards normal conditions. This study shows the anti-diabetic potential of *A. paniculata* plant extract and strengthens the idea of using this plant against the diabetes. Further classical genetic methods and state of the art molecular techniques could provide insights into the molecular mechanisms involved in the pathogenesis of diabetes mellitus and anti-diabetic effects of *A. paniculata* water extract.

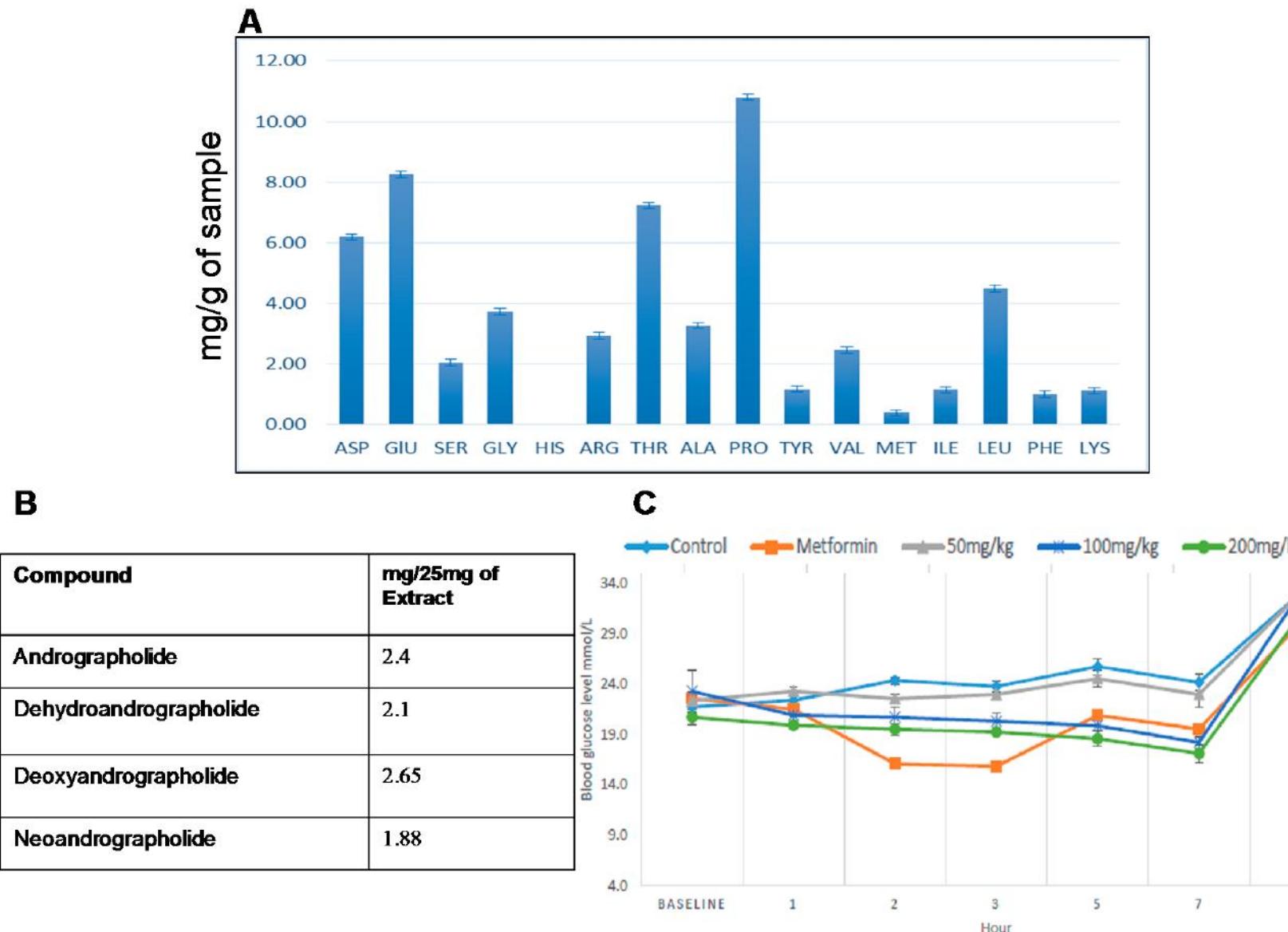
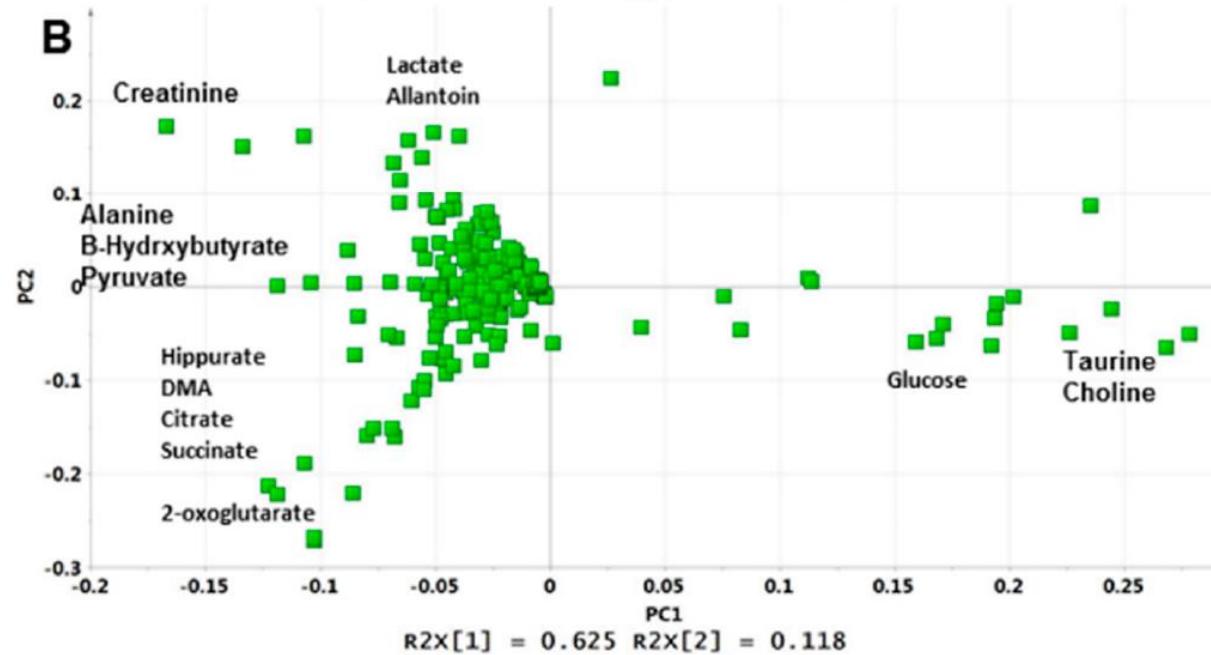
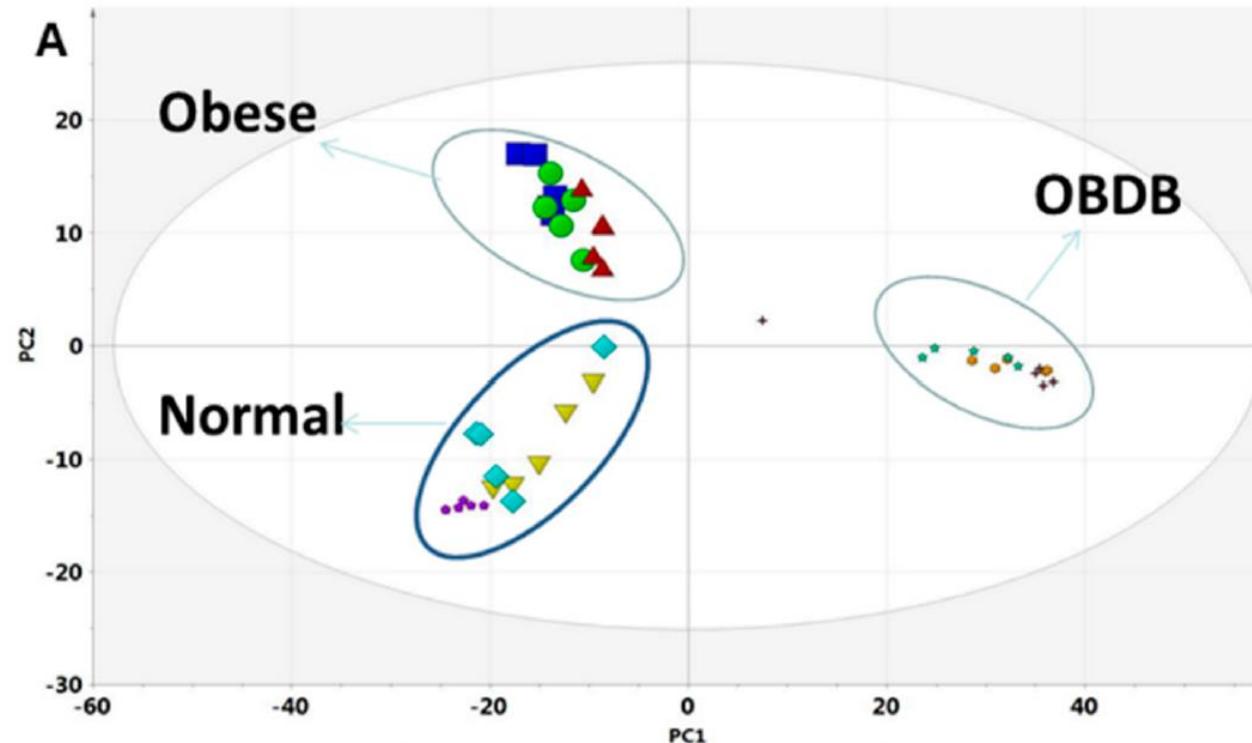


Figure 1. (A) Percentage content of individual amino acids of *A. paniculata* Harapan leaf water extract; (B) Quantitative ^1H NMR analysis of four major chemical markers of *A. paniculata* leaf water; (C) Oral glucose tolerance test for obese-diabetic group treated and non-treated with *A. paniculata* harapan leaf water extract.



(A) PCA score plot of normal, obese, and obese-diabetic (obdb) rats;
 (B) PCA loading plot of normal, obese and obese-diabetic (obdb) rats

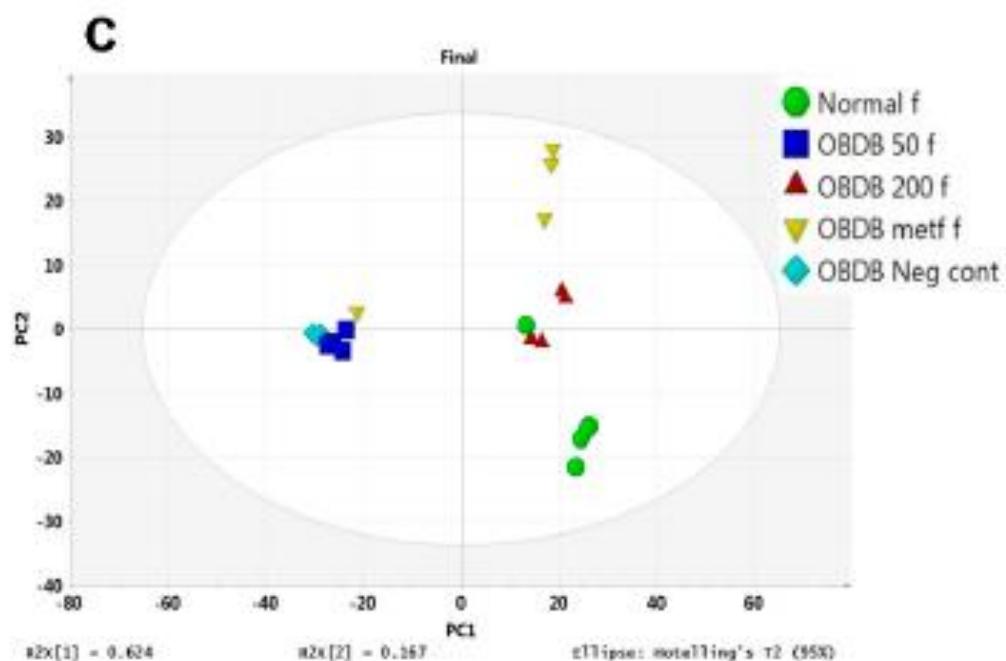
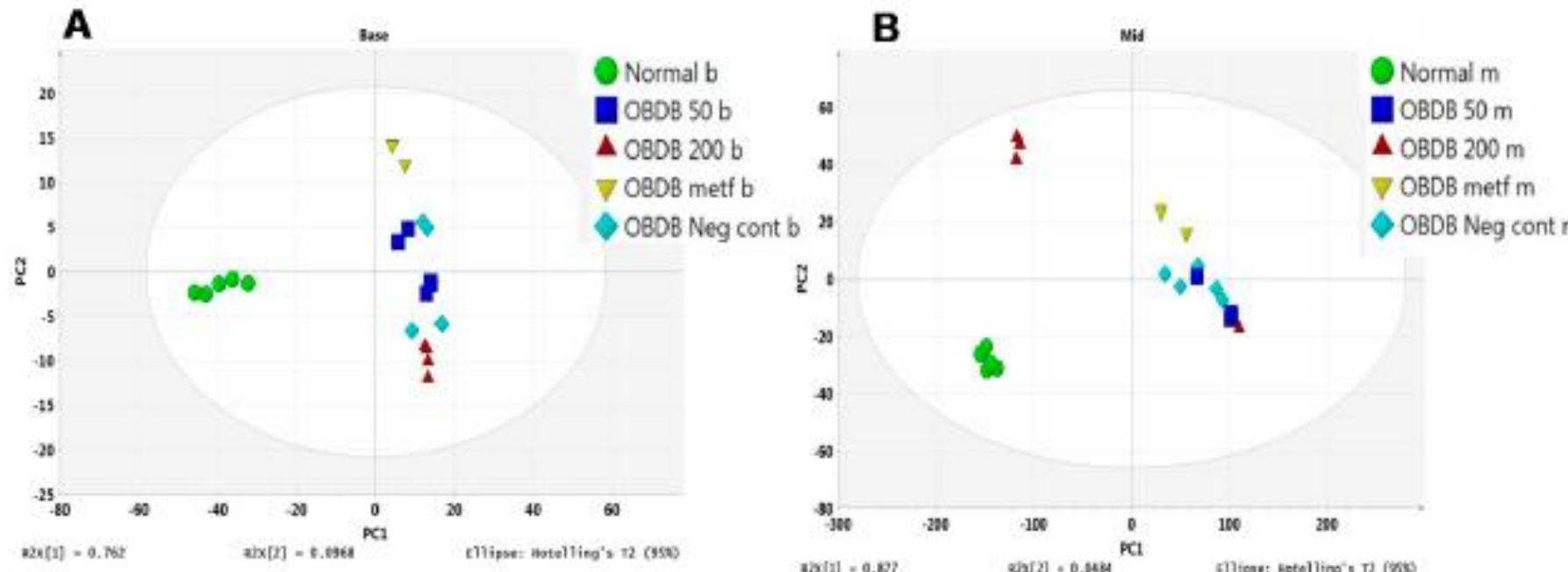
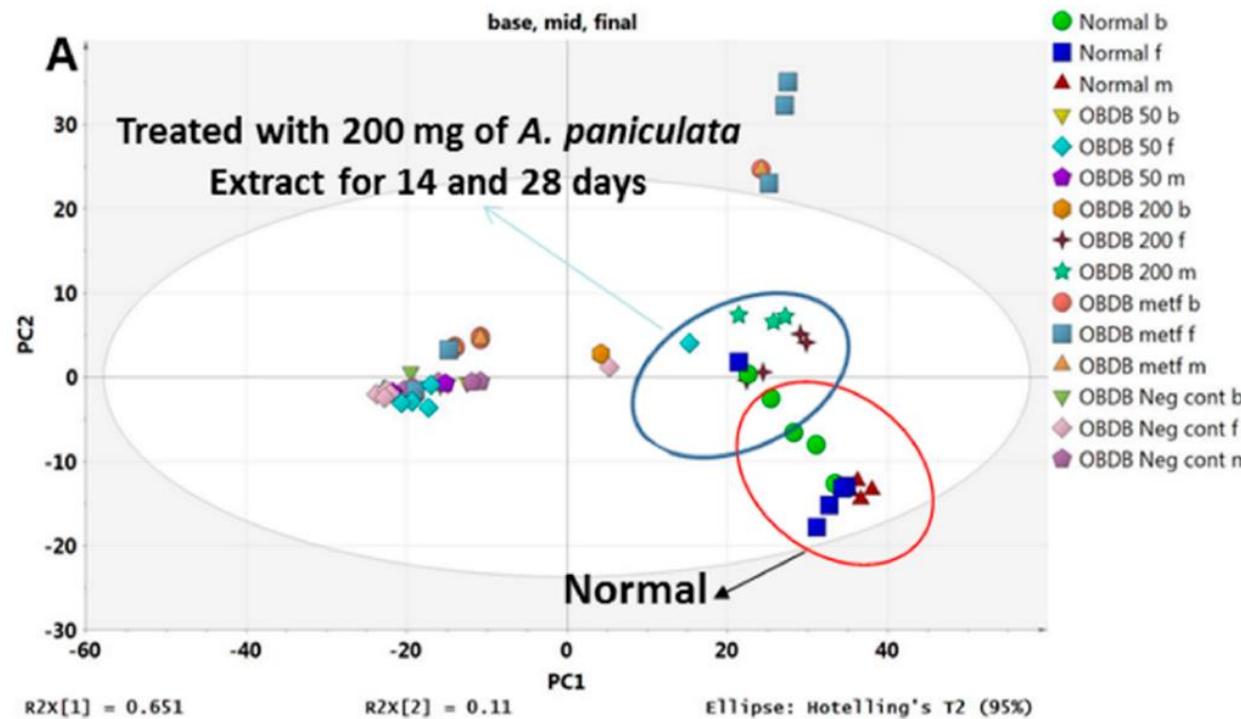
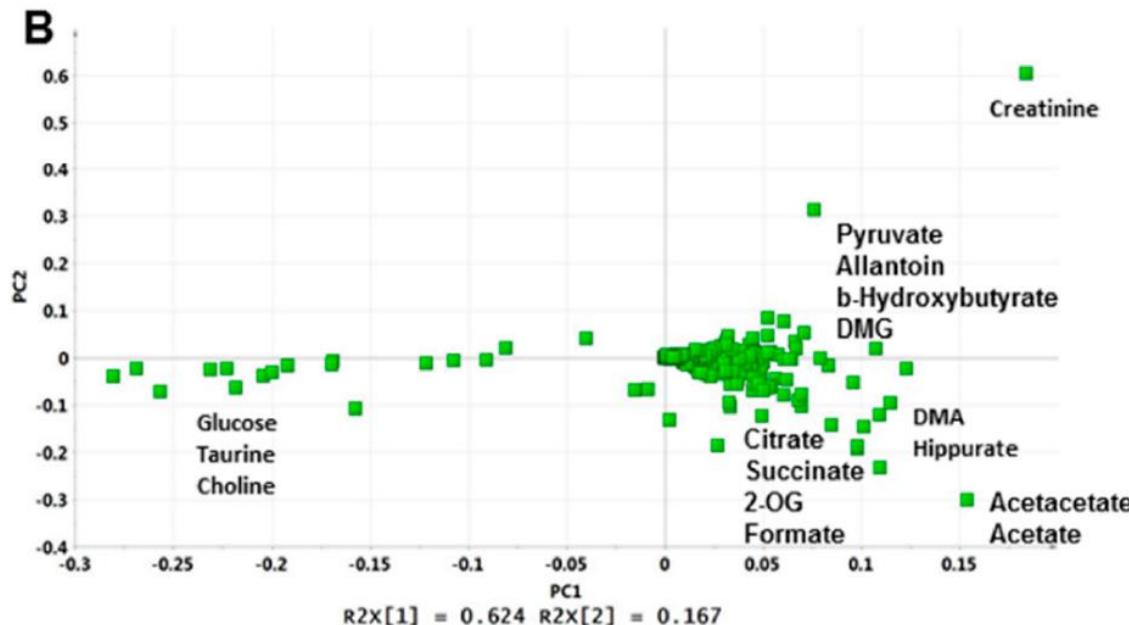


Figure 3. (A) PCA score plot of obese-diabetic and obese-diabetic treated rats' urine samples collected at the basal stage. Normal (normal b), obese-diabetic (obdb neg cont b), obdb rats treated with 50 mg of *A. paniculata* extract (obdb 50 b), obdb rats treated with 200 mg of *A. paniculata* extract (obdb 200 b), and obdb rats treated with metformin (obdb metf b); (B) PCA score plot of obese-diabetic and obese-diabetic treated rats' urine samples collected at the middle stage. Normal (normal m), obese-diabetic (obdb Neg cont m), obdb rats treated with 50 mg of *A. paniculata* extract (obdb 50 m), obdb rats treated with 200 mg of *A. paniculata* extract (obdb 200 m), and obdb rats treated with metformin (obdb metf m); (C) PCA score plot of obese-diabetic and obese-diabetic treated rats' urine samples collected at the final stage. Normal (normal f), obese-diabetic (obdb Neg cont f), obdb rats treated with 50 mg of *A. paniculata* extract (obdb 50 f), obdb rats treated with 200 mg of *A. paniculata* extract (obdb 200 f), and obdb rats treated with metformin (obdb metf f). ("b" = basal stage, "m" = middle stage, "f" = final stage, "Neg cont" = Negative control).



(A) PCA score plot of normal, obese-diabetic, and obese-diabetic treated (50 or 200 mg of *A. paniculata* extract, or metformin) rats' urine samples collected at the basal, middle, and final stages;

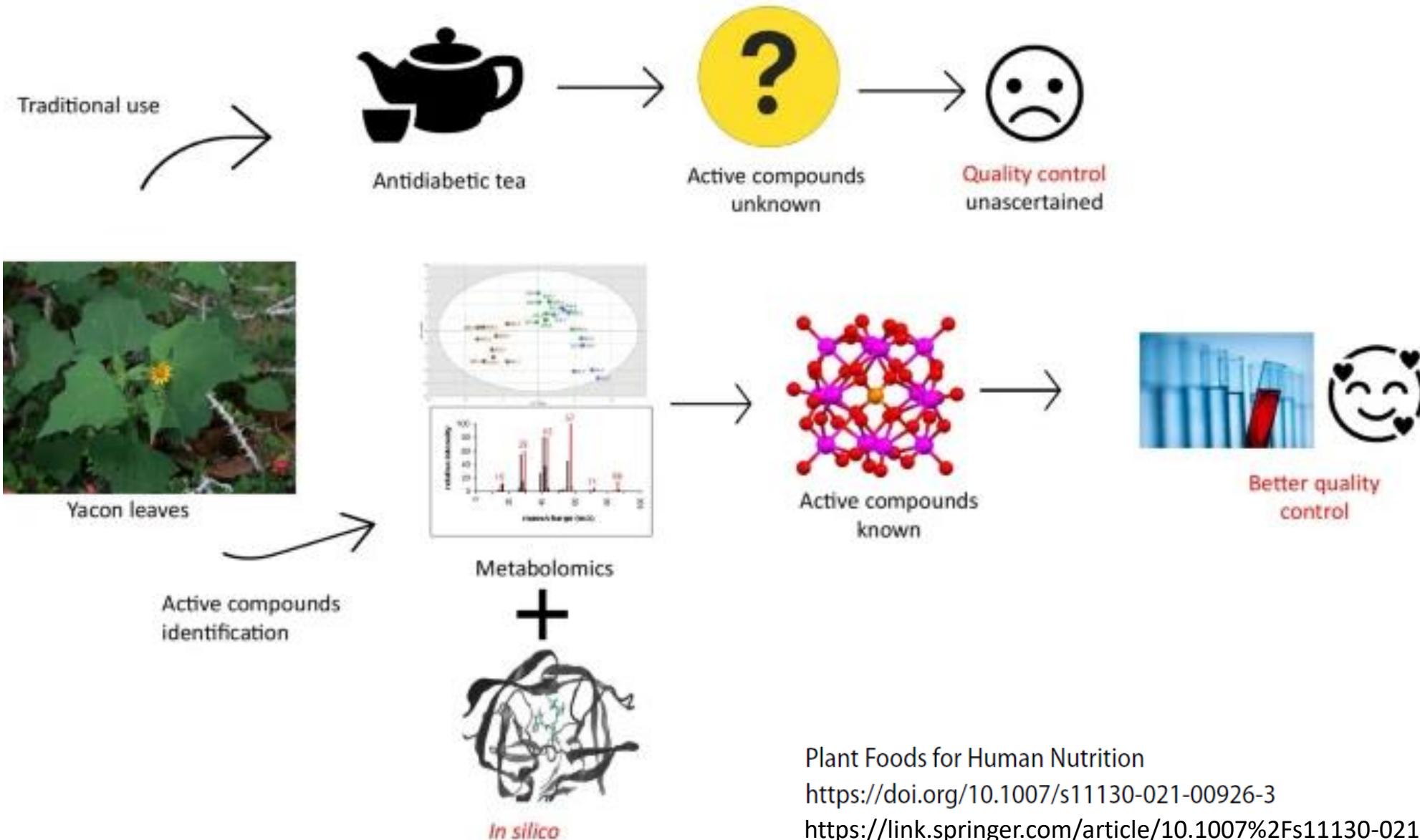


(B) PCA loading plot of normal, obese-diabetic, and obese-diabetic treated (50 or 200 mg of *A. paniculata* extract, or metformin) rats' urine samples collected at the basal, middle, and final stages.

Investigasi inhibisi α -glucosidase daun yakon



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Investigasi inhibisi α -glucosidase daun yakon

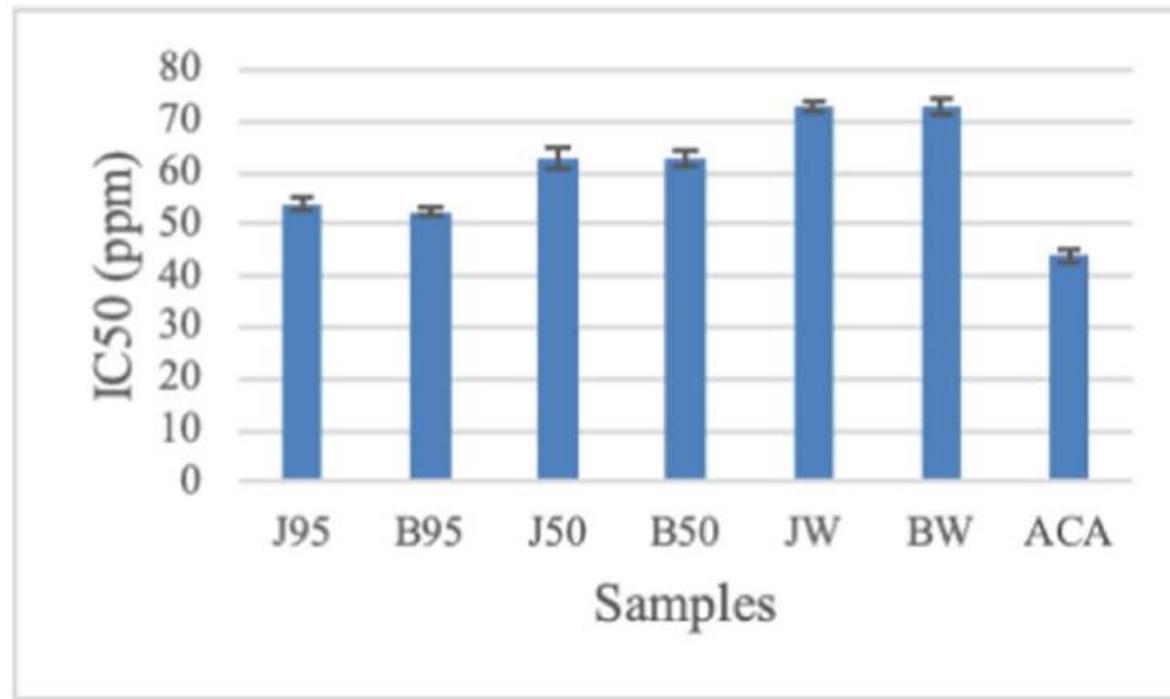


Fig. 1 *In vitro* α -glucosidase inhibitor activity profile of Yacon leaf extracts, expressed in IC₅₀ value (J=Yacon leaves from Wonosobo-Central Java, B=Yacon leaves from Lembang-West Java, 50 and 95 reflecting ethanol 50 and 95% as extraction solvent, W=water extract, ACA=Acarbose). The values were the mean from five replications

Analisis OPLS --- spektrum FTIR

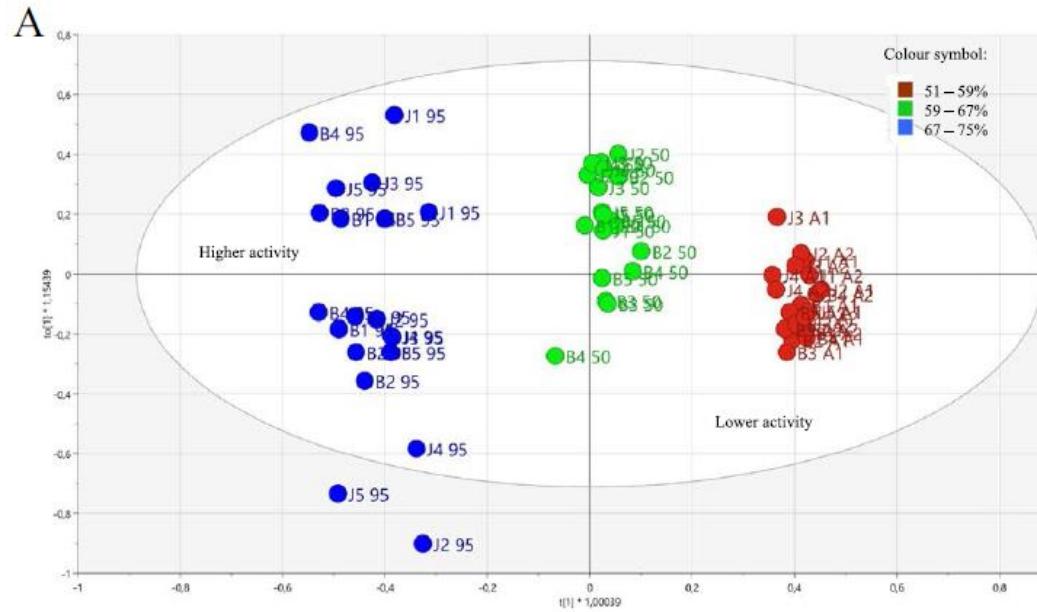
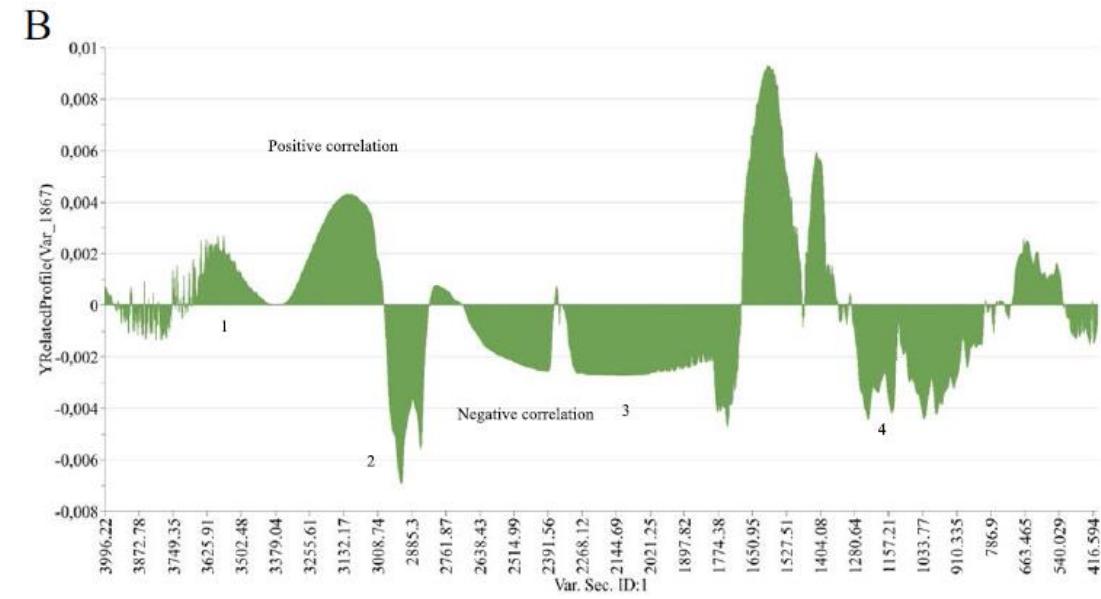
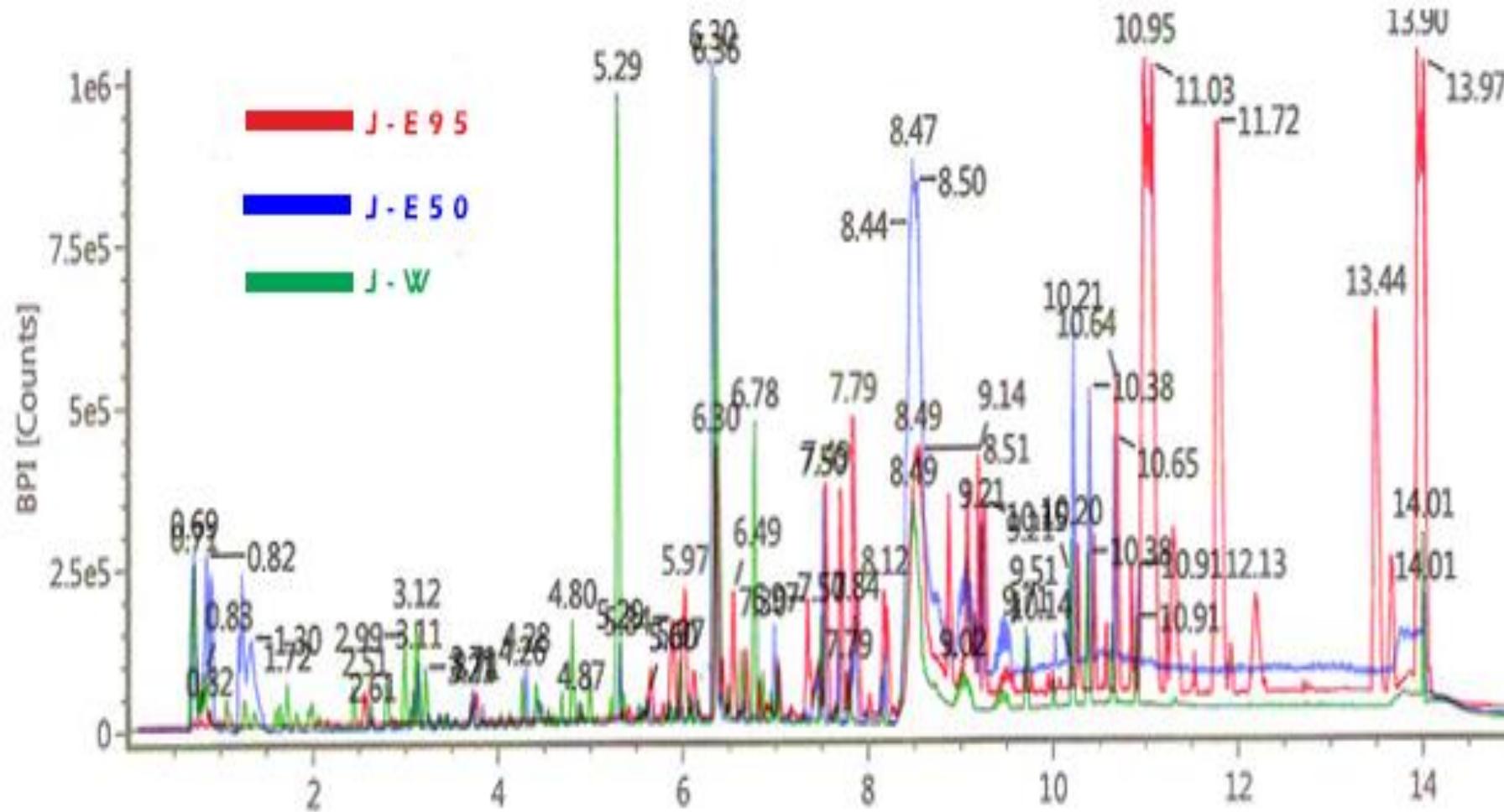


Fig. 2 A FTIR OPLS scores scatter plot of Yacon extracts. B = Yacon from Lembang (West Java), J = Yacon from Wonosobo (Central Java). The numbers following B and J represented extraction solvents (95=ethanol 95%, 50=ethanol 50%, A=water) and replication, respectively. Red to blue colour representing lower to higher



α -glucosidase inhibitor activity B: Y-related Profile Coefficient Plot to determine which wavenumber regions contribute to samples separation in the score scatter plot (1 = 3872.78–3749.35 cm⁻¹, 2 = 2983.66–2831.36 cm⁻¹, 3 = 2638.43–1774.38 cm⁻¹, 4 = 1774.38–786.9 cm⁻¹)

LC-MS/MS

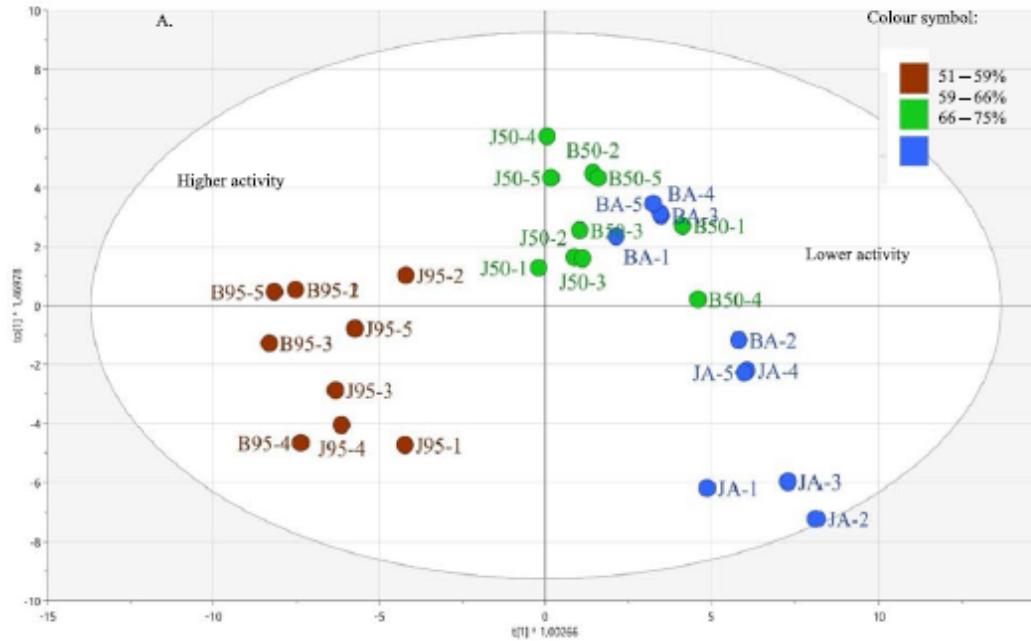


Representative Chromatogram of LC-MS/MS water extract (JW), 95% ethanol extract (J95) and 50% ethanol extract (J50) from Yacon J leaf (The data was obtained using LC-MS/MS Water Acquity Xevo G2 XS, Column acquity UPLC C18 HSS T3 1mm, or BEH C18 50mm). The mobile phase system is explained in Table S1.

Analisis OPLS --- LC-MS/MS



A



B

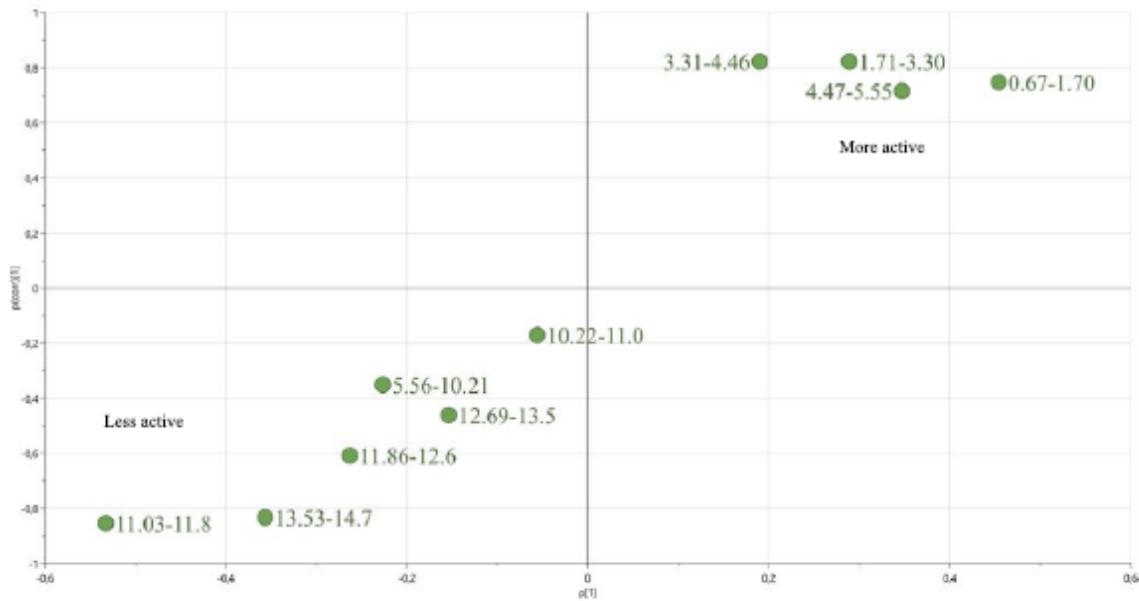


Fig. 3 OPLS analysis of LC-MS data **A:** Score scatter plot, showing samples grouping based on their α -glucosidase inhibitor activity **B:** S-plot to identify LC-retention time responsible for the sample

separation based on the IC_{50} in the score scatter plot. Retention times located in the left side of the plot are the dominant peaks in the chromatogram of the sample with higher α -glucosidase inhibitor activity

Analisis OPLS --- LC-MS/MS



Table 1 Peaks with retention time predicted to be associated with α -glucosidase inhibitor activity based on Y-related coefficient and VIP value

Retention time (minutes)	Y-related coefficient	VIP
13,53–14,70	– 0.91147	1.22002*
11,03–11,85	– 0.91147	1.5237*
11,86–12,86	– 0.647327	0.901493
12,69–13,50	– 0.559524	0.760514

*VIP ≥ 0.5 is relevant, VIP > 1 is highly relevant correlates with α -glucosidase inhibitor activity

Table 2 Docking score and amino acids residue involved in H-bond (kcal/mol) of the four compounds obtained from OPLS

No	Compounds	Docking Score (kcal/mol)	H-bond interacting residues
1	Nystose	-115,657	Lys422, Asp420, Asp73, Glu481
2	1-Kestose	-112,641	Asp73, Asp236, Asp197
3	Luteolin-3'-7-di-O-glucoside	-111,986	Glu481, Lys422, Asp455, Phe314, Gln344
4	1,3-O-Dicaffeoilquinic acid	-100,216	Asp197, Asp420, His478
5	Native ligand/isomaltase	-99.4922	Asp197, His478, Asp420, Arg404, Asp73
6	Acarbose/reference drug	-115,774	Asp420, His478, Asp197, Arg404, Gln344, Trp341

Ringkasan



Kemometrik dan metabolomik menjadi salah satu bagian penting dalam pengembangan obat herbal yang memiliki sistem yang kompleks dengan multivariat variabel yang ada



Kemometrik dan metabolomik dapat digunakan mulai dari standardisasi bahan baku hingga sampai produk jadi obat herbal



Analytical chemistry is what analytical chemist do (CN Reilly)

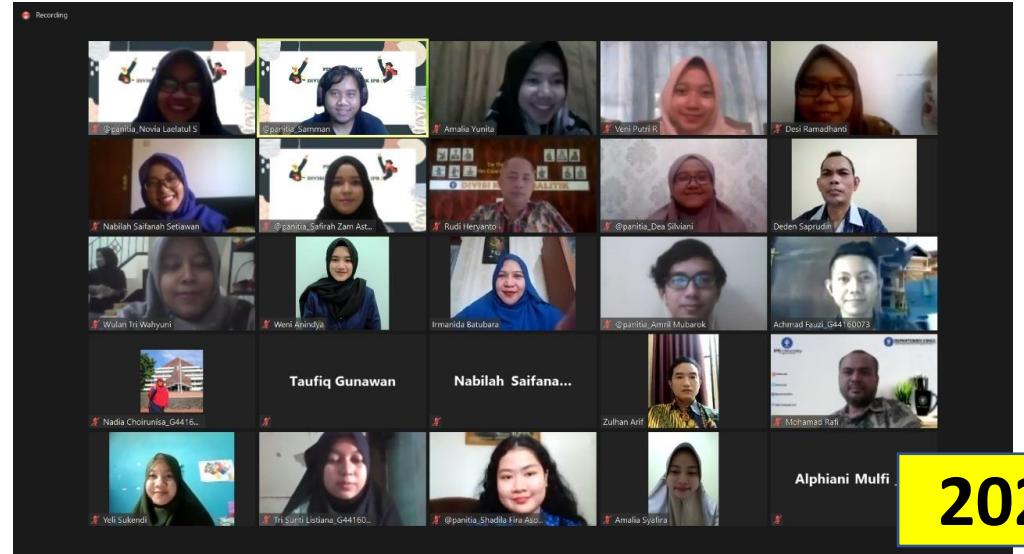
2018



Analytical Chemistry Research Group, IPB University:

- Metabolomics and chemometrics
- Natural product analytical chemistry
- Sensor and material for analytical chemistry

2019



2020

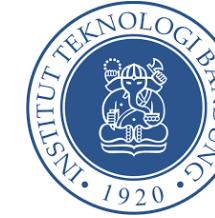


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Kolaborator



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Mari kita berkolaborasi.....



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