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Trends in bioprospecting of biodiversity in new drug design

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Abstract

Medicinal plants constitute main resource base of almost all the traditional healthcare systems. Most of the herbal drugs produced currently in majority of the developing countries lack proper quality specification and standards. The technological advancement of 20th century saw the collaboration of engineering science and biological science, thus explore the genomic basis and molecular structure of the active principles. In the 21st century with the availability of sophisticated instruments led to the detailed structural and functional dynamics of the organism/ genes become so handy. This led to the newer opportunities in creating different pharmaceuticals with lesser side effects, and food with more nutritional value and emergence of novel field of research. The understanding of the genomics, proteomics, transcriptomics and metabolomics has contributed a great deal in pharamaceutical and nutraceutical science. The giant strides made by analytical and synthetic chemistry, electronics and science in general, have immensely contributed to the development of the science or biomedicine that has achieved miracles in medical practice. A new thinking centered on the concept of 'knowledge engineering' for building up future 'knowledge societies' and 'knowledge industries' is now gaining attention and acceptance both nationally and internationally.

***Key words*:** Biodiversity, bioprospecting, metabolomics, knowledge engineering, high-throughput screening

INTRODUCTION

Biodiversity and traditional knowledge together formed the cradle for bio-prospecting and the technological advancements in the modern world provided the essential nourishment to the mammoth development of this new age field. The prospects of exploring biodiversity for new medicines, foods, crops, insecticides, pesticides and other commercially valuable genetic and biological products and processes are booming, thanks to the rapid development in biotechnology - particularly genomics, proteomics, transcriptomics, enzymatic and transgenic technologies, herbal technology and information technology. This exploration of biodiversity for commercially valuable genetic and biochemical resources is termed as "bio-prospecting" - a concept pioneered by Thomas T Eisner as "chemical prospecting" (Eisner 1989; Reid *et al*. 1993). The advancements in biotechnologies have further redefined the overall scope and utility of bio-prospecting to encompass all relevant activities related to systematic search for genes, natural compounds, designs and whole organisms in wildlife with a potential for product development by biological observations and biophysical, biochemical and genetic methods without disruption to nature (Mateo *et al*. 2001). In short, bio-prospecting involves investigation of genetic resources or bio-chemicals for new commercial leads (Laird & ten

Kate 2002) and includes three major areas such as chemical prospecting, gene prospecting and bionic prospecting (Maeto *et al*. 2001).

Chemical prospecting

Modern high-throughput chemical screening and automated bioassay programs for identifying, isolating, characterizing novel bioactive compounds from wild plants, fungi, animals (insects and invertebrates especially) and microbes have opened up new vistas in natural product research in general and drug and pharmaceutical discoveries in particular. Chemical prospecting of wild plant resources is becoming increasingly applicable in agro-chemistry (biopesticides and insecticides), drugs and pharmaceuticals, cosmetics, proteins, enzymes, food additives and other industrially valuable chemical products (Eisner 1997).

Gene prospecting

Modern molecular technologies like DNA recombinant techniques and transgenic technologies made it possible to identify, isolate and introduce desirable gene(s) from one organism to another, transcending the biological/taxonomic barrier. Transgenic technologies are making significant headways by facilitating transfer of the desirable agronomic traits or chemicals from one organism (e.g., plants; animals, including humans) to bacteria and converting the resultant transgenic bacteria to potential chemical factories producing desired products such as enzymes, drugs, pharmaceuticals and other valuable products. Similarly, genetically modified fungi are now proved to be potential sources for mass production of enzymes, proteins and other biomolecules. New and improved crop varieties are developed through genetic engineering techniques with desired genes that confer resistance/tolerance to pest, disease and climatic/environmental stresses. Isolation of Bt gene (isolated from the bacterium, *Bacillus thuringiensis*) having insect resistance and introducing this gene into crop plants such as potato, cotton, corn, wheat, rice, tomato, etc., through transgenic techniques is a classic example to demonstrate the prospects of gene prospecting for crop improvement and sustainable agriculture. Proteomics is an interesting field of gene prospecting. This deals with the identification and patterns of expression of gene(s) that encodes for the synthesis of a specific protein of interest. Bioinformatics provide the key source for mining DNA / nucleotide sequences data, based on which new genes responsible for the synthesis, expression and function of a particular protein or enzyme can be made. Proteomics offer new promises to gene therapy and enzymatic technologies.

Bionic prospecting

Bionic prospecting is a new area by which new designs, patterns, models and techniques are evolved based on natural biodiversity. New sensor technologies, architecture, bioengineering and bio- modeling are some of the interesting fields in bionic prospecting.

Major sectors of bioprospecting

Bio-prospecting covers wide range of commercial activities, including the pharmaceutical, biotechnology, seed, crop protection, horticulture, botanical medicine, cosmetic and personal care, and food and beverage sectors (ten Kate & Laird 1999; Laird & ten Kate 2002). The major players of bio-prospecting include multinational companies (in private and public sectors), R&D institutions, universities, botanic gardens, etc. Genetic resources and associated traditional knowledge provide the key resources and biotechnologies offer the key tools relevant for these bio-prospecting sectors. The ways in which they use genetic resources and biotechnologies would vary among and between these sectors depending upon the ultimate

aim and targets of each bio-prospecting activity. The quantum of genetic resources or their derivatives used, the leads from associated traditional knowledge accessed or utilized, and the methodological framework of various techniques and tools employed would differ significantly in each bio-prospecting activity. These are guided by a number of requisite factors such as the capability of the bio-prospecting companies or institutions in terms of infrastructure, human resources and technological capabilities, as well as the existing national and international policy and legal frameworks that facilitate free and regulated access to genetic resources, or their derivatives and/or the associated traditional knowledge, and more importantly the ultimate objectives of the bio-prospecting mission envisaged. For example, among the above-mentioned major players in bio-prospecting programs, the pharmaceutical and agro-technology industries are the prominent ones and have a major stake in the global bio-industrial regimes. They use genetic resources in significantly different ways. There is diversity of genetic resources use and biotechnological interventions within and between the bio-prospecting sectors, which is influenced greatly by the following factors such as (1) size of industries and markets for the products, (2) role of natural products in these markets and percentage of sales contributed by genetic resources, (3) relationship between commercial products and the genetic resources from which they are developed (Laird & ten Kate 2002).

The global estimates show a 900 billion in the annual sale values of pharmaceuticals, crop protection, agricultural seed, horticulture, botanical medicines, cosmetics and personal care. Within each sector, the share of sale value derived from genetic resources varies. For example, in the pharmaceutical industry, natural products contributes about 25 – 50 percent of medicines, whereas ornamental horticultural products and sales of agricultural seeds are 100 percent natural products and make up less than 10 percent of global sales. Biotechnology is yet another largest sector wherein genetic resources are found increasingly used for the development and manufacture of a plethora of products, from enzymes and metabolites to process such as bioremediation systems.

The relationship between commercial products and genetic resources also varies within each bio-prospecting sector. In the pharmaceutical and crop protection industries, the final commercial product might be: (i) chemically identical to the pure natural product, and (ii) start with a natural synthesized to a design based on a natural template. Similarly, natural personal care and cosmetics products might be derived directly from natural genetic resources through high-throughput chemical screening, standardized chemical extracts (with set amounts of chemical markers), or extracts of whole plants containing all the constituents found in a given plant (Laird & ten Kate 2002).

Bioprospecting methods and strategies

The modern bio-prospecting sectors employ all the latest tools and technologies in biotechnologies along with appropriate designs and models of high-throughput screening, combinatorial chemistry, computational biology, bioinformatics, biosynthesis, and production and marketing strategies. While the inventive steps and technical inputs involved in development of a new product or process in each of the above sector would vary significantly, the general framework and overall strategies required by each sector contain certain essential common elements. For example, all bio-prospecting enterprises would require collection and acquisition of the essential raw or value-added or derivatives materials including naturally occurring genetic resources and/or associated traditional knowledge, which would be followed by random or selective screening for targeting a desired candidate/precursor or new leads for developing the targeted product or process. Once the leads are obtained, these need to be characterized and evaluated using appropriate techniques so as to pin point the most ideal

and desirable hit for further evaluation, standardization, quality tests and trials (clinical trials in case of biopharmaceuticals), synthesis, production, and marketing.

The success of any bio-prospecting programme depends on a number of factors such as (i) demand for and constraints on access to biological resources and associated traditional knowledge by potential users (ii) capability of providers of genetic resources to supply a good service and constraints on capacity to supply (iii) legislation, policy and a political climate suitable for bio-prospecting (iv) economic contribution that facilitates bio-prospecting and (v) ability to guarantee enforcement of agreements (ten Kate 1995).

There are also a number of other factors that contribute to better choice of bio-prospecting venues and partners. These, according to ten Kate & Laird (1999) include: (i) "biological diversity of the region; (ii) confidence that partner collects and supplies specimens in strict compliance with international and national law; (iii) ability of partner to obtain relevant permits and prior informed consents; (iv) clear, simple, and unbureaucratic procedure for securing permits and access agreements; (v) capacity of partner to ensure quality and quantity of supply and resupply; (vi) caliber of scientific staff in partner institute; (vii) political stability of the country and region in which collection takes place and where partners collaborate in value – adding activities, law and policy on protection of IPRs, taxation, and foreign ownership conducive to foreign investments.

Development in chemistry

Unfortunately, most of this modern therapeutics are so expensive that they are beyond the reach of the vast majority of the world's population. Also there are many ailments like cancer, liver disorders and arthritis etc, which has no satisfactory cure in modern medicine but traditional medicines like Ayurveda and Siddha claim to have satisfactory cure and management of such dreadful diseases. Modern medicine generally serves only a minority (about 30 to 35 per cent) of the total population in the developing countries (Naranjo 1981, 1995). The rest of the population attends to its health needs through the traditional medicine, which is essentially based on the use of easily accessible low-cost medicinal plants. Several considerations make the use of medicinal plants desirable. Among them are: a) their low cost, while the new synthetic drugs are becoming increasingly inaccessible to the vast majority of people; b) often they are the only recourse available; c) research has confirmed the presence of therapeutically active compounds such as alkaloids, glycosides and others, justifying a good many practices of folk medicine; and c) they have few, if at all, harmful side effects and hence their direct administration in traditional medicine offers little risk of causing iatrogenic (drug induced) disorders, unlike the modern synthetic drugs (Pushpangadan & Govindarajan 2005).

The capacity of chemists to modify a molecular structure is almost unlimited, but the capacity to create new structures with therapeutic properties has been found to be limited (Naranjo 1995). Plants and animals offer thousands of new molecules (Evans *et al*. 1982; Gottlieb 1982). An intensive and extensive study of the naturally occurring molecules identified as 'therapeutically active' is desired urgently to come out with new therapeutic entities. The very large number of alkaloids and several other classes of chemical compounds discovered during the 1970s and 1980s found to be pharmacologically active, serve as models for new synthetic compounds (Barz & Ellis 1981).

A number of plant-based drugs, such as vincristine, taxol, digoxin, quinine, reserpine, ergotine, opioids, ephedrine, colchicine, rutin, coumarins, anthraquinones, etc., are still a part of standard therapy. Most of these do not have any synthetic substitutes. Several other plant products are used in formulations that are sold over the counter (OTC) in several countries.

The role of plants in standard therapy will certainly be enhanced several fold in future, provided we make the move in the right direction.

Chemo-profiling using HPLC, HPTLC, GC-MS, LC-MS, UV-Vis, FT-IR, LC-NMR-MS, LC-ToF etc. have a great role in quality control of medicinal plants (raw drugs) and in the finished herbal drugs. European Pharmacopoeia gives assay of quinine type alkaloids and cinchonine type alkaloids. *Cinchona* bark using UV spectroscopy and US Pharmacopoeia include an UV absorption test for the absence of foreign oils in oil of lemon and orange. UV spectroscopic analysis has been used for the quantification and qualitative detection of marker compounds from the herbal material. Infrared spectroscopy, NMR and mass spectroscopy have been used for the structure elucidation of marker or active components of medicinal plants. As mentioned earlier the active principles in most medicinal plants are highly variable. This include intrinsic factors such as the genetic variations particularly in cross pollinated plants and intrinsic factors such as agroclimatic, edaphic conditions, stage of growth and developmental stage of the plant etc. to ensure a reasonable consistency in quality and efficacy one needs to identify the right genotype and correct growing, collection and post harvest handling practises. *Withania somnifera* an important medicinal plant of Ayurveda is reported to have three chemotypes depending upon the presence of a class of closely related steroidal lactones like withanolides, withaferin A etc. The content of withanolides and withaferin A and other biological active compounds may vary depending upon the genotype, micro and macro environment and developmental stage of the plant. All such finer details of the medicinal plant species when compiled together, it is referred as the passport data of the plant.

DNA based molecular markers are proving to be a versatile tool in the plant genome analysis and in differentiating different genotypes. Various techniques like Random Amplified Polymorphic DNA (RAPD), Fragment Length Polymorphic DNA (AFLP), Restricted Length Polymorphic DNA (RFLP) and Inter Simple Sequence repeat (ISSR) etc are now being successfully used for such genetic analysis of medicinal plants and also for the characterization of semi-processed and even fully processed herbal products. DNA markers are highly stable and specific. It has immense applications in the standardization of medicinal plants and its products.

Minimum requirements for medicinal plants and its products for global acceptance should have:

- Demonstrated safety
- Mapped efficacy (Pharmacologically credible formulation)
- Consistency in batch to batch quality
- For polyherbals, contribution of each herb to be proved along with synergims. It should be synergistic rather than additive
- Avoidance of endangered species
- Easy availability and accessibility of the plant

New technologies are constantly being developed to isolate and identify the components responsible for the activity of these plants. But these technologies should consider and possibly use the fact that the biological activity of plant extracts often results from additive or synergistic effects of its components. Another possibility is the qualitative and quantitative variations in the content of bioactive phytochemicals, which are currently considered major detriments in their use as medicine. Different stresses, locations, climates, microenvironments and physical and chemical stimuli, often called elicitors, qualitatively and quantitatively alter the content of

bioactive secondary metabolites. Enzymatic pathways leading to the synthesis of these phytochemicals are highly inducible (Ebel & Costa 1994). This is particularly true for phytochemicals that are well documented for their pharmacological activity, such as alkaloids (Facchini 2001), phenylpropanoids (Dixon & Paiva 1995) and terpenoids (Trapp & Croteau 2001) whose levels often increase by two to three orders of magnitude following stress or elicitation. Thus, elicitation-induced, reproducible increases in bioactive molecules, which might otherwise be undetected in screens, should significantly improve reliability and efficiency of plant extracts in drug discovery while at the same time preserving wild species and their habitats. Standardization, optimization and full control of growing conditions should guarantee a cost-effective and quality-controlled production of many plant-derived compounds. This kind of standardization and quality control of the plant-based drugs will improve safety of these drugs and promote their usage.

Alarming levels of antibiotic resistance in many human pathogens is likely to provoke an increase in pharmaceutical bioprospecting, which remains a vital source of lead drug discovery. Malaria, one of the world's most deadly diseases, has been treated historically with drugs derived from natural products - quinine, chloroquine, meoquine, and doxycycline and today the artemisinins derived from the Chinese herb Qinghao (*Artemesia annua*) are at the forefront of the battle against this parasite. Ethnomedical knowledge of some plants led to the development of drugs like Aspirin. It was rst isolated from *Filipendula ulmaria* because it had long been used in folk medicine of Europe to treat pain and fevers. When the Bayer company developed a synthetic derivative of salicylic acid called acetylsalicylic acid, they named it Aspirin - ''a'' for ''acetyl'' and ''spirin'' for *Spiraea*, the former Latin name for the genus. Another European folk cure that became a drug was derived from *Digitalis purpurea*, the leaves of which were rst used to treat congestive heart failure. The active ingredients, digitoxin and diyoxin, remain an important treatment for heart ailments.

The potential of plant metabolomics in drug development

Plant Metabolomics provides one of the pillars for studying the relation between the composition of complex and variable mixtures of plant derived remedies and their - also complex - biological effects. Plant metabolomics starts with the analysis of as many as possible detectable individual components that are present in the material. Extracts made from individual herbs/ plants, total mixtures or combinations of individual herbs/plants and extraction/mixing/preparation methods as used in phyto medicine can be analyzed by means of different techniques (LC-MS, GC-MS, NMR, LC-NMR etc.) resulting in total metabolite profiles.

Next, extracts (individual, total, combinations) are investigated for bioactivity (effects in cell lines, animal models, human volunteers) studying their effects at various biological levels using the body fluid studies. The databases from plant metabolic profiles, bioactivity and animal studies can be linked and analyzed by means of Multivariate Data Analysis (MVDA). MVDA is a powerful technique for the analysis of data sets with a large number of variables. It enables, for example the visualization and interpretations of patterns in NMR data that correlate with a target variable such as bioactivity. In this way, a plant metabolic data base can be constructed which will be extended with single compounds and metabolic profiles of all types of commercial extracts available from vegetables/crops/herbs that have grown, harvested and stored under different conditions. In the past a major tool in identifying new activity was testing unknown compounds or extracts on whole animals and making a whole set of observations at regular time intervals (Hippocratic screening). By validating this method with various known compounds, it was very useful for the identification of novel activities. However, for determining which were the active compounds this approach had the disadvantage that it was elaborate and quiet large samples were needed, something

which is difficult to realize with bio-assay guided fractionation. By administering plant extracts of different composition and using MVDA, it will be possible to calculate which compounds or groups of compounds are associated with the highest bioactivity.

Reverse pharmacology

Contrary to what has been said earlier the first step in Reverse Pharmacology (RP) is to select a remedy for development, through a retrospective treatment – outcome study. The second step is a dose escalating clinical trial that shows a dose – response phenomenon which helps in selecting the safest and most efficacious dose. The third step is a randomized controlled trial to compare the phytomedicine to the standard first-line treatment. The final step is to identify active compounds which can be used as marker for standardization and quality control. Thus RP approach can help in development of phytomedicines more economically and cheaply. The advantage of RP method in development of herbal phytomedicine can be appreciated when we consider that the development of conventional drugs is a slow and expensive processes taking up to 15 years and about $ 800 million (Nwaka *et al*. 2009; DiMasi *et al*. 2003). The various steps involved in dose optimization in RP studies are depicted in Fig. 1 and 2.

Vaidya *et al*. (2003) proposed a new discipline called Ayurvedic pharmacoepidemiology. Pharmacoepidemiology is a new field developed by synergy of fields of clinical pharmacology and epidemiology taking roots in the Western countries. He felt that with the ever-growing global interest in the Ayurvedic system of medicine, Ayurvedic Pharmacoepidemiology could emerge in India. This was indeed the result of a novel highly competitive research programme launched under NMITLI to develop globally acceptable herbal drugs from the Ayurvedic therapeutic heritage. There projects have been already initiated in – diabetes mellitus, osteoarthiritis and hepatitis.

Modern molecular technologies like DNA recombinant techniques and transgenic technologies make it possible to identify, isolate and introduce desirable gene(s) from one organism to another, transcending the biological/taxonomic barrier. Proteomics and metabolomics are interesting field of gene and drug prospecting. This deals with the identification and patterns of expression of gene(s) that encodes for the synthesis of a specific protein of interest. Bioinformatics provide the key source for mining DNA / nucleotide sequences data, based on which new genes responsible for the synthesis, expression and function of a particular protein or enzyme can be made. Proteomics offers new promises to gene therapy and enzymatic technologies.

Council of Scientific and Industrial Research (CSIR) has initiated a coordinated program on drug discovery with a network of 19 CSIR laboratories and other R&D institutions working in the area of traditional systems of medicines and universities. The program which was initiated in 1996 aims at discovering new bioactive molecules from plants, fungi, microbes, insects, etc. using new techniques of both synthesis (combinatorial chemistry) and bio-evaluation (medium and high through put screening). The program also includes discovery of molecules based on mechanism of the disease, functional genomics, anti-sense agents, etc. Currently bio-evaluation of the following eleven major diseases is in progress: 1. Bacterial infections 2. Malaria 3. Tuberculosis 4. Filaria 5. Hepatitis 6. Hypertension 7. Memory 8. Leishmania 9. Inflammation and Arthritis 10. Diabetes 11. Cancer.

The Planning Commission, Govt. of India and CSIR has embarked on a few bio prospecting programs with some specific targets/goals – such as the inter laboratory collaborative programs on biomolecule/drugs, drug prospecting. The Planning Commission

sponsored the NMITLI (New Millennium Indian Technology Leadership Initiative), which is one of the most innovative bioprospecting programs. NMITLI has major herbal drug development program for developing effective herbal remedies for hepatic disorders, arthritis and diabetes, which has shown highly encouraging results.

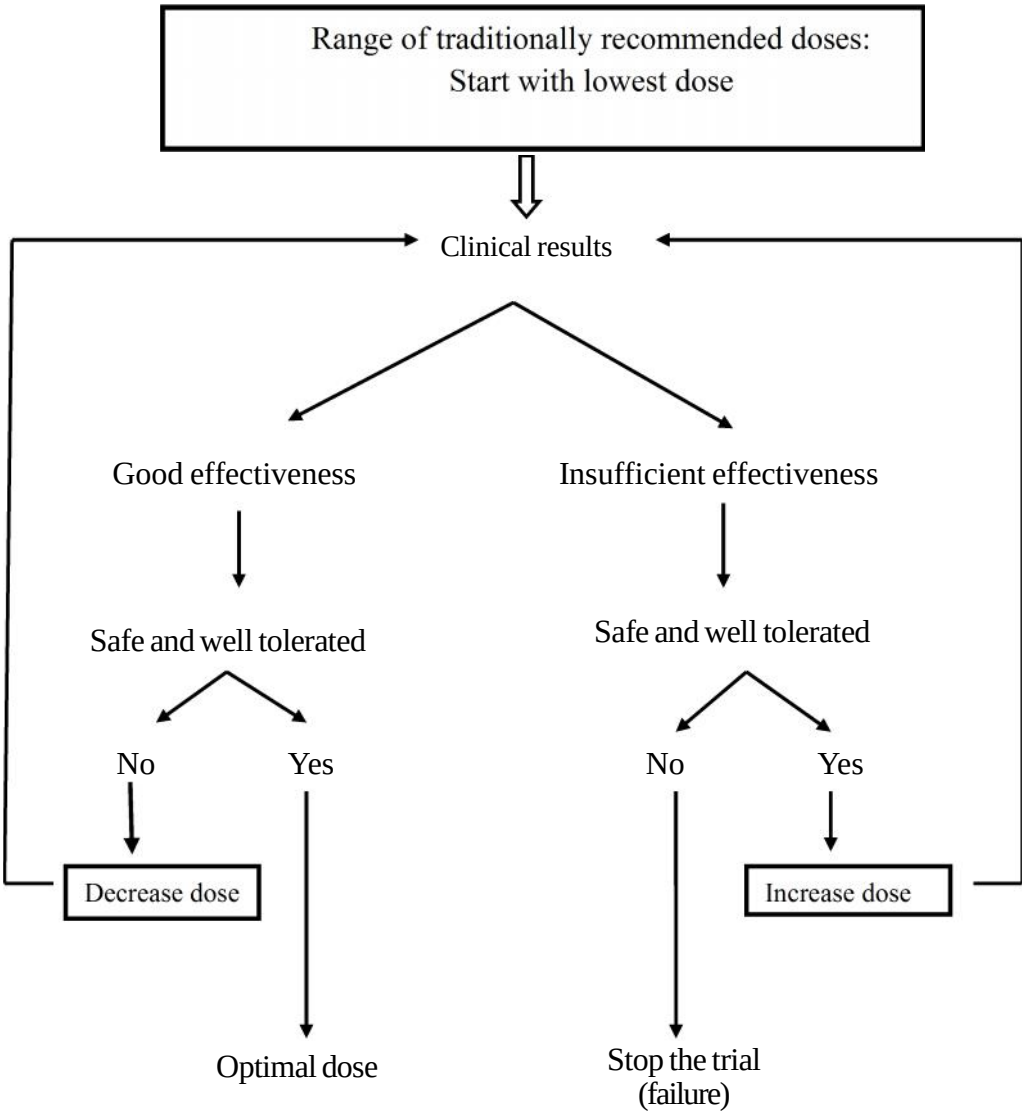

Fig. 1. Dose optimization [Adapted from Willcox *et al*. 2011; George & Ijinu 2011]

Systems biology and traditional medicine

It is now seen that systems biology can provide an important bridge function between TM and Western Medicine, because it can reveal the effects of simple perturbations such as single drug and/or complex perturbations such as TM or food. The use of systems biology to study the effect of TM on humans is very promising but also one of the most complex challenges in life science research. Moreover, within TM there is the challenge of the quality control of the production.

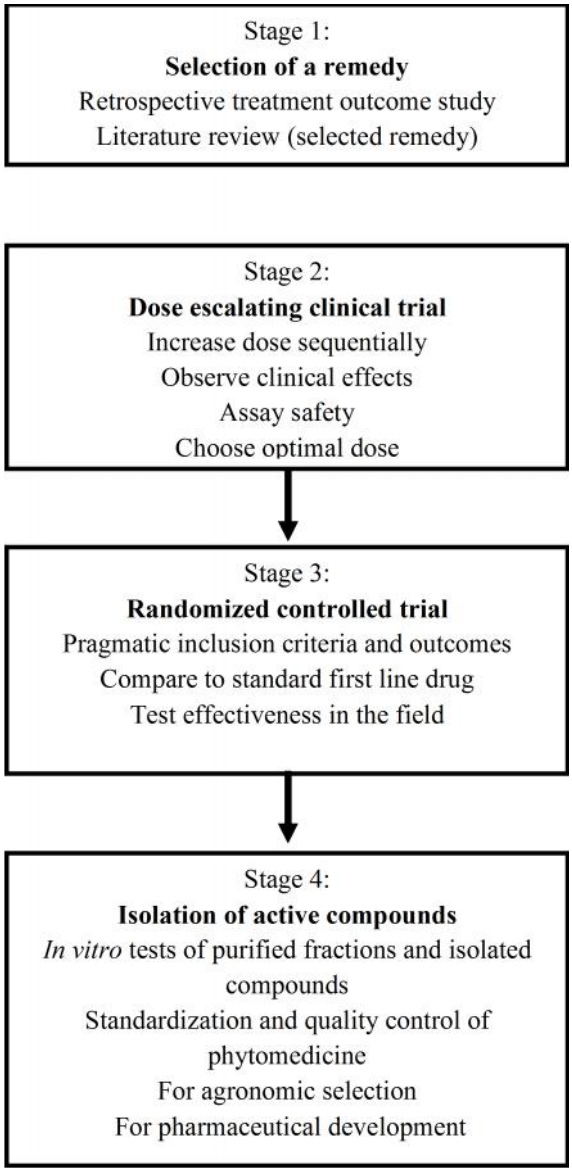

Fig. 2. Summary of the methodology used to develop phytomedicine by Reverse Pharmacology [Adapted from Willcox *et al*. 2011; George & Ijinu 2011]

The optimal bioactive fingerprint of the many components in a TM preparation is not known. There is no direct control mechanism on the production and processing of plants and therefore it may cause a varying success factor in the evaluation process. In fact differences in harvesting conditions that occur even during intervals as short as less than one day may already generate differences between batches. A more basic consideration in the evaluation of TM comes from the fact that it is prescribed as a personalized preparation. Consequently it cannot be evaluated in the more conventional way by applying clinical trials and generic treatments.

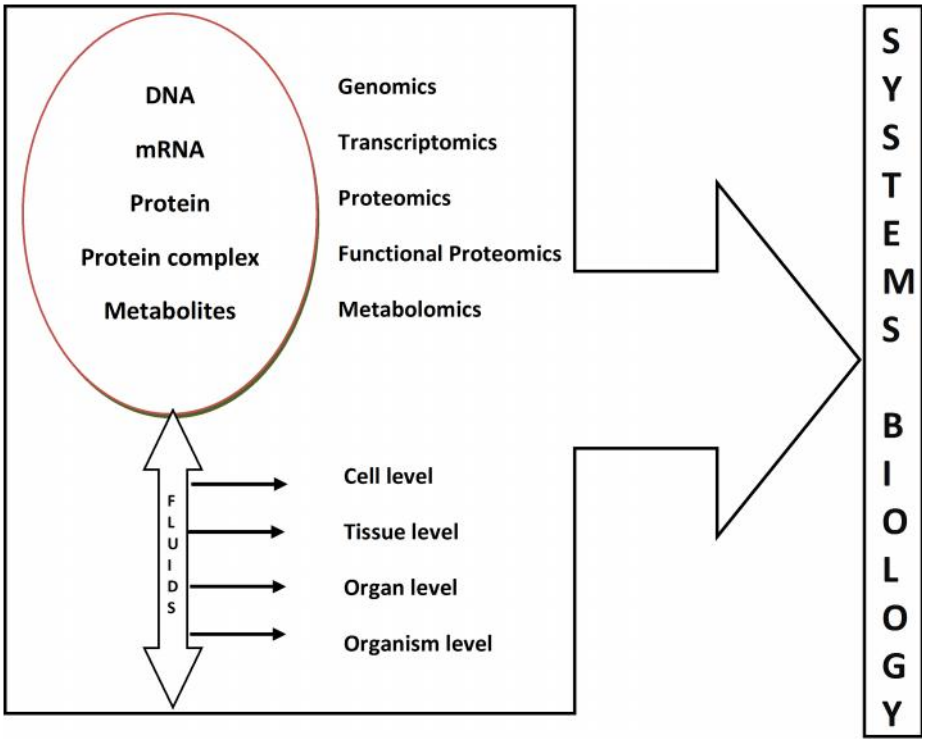

Fig. 3. The different levels of measurement in a systems approach [Adapted from Wang *et al*. 2005]

All the complicating factors mentioned above can be addressed in well designed studies if technologies are used based on finger printing of not only the system but also the complex perturbation mixtures and their linking both together using non-linear multivariate approaches.

The technology platform used in system biology comprises the elements as given in Fig.3: transcriptomics, proteomics and metabolomics at different levels: cell, tissue, organ, organism and system level. In addition, special attention is paid to profiling body fluids being an important source of information regarding the systems control functions or its biochemical "body" language. The extensive mapping of body fluids brings a new dimension into human physiology and is especially suited for the evaluation of the effects of treatments. Typical fluids are urine, blood, cerebrospinal fluid (CSF), saliva, lymph, synovial fluid etc. The ethical consideration favors urine and blood, which opens up especially the elements of proteomics and metabolomics measurements. In selected cases transcriptomics can be used in monitoring studies when blood cells are harvested or tissue biopsies are available. As for TM, the medical preparation itself is also of a complex and changing character. This introduces a second set of variables. To make it even more challenging, not only the relative composition of a remedy is subjected to variation, but the quality of the starting materials may also differ.

In TM most of the constituents of the preparations are derived from plants. Plants constantly interact with their changing and often harsh environment during the different phases of their life cycle. Plant secondary metabolites provide chemical protection against invading pathogens and predators to attract, for e.g., pollinators and physical stress but may also act to give a typical smell or color. Plants can make several thousands of these secondary metabolites. This has resulted in a natural treasure house with highly diverse and often very

potent compounds with a wide diversity of application in human health. A complicating factor when using plants is the variability of the material. This can be caused by differences occurring during growth, but also after harvesting the plant, due to decomposition during post harvest processing, extraction and preparation. Quality control and standardization are therefore highly relevant to assure proper preparation and standardization of a medication. In India the Indian Council of Medical Research has developed quality standards for a large number of Indian medicinal plants and are published in a series of volumes. However, standardization is still a matter of debate. It has been demonstrated that the secondary metabolite concentration in plants like *Ginkgo biloba* leaves vary considerably between the leaves harvested in the morning and in the evening from the same tree. It is noted that the ginkgolide and bilobalide concentration in the leaves were much higher when they are harvested in the evening. Also it has been noticed that there is cultivar dependent variation on metabolites in plants such as *Cannabis sativa*. In fact, in many studies on the activity of medicinal plants, and in particular in clinical studies, the plant material was not properly defined, making the results very doubtful. At the same time with all the possible variables it is almost impossible to measure each separately to determine which one is more active. Again by using the multivariate analysis of the data from either the treated animals or patients and of the metabolomes of the different preparations tested, the optimal composition can probably be calculated.

More recently novel techniques such as Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-MS) represent a quantum leap forward in the capabilities of mass spectrometers for metabolite analysis. Due to the exceptionally high resolution of these instruments metabolites with mass differences of less than 2ppm can be separated on a chromatographic scale. The accurate masses obtained give elemental compositions, which enable unequivocal metabolite identification.

Nutrigenomics, nutrigenetics and personalized nutrition

With the revolutionary advancement now making in genomics and proteomics a new type of food and nutrition under the head nutrigenomics are expected to emerge. With genome analysis the genetic predisposition of an individual can be made and thereby it is possible to know what kind of proteins or nutrients will suit to his/her constitutional type. Based on such genetic/constitutional analysis it is possible to recommend specific food items or even can breed or develop genetically modified food(s) that suit his/her genetic predisposition. Alternatively it is also possible to introduce raw or modified functional gene(s) or administer desired protein(s) / enzymes to human so that he/she may be able to assimilate/avoid the allergy or certain proteins in the food. Thus one look forward for a custom-made or personalized food and medicine that is genetically modified can be very soon expected to flood in the market. In fact the concept of personalized/ person specific food/nutrition and medicine is not a new one ancient Ayurvedic / Siddha masters have given top priority to this concept.

The Human Genome Project (HGP) is the largest ever international collaboration in biology. The result has been that the sequence of three billion chemical coding units in human DNA is now known. The next challenge is to identify each of the sequences of codes that are responsible for a specific activity or outcome. Genes are turned on and off according to metabolic signals that the nucleus receives from internal factors (e.g., hormones), and external factors (e.g., nutrients), which are among the most influential of environmental stimuli (Harland 2005). Unbalanced diets alter nutrient gene interactions, thereby increasing the risk of developing chronic diseases. Numerous dietary components can alter genetic events, and thereby influence health. In addition to the essential nutrients, such as carbohydrates, amino acids, fatty acids, calcium, zinc, selenium, folate, and vitamins A, C and E, there is a variety

of non-essential bioactive components that seem to significantly influence health (Corthésy-Theulaz *et al*. 2005; Trujillo *et al*. 2006).

Nutritional genomics or nutrigenomics, is the study of how food and genes interact and aims to understand the effects of diet on an individual's genes and health. It attempts to study the genome wide influences of nutrition and identify the genes that influence the risk of diet related diseses on a genome wide scale, and to understand the mechanisms that underlie these genetic predispositions (Muller & Kersten 2003). More practically, nutrigenomics describes the use of functional genomic tools to probe a biological system following a nutritional stimulus that will permit an increased understanding of how nutritional molecules affect metabolic pathways and homeostatic control. Nutrigenetics, on the other hand, aims to understand how the genetic makeup of an individual coordinates their response to diet, and thus considers underlying genetic polymorphisms. It embodies the science of identifying and characterizing gene variants associated with differential responses to nutrients, and relating this variation to disease states. Therefore, both disciplines aim to unravel diet/genome interactions; however, their approaches and immediate goals are distinct. Nutrigenomics will unravel the optimal diet from within a series of nutritional alternatives, whereas nutrigenetics will yield critically important information that will assist clinicians in identifying the optimal diet for a given individual, i.e., personalized nutrition (Mutch *et al*. 2005).

Ayurgenomics

Many rare diseases like hemophilia, beta-thallasemia etc. are monogenic, caused due to mutations in single genes. Most of the common diseases such as diabetes, asthma, cardiovascular disease etc. are multigenic complex disorders involving many genes. It is generally observed that common diseases are a consequence of cumulative effect of a large number of variations in the genome which independently have small effects that are not sufficient to cause the disease. However, it is now being increasingly realized that even those diseases that were considered to be monogenic sometimes exhibit differences in manifestation of disease in different individuals in spite of carrying the same mutations. This is thought to be due to presence of variations in other genes that could modify the effect of the primary mutation. Further, there is a complex interplay of gene and environment involved in the majority of the diseases. Most of these diseases require long term drug administration and there is a high variability in individual response to drug dosage and adverse effects due to mainly variations in the genes responsible for drug transport and drug metabolism within the individual's system. Therefore, design of optimum dosage with least side-effects is difficult to establish.

Tridoshas are not only genetically determined (Shukra Shonita) but also influenced by the environment during development, especially maternal diet and lifestyle. Prakriti is fixed at the time of birth and remains invariant throughout the individual's lifespan. Ethnicity (Jatiprasakta), familial characteristics (Kulanupatini), and geoclimatic regions (Deshanupatini) are also implicated in influencing phenotypic variability through their effect on Tridoshas and Prakriti. Thus, most of the factors such as ethnicity, geography, and environment that contribute to inter-individual variability at the genetic or epigenetic levels are embedded in Ayurveda's concept of Prakriti. In an individual, the Tridoshas work in conjunction and maintain homeostasis throughout the lifetime of the individual (Sethi *et al*. 2011).

There is no modern methods available to look at inter-individual differences within ethnically matched healthy populations and no studies at the genome-wide scale have, however, been attempted before. Mukherji and her team at the Institute of Genomics and Integrative Biology, have been exploring the concept whether Ayurveda, can fill this gap and help in

identification of predictive markers for some of these complex diseases (Mukherji & Prasher 2011).

In this regard, recently the Council of Scientific and Industrial Research (CSIR) in association with Indian Centre for Social Transformation (Indian CST) has started major program called TRISUTRA (Translational Research and Innovative Science Through Ayurgenomics) at the Institute of Genomics and Integrative Biology (IGIB), New Delhi in March, 2009. The European Institute of Systems Biology and Medicine (EISBM) and the Institute of Genomics and Integrative Biology (IGIB) are already planning a research exchange programme in Ayurgenomics.

Time and risk factors in high-tech bioprospecting

The underlying premise of any meaning bio-prospecting ventures should be conservation of biological and sustainable human development. Acknowledging this fact, the partners need to appreciate the strength and constraints on both parties and certainly have to make benign compromises on acceptable realities and risks involved in long – term bio-prospecting programmes. For example, drug prospecting is a multi – billion-dollar industry. The cost of developing a single modern drug is to the tune of US $ 500 – 575 millions. It is well accepted that the possibility of finding a potential bioactive compound is 1 in 10,000 samples and that of discovering a marketable drug is a 1 in 4 bioactive compound. Moreover, drug-screening programmes take a long term of say 15 to 18 years. The market opportunities prevailing at the time of a drug discovery is also vital factor that prompts the drug or pharmaceutical prospectors to compete for marketing their products. Under such circumstances, the drug prospecting companies would neither be sure about the royalty to be fixed for its future product nor would they like to forfeit their investments by taking sheer risks. It is therefore recommended and followed in many bio-prospecting partnerships, like the In Bio – Merck, that an 'up – front' payment may be made by the prospectors to the source countries to support research and development programs, infrastructure development and human resource development through training and capacity building in biotechnology and bio-prospecting. Likewise, the biotechnology–rich countries should make certain commitments and concessions to the developing countries to access the relevant biotechnologies required for sustainable use of genetic resources.

Bioprospecting and biodiversity conservation

It may, however be recalled that the pros and cons of bio-prospecting need to be evaluated against the backdrop of the increasing incidence of bio-piracy and more seriously against the current crisis of bio-depletion and the likely impacts of predicted mass extinction spasm impending in the tropical biomes (Myers 1987; Pimm *et al*. 1995). About 5 % of the earth's land surface is in protected area networks, and if human activities continue in the rest of the 95 % of the unprotected wild land habitats, about 50 % of the species would go extinct (Pimm & Lawton 1998). This is a ground reality and any bio-prospecting program should, therefore, be carried out with the end in view that apart from direct economic benefits, such activities would contribute directly or indirectly to fund conservation, inventorying and monitoring of biodiversity, both *in- situ* and *ex- situ*.

The practices and strategies for bio-prospecting should, therefore, focus on the above mentioned components of (1) assessments (2) national policy and legislation (3) capacity building (4) equitable benefit sharing mechanisms (5) participatory management involving all stake holders in bio-prospecting, including local and indigenous communities (6) mobilizing financial resources for bio-prospecting and other sustainable uses of biodiversity (ten Kate & Laird 1999).

Relationship between bioprospecting and access and benefit sharing

The recent trends in bio-prospecting necessitate an ever-increasing demand for access to genetic resources and traditional knowledge that are available in in situ and ex situ sources. This has also triggered conflicting interests and common concerns among all stakeholder states, organizations, institutions, individuals, and communities involved in collection, characterization, conservation, sustainable utilization, and documentation of genetic resources and traditional knowledge at local, national, regional and global levels. The United Nations Convention on Biological Diversity (CBD 2001) is the first international legal instrument that brought out a radical change from the then prevailing common perception on genetic resources as "common heritage of mankind" to a legally binding regime that confers "sovereign rights" to the states over their own biological resources (including genetic resources and traditional knowledge).

CONCLUSION

With the emerging area of biotechnological intervention in medicinal plants for pharmaceutical and nutraceutical discoveries include: "systematic search for genes, natural compounds and designs with a potential for product development of biochemical and genetic methods without disruption to nature". Modern high-throughput chemical screening and automated bioassay programs for identifying, isolating, characterizing novel bioactive compounds from wild medicinal plants, fungi, animals (insects and invertebrates especially) and microbes have opened up new vistas in natural product research in general and drug and pharmaceuticals research in particular. The complexity of ingredients and the aspects of synergistic bioactivities of poly-herbal medicines could now be well explained by system biology approach that enables linking of the complex metabolic profile of herb with biological effects.

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