# AUTHENTICATION OF BOTANICALS AND HERBAL PRODUCTS USING UPLC/ION MOBILITY QTOF-MS AND A METABOLOMICS APPROACH

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## INTRODUCTION

### BACKGROUND

- ***Fadogia agrestis* is a small shrub indigenous to Africa.**
- **The aqueous stem extract contains mainly saponins, flavonoids, alkaloids, and anthraquinones.**
- **The aqueous extract is extensively used to manage sexual dysfunction, antimalarial drug, kidney pain, stomachache and toothache (1).**
- **A major concern of *F. agrestis* commercial products is adulteration either deliberately to substitute with cheaper products for financial incentive or to increase the efficacy of the commercial product. The main objectives of the study are:**

1. **To identify the main chemical markers that differentiate between authentic *F. agrestis* and commercial products from the market.**
2. **To build a database of potential multi-marker metabolites for targeted routine authentication using low resolution nominal mass MS or UV.**

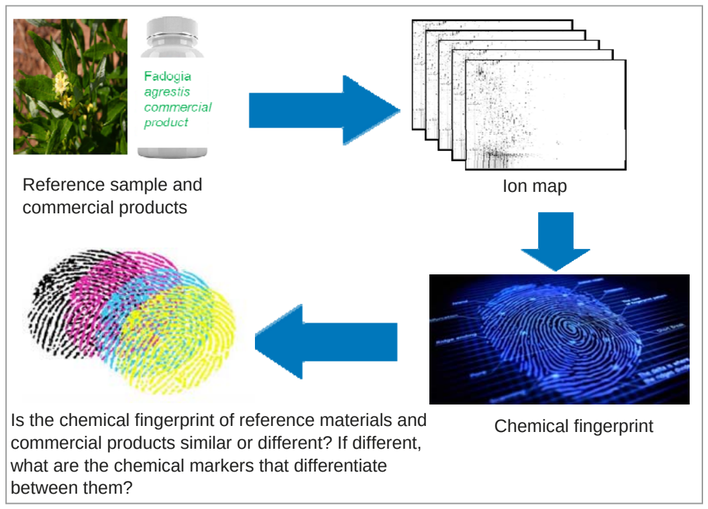


*Figure 1. Non targeted metabolomics chemical fingerprinting with multivariate statistical analysis to correctly distinguish authentic F. agrestis and Fadogia commercial products.*

## METHODS

### SAMPLE PREPARATION

- 500 mg of dry grounded *F. agrestis* sample or an adequate amount of capsule were weighed and extracted with methanol.
- The methanol solution was vortexed for 30 min and centrifuged at 1000 g. The supernant was transferred to a 10 mL tube.
- The procedure was repeated four times and the supernant were combined.
- The final volume was adjusted to 10 mL with methanol and passed through 0.2 µm PTFE membrane. The first 1.0 mL was discarded and the remaining volume was collected in an LC/MS certified sample vial.
- A total of 27 samples (10 *F. agrestis* and 17 *F. agrestis* commercial products).
- QC sample was created by mixing 10 µL of each sample.

### LC CONDITIONS

| | |
|---|---|
| UPLC: | ACQUITY UPLC I-Class FTN sample manager |
| Column: | HSS T3 1.8 µm 2.1 x 100 mm @ 40 ºC |
| Mobile phase A: | Water (0.1% FA) and B: Acetonitrile (0.1% FA) |
| Flow rate: | 0.5 mL/min; Injection volume: 1 µL |

### MS CONDITIONS

| | |
|---|---|
| MS System: | Vion IMS QTof |
| Acquisition mode: | HDMS$^E$, ESI$^+$ and ESI$^-$ mode |
| Capillary voltage: | 3.0 KV (ESI$^+$); 2.0 (ESI$^-$) |
| Collision Energy: | Low CE: 6eV; High CE: 25-45 |
| Tempertaures: | Source: 120ºC; Desolvation: 550ºC |
| Acquisition mode: | 100-1200 Da (0.1s scan rate) |

## RESULTS AND DISCUSSIONS

### DATA ANALYSIS USING PROGENESIS QI


### Intuitive Step by Step Workflow

Progenesis QI adopts an intuitive step by step workflow to perform comparative high resolution UPLC-IMS metabolomics data analysis. The key to data processing and analysis is the ability of the software to distinguish biological variation and metabolic changes from analytical interferences. It is crucial that each sample is randomized and injected a minimum of three times to ensure that the data analysis is statistically valid. For this study the *F. agrestis* and the commercial products were randomized and injected three times with a set of QC pooled sample runs.

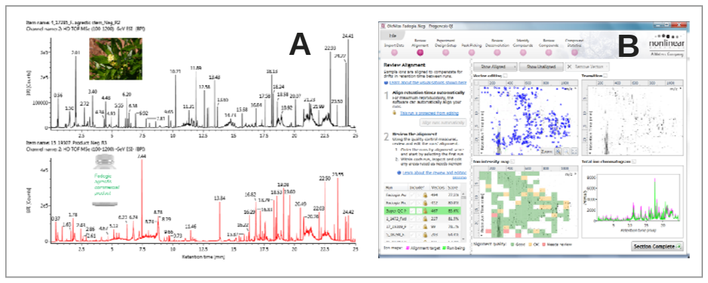


*Figure 2. (A) Representative LC/MS chromatogram of F. agrestis and commercial product extracts analyzed using UPLC-Vion IMS QTof in negative ion ESI mode. (B) Progenesis QI software provides an integrated workflow for raw data processing, chromatographic alignment, peak peaking, compound identification and multivariate statistical analysis.*

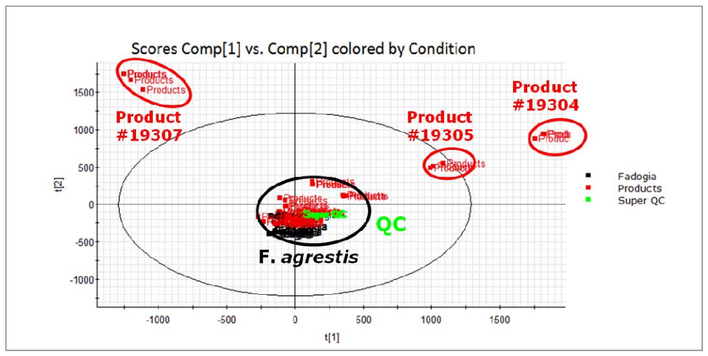


*Figure 3. PCA scores plot of F. agrestis and commercial products in negative ion mode. The placement of the QC in the center indicates the robustness of the LC/MS analysis. All F. agrestis reference samples are within 95% confidence interval. Most of the commercial products clustered with reference F. agrestis samples.*

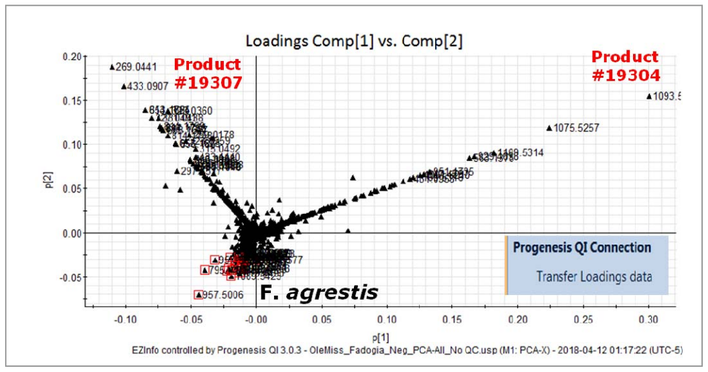


*Figure 4. Loadings plot showing the significantly changing features (in red box) corresponding to the PCA plot from figure 3. The red highlighted markers were imported back to Progenesis QI for automatic compound identification.*

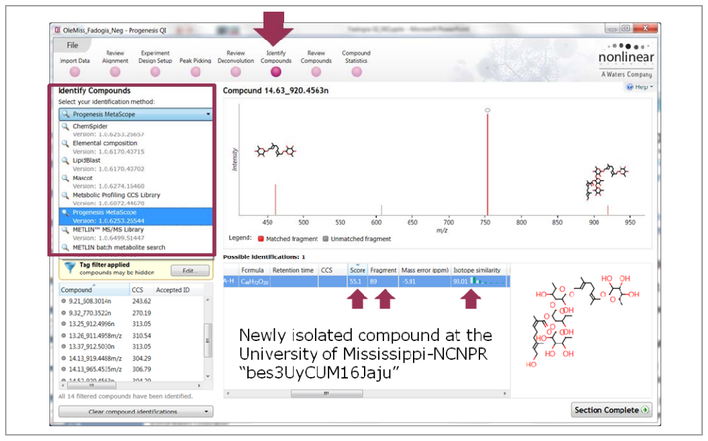


*Figure 5. Fragmentation trace for compound 14.63_920.4563 in the negative ion mode with the identification of newly isolated significant marker. Identification based on precursor exact mass, theoretical isotope distribution and by matching experimental ions with in-silico fragment ions with a total score of 51.1/60.*

## CONCLUSION

- **Large scale LC-MS metabolomics data analysis is complex and very time consuming.**
- **A simple step by step non-targeted metabolomics chemical fingerprinting is described for the authentication of botanicals and commercial products.**
- **All 10 reference *F. agrestis* samples analyzed are within 95% confidence interval and clustered more closely.**
- **Most of the commercial products (14 out of 17 samples) clustered with reference *F. agrestis* samples.**
- **The significantly changing major chemical markers that differentiate between reference *F. agrestis* and commercial products were identified.**
- **Identification based on precursor exact mass, theoretical isotopic distribution and fragmentation pattern provides confidence in compound identification.**

**References**

1. Yakubu, M.; Oladiji, A., Akanji, M.; Evaluation of biochemical indices of male rat reproductive function and testicular histology in wistar rats following chronic administration of aqueous extract of *F. agrestis* stem. *African Journal of Biochemistry* **2007,** *1*, 156-163.

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