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Review Article

Natural products drug discovery: Accelerating the clinical candidate development using reverse pharmacology approaches

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The pharmaceutical industry is facing serious challenges as the drug discovery process is becoming extremely expensive, riskier and critically inefficient. A significant shift from single to multi targeted drugs especially for polygenic syndromes is being witnessed. Strategic options based on natural product drug discovery, ethnopharmacology and traditional medicines are re-emerging to offer good base as an attractive discovery engine. Approaches based on reverse pharmacology may offer efficient development platforms for herbal formulations. Relevant case studies from India and other countries where such approaches have expedited the drug discovery and development process by reducing time and economizing investments with better safety are discussed.

Keywords: Ayurveda, Ethnopharmacology, Herbal drugs, Natural products, Reverse pharmacology, Traditional medicine

History of medicine revels that most of early discoveries resulted from serendipity based on poisonous sources and not really from traditional medicines. The mass screening of plants in the search for new leads or drugs is vastly expensive and inefficient, but traditional knowledge offered better leads. It is estimated that over hundred new natural product-based leads are in clinical development[1]. About 60% of anticancer and 75% of anti-infective drugs approved from 1981-2002 could be traced to natural origins[2]. It would be cheaper and perhaps more productive to re-examine plant remedies described in ancient texts[3].

Many active compounds from traditional medicine sources could serve as good scaffolds for rational drug design. Combinatorial chemistry approaches based on natural product scaffolds are being used to create screening libraries that closely resemble drug-like compounds[4]. Most of these compounds are part of routinely used traditional medicines and hence their tolerance and safety are relatively better known than any other chemical entities that are new for human use[5]. Thus, traditional medicine based bioprospecting offers unmatched structural variety as promising new leads[6].

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Large numbers of promising lead molecules have come out of Ayurvedic experiential base including *Rauwolfia* alkaloids for hypertension, Psoralens in Vitiligo, *Holarrhena* alkaloids in Amoebiasis, Guggulsterons as hypolipidemic agents, *Mucuna pruriens* for Parkinson's disease, Piperidines as bioavailability enhancers, Baccosides in mental retention, Picrosides in hepatic protection, Phyllanthins as antivirals, Curcumines in inflammation, Withanolides, and many other steroidal lactones and glycosides as immunomodulators[7].

There are growing incidences where the old molecules are finding new applications through better understanding of traditional knowledge and clinical observations. For instance, forskolin an alkaloid isolated by Hoechst and coleonol by Central Drug research Institute (CDRI), CSIR, Lucknow a few decades ago from *Coleus forskohlii*[8] and phytochemicals from *Stephania glabra*, which were shelved for a considerable time are now being rediscovered as adenylate cylase and nitric oxide activators, which may help in preventing conditions including obesity and atherosclerosis[9]. Antimicrobial berberine alkaloids are now being rediscovered as novel cholesterol-lowering drugs working through different mechanism than statins[10]. Potent anti microbial[11-13] antirheumatic and cyclooxygenase inhibitory activities of phenolics, catechols and flavonoids from an important Ayurvedic plant

Semecarpus anacardium have been reported as promising leads. Small-molecule drugs that can regulate TNF-α levels or activity may provide a cost-effective alternative to protein-based therapeutics[14]. Natural products also provide a vast pool of pancreatic lipase inhibitors as potential candidates, which can be developed into new drugs for treatment of conditions like obesity[15]. A large number of promising leads for development of newer antiinflammatory drugs are also available in medicinal plants[16].

Drug discovery: Current scenario

The pharmaceutical industry has historically seen an incredible growth primarily due to the discovery of blockbuster drugs with the potential to generate over 1 billion US $ sales. However, recent trends indicate that this model may no longer lead to high growth rates. The average cost and time of discovering, developing and launching a new drug is consistently increasing without an expected corresponding increase in the number of newer, safer and better drugs. As compared to the previous years the numbers of New Molecular/ Chemical Entities produced per company have declined. Moreover, the number of approvals for new drugs has steadily declined from 53 in the year 1996 to 17 in 2007[17]. Clearly, drug discovery is no more a game of chance or just limited to technology availability today. The strategies that awarded success during the past may not guarantee success in the future[18]. The industry is really facing a major challenge to sustain and grow, which is resulting in many mergers, acquisitions or closures[19]. The situation is progressively deteriorating and analyst predict that worst is yet to come[20]. The global market situation and current financial crisis is bound to compound these pressures even to higher intensity.

The age of the blockbuster drug seems to be over or at least in its last days. The usual distinctions drawn between breakthrough and me-too drugs may not be very relevant today[21]. Critiques argue that the pharmaceutical industry has not been as innovative as it claims to be. Despite very stringent and tedious regulatory processes the industry has agonizingly experienced increased incidences of post approval or post marketing withdrawal of new drugs. Increasingly cautious regulatory processes are adding more risk and years for the pharmaceutical companies. The United States Food and Drug Administration (US FDA)'s Drug Watch and Drug Advisory Committee briefings on new anticoagulant Ximelagatran of Astra Zeneca or Cox II inhibitor Vioxx of Pfizer are very indicative of the impasse[22].

Drug discovery and development process involves a 10-15 years of investigation period and investments of the order of US $ 1 to 1.5 billion are not uncommon. This extremely complex, technology based and capital-intensive process has resulted in 'target rich lead poor' performance. Obviously, the pharmaceutical companies are looking beyond conventional drug discovery and development approaches not only to expedite the process, but also to ensure that safer and effective drugs could be launched faster and sustained (Fig. 1).

Over this background the importance of experiential wisdom and holistic approach is intensifying to offer good base as an attractive discovery engine[5]. Natural product drug discovery, ethnopharmacology, traditional, complementary and alternative medicines are re-emerging as new strategic options[23]. The World Health Organization's Commission on Intellectual Property and Innovation in Public Health also has duly recognized the promise and role of traditional medicine in drug development for affordable health solutions[24].

Reverse pharmacology

Reverse pharmacology is defined as the science of integrating documented clinical experiences and experiential observations into leads by transdisciplinary exploratory studies and further developing these into drug candidates or formulations through robust preclinical and clinical research[25]. The traditional knowledge inspired reverse pharmacology described here relates to reversing the routine 'laboratory to clinic' progress of discovery pipeline to 'clinics to laboratories'[26]. In this process 'safety' remains the most important starting point and the efficacy becomes a matter of validation.

Sir Ram Nath Chopra and Gananath Sen laid the foundation of reverse pharmacology of medicinal plants by pursuing clinically documented effects of Ayurvedic drugs[27]. *Rauwolfia serpentina* Benth, was a major discovery via this approach. Sen and Bose in 1931 convincingly demonstrated the antihypertensive and tranquillizing effects of the plant and also observed unique side effects such as depression, extra pyramidal syndrome, gynecomastia and peptic ulcer[28]. This effort led to a watershed for new antidepressants, anti Parkinson's drugs and prolactin–reducing drugs[29].

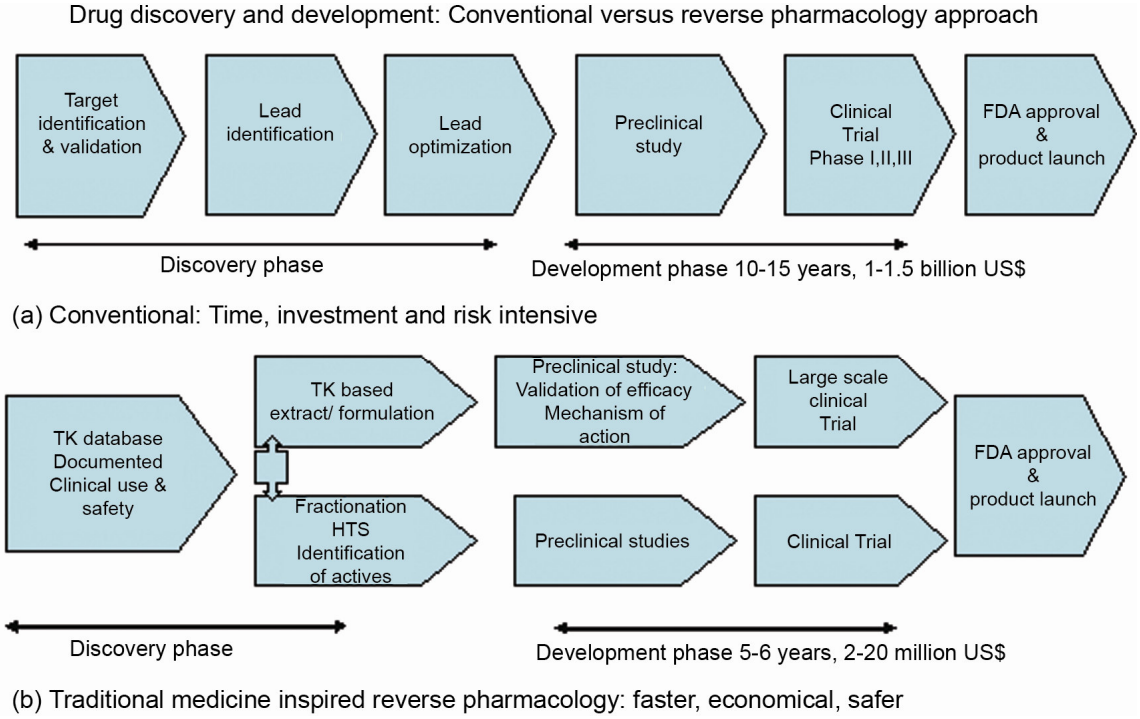

Fig. 1—Drug discovery and development: Conventional versus reverse pharmacology. (a) Conventional: Time, investment and risk intensive; (b) Traditional medicine inspired reverse pharmacology: Faster, economical and safe.

Reserpine, an anti-hypertensive alkaloid from *R. serpentina* became available for the treatment of hyper-tension by Ciba-Geigy.

Concept of reverse pharmacology was practiced for several years at Ciba-Geigy and Podar Ayurveda Hospital, Mumbai. Some promising work was undertaken almost 2-3 decades ago through composite drug research program jointly conducted by Indian Council of Medical Research (ICMR) and Council for Scientific and Industrial Research (CSIR) of Government of India. A cholesterol lowering drug Guggulipid was developed from *Commiphora mukul* taking the lead from Ayurveda[30]. Drug Controller General of India (DCGI) approved the drug for marketing in 1986. Guggulipid is being manufactured and marketed by Cipla Ltd, Mumbai under the brand name Guglip, however availability of authentic raw material has remained a limiting factor. A memory enhancer developed from *Bacopa monnieri* by CDRI, Lukhnow is also available in market.

Regrettably, most of such efforts remained more academic and could not be sufficiently pursued for the advanced molecular mechanistic work. Potential of such effort could not be optimally explored to make them globally successful products probably due to inadequate industry involvement during the development cycle.

CSIR, under the national network project known as New Millennium Indian Technology Leadership Initiative (NMITLI) attempted to bridge this gap by bringing industry and academia together right from the beginning where herbal drug development projects on psoriasis, osteoarthritis, hepatitis and diabetes were undertaken[31]. Recognizing timely importance, the ICMR has recently established an advanced center of reverse pharmacology with initial focus on malaria, sarcopenia and cognitive decline. Few case studies from India and abroad where the reverse pharmacology approach has been attempted to expedite the drug development process are presented here. Cases such as taxol or vinblastin, which are more coincidental rather than intentional discoveries and other examples which tend more towards health foods than drugs such as Ginseng or *Gingko biloba,* are excluded in this article.

Artemisinin—The herb *Artemisia annua* has been used for many centuries in Chinese traditional medicine as a treatment for fever and malaria. In 1971, Chinese chemists isolated active substance from the leafy portions of the plant responsible for its

reputed medicinal action. This compound, known as qinghaosu or artemisinin is a sesquiterpene lactone. The compound has been used successfully in several thousand malaria patients in China, including those with both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. Its derivatives have been shown to act rapidly in restoring to consciousness comatose patients with cerebral malaria. Thus artimisinin and its derivatives offer promise as a totally new class of antimalarials[32]. This discovery of Artimisinin is a result of scientific work based knowledge from Traditional Chinese Medicine (TCM) and presents best case for reverse pharmacology approach.

Psoriasis—Psoriasis is one of the common dermatological diseases affecting around 2% of the world population with no preventive or curative therapy except the symptomatic management. Under the NMITLI project, Lupin Laboratories in India attempted development of a single plant based oral herbal formulation through reverse pharmacology approach[33]. The drug candidate (Desoris) is an herbal beneficiated extract of a single plant that has a novel mechanism of action and effectively modulates the cellular function leading to psoriatic lesion improvement. Extensive studies comprising fingerprinting, activity guided fractionation, pharmacology, toxicology, efficacy, safety pharmacokinetics and toxicokinetics helped the company filing an Investigational New Drug (IND) application. Lupin commenced the Phase I clinical trial in September 2004 and successfully completed it. This drug has been developed conforming to the US FDA guidelines for botanicals and DCGI norms on new drug development. This is expected to take 5-6 years and cost US $ 5 million as against routine drug discovery path of 10-15 years and US $ 1-1.5 billion. If successful, the resulting treatment with Desoris may cost US $ 50, quite a step down from a new US $ 20,000 antibody injection treatment developed by a western biopharmaceutical company[34]. The estimated market for psoriasis therapeutics is around US $4 billion and this development may capture a significant part.

Vaccine adjuvant—Despite centuries of vaccine use, still alum salts remain universal vaccine adjuvants licensed for human use. However, they have limitations in engaging cellular immunity. Various immunostimulants from natural and synthetic origin are being studied either to replace or complement alum salts in vaccine formulations[35]. A project supported by Department of Science & Technology (DST), Govt. of India to develop herbal vaccine adjuvant was undertaken by the Interdisciplinary School of Health Sciences, University of Pune with Serum Institute of India as an industry partner. This project generally follows the reverse pharmacology approach based on Ayurvedic knowledge and previous scientific studies[36,37]. The project used flow cytometry to monitor effects of fractions on antigen specific protective immunity. Test materials were screened using *in vivo* potency assays for pertussis or diphtheria. These models are known to have relevant correlations with clinically established protective levels and routinely used in potency testing of vaccines[38,39]. Just within three years, the project has resulted in identification of few semi-pure leads that have considerable efficacy against polysaccharide, toxoid and recombinant group of vaccines. Further, these leads were found to modulate T-helper cell immunity, which is crucial for efficient cellular and humoral immunity. Detailed safety profile of one of the lead has been established, which is over 20 times than its effective pre-clinical immunomodulatory dose. Thus in a time span of three years a chemically characterized herbal fraction is ready to enter human clinical trial.

Parkinson's disease—Ayurvedic physicians in ancient India first used *Mucuna pruriens* seeds for the treatment of Parkinson's disease. Specially processed powder of Mucuna is used for treating Parkinson's disease. Untoward effects of Mucuna such as headache, dystonia, fatigue, tremor, syncope, and thirst are known in Ayurveda. The dose used by Ayurvedic physicians is small as compared to synthetic L-DOPA. These observations inspired scientists to further study and led to collaboration between academia and Zandu Pharmaceutical Works from Mumbai[40,41]. Their team conducted series of experiments on Mucuna to develop a natural drug for Parkinson's disease[42]. The United States Food and Drug Administration have approved New Drug Application for clinical studies. A Patent Cooperation Treaty application has been filed as a novel method extracting *Mucuna prurients* cotyledons and a composition for treatment of Parkinson's disease. Zandopa is now approved by the Indian Food and Drug Administration. This standardized, safe and economical natural product can effectively replace synthetic L-DOPA formulations.

Traditional herbal formulations

It is suggested that drug discovery need not be always confined to discovery of single molecule. Many analysts believe that the current assumption of one drug can fit to all will be unsustainable in future. Moreover, we are dealing with polygenic syndromes and not just isolated diseases. Multi-target approaches are in main stream with renewed interest in multi ingredient synergistic formulations[43]. Due to diversity of structures, herbal extracts can deal with multiple targets simultaneously and may give synergistic effect. Therefore development of standardized, synergetic, safe and effective herbal formulations with sufficient robust scientific evidence support also can offer faster and much economical alternative.

For instance, the Ayurvedic texts include few thousands of single or poly herbal formulations. These are rationally designed and are in therapeutic use for several years. Sufficient pharmacoepidemiological evidence could be generated to support their safety and efficacy[44]. Systematic data mining of the huge existing formulations database can certainly expedite the drug discovery processes where real effective and safe candidates could be identified. One of the pioneering series of clinical studies on Ayurvedic anti arthritis formulation named Rheumayog concluded the disease modifying activity, which was comparable with modern drug Auranofin[45]. Many more examples based on traditional medicines even for nutraceutical[46] and veterinary[47] applications seem promising. The US FDA and few other regulators do have very practical guidelines for botanical 'drug' development and herbals are no more restricted to nutraceuticals. Traditional herbal formulations could follow this route to create scientific evidence base with robust chemistry, manufacturing and controls. Department of Ayurveda, Yoga, Unani, Siddha, Homoeopathy (AYUSH) has recently established a research center at the University of Mississippi Oxford for to facilitate scientific investigations on Indian herbal drugs. In such process there may be exciting spin offs where promising molecular entities could be discovered.

Thus, if safe and effective herbal formulations are developed in accordance with stringent regulatory requirements on par with any modern drug, it is hoped that the conventional skepticism against herbals may slowly wane. However, issues related to appropriateness of conventional biomedical and clinical models for evaluating efficacy of traditional medicine remain very critical. A holistic approach based on systems biology seems much more suited to study therapeutic efficacy and pharmacodynamics of traditional medicine based drug development[48]. It is also argued that instead of randomized controlled trials normally used as gold standard in routine biomedical research, strategies of pragmatic or management clinical trials may be better suited for traditional medicine inspired reverse pharmacology approaches[49]. Two case studies where herbal formulations have been successfully developed using these strategies in much lesser time and resources are as follows:

Osteoarthritis—For osteoarthritis herbal drug development NMITLI project involved a network of 16 national research institutions, modern medicine hospitals and pharmaceutical industries from India. Following prior art[50] and several rounds of national level consultations with Ayurvedic physicians and scholars, short listed botanical drugs entered a parallel track of animal pharmacology and open label observational studies by clinicians. The project used traditional knowledge guided platform where the base formulation was optimized with additional ingredients to obtain desired therapeutic activities. All the formulations were manufactured under Good Manufacturing Practices in accordance with US FDA guidance to industry for botanical drugs. The preclinical evaluation was designed on the basis of systems approach, wherein the assay battery involved targets relevant to inflammation, pain, immunomodulation and chondroprotection (proteoglycan release, nitric oxide release, aggrecan release and hyaluronidase inhibition as markers) in human explant model of OA cartilage damage[51]. This led to design of synergistic poly herbal formulations that were found to be safe and devoid of any genotoxicity or mutagenic activity. Short listed formulations entered series of randomized clinical trials compared with known drugs glucosamine and celecoxib. Finally one best formulation was selected that led to one Indian and one Patent Cooperation Treaty applications with a dossier of necessary data required for possible regulatory submissions[52]. Thus, this project was completed in five years with expenditure of over US $ 2 million. This treatment may cost just US $ 25 a month for patients with much better therapeutic benefits including chondroprotection that no other modern drug offers. Currently, CSIR is in the process of identifying

suitable industrial partner for further development, optimization, manufacturing, registrations and marketing.

Tanga project—In Tanzania, The Tanga AIDS Working Group (TAWG) has innovatively used indigenous knowledge (IK) to alleviate suffering from HIV/AIDS. The group has treated over 4000 AIDS patients with herbs prescribed by local healers. The impact has been most significant in alleviating the opportunistic diseases brought on by the AIDS virus. The patients who have responded most positively have lived longer by up to five years. The Tanga regional hospital, which is a modern medicine facility, has been involved to test patients for HIV, treat them and provide counseling. With support from the World Bank's Indigenous Knowledge for Development Program, TAWG has organized community-to-community exchanges, involving their healers, people living with AIDS and staff working with patients to provide medical care and alternative income generating opportunities, in exchanges of IK with other communities in Tanzania[53]. Such experiences also are important in scientific or rational drug discovery process. A critical challenge is to leverage local and global knowledge systems to effectively resolve development challenges. To facilitate this process, the Global Research Alliance and the World Bank have initiated a partnership between the Tanga AIDS Working Group and the US National Institutes of Health to cooperate on the scientific validation of the efficacy of these herbal treatments.

Future perspectives

For several compelling reasons researchers involved in the modern drug discovery processes have started revisiting traditional knowledge and ethnopharmacology to reduce the typical innovation deficit faced today. Synergistic combinations and improvements in bioavailability are innovative strategies that can play an important role in drug development. For instance, in animal studies, artemisinin derivative and curcumin combination has been reported to show an additive interaction in killing *Plasmodium falciparum* to ensure total survivals[54]. There have been several studies on piperine showing improved bioavailability of synthetic drugs such as propranolol, theophylline and rifampicin. The clue for piperine as bioenhancer came from Ayurveda[55]. Such bioavailability enhancing activity may have numerous advantages in drug development including reduction in dose, toxicity and cost of the treatment.

Multi site mechanisms of action of herbal preparations from the crude extracts may offer greater chances for success where conventional single site agents have been disappointing. However, a single drug may not be an optimal way to treat every patient with vast genetic diversities. Genome-wide functional screenings for targets for diseases may be the practical approach. Combining Ayurveda and functional genomics in a systems biology scenario may reveal the pathway analysis of crude and active components[56]. The pharmacogenmics is now influencing drug discovery processes. Efforts to correlate genotype and phenoptype based on traditional methodology of classifying human as three major *Prakriti* types or constitutions described in Ayurveda have opened an exciting scientific chapter and will help progress of individualized medicine approaches[57].

Many countries are becoming increasingly aware of the value of their traditional knowledge, while global pharmaceutical industry is looking for innovative solutions to their existing impasse on innovation deficit to re-activate and re-energize discovery pipeline. Therefore, innovative approaches inspired by traditional knowledge will remain important to fast forward the discovery process and add new life especially in the existing global economic environment.

However, despite a vast potential and possibilities very few success stories are available as of now. This may be because most of the work in this field has remained within clinics of traditional practitioners or confined to academic research laboratories and not taken by industries that are strong in research and development. The earlier successes have been achieved distinctly when the industry effort was intensive. History indicates that majority of the drug discoveries would not have been developed or their development would have been delayed significantly in the absence of the scientific or technical contributions from the pharmaceutical companies[58]. Therefore, national efforts like NMITLI will remain important. The Government of India's golden triangle project integrating biomedicine, modern sciences and traditional medicine is indicative of trend where traditional sciences like Ayurveda are aggressively embracing scientific evidence base and integrated research[59-60].

The pharmaceutical industry needs many more successes like artemisinin and reserpine. Many promising leads like curcumins and withanoloides are available but such R & D cannot ensue in isolation. Best of public and private sector partners comprising academia and industry should come together to reap significant benefits from these seemingly low fashionable but highly gifted explorations based on traditional knowledge.

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