Metabolomics and Ethnopharmacology: A multidisciplinary approach for herbal drugs research

U.K. Patil

Department of Pharmaceutical Sciences

Dr. H.S. Gour Central University, Sagar

Metabolomics and Ethnopharmacology: A perfect holistic match

Our ancestors did not only discover active plants, but they also developed a holistic approach in their medical systems. Ex. Ayurveda and TCM

Fractionation of an active extract will not necessarily produce active compounds.

The activity shown by an extract, is due to the sum of the activities of the individual constituents.

It is not unusual for original extract to have an **activity greater than any of the fractions** obtained by process such as CC.

Two possible reasons why this loss may occur are;

1. Decomposition transformation of compounds to lees active substance may take place due to solvent used for fractionation

   Ex.: Esterification of acid groups on the molecule if alcoholic solvents are used.
   Oxidation of phenolic substances in presence of silica gel exposure to atmosphere.

Many plant extracts contain antioxident compounds, which might protect labile substances in a crude extract. However, upon fractionation such protective substances might become separated from the susceptible compound, so that the latter are quickly oxidized.

2. Synergy

Most active compound found is not necessarily responsible for the major part of the effect

Whole extract should be considered for its activity

It is unusual for a single compound to be responsible for the activity observed, and very often several compounds are isolated which exert the same effect.

Ex. Senna; Several dianthrone glycosides are responsible for overall laxative effect

It should not be assumed that most active compound accounts for the bulk of the activity of the extract.

It is important to determine the concentration of each compound and correlate it with dose response characteristics in the test system.

Ethnopharmacology with multidimensional therapeutic goals

1. Wound Healing;

   WH is a mixture of process; i. Cell proliferation,
   ii. Collagen formation,
   iii. Contraction of collagen lattice.

   In addition WH is impaired by;

   i. Microbial infection
   ii. Destruction of cell and tissue by reactive oxygen species

   Therefore the therapeutic target must be multidimensional in drug development from natural sources.

2. Malaria;

   For antimalarial activity of natural drug, the goal of new drug discovery must be killing of Plasmodium species along with antiinflammatory or antipyretic activity that might help to reduce the feverish state associated with disease.

Ethnopharmacology

Ethnopharmacology is an amalgam of perspectives, primarily those of;

Pharmacology,
Pharmacognosy,
Anthropology,
Botany.

Contributions are also made by;

Historians of science,
Clinicians,
Agronomists,
Biochemists ,
Researchers in veterinary medicine.

Ethnopharmacological investigation…?

Becoming increasingly important in defining strategies and actions for conservation of medicinal plants.

Profiling of traditionally important herbs;

Use of medicinal plants.

Identify the most important species used.

Determination of the relative importance of the species surveyed.

Calculation of the informant consensus factor (ICF) in relation to medicinal plant use.

Traditional medicines turned into modern drugs

Artemisinin, Triptolide, Celastrol, Capsaicin, Curcumin are "poster children" for the power and promise of turning traditional medicines into modern drugs.

Success stories highlight the ongoing interdisciplinary research efforts that continue to be necessary to realize the pharmaceutical potential of traditional therapeutics.

Herbal products;
basic requirements of being safe and effective.

Challenges

Herbal products are usually mixtures of many constituents.

The active principles are unknown in most cases.

Selective analytical methods or reference compounds may not be available commercially.

Challenges

Plant materials are chemically and naturally variable.

Chemo-varieties exist.

The source and quality of the raw material are variable.

Trends

In herbal products;

Herb's actions often are recognized long before mechanism of action and active principles are appreciated.

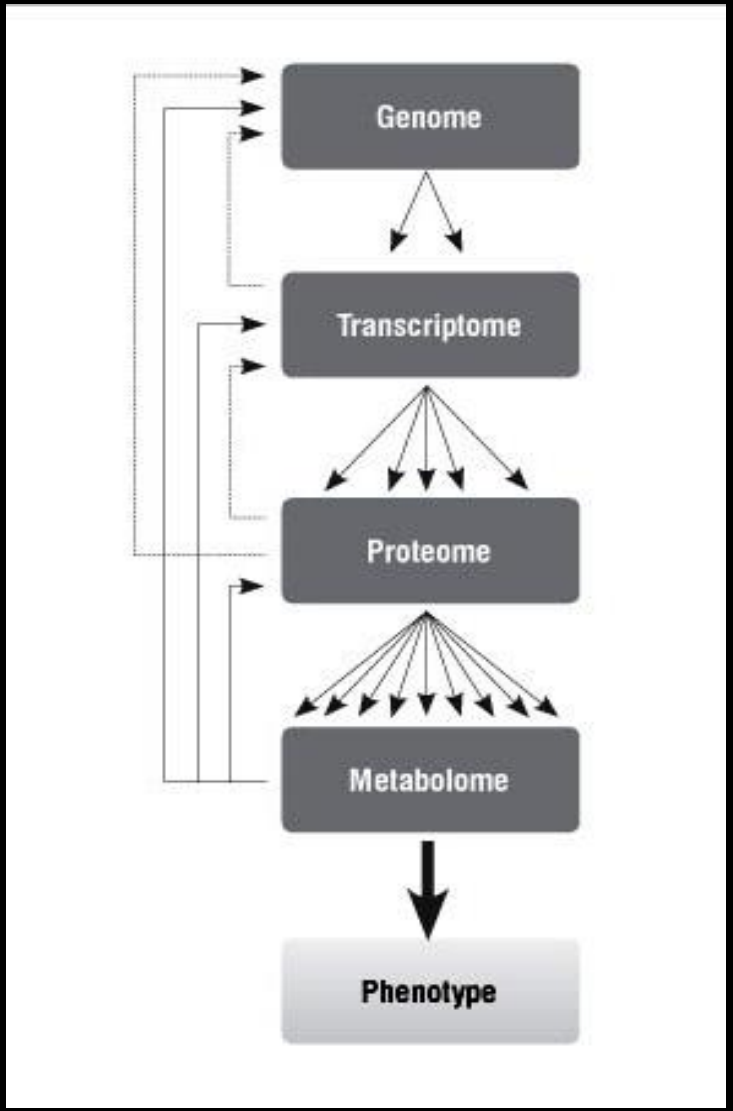
The primary and secondary metabolites

The primary and secondary metabolites found in plant cells are the final recipients of biological information flow.

Can influence gene expression and protein stability.

Qualitative and quantitative measurements of these metabolites reflect the cellular state under defined conditions, and yield critical insights into the cellular processes that control the biochemical phenotype of the cell, tissue or whole organism.

Biological information Flow: Genome to Metabolome



Biological information flow from genome to metabolome. The metabolome is complementary to the transcriptome and proteome, captures the functional or physiological state of the cell, and provides a link between genotypes and phenotypes. Altered gene expression is ultimately reflected in changes in the pattern and/or concentration of metabolites. There are an estimated 200 000 plant metabolites and many remain unknown.

Metabolomics

- Represents the exhaustive profiling of metabolites contained in organism.

- Systematic study of the unique fingerprints that specific cellular processes leave behind.

Metabolomics

Differs from traditional targeted phytochemical analysis in various fundamental aspects;

It is a data-driven approach with predictive power that aims to assess all measurable metabolites without any pre-conception or pre-selection.

Providing new dimensions in the study of systems biology, enabling the in-depth understanding of the intra- and extracellular interactions of plant cells.

Metabolomics

- Study of all the naturally occurring small molecules, called metabolites, in biological samples such as cells, bio fluids or tissues.

- These small molecules are the products of metabolism and include;
  - sugars (or carbohydrates)
  - fats (or lipids)
  - amino acids.

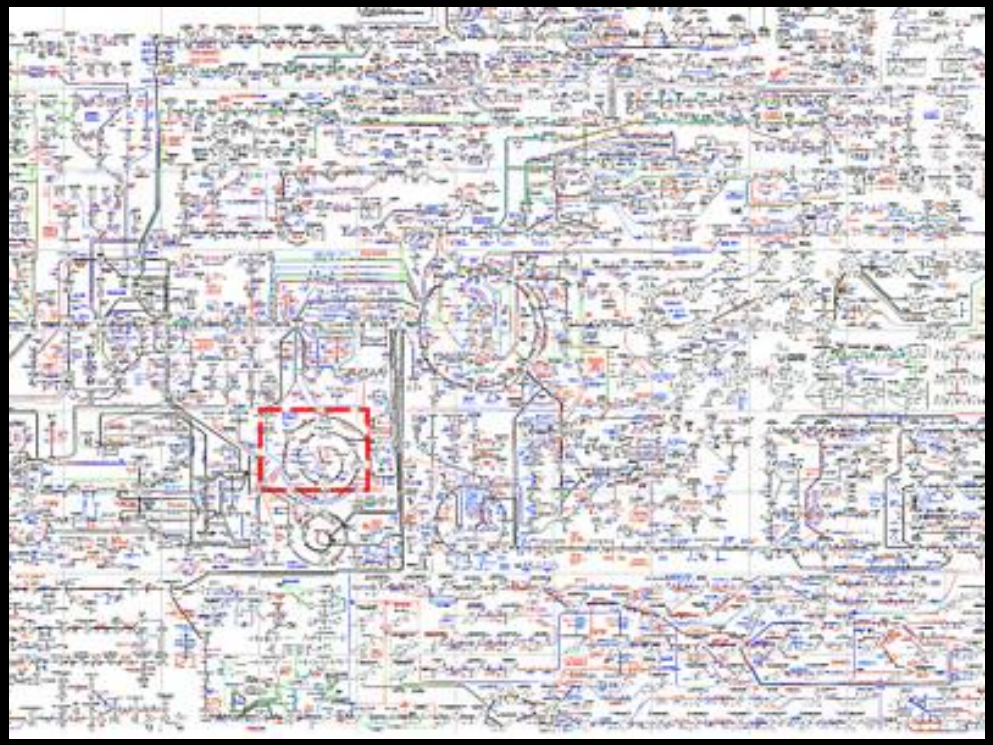
The collection of all the metabolites within a cell is called the metabolome.

Metabolomics

Metabolome = the total metabolite pool

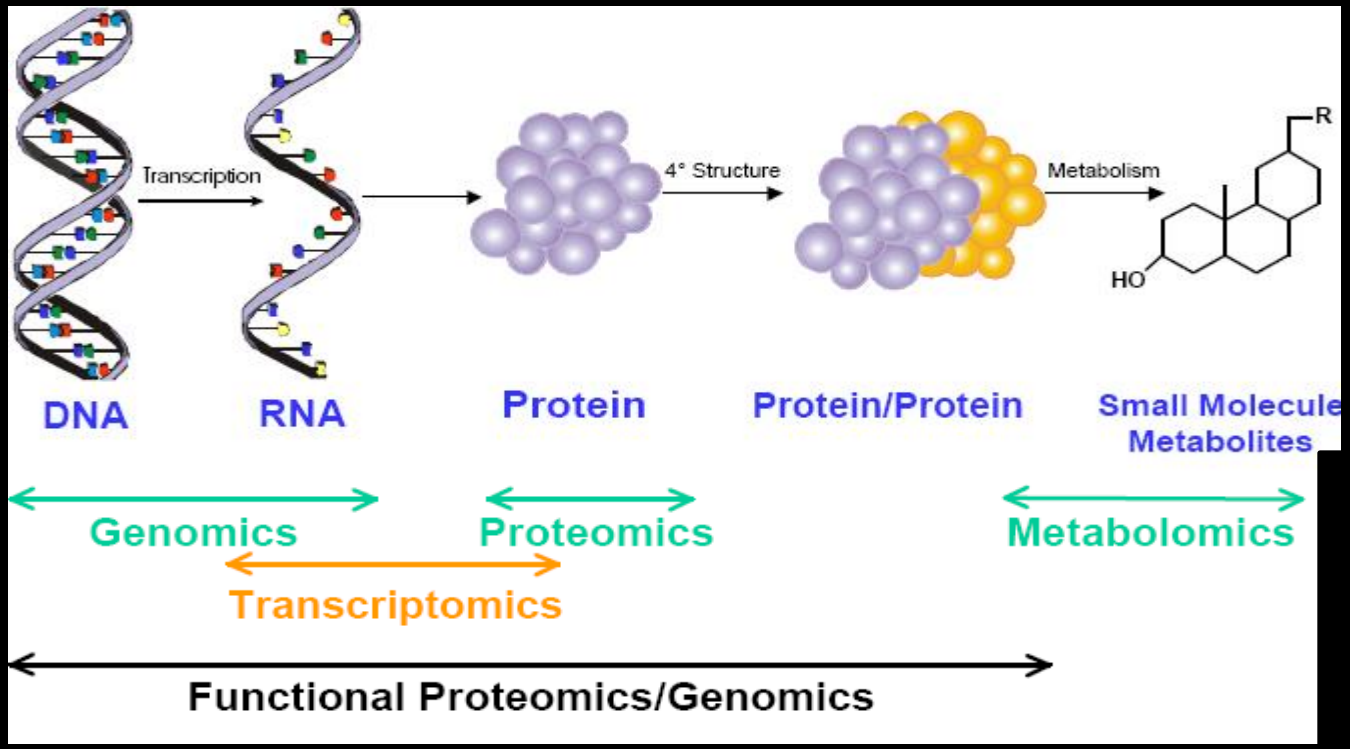
All low molecular weight (MW < 1000 Da) organic molecules in a sample such as a leaf, fruit, or tuber.

- Peptides
- Oligonucleotides
- Sugars
- Nucleosides
- Organic acids
- Ketones
- Aldehydes
- Amines
- Amino acids
- Lipids
- Steroids
- Alkaloids
- Drugs (xenobiotics)

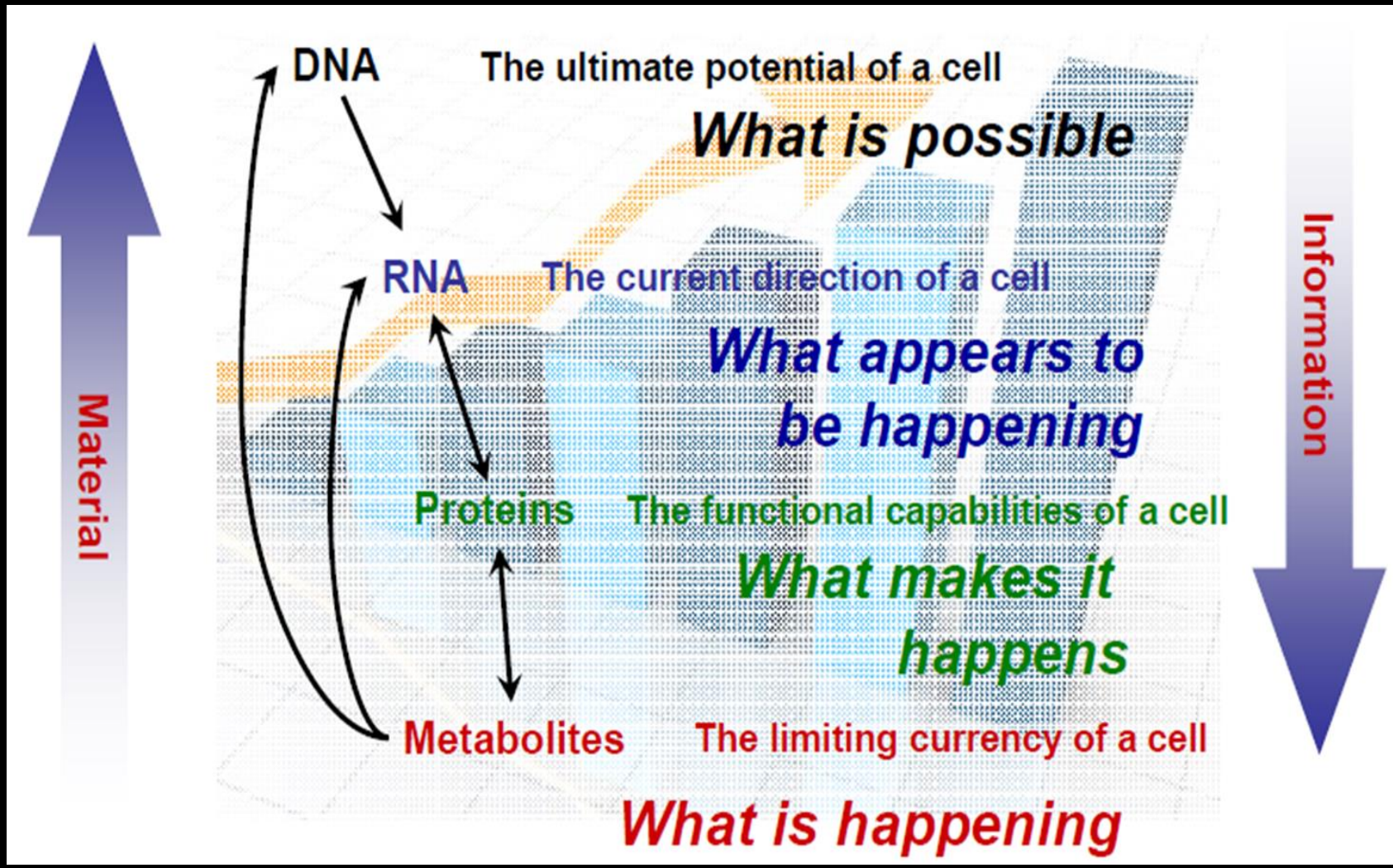


A high-throughput analysis of metabolites

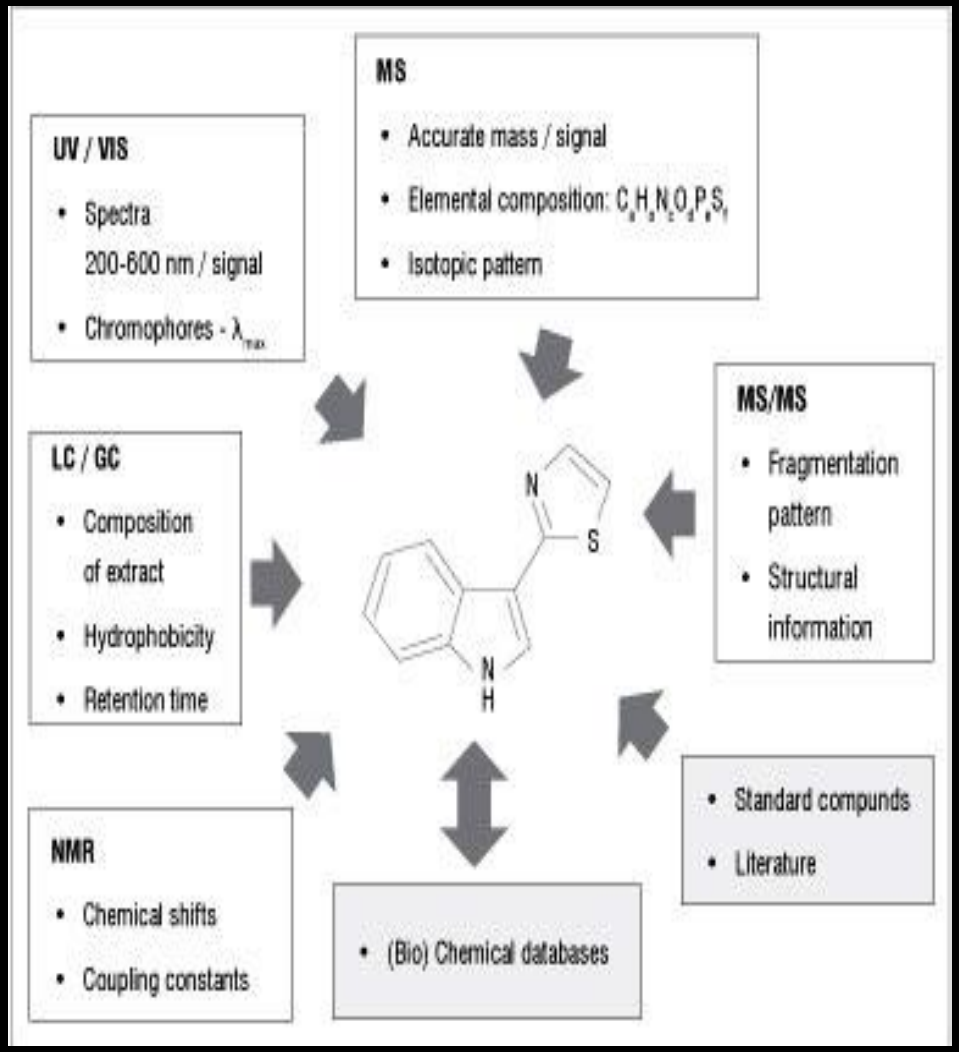
Metabolomics is the simultaneous ('multiparallel') measurement of the levels of a large number of cellular metabolites (typically several hundred). Many of these are not identified (i.e. are just peaks in a profile).



Background and scope



Identification of metabolite



The identification of a metabolite. Typical information generated by analytical technologies and knowledge resources that are used in the identification of a metabolite. Experimental validation is by means of standard compounds and information present in literature and databases. Resources such as species databases, literature, spectral databases and chemical databases help in narrowing the number of ambiguities for candidate metabolites.

Goal

The ultimate goal of integrated techniques is to qualitatively and quantitatively analyze all metabolites in traditionally claimed MAPs.

Metabolomics

As per ICMAP, Metabolomics is able to describe "Plant-to-Patient platform" i.e. comprises of novel approaches based on Systems Biology to tackle safety issues.

New Opportunity for System Biology

System Biology: Scientific evidence and a novel quality control for HPs

Key for worldwide registration and acceptance of any Herbal Product is the ability to provide scientific evidence in combination with a quality control system based on the bioactive ingredients.

The system biology approach, a multi dimensional chemical and pharmacological approach enables;

linking of the complex metabolic profile of herbs + biological effects and is therefore a key for quality control of HPs *while providing simultaneous scientific evidence for the underlying efficacy*.

Current status of Metabolomics

System biology → is revolutionizing science

[different approach to study biological systems]

Key technologies in systems biology → approach to study biological systems

- **Transcriptomics**
- **Proteomics**
- **Metabolomics**

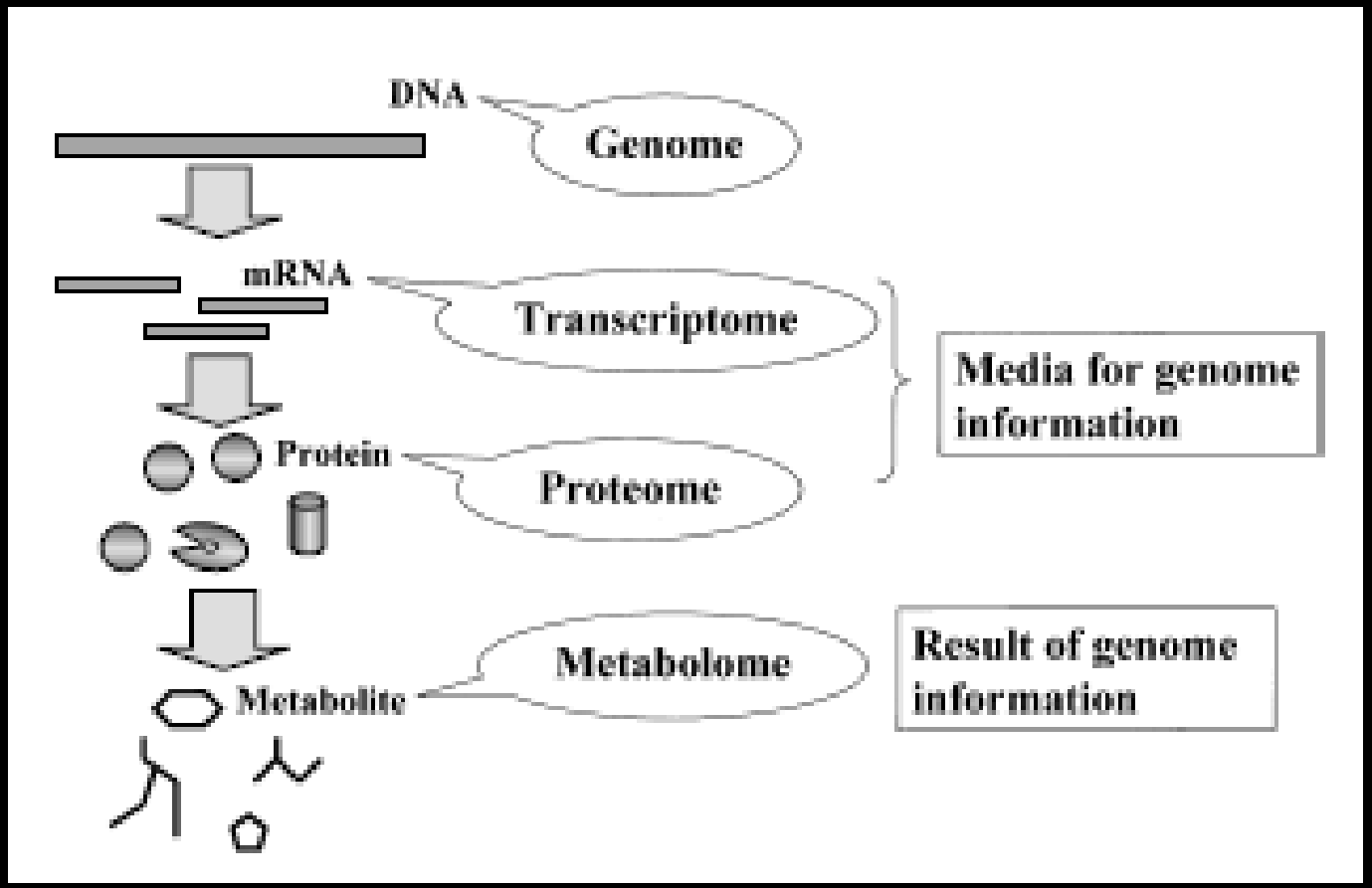
→ **great interest for finding compounds that correlate with biological activity**

- **Study the systems in a more holistic way.**
- **In resistance against insects.**
- **Pharmacological activity.**

Potentials

- In postgenomic era, integrated technique is expected to be the newest useful omics science for functional genomics.
- The technique is used for the analysis of large mutant or transgenic libraries.
- An important tool for quality control of plant products;
    1. Showing equivalence of marker components.
    2. Control of consistency of the composition of botanicals.

Metabolomics in functional genomics



Analytical platforms and methodologies

1. Metabolomics; sampling

Unbiased identification and quantification of all metabolites in a biological system. Sample preparation must not exclude metabolites, and selectivity and sensitivity of the analytical technique must be high.

2. Metabolite profiling

Identification and quantification of a selected number of pre-defined metabolites, generally related to a specific metabolic pathway(s). Sample preparation and instrumentation are employed so to isolate those compounds of interest from possible matrix effects prior to detection, normally with chromatographic separation prior to detection with MS.

Analytical platforms and methodologies

3. Metabolic fingerprinting

High-throughput, rapid, global analysis of samples to provide sample classification. Quantification and metabolic identification are generally not employed. A screening tool to discriminate between samples of different biological status or origin.

4. Metabolite target analysis

Qualitative and quantitative analysis of one or a few metabolites related to a specific metabolic reaction. Extensive sample preparation and separation from other metabolites is required and this approach is especially employed when low limits of detection are required. Generally, chromatographic separation is used followed by sensitive MS or UV detection.

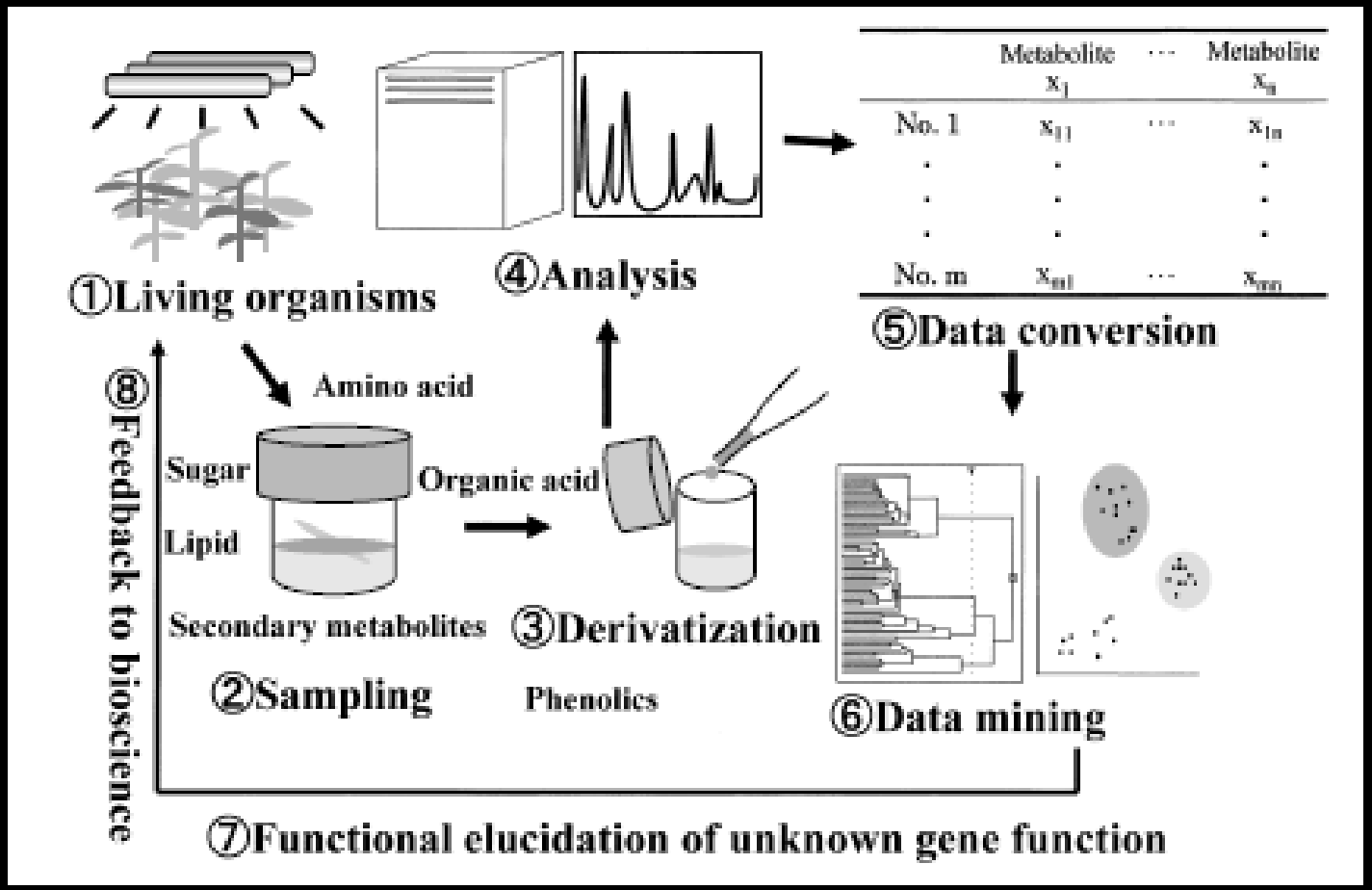
Analytical platforms and methodologies

5. Metabonomics

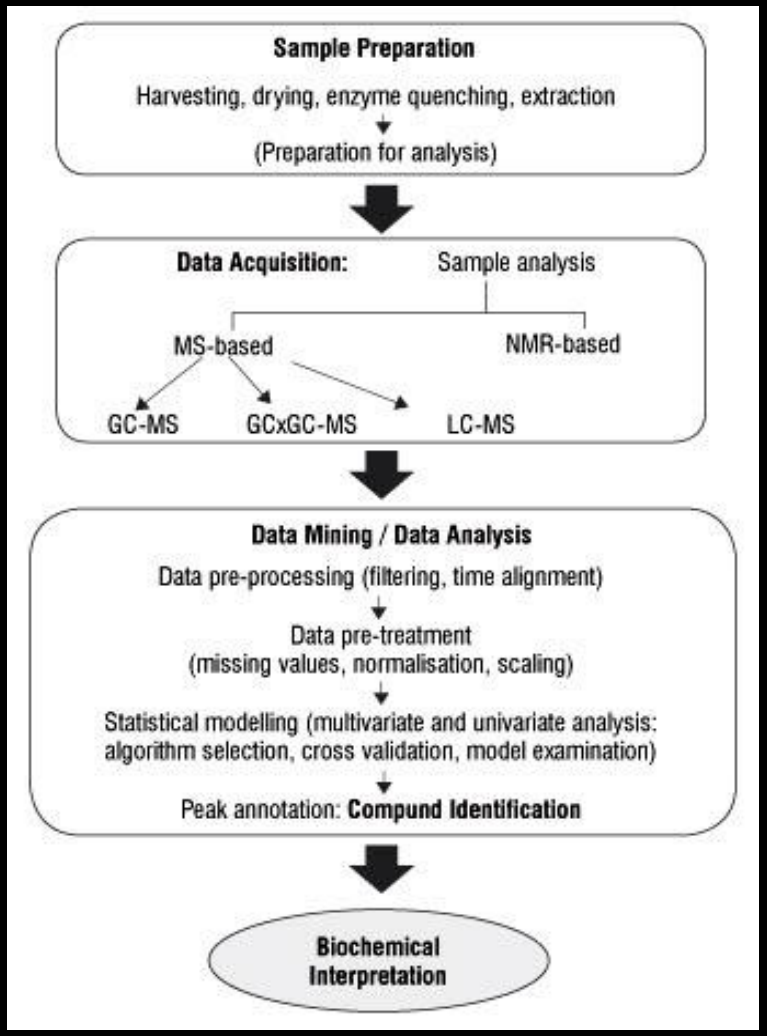
Evaluation of **tissues and biological fluids for changes** in endogenous metabolite levels that **result from disease or therapeutic treatments**.

Toxicity evaluation is done.

General scheme for metabolomics



Flow chart of Plant Metabolomics

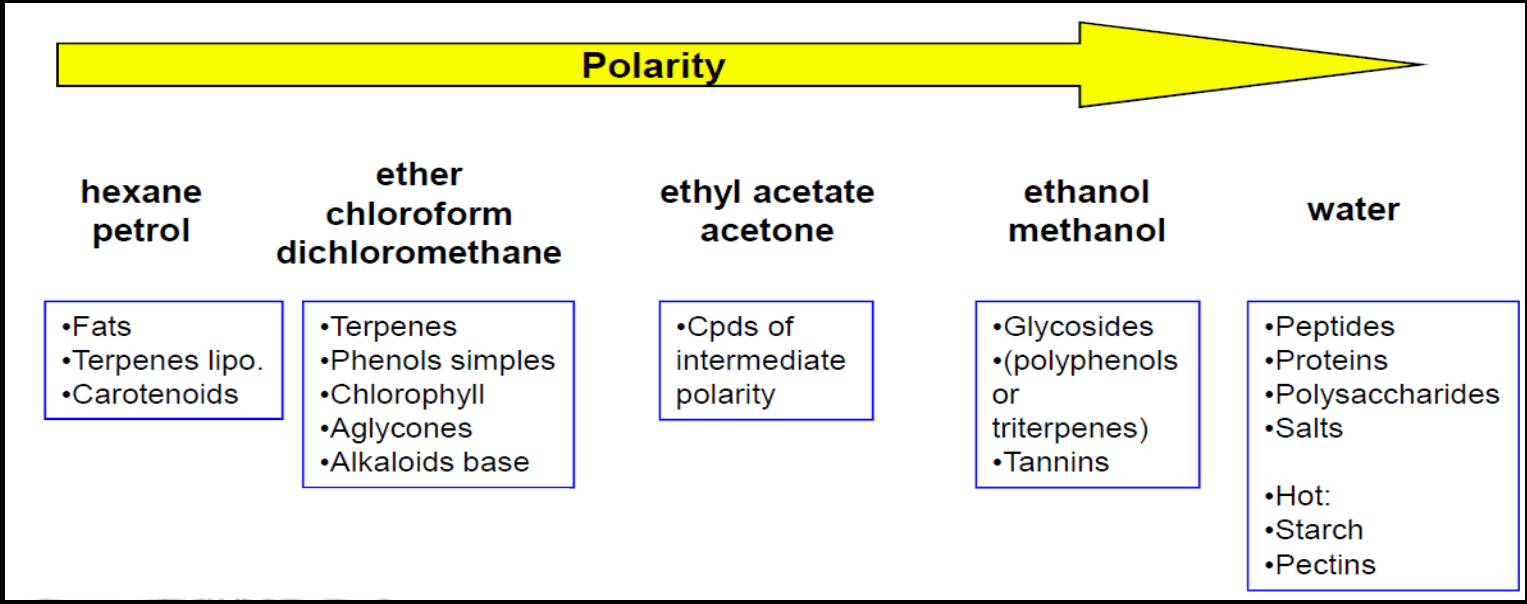


Flowchart for plant metabolomic studies. The three main steps of a metabolomic analysis are sample preparation, data acquisition and data mining. A data handling pipeline is established from data acquisition to data mining. These three steps are interrelated and lead to the biochemical interpretations.

Metabolic Profiling: Sample Preparation

Metabolites are typically extracted in aqueous or methanolic media, then fractionated into lipophilic and polar phases that are then analyzed separately.

Further fractionation of each phase may follow to split metabolites into classes prior to analysis.



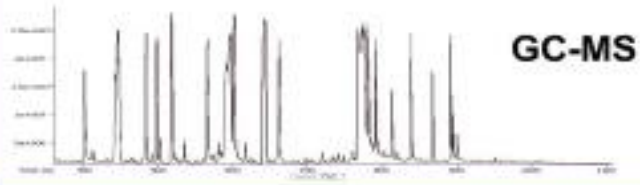
Summary of the different metabolomics based strategies for sample preparation and sample analysis

METABOLITE PROFILING & METABOLITE TARGET ANALYSIS (& METABOLOMICS)

Automated, sensitive detection, chromatographic separation, quantification and metabolite identification. Analysis times of 5-140 minutes

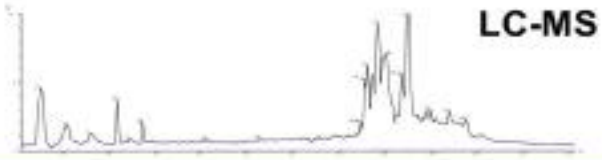
Sample dried and chemical derivatisation

GC-MS



Sample dilution or further metabolite isolation (SPE, liquid-liquid extraction)

LC-MS



Biofluids

Urine, plasma, serum. Requires protein precipitation

Intra-cellular metabolites

Microbial, plant or animal. Require extraction using polar and non-polar solvents

METABOLOME

Metabolic footprint

Natural secretion of intra-cellular metabolites to extra-cellular volume

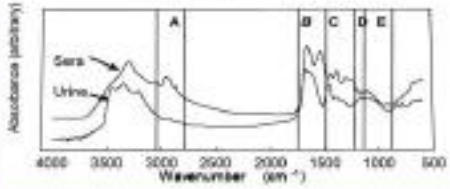
Tissues

No extraction or minimal preparation required

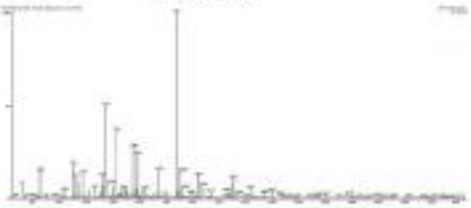
METABOLIC FINGERPRINTING & METABONOMICS

Automated, rapid, high throughput global analyses with minimal sample preparation, used for sample classification. Limited ability for metabolite identification and quantification except for NMR

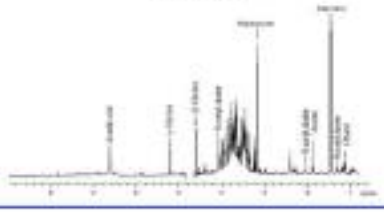
FTIR



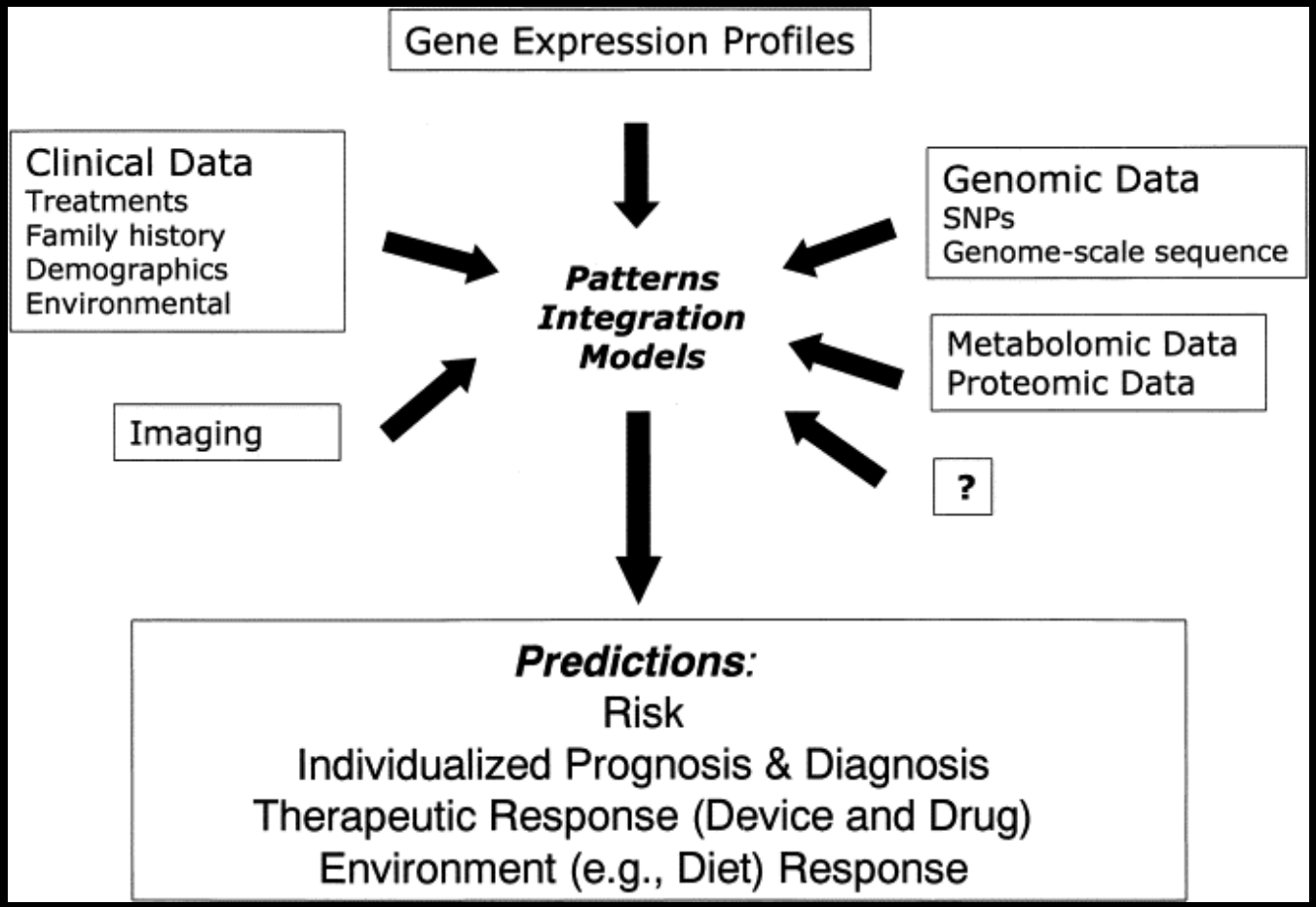
DIMS



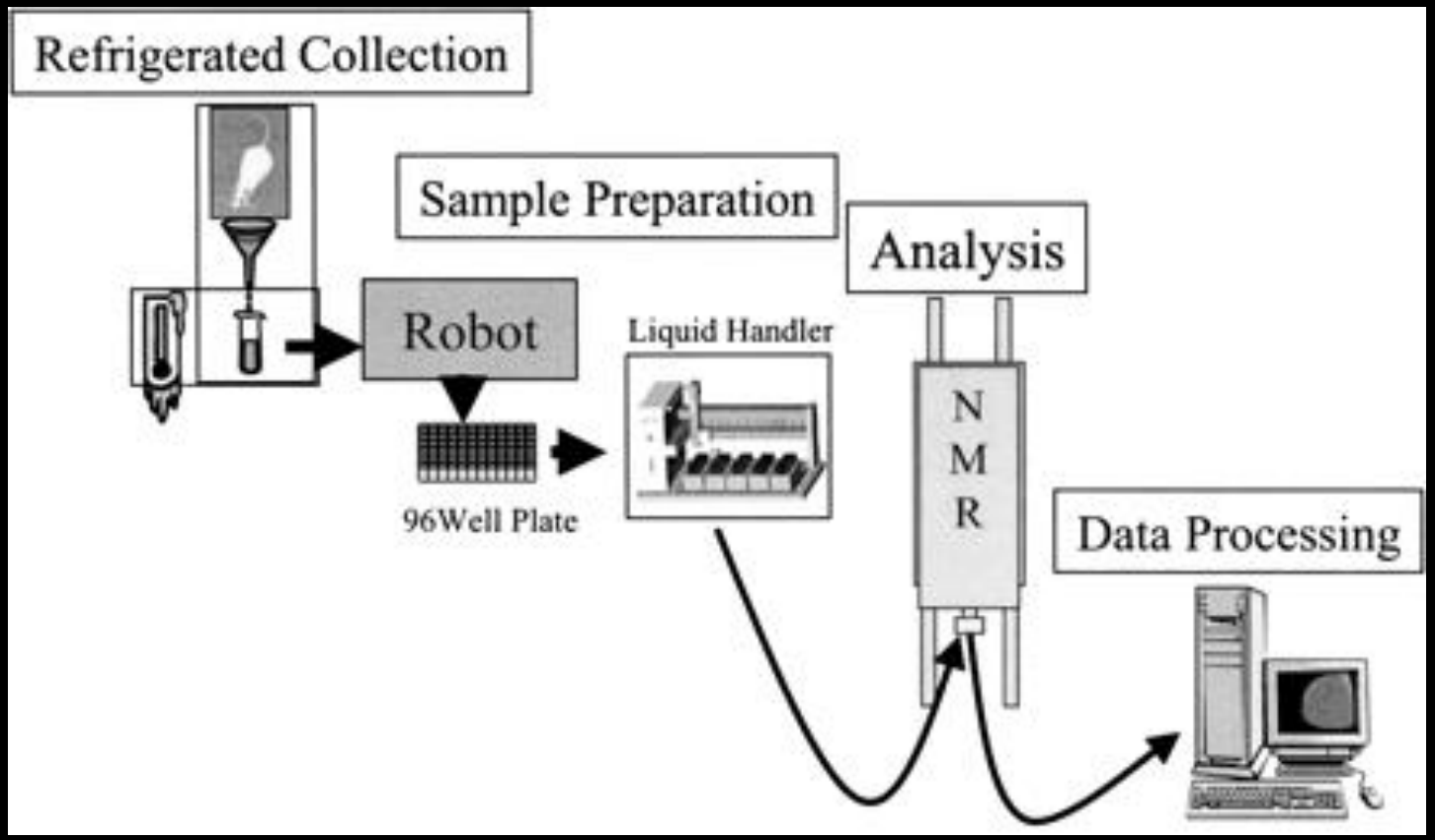
NMR



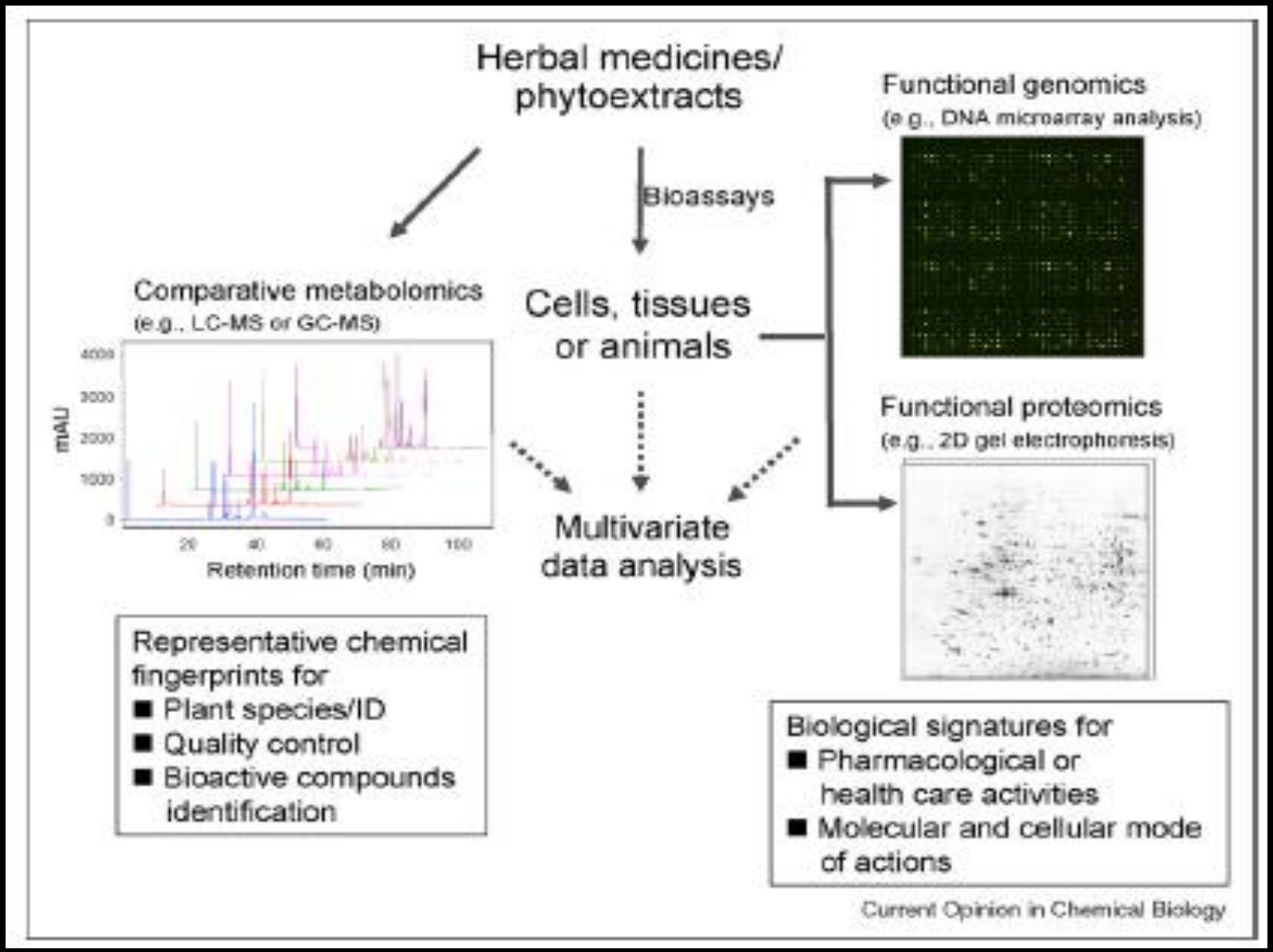
Metabolomics- A tool for study of safety of drugs



Metabolomics- A tool for study of toxicity of drugs



Metabolomics for HPs research and drug development



Merits

- Metabolite profiling as a diagnostic aid.
- Characterization of the metabolic response to stress.
- As an integral component of genomics.
- Genetic metabolic regulation.
- Localization of activity and biomarker.
- Reverse Pharmacology.
- A holistic approach in NPR; Natural Drug Discovery with ethnopharmacology and metabolomics.

Conclusion

Ethnopharmacology is entering an exciting era of metabolomics.

Extensive chemical characterization of clinically efficacious preparations appears to be currently the only sensible path towards establishing a reliable basis for quality control and advancing the expectation of consistent pharmacological responses of herbal products.

Metabolomics with Ethnopharmacology: valuable tool that can be used to monitor and assess gene function, and to characterise post-genomic processes.

Future Perspectives

- For the researchers, ethnopharmacology in metabolomics age and systems biology are a **great opportunity in HMPs.**

- The coupling of ethnopharmacology with the other -omics can be made with the help of **chemometrics** and **bio-informatics.**

- In a multidisciplinary collaboration we can make a major contribution to developing medicinal plants, by delivering the evidence that they are active and contribute to the understanding of the mechanism of action.

Thanks…..