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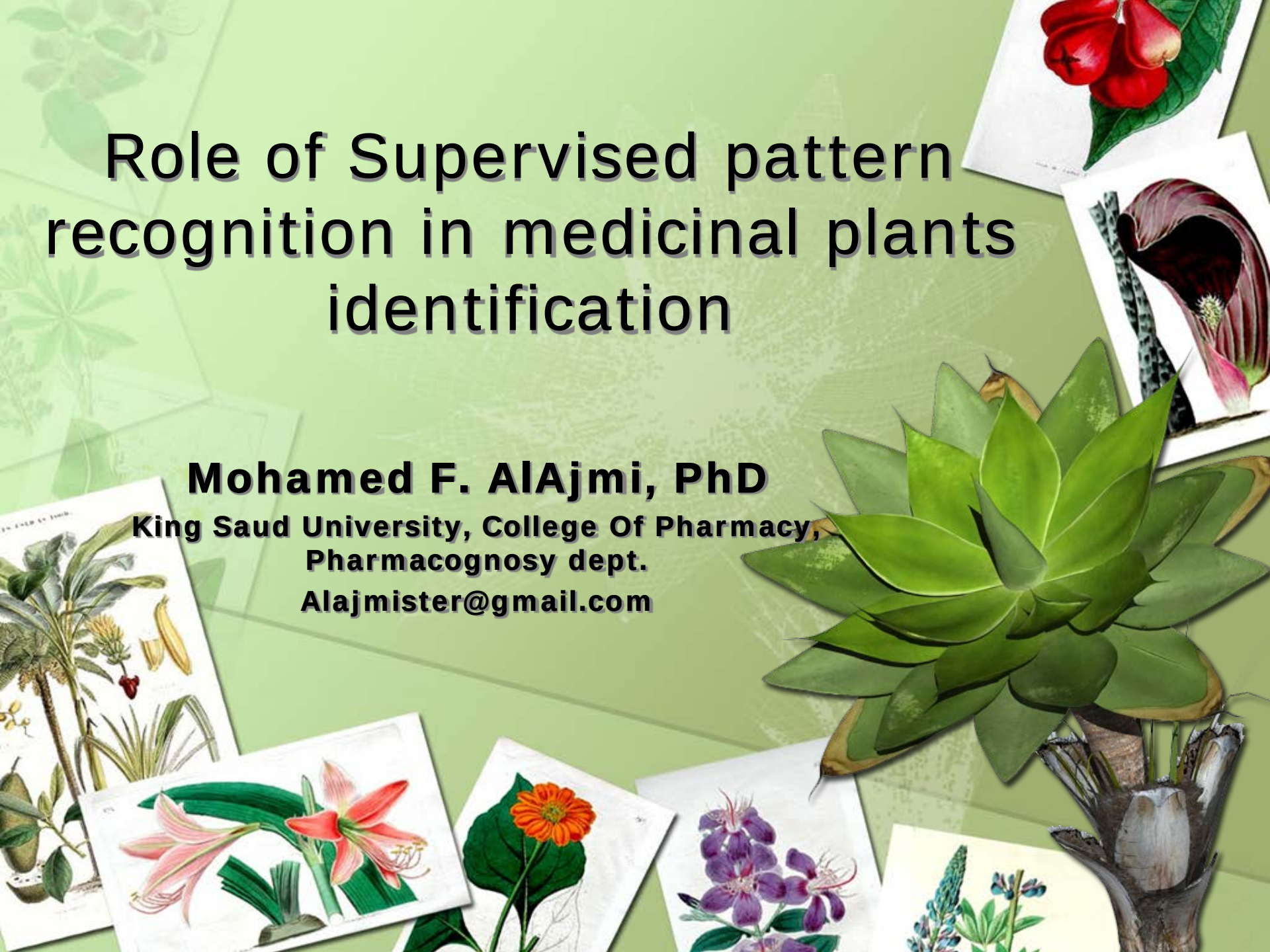
Role of Supervised pattern recognition in medicinal plants identification

Mohamed F. AlAjmi, PhD

King Saud University, College Of Pharmacy, Pharmacognosy dept.

Alajmister@gmail.com



Objectives

- To explore the power of Supervised pattern recognition in medicinal plants authentication.
- To study the relationship between sample preparation and quality of data.

Medicinal plants variability

- All the biochemical and medicinal ingredients in *Mentha longifolia varied* significant during different seasons

Medicinal plants variability

- Adhatoda vasica (Pandita et al., 1983), Mentha pulegium (Stengele et al., 1993), Sargassum wightii (Reeta, 1993), Mentha spicata (Kofidis et al., 2004), Toona sinensis (Wang etal., 2007), Adiantum capillus-veneris (Ahmad et al., 2008), Ocimum basilicum (Hussain et al., 2008) and Ulva reticulata (Shanrnugam & Palpandi, 2008).

Classification methods

- Several classification techniques have been proposed, each of them with different properties and skills, offering to the scientist different approaches for solving classification problems.

Classification methods

- Macroscopic features e.g inflorescence description, Linnaeus system.
- Microscopic features e.g Ca oxalate crystals.
- Biomarkers e.g Naringinin in *Rumex* and Menthol in *Mentha* (Peppermint)
- Genotyping

Classification methods

- Tedious work!
- Big variation!
- Potential for misidentification especially varieties (same species but small difference e.g Catha varieties)
- Useless in Herbal powdered mixtures.
- Remember! Herbs might be a treatment and might be poisonous?!
- Can Multivariate analysis help in solving these problems?

Medicinal plants variability

- *Ameyaw et al (Phytochemistry Department and Microbiology Department, Centre for Scientific Research into Plant Medicine, Mampong- Akuapem)*
- *Ghana*

Medicinal plants variability

1. *Alchornea cordifolia (Schum.& Thonn.) Stapf Leaves ACl*
2. *Cinnamomum zeylanium Nees Leaves CVl*
3. *Clausena anisata (Will.) Hook.f.ex Benth Root bark CArb*
4. *Cryptolepis sanguinolenta (Lindl.) Schtr. Root bark CSrb*
5. *Cnestis ferruginea DC Leaves CFl*
6. *Hoslundia opposita Vahl Leaves HOl*
7. *Lippia multifolia Moldenke Leaves LMl*

Medicinal plants variability

8. *Morinda lucida Benth Leaves MLl*
9. *Ocimum gratissimum Linn. Leaves OGl*
10. *Psidium guajava Linn. Leaves PGl*
11. *Spondias mombin Linn. Leaves SMl*
12. *Tridax procumbens Whole plant TPwp*
13. *Xylopia aethiopica (Dunal) A. Rich Fruits XAf*
14. *Zanthoxylum xanthoxyloides (Lam.) Waterm Root bark ZXrb*

Medicinal plants variability

- Five replicates were prepared for each plant organ per location and the mean value computed.

Medicinal plants variability

- The Duncan’s Multiple Range Test and Principal Component Analysis were applied to assess the level of significant differences and weighting of the medicinal plant species.

Medicinal plants variability

Name of plant species	Family	Local name	Plant part used	Total extraction (g)
Alchornea cordifolia	Euphorbiaceae	Agyamma, Gyeka, Gboo, Gbloo, Ahama	Leaves	28.30(g)
Cinnamomum zeylanium	Lauraceae	---------	Leaves	83.10 (b)
Clausena anisata	Rutaceae	Sesadua, Samanɔbere, Duawonsi, Ayira	Leaves	47.59 (c)
Cryptolepis sanguinolenta	Periploaceae	Nibima, Kadze, Gangamau	Root bark	19.68 (j)
Cnestis ferruginea	Connaraceae	Apɔsẽ, Akitase, Pudaegye	Leaves	18.43 (k)
Hoslundia opposita	Labiate	Aberewa-ani-nsu, Nunum-	Leaves	

Medicinal plants variability

1. Results:

- Significant difference among the medicinal plant species using Duncan’s Multiple Range Test and the Principal Coponent Analysis (PCA).

Medicinal plants variability

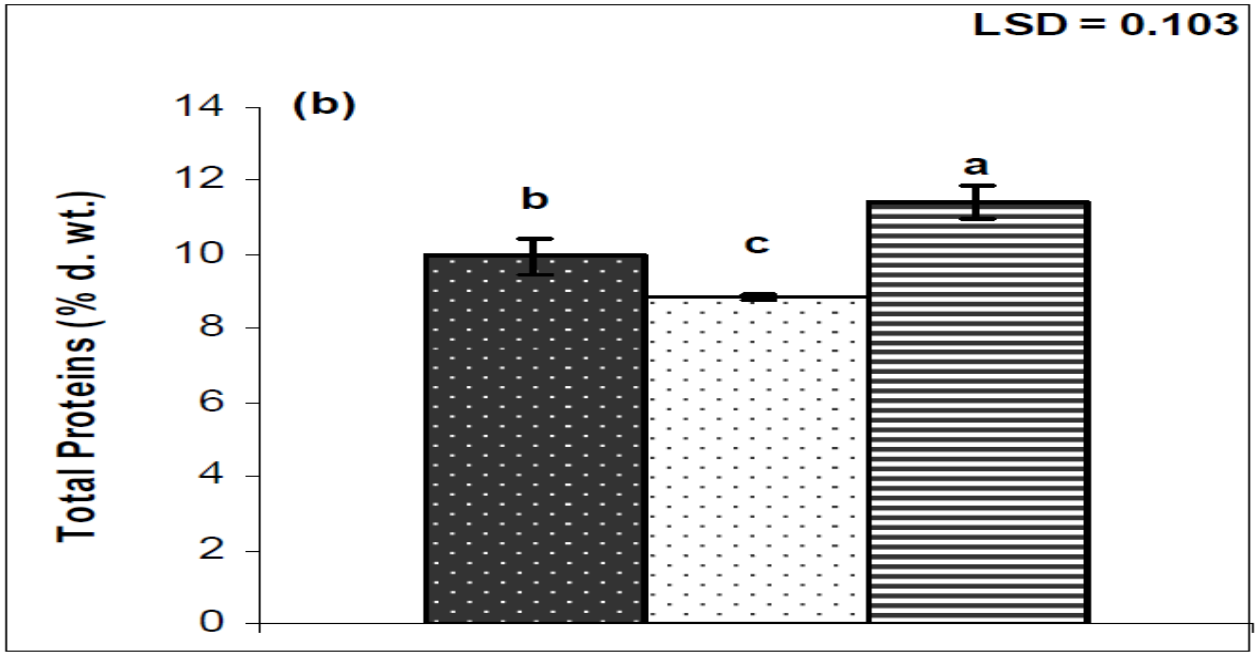
Plant species	PC 1	PC 2	PC 3	PC 4
ACl	0.080	0.383	-0.094	0.390
CZl	0.346	-0.016	-0.147	-0.093
CArb	-0.162	-0.436	-0.054	0.064
CSrb	-0.405	0.070	-0.120	-0.139
CFl	0.167	-0.339	0.092	-0.310
HOl	0.361	0.025	-0.024	-0.201
LMl	-0.047	0.175	-0.558	0.059
MMs	0.158	0.078	-0.610	0.070
MLl	0.169	-0.201	-0.437	-0.381
OGl	-0.405	0.070	-0.120	-0.139
PGrb	-0.405	0.070	-0.120	-0.139
SMl	-0.317	-0.040	-0.089	0.132
TPwp	0.135	-0.239	-0.068	0.649
XAf	-0.065	-0.450	-0.127	0.209

Medicinal plants variability

- They conclude that some of the medicinal plant species can be rated as highly variable more than others.

Medicinal plants variability

- Another example:
- *Mentha longifolia:* The shoots of *M. longifolia collected during different* seasons differed significantly for



IFTIKHAR AHMED *et al*., 2011

Supervised Pattern Recognition

- Data analysis has become a fundamental task in analytical chemistry due to the great quantity of analytical information provided by modern analytical instruments.

Supervised Pattern Recognition

- Establish a classification model based on experimental data in order to assign unknown samples to a previously defined sample class based on its pattern of measured features.

Supervised Pattern Recognition

- Multivariate classification is one of the basic methodologies in chemometrics and aims in finding mathematical relationships between a set of descriptive variables and a qualitative variable.

Supervised Pattern Recognition

- Is good alternative to other classifying methods i.e. cellular key element... etc

Comparison between different types of coffee as an example

- The more popular types of coffee are: Espresso (strong black coffee), Turkish coffee, Arabic mildly roasted coffee… etc.
- The Caffeine ;main content of the coffee; varies depending on the brewing method, variety of seed etc.
- The strength of the coffee is usually interpreted with respect to the taste of the coffee users.

Comparison between different types of coffee as an example

- Method:
- Coffee samples (Arabic coffee and wild coffee) were purchased from the market. The samples have been authenticated by smart coffee drinkers. Water was double distilled and methanol was of mass grade. Formic acid is analytical grade.

Comparison between different types of coffee as an example

- Method:
- Sample preparation: 200 mg commercial coffee of each samples have been extracted three times each with boiling water. The samples were then filtered through wattmann filter paper and 1 ml has been withdrawn through 0.45 filter. Each sample were analysed in triplicate

Comparison between different types of coffee as an example

- Method:
- Analysis: LC-QTOF (Agilant 6540) with C18 Zorbax column (100 mm, 1.8 um particle size) have been used. The samples have eluted in triplicate using the mobile phase consisting of 100% methanol (A) and 1% formic acid in water (B). The gradient was 1:1 at zero time for 0.5 min and ramping up to 95 % of A. The results were analysed using Mass hunter software 5.0 and multivariate analysis was done using MPP.

Comparison between different types of coffee as an example

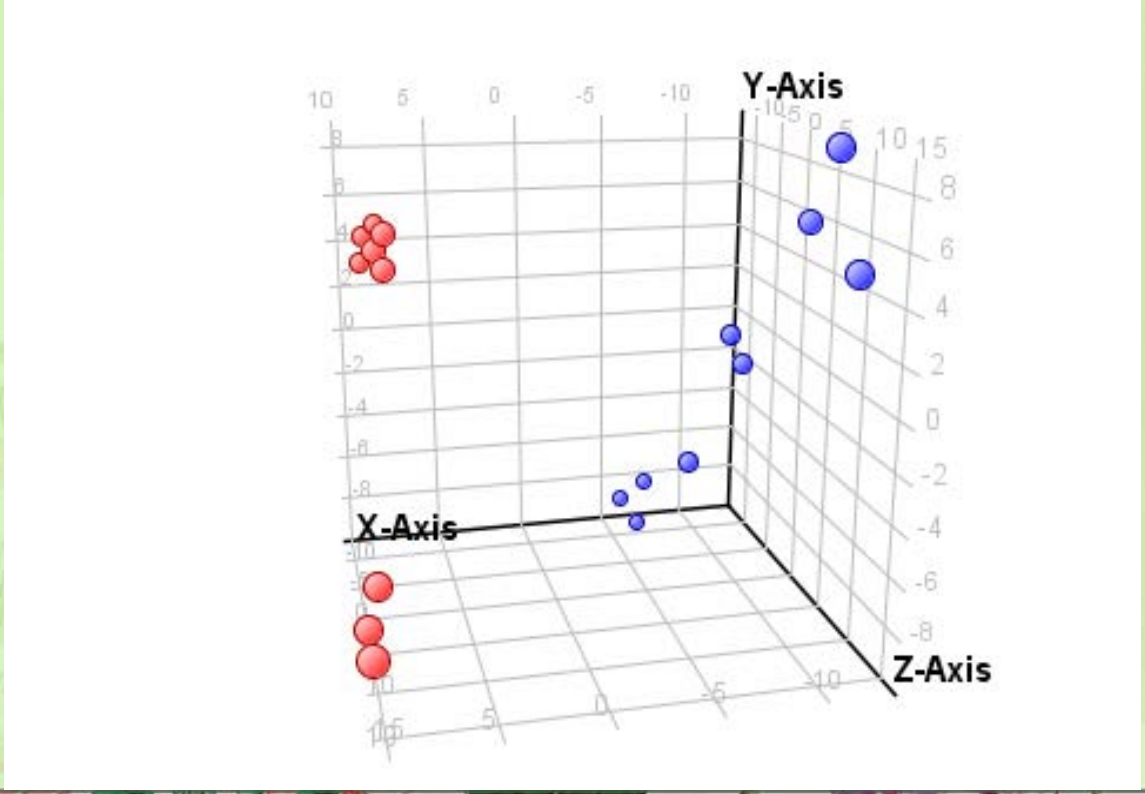
- Results:
- PCA (Fig 1)shows clearly the different pattern of Arabic coffee (AC) and Wild coffee (WC). This difference is presented in terms of the different compounds present in each type of coffee (Fig 2). Analysis of these compounds revealed a list of potential

  differentiating compounds

  listed in Fig 3.

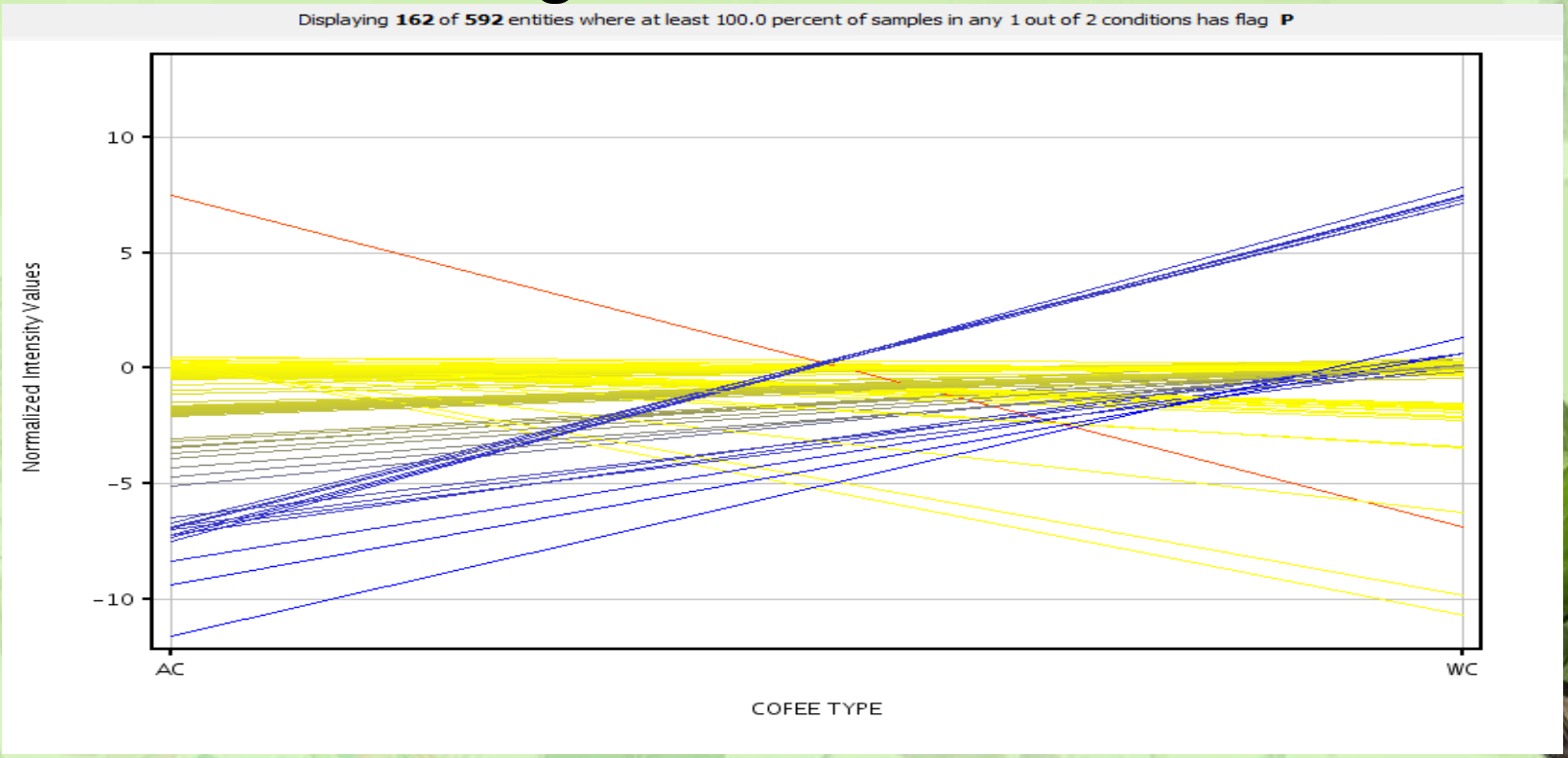
Comparison between different types of coffee as an example

- Results: Fig 1



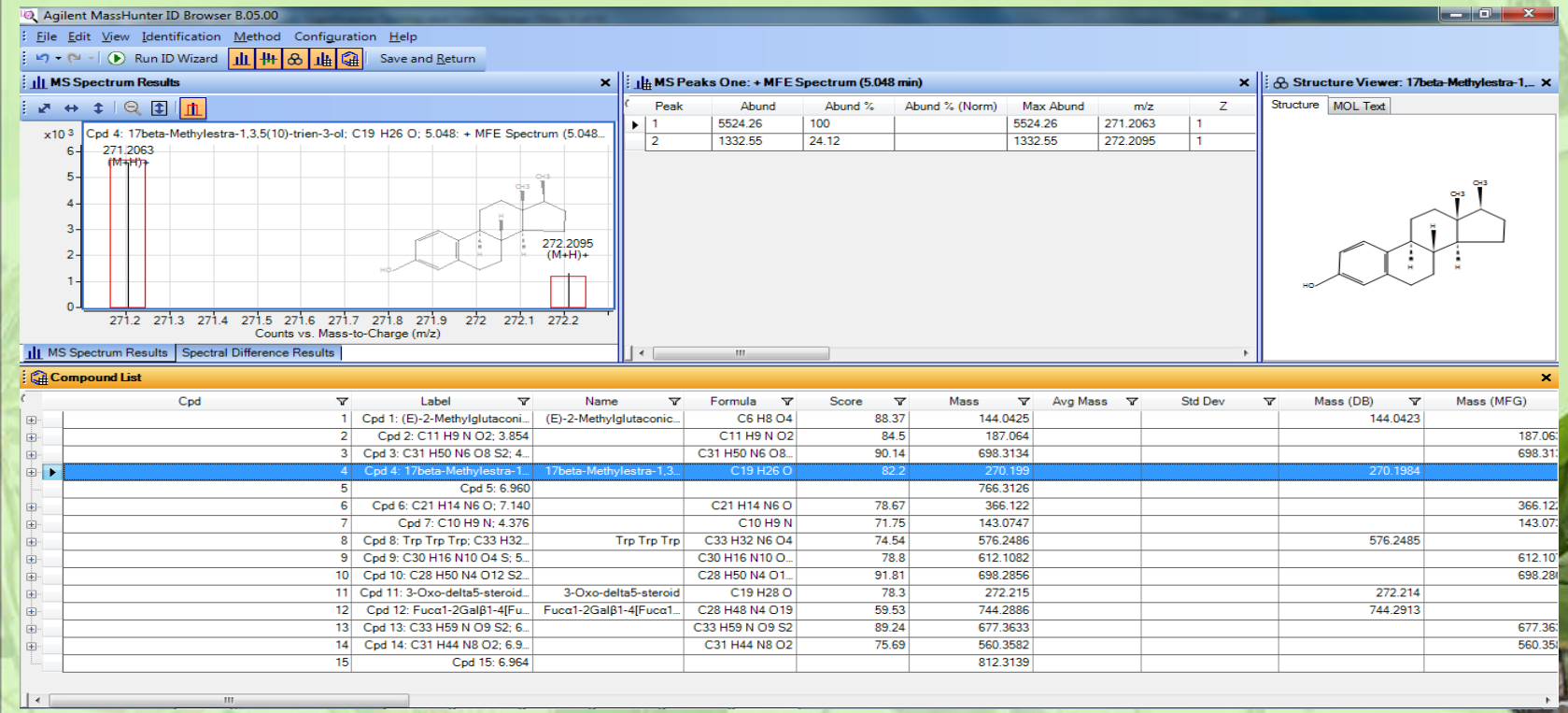
Comparison between different types of coffee as an example

- Results: Fig 2 OPLS



Comparison between different types of coffee as an example

- Results: Fig 3



Conclusions

- The study shows clear difference between Arabic coffee and wild coffee in terms of certain chemical constituents, caffeine is not one of them.
- The taste has no correlation with any biological active chemical constituents.

Conclusions

- Multivariate analysis may help in authentication of herbal samples provided that the preparation method is standardized and there is a strict GACP guidelines.
- The problem what standard sample should be regarded as standard.
- This study showed that caffeine has no role in determining the taste strength.

Conclusions

- Supervised pattern recognition may help not only in the authentication of the herbal samples but also in the quality of the preparation.

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