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## Developments in Phytochemistry

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### 1. Introduction

Carbohydrates, proteins, fats and oils are utilized as food by man and animals. Other chemical compounds in plants apart from these listed above are phytochemical. Such compounds usually exert peculiar, unique and specific active physiological effects responsible for their therapeutic and pharmacological functions. Activities of such naturally occurring compounds are generally responsible for changes, which are utilized to satisfy man's desires. Phytochemical studies afford revelation and understanding of phytoconstituents, as much as possible conserving their bioactivities, and are on how to standardize them; compared with the crude herbal methods that are not easily standardized. These complex substances of diverse nature occur mostly in plant based foods; they are in very small amounts in grams or mg or  $\mu\text{g}/\text{Kg}$  of samples. They do not add to body calorie and are numerous in types. These phytochemical are applied mostly for preventive and healing purposes. About 25% of prescribed drugs are obtained from phytochemical in higher plants. Plants are safe means of obtaining drugs. About 250,000 higher plants have promising phytochemical, half of which are located in tropical forests; 60% of these have their biological activities established, while about 15% of them have their phyto-compounds isolated and reported [Hamburger and Hostettman, 1991].

Studies and researches into medicinal constituents of plants, involve qualitative and quantitative analyses. There is rationale behind each experimental work involving definite steps and processes; having in mind properties of compounds analyzed in conjunction with procedures utilized. Also our desired active metabolite to be isolated and studied as interested lead compound, many times is in very complex mixtures of many unwanted and undesired materials [known as contaminants], which have close properties to our desired bioactive molecules.

### 2. General and specific techniques, procedures and methods in phytochemical analyses, with highlight on recent developments in phytochemistry

Most of the techniques and procedures in phytochemical analyses are cumbersome and tasking, to have detailed understanding of phytoconstituents- [their activities, structures, how to improve on them and standardize them]. If they are carefully followed one achieve

the aim of isolations, characterizations and better establish bioactivities of active metabolites.

Phytochemical methods mainly involve EXTRACTIONS, PURIFICATIONS and ISOLATIONS of the active compounds in plants. Procedures are ways of carrying out the methods and techniques. There are numerous methods some specific for interested compounds one is looking for, duly modified to meet the required aim and focus of work. There are daily modifications of techniques and procedures to suit individual purposes of having the phytochemical compound(s) of interest. It is important to say that some natural product may (to variable extent) or may not possess their pharmacological properties and activities when in isolation compared to when among mixture of compounds (synergy) in the natural setting in organism. Recently in genomics whole plant is analyzed which afford easier and truthful analyses of contents in the whole plant as in their natural state.

It is important to first establish proper botanical taxonomic identifications and classification of plant of study. Scientific names must be established, common and local names must be sought. Right choice of study plants or part of plant to study may be from local or traditional surveys i.e. ethno-medicine, ethno-pharmacology or ethno-botanical uses and applications. Geographical location and environmental effects [time and period of plant collection], must be considered also, which may be responsible for variations. Voucher samples of plant of study may be filed in local and national herbaria for accurate authentications. Usually plants are richer in active metabolites during their flowering and fruiting stages [Mendonca-Filho, 2006].

Procedures involve first the analytical stages. Most times our desired active metabolite to be isolated and studied as interested lead compound, is in very complex mixtures of many unwanted and undesired materials [known as contaminants], more so they usually have close properties to our desired bioactive molecules.

Preliminary tests and screenings on plant extracts are faster and easily done following standard procedures and methods in manuals and literature. They detect the presence and amount of basic phytoconstituents like terpenoids, alkaloids, flavonoids, saponin, glycosides, steroids, tannins, phlobatannins and anthraquinones to mention few.

More common and familiar separation and isolation techniques in phytochemical studies are distillation, crystallization, solvent extractions, continuous and liquid-liquid extractions, partitioning using separatory funnels, and chromatography. For accuracy characterizations follow side by side with the above techniques. Bioactivities can also be tested along the above, such as antibacterial, antifungal and antioxidant. The followings are important to consider during choice of procedures for separations and isolation of interested biomolecules of interest:

- Availability of necessary materials, equipments and chemicals. Also economically cheap methods.
- Ease and simplicity of procedures, and risk involved.
- Compatibility of interested solute with phases or solvents such as it distilling out [more volatile phyto-compound], crystallizing and recrystallizing out from phases/ solvents etc.
- Possibility of retrieval methods.

- Selectivity and sensitivity of chosen method(s) and equipments.
- Feasibility of chromatographing out our desired biomolecules from stationary phase using appropriate and suitable solvent or mixture of solvents for elution. Also consider factors like polarity, temperature, agitations etc.
- Provision of rapid online information on activities and structures of the phyto-compound. [Specific methods can be better studied by consulting literature and appropriate textbooks].

### **3. Isolation, characterization and identification of phytochemical in natural products**

Phytochemical are active metabolites that necessarily require extraction and isolation from their natural sources with many unwanted materials. The phytochemical can come singly or as a mixture of important substances to form active principle responsible for its activity (synergy). When singly active, the processes of their separations are of great practical advantages, which in many cases the isolated phytochemical have better and higher activity. We will consider genomics and metabolomics later as more efficient methods of rapid phytochemical screening and characterizations of plant extracts to study their chemical constituents, using NMR-based metabolomics. It utilizes mathematical data; NMR is used directly on extracts before commencing detailed work on plant. It makes it easy to determine which plants are more promising to research into. A large number of variables are collected, then choices on which are important are made, followed by selection procedures.

#### **3.1 More modern spectroscopy utilized in phytochemical studies**

Once preliminary separations and detections have confirmed presence of active secondary metabolites, their characterizations as they are separated follows. Chromatographic techniques are utilized in separations and purifications to isolate bioactive constituents based on polarity or other gradient factors. The isolated compound is characterized by spectroscopic methods. The four basic types of spectroscopy are utilized in the characterizations of purified natural product compounds. They are ultraviolet (UV), infrared (IR), mass-spectroscopy (MS) and nuclear magnetic resonance (NMR) techniques. MS is an instrumental technique, while the other three utilizes different parts of the broad electromagnetic radiation spectrum. UV spectroscopy discovered and utilized in the 1930s gives detailed information on detecting presence of conjugation in molecules and the extents of conjugation. By 1940s the infrared (IR) region of EMR was utilized to detect different vibration frequencies of different chemical bonds present in the molecule. Combination of these two types of spectroscopy [UV & IR] gave information about the functional groups present in the molecule. MS was introduced a decade after by 1950s, involving three important steps: Ionization and vaporization; Separation of ions by  $m/z$ ; and Detections. The analytical technique provides information which determines the molecular ion. Compounds are ionized for analysis, and also fragments are produced useful for structural characterizations. Almost all compounds can be analyzed by MS, but modes of ionization and type of instruments determine the results. Recent developments have shown the use of others like MALDI[matrix-assisted laser desorption], EI[electron impact], CI[chemical

ionization], API[atmospheric pressure ionization], LRMS[low-resolution MS], HRMS[high resolution MS], IT[ion traps], TOF[time of flight] and QQQ[triple quadrupoles]. MS is a destructive technique. In conjunction with UV and IR, and tandem and hyphenations MS is able to give detailed information on molecular formula of the molecule. Recently by 1960s NMR made an easier way of detecting and confirming structures of pure metabolites, and has grown so fast, almost becoming a scientific discipline today.

NMR is a type of absorption chromatography which reveals connectivity of nuclei in the metabolite. Superficially and most common,  $^1\text{H}$  and  $^{13}\text{C}$ -NMR [1D] techniques [earlier used] are unambiguously and widely utilized in elucidation of structures of naturally occurring metabolites usually isolated and purified from their natural sources. Recently the 2D and 3D-NMR are utilized [as in use of HSCQ, TOCSY, COSY, HMBC and NOESY etc]. Fundamentally NMR reveal (a)information on types of chemical environments in the metabolite from the frequency absorption chemical shift values; (b)number of protons in each type of environment from integral values; (c)details on type of nuclei/ protons on adjacent and neighbouring positions in the metabolite, giving details on the stereochemistry and 3-dimensional structure of metabolites.

The theory of NMR is based on magnetic atomic nuclei with net nuclear spin 'I', capable of having  $(2I+1)$  patterns of orientations. Such NMR-active atomic nuclei have odd atomic number and/ or odd mass number. An internal standard, usually TMS [ $\text{Si}(\text{CH}_3)_4$ ] with equivalent twelve protons and arbitrarily have absorption at  $\delta 0$ , is used in calibrating NMR spectrum for easy interpretations and evaluation of resonances and absorptions. Most used unit is  $\delta$  (delta), the other unit is  $\tau$  (tau). Relationship between both is expressed thus:  $\delta=10-\tau$  or  $\tau=10-\delta$ . At high resolutions the splitting patterns (multiplicity) of protons are due to protons on adjacent group of protons; peak is split into  $(n+1)$  by  $n$  equivalent and adjacent H-atoms in the metabolite.

There are now more rapid strategies for chemical characterizations of phytoconstituents of natural products as well as assessing the bioactivities of the natural products. Coupled or hyphenated methods of separations, isolations, purifications and characterizations are now very appropriate. These include LC/UV, LC/MS, LC-FTIR, LC-NMR, LC/UV-DAD, MS/MS, LC-MS/MS, Q-TOF-MS, CE- capillary electrophoresis, with its added advantage of use of very little solvent consumption, lower costs, short time of analysis and its generally economical; MECC- micellar electrokinetic capillary chromatography, this is when the capillary electrophoresis is in conjunction with electrochemical detections usually along with assay experiments; HSCCC- high speed counter-current chromatography; SPME- solid phase micro extraction; SCFEC- supercritical fluid extraction chromatography; ESI; HPLC-MS/ESI. Introduction of FT [fourier transform] in structural elucidations have increased the enormous power of spectroscopies like IR and NMR.

#### **4. Techniques of establishing phytochemical bio-activities by bio-assay**

Bio-assay of extracts or fractions and bioactivity guided fractionations are important and are major steps in phytochemical studies. Bioassay combines biological and chemical screenings to obtain important information on and about plant constituents and chemical compositions. It investigates, establish and estimate biological activities of biomolecules, involving chemical screening techniques. The amount of material to be tested is important

determinant of method to be used. There are many methods of in-vitro assays for assessing different activities like antimicrobial or cytotoxic activities. Such have advantages of easy automations with robotics and miniaturized techniques resulting in rapid through-put screening of large numbers of samples. They are more common as their materials are easy to get. Other assays which utilize affected organisms or living cells directly, give more reliable results, though may not be cheap. Such include use of brine shrimps, ants and insects like *Drosophila* sp., cell lines in different media, tissue culturing, ligand bindings, use of rabbits and rats. Assays may identify promising molecular structures.

Usually many biological activities are screened and tested during a particular bioassay. It is important to note that results of bioassays are not strong enough to establish uses and dosage of compounds found to be bioactive; also they cannot replace pharmacological discovery and establishment of potent drugs in development of lead compounds to consumables and marketed substances. They may be seen as alternatives. Also one must be extremely careful when interpreting in bioassays to get results, especially in cases of clinical studies and investigations. It is best and more reliable for effective results to perform and run bioassays alongside with chemical separations and characterizations. These in modern times are achieved by using hyphenated processes. Particular constituents of extracts or plants as they are separated are characterized as well as assessing their activities side by side. The information obtained from the bioassay and chemical analyses (separations and characterizations) give full description of the bioactive compound, and afford easy and appropriate detection of specific targeted bioactive metabolite(s).

To get pure constituents, modify structures, and carry out toxicological tests bioassay results are very important.

## **5. General biosynthetic relationships between primary metabolites with interlink precursors and secondary metabolites**

The whole plant or organism serves as an active laboratory for the production of natural products from primary metabolites such as proteins, amino acids, carbohydrates, fats and oils, which are mostly obtained from food items. The primary metabolites are basic biological molecules also called biochemist molecules, which are functional compounds found virtually in all plants and organisms. Secondary metabolites are varieties of simple to sophisticated bizarre molecules also called natural products. They are fascinating chemical molecules, very useful and of great importance in nature, as well as highly diversified in structures, properties, uses, chemistry etc. These varied properties and characters emerge from their biological generation, production and formation from basic primary metabolite sources and origin. Natural products are in restricted taxonomic groups and species of organisms. They are from secondary metabolic processes and express individualities of organisms. These are the areas of interest in phytochemistry and pharmacognosy.

We will be examining the underlining principles behind formation, production and generation of natural products syntheses in plants. Primary metabolites are first formed in the first phase (primary metabolism), which is followed by secondary metabolism processes to give the more sophisticated and complicated more specific secondary metabolites.

Successive enzymes which are proteinous organic biocatalysts are utilized in catalyzing specific metabolic reactions and processes, all coded by specific genes in plant's DNA in the

nucleus which controls all activities leading to creation of new substances and new organisms. Organelles in cells of the plant carry out specific biochemical functions. Transcriptional processes are involved to get particular enzymes; common reactions in the plant include syntheses, breakdowns, isomerizations, cyclizations, regulations, hydrolysis etc; the key energy molecules in the cell are ATP, ADP, AMP, GTP and derivatives. There are many biosynthetic pathways occurring in plants, initial pathways such as carbon-reduction cycles, pentose phosphate pathways, glycolysis, Krebs cycle, shikimic acid pathway and tricarboxylic acid cycles lead to biosyntheses of primary metabolites, which are precursors of the diverse secondary metabolites. There is need for continuous supply and flow of energy for the ordered transformations of substances in cells. Metabolic activities in specific pathways occur vegetative in cells producing precursors for components of cells to further react and produce simple to complex natural product metabolites. Generally biosynthetic procedures can be viewed as starting (primary metabolism) from biosynthetic activities to produce carbohydrates. From it more complex metabolites are formed. Main metabolites as precursors of specific secondary metabolites include fatty acids and lipids to give the polyketides, amino acids and sulphur containing metabolites to form the peptides and alkaloids, phenyl propanoid and cinnamic acid metabolites, isoprenoids which yield terpenoids, carotenoids, steroids etc. There are now genomic approaches to studies of biosyntheses of natural products, which will be discussed shortly.

## 6. Importance of the phytochemical to plant producing them and man

Plants are energetic organisms that carry out specific oriented processes to produce useful compounds. They do not waste time to form substances that have no use to the plant. The wide categories of phytochemical produced have their importances to the plant generating them. Some of these are as follows-

1. Starch in plants is hydrolyzed to D-glucose units utilized in syntheses of ATP by aerobic respiration for vigor in growing cells of plants. Starch and sugars are also utilized by plants in development of their storage organs as in rice, tubers of yam and potatoes.
2. Cellulose is the most common naturally synthesized polymer in plants, it is made of glucose units, and it is the main component of plant cell walls, which provides structural supports along with other polysaccharides for the plant.
3. Chlorophyll in plants is photo-receptors which afford the plant important photosynthetic activities to take place.
4. Gums and mucins are hydrophobic acidic residues of hetero-polysaccharides produced by plants. They serve as matrix in cell walls to protect plant against attack of microbes. The gum is also used in sealing up wounds in leaves and stems of plants, and also prevents infections on plants.
5. Lipids are the main constituents of membranes of plasma and cell organelles. Fats and oils are lipid bodies; they are the stored energy forms in fruits and seeds of plants.
6. Proteins are responsible for main cell structures and main constituents of enzymes involved in biochemical reactions and biosynthetic activities.
7. Nucleic acids and nucleotides are for protein coding. They supply metabolic energy molecules like ATP, ADP, AMP, and GTP.

8. Inulins and fructans are soluble polysaccharides made mostly of fructose sugar with some glucose in the chain. Hydrolyses of the stored fructans in plants at spring, provide energy to plant for commencement of its growth in early spring.
9. Lignin are hydrophobic complex polymers in secondary cell walls made of units of aromatics like phenylpropanoids, coumaryl, coniferyl and sinapyl alcohols via shikimic acid pathway. It provides additional supports with rigidity, impermeability to water and prevent water loss from plant, also give compressive strength to cell walls. Lignins resist intrusion of herbivores, but prevent growth and bending of plant tissues.
10. Anthocyanins are flavonoids responsible for colored pigments like blue, red, pink, purple in plant parts like flowers, fruits, stems, leaves, roots, seeds etc. They are flower attractants for birds and insect pollinators, as well as attractants to animals and birds that disperse their fruits and seeds. They also protect plant from UV irradiation.
11. Alkaloids are nitrogen-containing heterocyclic organic bases with complicated structures usually with specific physiological functions. They are biosynthesized and derived from amino acids through mevalonic acid pathways. Most alkaloids are toxic to man and animals, hence prevent herbivores from consuming them; so acts as defensive compounds in plants.
12. Carotenoids are responsible for the bright colours observed in plant pigments like flowers, fruits and seeds such as yellow, orange, red etc. The bright flowers in particular are attractions for pollinators. Carotenoids in fruits and seeds serve to attract animals that disperse them. Usually odoriferous (C<sub>10</sub> and C<sub>15</sub> terpenoids) are produced along with the carotenoids.

To man, phytochemical have some direct and indirect importance, few are highlighted thus:

- a. Cellulose is important industrially in fabrics like cotton. Other plant fibers are used lumber, paper and cardboards. Some are even modified in matrix forms in columns and TLC for chromatographic separation processes.
- b. Carotenoids are important in man's diet; they are known to prevent cancer and are important sources of vitamin A.
- c. Starch which are stored polysaccharides (of sugar units) in plants serve as primary source of food for man, microbes, insects, birds and other animals. Man consumes it directly and as processed forms like drinks of malt and beers.
- d. Anthocyanins are utilized as ornamentals for beautifying man's environment.
- e. Fructans and Inulins are beneficial to swine and poultry diets.
- f. Alkaloids are generally toxic to man and animals especially in large doses. But in lower doses have been reported to have great medicinal uses e.g. pain relievers, stimulants, antimalaria etc.

## **7. Aspects of biotechnology in biochemical and molecular regulations in the industrial development of plant phytochemical with the syntheses of metabolites**

Biotechnology in phytochemical studies involve bio-reactions and manipulations in plants for producing better and healthier plant growth, developments, protections, expansions and improved potentials of its phytochemical constituents with higher productivity. It applies



recent areas of studies like genomics, metabolomics, system-biology and proteomics for producing beneficial natural products. Generally development of plant cells biotechnologically is for economic and industrial purposes. Current methods and techniques utilize high-throughput applications on genomic modifications by homologous and recombinations at specific sites. It applies basic principles of plant and molecular biology, involving recombinant DNA technology modifying functional genes in natural product biosyntheses. The DNA and molecular biomarkers are involved; favourable traits are recognized, identified and isolated, then the selection of the genotype. Results from these assist in appropriate creation of transgenic plants that yield important and economic natural products using plant cells and tissues to get genetically modified plants and natural products. Such results are also utilized in assessments of biodiversities and chemotaxonomy. It is explorative applying foreign genes into plant genomics, so creating improved metabolic biosyntheses with genetic modifications to have faster and better production of active secondary metabolites, than from the conventional ways. But some are opposed to it because of the risk involved, and suggested it should not be applied to food developments. Well it requires first understanding details of genetic information on the plant and its natural product also know and identify the marker genes to be able to successfully transfer its genetic culture, so positively manipulating the plant with beneficial characters which last longer, and so affords better strategies in natural product formation and studies.

Incorporation methods (in-vivo) can be used as well as in-vitro cultivation and regeneration of excised or cultured whole plant provided necessary nutrients and hormones are available. Phytochemical researches in most part of the world utilize wild field cultivated plants or plants in the wilderness. It is more tasking to form plants and natural products from biotechnology, and the yield may even be too low, making it to be more costly and uneconomical. But biotechnology methods are very appropriate for endangered plant species and their natural products. Stereo- and regiospecific bio-transformations and bio-conversions afford in cultured cell suspension cultivations the discovery of new biomolecules which are not in the intact plant, so need to identify the particular enzymes causing this synthesis of new natural products which can be applied on large scale productions.

Molecular farming afford massive production of phytochemical from bioreactor plants to give cheaper and safer ways of forming recombinant proteins of higher values to give more valuable natural products and pharmaceuticals. It is a new area of bioengineering. It has the advantage of expressing gene at specific organs like leaves, fruits, roots or seeds. Gene of the host plant is modified so it forms stable products. These methods are important in fermentation processes, commercial proteins and products, in the generation of therapeutics and vaccines, as well as in diagnostics.

Biotechnology in natural products research afford discovery of bioactive natural products from sources outside the already known conventional plants, so reveals wider diversities of phyto-compounds. This is an important aspect of bio-prospecting.

## **8. Practical involvements of specific phytochemical in health and treatments as for example antimalaria, antibacterial, anti-fungal, antioxidant, anti-ulcer, anticancer**

Active metabolites from natural sources usually have very minimal or no toxic effect on organism using them; hence they are more useful and promising. The great diversity of

tropical forest plants are good sources of great number of bioactive substances with many therapeutic uses from which drugs can be discovered and processed. Many lead compounds have been isolated and derived from plants, which are now very useful drugs. [See Table 1]. Optimization follows after identification and establishment of a lead compound.

S/N	Specific use(s)	Active phytochemical	Plant/ Sources
1	Analgesic	Distylin, Rutin flavonoids; Morphine & Papaverine alkaloids.	<i>Saccharum officinarum</i> , <i>Leersia hexandra</i> , <i>Schrobera arborea</i> ,
2	Antibacteria	Tannins, Saponins	<i>Nesogordonia papaverifera</i> ,
3	Antifungal	Sterols, Saponins	<i>Centrosema pubescens</i> , <i>Parinari curatellaefolia</i> , <i>Anthocleista djalensis</i> , <i>Hygrophila auriculata</i> , <i>Dacryodes edulis</i> ,
4	Antibiotic	Macrolides, Penicillin, Tetracyclines, Cephalosporin, Gentamycins	<i>Chromolaena odorata</i> , <i>Acalypha wilkesiana/ hispida</i> & sps., <i>Mundulea sericera</i> ,
5	Antioxidant	Flavonoids	<i>Allium sativum</i> , Apple, Grape, Soyabean,
6	Anti-inflammatory	Flavonoids, Saponins	<i>Vitex doniana</i>
7	Antimalaria	Quinines, Pamaquines, Phenacetin Alkaloids, Flavonoids	<i>Rauwolfia vomitoria</i> , <i>Alstonia boonei</i> , <i>Cinchona officinalis</i> , <i>Polyalthia suaveolens</i> ,
8	Antidepressant	Flavonoids	<i>Marsderua latifolia</i> ,
9	Anti-tumor	Lignans, Saponins	<i>Pteris togoensis</i> , <i>Harrisonia abyssinica</i> ,
10	Stimulant	Nicotine & Caffein Alkaloids	<i>Haemanthus multiflorus</i> , <i>Lagerstroemia speciosa</i> , <i>Vitex cryosocarpa</i> , <i>Leucaena glauca</i> , <i>Cola acuminata</i> ,
11	Antiviral	Ginseng & Saikosaponins	<i>Hedranthera barteri</i> , <i>Bambusa vulgaris</i> , <i>Dissotis rotundifolia</i> ,
12	Antihypertensive	Reserpine, Flavonoids,	<i>Voacanga africana</i> , <i>Tapinanthus bangwensis</i> , <i>Adenia cissampeloides</i> ,
13	Tranquilizer	Schizandrins	<i>Kaempferia nigerica</i> ,
14	Aphrodisiac	Steroids	<i>Brunfelsia uniflora</i> , <i>Euphorbia deightonii</i> , <i>Prosopis Africana</i> , <i>Rhigiocarya racemifera</i> ,

Table 1. Active phytochemicals from plants and their medicinal uses.

## 9. Bioinformatics, genomics, and synergies of phytochemical with pathogens

We face challenges on how do plants with its enzymes and regulatory genes biosynthesize specific natural product compounds. It gives deeper and clearer understanding of processes involved in generating our highly interested natural products from perspectives of

molecular biology. Studies here are on characterization of functional genes and sequencing of genome because they express and regulate syntheses of natural product which is our main concern in phytochemistry and pharmacognosy. Important areas for molecular biology techniques of studying genomics of natural products in plants include identifying and expressing genes, functional genes and silencing. DNA and RNA isolations and clones with proteins derived from them. The important aspect of phytochemistry is the interest in processes occurring in each plant species for the biosyntheses of the metabolites. Techniques here are very sensitive high-through put plant metabolomics screenings with better separation, purification and structure characterization methods and instruments, as well as very sensitive methods of detections. These will reveal total and detailed natural product constituents in plants, which is our focus, and due to the great importances of natural products in isolated form and as synergies. With great diversities of natural product compounds, this method is highly reliable. The applications of these model plant species to non-model medicinal plants are the new trends in phytochemistry and pharmacognosy. Total genes' content of an organism is its genome, giving understanding of functions of genes proteins on the wide plant genome. The genetic studies are integrated approaches having both experimental and computational sides, later has genomic library with databases called 'bioinformatics'. Experimental work utilizes mutants, gene microarrays in cells and tissues, and spectrometry for analyses to complement the molecular techniques. Very large amount of data is generated from these studies, which are analyzed and interpreted with the computational bioinformatics which are available resources for studying metabolomics pathways from the characterized functional gene, in which a plant can have 20,000 to 50,000 genes responsible for generating its divers metabolites. There are presentations of large size of genome-sequencing projects of great number of plants serving as models with information, maps of genes and molecular markers in known websites. These are mostly on crop plants. Examples are:

[www.genome.ad.jp/dbget/ligand.html](http://www.genome.ad.jp/dbget/ligand.html) ; [www.p450.kvl.dk](http://www.p450.kvl.dk); [www.stke.org](http://www.stke.org);  
[www.genome.ad.jp/kegtg](http://www.genome.ad.jp/kegtg); [www.arabidopsis.org/tools/aracyc](http://www.arabidopsis.org/tools/aracyc);  
[www.genome.ad.jp/brite/brite.html](http://www.genome.ad.jp/brite/brite.html); [www.signalinggateway.org](http://www.signalinggateway.org);

The genes which govern the regulations for the different metabolic pathways and biosynthetic enzymes involved are identified and studied. Understanding families or categories of different biosynthetic genes leading to the production of many specific secondary metabolites is the main focus of natural product metabolomics and its processes. Families of genes function differently and we can even have evolutionary trends where constitution of a group of new genes with same function evolves independently by gene duplications, giving a composite gene evolved from recombination events between two different types. Now combining evolutionary ways of duplications with divergence and domain swapping are most likely the reasons for the very vast diversities of secondary metabolites in plants. Plant metabolomics pathway leadings leading to secondary metabolites have three types of genes which are (1) Glycosyl transferases (2) Acyl transferases and (3) Cytochrome P450s genes.

1. Glycosyl transferases: Here enzymes that cause glycolysation of secondary metabolites see to and ensure sugar moieties are added to organic molecules, so maintains the metabolic homeostasis. The effect causes increase in chemical stability and water

solubility, which may influence or even change the bioactivities. Nucleotide activated sugars are utilized as substrates on many secondary metabolites like flavonoids, carotenoids, steroids, lignin etc.

2. Acyl transferases: They are enzymes which make available the acyl group [RC=O] to other molecules [alcohols, amines, phosphates, carboxylates etc] in different pathways, so controls metabolite levels in biosyntheses of, for examples anthocyanins, alkaloids, cystein and phenylpropanoids.
3. Cytochrome P450 genes: They are heme-thiolate proteins responsible for electron transfers. They cause bio-transformations of xenobiotics and endobiotics in processes like detoxifications, signalling, growth and defenses. They are also important in drugs metabolisms.

Genomics and metabolomics studies will be able to reveal and decipher detail phytochemical in a particular medicinal plant species. Probably in the near future, transgenic methods may also provide good alternative biosynthetic methods of forming new active secondary metabolites not known now. Therefore this will demonstrate biotechnology in natural product studies, and it may even be a potential application in natural product researches, moreso plants are easy organisms to manipulate their genetics so as to obtain diverse natural products. These will also lead to safe nutraceutical natural products.

## 10. Highlights on thigmonastics, polygraphs, taoism, ancestral aspects of plants with quarantine

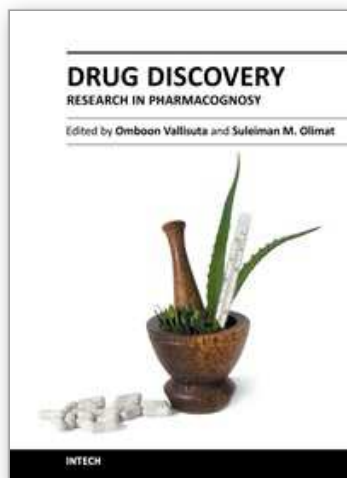
'Wellbeing' entails perfect maintenance of man's social, mental, physical and spiritual health. Plants have been highly beneficial to man's health and maintenance. Utilization of herbs for curative applications have been before 2000BC. Some of our ethical believe, behaviour and handlings have effects on plants, and results we get from plants. Man consume plants inform of foods, drinks, fruits and vegetables. The phytochemical in plants determine its smells, flavours, fragrances and colours. It also reduces a lot of risk in man. Man's aggressions and violence are known to decrease with his social interactions with his green environment. Through electromagnetic waves induced by man's activities such as words and music he pronounce in love or show of gratitude, have impact on plants, and vice versa. For examples insectivorous plants trap insects that rest on it; a plant like *Mimosa pudica* re-align its leaflets to a limp up position when touched by man. Likewise, man and animals influence health, pollination and survival of plants (**quarantine**). Experiments on tropisms indicate reactions of plants to factors like applied light, heat, electricity and gravity. Man's physical contact [e.g. applying strokes, rubbings] with plant parts such as flowers, leaves and stems affects its wellness. All described above refers to plant's THIGMONASTICS. Prove of its truthfulness is referred to as **Polygraph**. Emphasis on the natural and simple way of life with the interactions is called **Taoism**. It is believed and proposed that plants have spirits.

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This book, Drug Discovery Research in Pharmacognosy provides a full picture of research in the area of pharmacognosy with the goal of drug discovery from natural products based on the traditional knowledge or practices. Several plants that have been used as food show their potential as chemopreventive agents and the claims of many medicinal plants used in traditional medicine are now supported by scientific studies. Drug Discovery Research in Pharmacognosy is a promising road map which will help us find medicine for all!

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