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BIOTECHNOLOGICAL APPROACH FOR MEDICINAL PLANT CONSERVATION AND ENHANCED PRODUCTION OF SECONDARY METABOLITES

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ABSTRACT: Growing awareness and interest towards traditional health care system coupled with phenomenal increase in demand and trade of medicinal plant has caused severe threat to the very existence of medicinal plants, which can be gauged from the fact that a number of important medicinal plants are becoming threatened or extinct and an increasing number of species are being substituted in herbal preparations. There is, therefore, a need to strike a balance between conservation and use. It is imperative to evolve viable strategies to conserve the surviving populations and their genetic resource of critically important species to avoid further loss. Further, spurt demand for herbal drugs has led to renewed interest in screening plants for novel biologically active compounds particularly to combat such ailments which have defied synthetic drugs. Herbal sector is under constant pressure to develop and deliver effective natural drugs. Phytochemical and pharmacological investigations have led to the isolation and characterization of a significant number of active molecules with versatile therapeutic efficacy. Biotechnology has emerged as the frontier tool for *in-vitro* production of pharmacologically active phytochemicals. The paper describes the potential and prospects of chemo-biotechnological approach to conservation of medicinal plants and enhanced production of phytopharmaceuticals.

INTRODUCTION: Medicinal plants, the oldest source of pharmacologically active compounds continue to cater the therapeutic need of majority of population in the developing world. They remained to be the only source of useful medicinal compounds for centuries and constitute the principle basis of traditional medicine systems of the world's oldest civilizations. It is estimated that even today two third of the world population relies on plant-derived drugs. According to an estimate of World Health Organization (WHO), 80% of the population of developing countries relies on plant derived medicines for their primary health care requirements.

Medicinal plants have played a dominant role in the introduction of new therapeutic agents. Based on the knowledge of plants gathered from various ancient medical writings, folklores, cultures and civilizations, field observation and consultation with folk medicine men, research on medicinal plants have been undertaken all over the world for development of new herbal drugs.

Modern pharmacopoeia contains at least 25% drugs derived from plants and many other are synthetic analogues built on prototype compounds isolated from plants. Plant based drugs are included in the pharmacopoeias of Russia, Japan, Germany and other developed and developing countries¹. Presently plant-derived secondary metabolites (active constituents) are in use and the medicines made from them represent a significant part of the total therapeutic agents. Additionally a lot of important steroid compounds and hormones are derived semi synthetically from plant precursors. However, crude drugs still hold considerable

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importance for medical treatment. Recently, there is a tendency towards more natural way of living in the entire world resulting in growing focus on the importance of medicinal plants and traditional health care system. Because of this awareness, the international trade in plants of medicinal importance is growing phenomenally, often detrimental to their natural habitats. Indiscriminate harvesting leads to extinction of natural populations which are still the only source of raw material^{2, 3}. Many of the medicinal plants are under severe threat, which can be gauged from the fact that an increasing number of species are being substituted in herbal preparations⁴. There is, therefore, a need to strike a balance between conservation and use. It is imperative that viable strategies to conserve the surviving populations and their genetic resource of at least critically important species are formulated to avoid further loss.

Diminishing Resource: Research shows that collection from the wild continues to be the major source of supply. In India, most collection of medicinal plants is from the wild destructively leading to the declined status of a number of plants. Due to the reason, medicinal plants are being overexploited and many of them are pushed to the brink of extinction^{5, 6}. Similarly, the medicinal plants are collected without paying attention to the stage of maturity. They are processed and stored haphazardly for long period of time under unsuitable conditions. This results in deterioration of quality of source material and final product. Despite the wealth of resources (biological, human and financial) available, the sector has not developed in the absence of sustainable harvesting practices, suitable processing and storage facilities, quality control and efficacy of drugs⁷.

The current and future ability to generate novel industrial products is likely to be threatened by the loss of the basic floral resource at various levels. The loss of phytodiversity not only leads to the loss of commercial opportunity but also compromise ecosystem functions⁸⁻¹⁰. There is abundant evidence that such losses are widespread and there is little sign that the losses are slowing, except in circumstances specifically aimed at biodiversity protection, such as the establishment of effective protected areas¹¹. While there is much debate over exactly how many species are becoming extinct

each year, it is abundantly clear that a very high proportion of species are losing their constituent populations at an alarming rate¹². In some forested regions there is a direct conflict of interest between logging on the one hand and human health and bioprospecting on the other.

In Eastern Amazonia, for example, where native plants provide most of the medicines used locally, the removal of trees that supply medicinal leaves, fruits, bark or oils has critically diminished the supply of medicines required by both the rural and the urban poor¹³. It is ironic that the recent explosion of new techniques in the biological, chemical and physical sciences that has generated a vastly improved capacity to understand and use biodiversity has been accompanied by a global decline in this very resource. Shrinking population of medicinal plants diminishes the resource base for their value addition and commercial applications. Further, loss of related traditional knowledge in recent centuries has been well documented and it is very likely that much local knowledge of medicines has been lost to humanity in general and to pharmaceutical prospecting in particular^{14, 15}.

Conserving Medicinal Plant Resources: Conservation of threatened medicinal taxa is clearly one of the priorities towards which the available technologies must be directed. Cultivation is one way to prevent the genetic erosion of medicinal plants, which has been standardized for only a few numbers of species. In actual practice, however, very few species are commercially grown on a large scale. It is important to set critical parameters like their status, cost, therapeutic importance, traditional use, active constituents etc. for selected plants not likely to be developed by tissue culture¹⁶.

Conservation strategies need to be better categorized primarily by their specific objectives and approaches for attaining the same. The static conservation strategies seek to dramatically alter the original evolutionary trajectories of plant species so that a genetic snapshot of sorts is conserved. The *in-situ* strategies seek to conserve or reconstitute plant species with associated evolutionary trajectories and the biological, agro-ecological and/or human cultural processes that

comprise their original evolutionary milieu. *Ex-situ* strategy does not provide a panacea for conserving naturally occurring plants species and protecting the habitat in the phase of changing environmental conditions. *In-situ* strategy provides a complementary back up for *ex-situ* collections as insurance against total loss or extinction¹⁷.

Sustainability: The sustainability of medicinal plants harvest depends on the organs that are harvested but also on the life cycle of harvested species. Good collection/harvesting practices of some important medicinal plants like *i.e.*, Aonla (*Phyllanthus emblica*), Baividang (*Embelia tsjeriam-cottam*), Baheda (*Terminalia bellerica*), Gudmar (*Gymnema sylvestre*), Sarpagandha (*Rauwolfia serpentina*), Kalmegh (*Andrographis paniculata*) and bark of Arjuna (*Terminalia arjuna*) have been standardized¹⁸⁻²⁰.

Adoption of sustainable harvesting practices at right time of harvest showed positive impact on resource conservation, socio-economic status of community, quality of produce and economic returns. It is evident from our study that the medicinal plants collected at right time of maturity following sustainable harvesting practices possess better quality in terms of active ingredients concentration. Harvesting practices/standards are available only for few commercially important species. The sustainable techniques/ standards for other important species need to be developed²¹. People's participation is key to sustainability, therefore local communities must be involved for the conservation of medicinal plants².

Collection/Harvesting of Medicinal Plants: Medicinal plants should be collected/harvested during the appropriate season or time period to ensure the best possible quality of both source materials and finished products. It is well known that the quantitative concentration of biologically active phyto-constituents varies with the stage of plant growth and development.

The best time for collection/harvest (quality peak season/time of day) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts during harvest⁶⁻⁷. Care should be taken to

ensure that no foreign matter, weeds or toxic plants are mixed with the harvested medicinal plant materials.

Modern Conservation Approach: Seeds are best suited for storage in gene banks. However, such storage is difficult for the species which do not set seeds or produce sterile or recalcitrant seeds. Seeds of most of the medicinal plants belong to this category. Generally, field conservation of medicinal plants requires more space and is labour intensive and expansive. They also run the risk of being damaged by natural calamities and biotic stress factors.

In-vitro propagation of plants holds tremendous potential for the production of high-quality plant-based medicines. Micropropagation of various plant species, including many medicinal plants, has been reported. Plant regeneration from shoot and stem meristems has yielded encouraging results in medicinal plants like *Catharanthus roseus*, *Cinchona ledgeriana*, *Digitalis* sp, *Rehmannia glutinosa*, *Rauwolfia serpentina* and *Isoplexis canariensis*²²⁻²³.

Synthetic Seeds: Synthetic seed being a new concept in seed biotechnological research is mainly considered for micropropagation and delivery of tissue culture plants. The concept of synthetic seeds was introduced by Toshio Murashige in 1977 but was put into practice by Mishra *et al.*, (2011), Faisal *et al.*, (2013) and Guan *et al.*, (2016) while working on synthetic seeds of different crop species²⁴⁻²⁶. The technology employs encapsulation of *in-vitro* propagules (somatic embryos, axillary buds, shoot apices, cormlets, bulbs *etc.*) for preparation of functional seeds, which can develop into seedlings under suitable conditions.

Synthetic seed technology can be very useful for the propagation of a variety of medicinal plants in which true seeds readily not available for multiplication. It is also useful in case of expansive seeds, hybrid plants and vegetatively propagated plants that are more prone to infections. This newly emerging technology would also be useful for multiplying genetically engineered plants, somatic and cytoplasmic hybrids. The survivability of artificial seeds could be increased by packing them along with pesticides, fertilizers,

nitrogen fixing bacteria *etc.* The micropropagation through artificial seeds can be commercially exploited further on a large scale.

Pollen Storage: The application of pollen storage methods to several plant species has been adequately reviewed by Rajsekharan and Ganeshan (2002) and Barnabas and Kovacs (1997)^{27 - 28}. Their small size and desiccation tolerance render pollen grains particularly suitable for storage. Relatively limited use has been made thus far of pollen for long-term conservation of plant species, since the strategy cannot accomplish conservation of whole plants. However, it may be useful for storage of clonally propagated species, conserving intra-clonal genetic variation. The international transfer and exchange of plant germplasm in the form of dry pollen is not generally restricted, since most pollen does not transmit diseases organisms. With the advantages outweighing disadvantages, pollen collection and storage is likely to be most suitable option in foreseeable future as a complementary conservation strategy for species that are normally conserved *ex-situ* in field gene banks, having large and/or recalcitrant seeds.

Conservation of Tissues/Cells: There are a number of important plant species, which cannot be conserved as seeds and present different problems. Conservation of tuber, root, shrub and tree species becomes very difficult. Several techniques to conserve such vegetatively propagated species have recently been developed and some of them are undergoing rigorous testing. For some species, the *in-vitro* conservation is the only option available. Though tissue culture offers great potential for conservation of germplasm of vegetatively propagated material, however genetic instability of the material conserved due to somaclonal variation and length of storage, have been the major technical limitations to it. Once these techniques are further refined, their large-scale adoption will be possible²⁹.

DNA Storage: Storage of DNA is, in principle, simple to carry out and widely applicable. The storage of DNA seems to be relatively easy and cheap. The progress in genetic engineering has resulted in breaking down the species and genus barriers for transferring genes. Transgenic plants have been produced with genes transferred from

viruses, bacteria, fungi and even mice. Such efforts have led to the establishment of DNA libraries, which store total genomic information of germplasm²⁹. However, strategies and procedures have to be developed on how to use the material stored in the form of DNA. Therefore, the role and value of this method for PGR conservation are not completely clear yet^{30 - 32}.

Development of Herbal Drugs: Plants have been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. The search of new molecules has obviously led to drug development and a better understanding of unique and complex metabolic phenomena of living organisms.

In more recent times, natural products have continued to be significant sources of drugs and leads. During 1950-1970 approximately 100 plant-derived new drugs were introduced including deserpidine, reseinnamine, reserpine, vinblastine and vincristine. Afterwards, from 1971 to 1990 new drugs such as ectoposide, E-guggulsterone, Z-guggulsterone, teniposide, nabilone, plaunotol, lectinan, artemisinin and ginkgolides appeared all over the world. 2 % of drugs were introduced from 1991 to 1995 including pacitaxel, toptecan, gomishin, irinotecan *etc.* Plant based drugs provide outstanding contribution to modern therapeutics³³.

Plant derived bioactive constituents *e.g.* reserpine, morphine, codeine, caffeine papaverine, nicotine, berberine, chymopapain, colchicoside, hyoscyamin, quinine, quinidine, allicin, curcumin, aspirin, atropine, emetin, ephedrine, digitoxin, digitoxigenin, gitoxigenin, gomishin, digoxigenin, tenoposide, tubocurarine, camptothecin, pilocarpine, capscicine, diosgenin, vinblastine, vincristine, taxol, podophyllotoxin, artemesinin, sennoside, solasodine, strychnine, hecogenin, β -ionone and others are constituting central ingredients as important chemical intermediates for manufacturing of several life-saving drugs in modern medicines³⁴.

The discovery and introduction of reserpine, an alkaloid obtained from the roots of the Indian plant *Rauwolfia serpentina* as drug for the treatment of hypertension and as a tranquilizer was considered as a revolutionary event³⁵. Vinblastine and isolated

from *Catharanthus roseus* is used for the treatment of hodgkins, choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer whereas vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymphosarcoma, cervical and breast cancer³⁵. Phophyllotoxin is a constituent of *Phodophyllum emodi* currently used against testicular, small cell lung cancer and lymphomas. *Daboia russellii* and *Naja kaouthia* used as antidote activity. Venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus*³⁶.

Recently, Chatterjee *et al.*, (2004) reported that an active compound from the *Strychnus nux vomica* seed extract, inhibited viper venom induced lipid peroxidation in experimental animals³⁷. Teniposide and etoposide isolated from Podophyllum species are used for testicular and lung cancer. Taxol isolated from *Taxus brevifolius* is used for the treatment of metastatic ovarian cancer and lung cancer. The remarkable progress made in phytochemical studies on polyphenolics during the last three decades has revealed numerous novel polyphenols from a wide array of medicinal plants. Newly found biological functions of polyphenols that are associated with potent antioxidative effects have attracted increasing attention in recent years; this is because such polyphenol-rich foods and beverages are expected to be beneficial to human health³⁸, including prevention of life style-related diseases that are related to oxidative stress. The above drugs came into use through the screening study of medicinal plants because they showed lesser side effects, were cost effective and possessed better compatibility.

Plant derived drugs have been adopted in the modern medicine and are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer^{39, 40}. More than 64 plants have been found to possess significant antibacterial properties; and more than 24 plants have been found to possess antidiabetic properties. Their dominant role is evident in the approximately 60 % of anticancer compounds and 75 % of drugs for infectious diseases that are either natural products or natural product derivatives^{41, 42}. In the quest for new medicines to treat old and emergent

diseases such as malaria, cardiovascular, cancer and AIDS, attention is now being given to discovering active ingredients from medicinal plants used routinely and traditionally. More than 20,000 different chemicals have been so far been isolated from plants.

Natural compounds of pharmaceutical importance that were once obtained from plant sources, but now produced synthetically on large scale include caffeine, theophylline, theobromine, ephedrine, pseudoephedrine, emetine, papaverine, tetrahydrocannabinol, L-dopa and salicylic acid⁴³. These drugs played an inestimable role in the treatment of human diseases. Today more than 20 best selling pharmaceuticals all over the world are derived from plants. However, the number of new chemical compounds from higher plants, which found entry as drug, has been extremely low. But many of these complex biochemicals cannot be synthesized economically on a commercial basis.

Chemo-biological Exploration: Medicine from sources like plants and plant products which are traditional in nature or derived from folklore has become an important part of the pharmaceutical industry. Plants chemical constituents termed as 'Active Principles' that act upon the specific organ or on the body as a whole remain the basis for a large proportion of the commercial medications used today for the treatment of several challenging diseases like, heart problems, high blood pressure, pain, asthma, and other ailments.

The efficacy of a number of phytopharmaceuticals derived from plants, such as atropine (pupil dilator), berberine (gastrointestinal disorders), caffeine (stimulant), camptothecin (antitumour) digitoxin (cardiotonic), emetine (antiamebic), ephedrine (antiasthmatic), forskoline (hypotensive and antispasmodic), morphine (analgesic), papain (protein digestant and anthelmintic), quinine (antimalarial), reserpine (tranquillizer), vinblastine and vincristine (antileukemic) with rich folklore have been discovered. The recent discovery of bioactive compounds *viz.*, artemisinin (antimalarial) from *Artemisia annua*, gossypol (male contraceptive) from *Gossypium sp.*, hypericin (antiviral) from *Hypericum perforatum*, taxol (breast cancer) from *Taxus brevifolia* and yuechukene (antifertility agent) from *Murraya paniculata* triggered the

interest in medicinal plants all over the world with a view to discover potent herbal medicines based on ethotherapeutics prevalent in the tribal and aboriginal societies⁴³.

Phytochemical and pharmacological investigation on the bark of willow tree (*Salix alba*) used for centuries to treat inflammation, pain and led to the discovery of aspirin based on the chemical found in the bark which remained all time largest selling drug. Likewise extracts of *Cinchona calisaya* and *C. officinalis* containing the anti-malarial drug quinine has been known to healers in South America for hundreds of years.

Extensive study led to the identification of the active constituents, quinine. Quinine and its derivatives have become the major drugs of choice to fight against malaria. In the same direction, *Digitalis lanata* and *D. Purpurea* have been used by herbalists for a long time to treat heart problems. Digoxin, today, is the main component prescribed for the treatment of congestive heart failures. Forskolin, a labdane-diterpenoid isolated from *Coleus forskohlii* having cardiovascular activity has appeared into international prominence due to its adenylatecyclase activity and the species is now immortalized through its inclusion among such celebrated medicinal plants as *Atropa belladonna*, *Cinchona ledgeriana*, *Rauwolfia serpentina*, *Digitalis lanata*, *Dioscorea deltoidea* etc.

Clinical trials under the drug development programme in the recent past based on the clues from native uses established the pharmacological properties of certain traditional medicinal plants that include *Acanthus illicifolius* seeds for analgesic and anti-inflammatory activities; *Vernonia anthelmintica* for rheumatism, conjunctivitis, dysuria etc; *Garcinia xanthochymus* and *G. morella* for antiprotozoal and antibacterial activities (morellin and neomorellin); *Rhuss emialata* and related species for cardiotoxic activity; *Butea monosperma* seeds for antifertility and anthelmintic activities; *Zornia gibbosa* for diuretic; *Plantago ovata* (Isabgol) seeds and seed husks as emollient, demulcent and laxative; *Nardostachys jatamansi* (Spikenard) for ventricular tachycardia; *Chlorophytum arundinaceum* (roots for nervine and general tonic; *Cephaelis ipecacuanha* (Ipecac) Rhizome for amoebic dysentery; *Adhatoda vasica*

(Vasaka) leaves and roots as expectorant; *Rheum emodi* (Rhubarb) dried rhizomes for mild purgatives; *Urginia indica* (Indian squil) roots for cardiac glycosides; *Viola odorata* and *V. pilosa* (Banabsha) whole herb as diaphoretic and demulcent; *Solanum* species (Solasodine), *Dioscorea* species (Diosgenins), *Agave* species (Hecogenins) for corticosteroids; *Heliotropium indica* for antileukemic activities; *Withania somnifera* (Ashwagandha) leaves for antitumour activity against sarcoma 180 and Ehrlich ascites carcinoma; *Operculina turpethum* for cancer etc.

One of the recent discoveries and much talked about the drug is the Memory + developed by the Central Drug Research Institute, Lucknow, India from *Bacopa monnieri* (Brahmi) and its origin is again from the ancient practice and literature. Taxol from the epiphytic plants of *Taxus baccata* has revolutionized the natural product chemistry off the world for anti cancerous properties. Other important species for which we owe a lot for their uses by tribal are *Ephedra vulgaris* for Ephedrine (hay fever, asthma, etc.), *Claviceps purpurea* for ergot alkaloids, *Punica granatum* for pelletiame (anthelmintic), *Gloriosa superba* for colchicines (leukemia), *Bixaorellana* for bixin, *Phyllanthus niruri* for phyllanthin, hyperphyllanthin etc., *Cassia angustifolia* for sennosides, *Erythroxyton* for cocaine, *Commiphora* for guggulipid, *Artemisia* for artemisine etc.

Diabetes mellitus is a chronic hereditary disease from which millions of people are suffering all over the globe. A number of plants and plant products are mentioned as antidiabetic agents in literature. Plants like *Momordica charantia* (Karela), *Gymnema sylvestre* (Gurmar), *Syzygium cumin* (Jamun), *Tinospora cardifolia* (Gulu), *Clerodendron phlomoidis*, *Pterocarpus marsupium* etc. have been studied for their hypoglycemic effect. Besides, a large number of natural products especially plant derived drugs continues to be discovered based on traditional medical practices⁴³.

Andrographis paniculata (Kalmegh) is an important herb used in several ayurvedic formulations as hepatoprotective herb. A study conducted by Pandey and Mandal (2010) revealed that the maximum amount of andrographolide (2.85%) was found in *A. paniculata* harvested after

130-150 days of planting (at the time of initiation of flowering)²⁰. The stage of maturation of the plant parts to be collected is another important factor: the root of *Wihania somnifera* (Ashwaganda) is dug out just 130-180 days after planting⁷, while the stem of *Tinospora cordifolia* (Giloe) is collected at full maturity (after 15 months). Quantitative analysis revealed that all the phyto constituents increased with the increase in diameter of the stem except alkaloid content⁴⁴.

With the advent of combinatorial chemistry, genomics research, newer bioassays tools, cell-based assays, high through put screening (HTS) and computer aided *de-novo* drug design, greater number of new drugs leads may now be expected. Several strategies have been explored to discover and invent new therapeutic agents to meet the rapidly emerging challenges. Consequently, in recent years a notable number of natural products derived agents such as provastatin, lovastatin and FK-506 have been discovered by employing mechanism based screening approaches. Screening medicinal plants for dealing with AIDS, reported that three species of *Calophyllum* have already shown promise. An active principle called calonoli has proved very effective against viruses. Interestingly, one of the species screened and found promising is *C. inophyllum*, the Indian laurel.

Enhancing Secondary Metabolite Production: Biotechnology has played an important role in the area of secondary metabolite production. Distinct progress made in stimulation of synthesis of plant chemicals, screening and selection of high yielding cell lines, plant cell and tissue culture, cell immobilization, hairy root culture, use of elicitors, feeding metabolic precursors and biotransformations, genetic engineering coupled with modern sophisticated analytical tools such as NMR, HPLC, GC-MS, LC-MS *etc* have offered mankind the great potency of exploiting the totipotent biosynthetic and biotransformation capabilities of plant cells under *in-vitro* conditions providing much needed boost to secondary metabolite production^{45,46}.

New biotechnological approaches that came into existence in 1975 include techniques like tailoring of plant resource, plant cell and protoplast culture, manipulation of nuclear and plasmid genes, plant cell and enzyme immobilization and industrial scale

production through biotransformation are the major component of plant biotechnology.

The concept of *in-vitro* production of secondary metabolites initiated by Gautheret in 1941 involving culture of all types of plant cell, tissue, organs, excised embryos and protoplast under aseptic conditions has considerable scope for the production of choice drug. The cellular mass derived from an explant and grown under aseptic conditions on a nutrient medium can be repeatedly grown on a fresh medium to yield stable cell lines⁴⁷.

The concept of high yielding cell lines and cloning marked the beginning of manipulation of specialized cells. Subsequent research on growth, differentiation and metabolism of plant cells gave further insight into the function of a plant cell. Introduction of production media^{22,48} and stress exerting conditions facilitated the stimulation of products accumulation. To date several secondary metabolites belonging to various groups have been isolated in remarkable yield from tissue and cell suspension culture of higher plants (Ellis, 1988) either in levels equivalent to or higher than the parent plant. Three compounds produced on commercial scale are; shikonin, a pigment used in lipsticks from cell cultures of *Lithospermum erythrorhizon*; berberine, an alkaloid possessing antiseptic activity from *Coptis japonica* and ginsenosides used in gastric disorders from the cell cultures of *Panax ginseng*.

Application of this technique has been extended to the large scale production of some important and interesting phytochemicals *e.g.*, anticancer, taxol and camptothecin; antimalarial, artemisinin; anti-HIV, castanospermine; anti-platelet, forskolin; ginkgolides *etc*. The first report (Christen *et al.*, 1989)⁴⁹ of the tissue culture for production of tropane alkaloids by hairy root cultures of *Datura hybrid* and second from *Taxus brevifolia* cultures and patented (Christen *et al.*, 1991)⁵⁰. Withanoloids are some potential steroidal lactones obtained from *Withania somnifera* can be extracted using *in-vitro* cultures⁵¹.

Plant cell and tissue culture emerges as a viable biotechnological tool for the production of bioactive compounds that can be used in the most diversified areas and particularly with a view of an additional effort for sustainable conservation and rational utilization of biodiversity⁵²⁻⁵³.

Recovery of secondary compounds stored intracellularly in the plant cell culture is very expensive and time consuming, thus permeabilization of cell cultures to release the synthesized or stored products without lowering their ability is considered necessary. This techniques involves treatment with a solution of high ionic strength, change of external pH, transfer to medium lacking phosphate, permeabilization with organic solvents, chitosan, electroporation, ultrasonic or ultrahigh pressure treatments⁵⁴⁻⁵⁶.

Depending upon the voltage applied product release upto 100% has been reported during electroporation, though a substantial decrease in viability was also recorded. Further, to achieve maximum output, cultured plant cell are to be kept in the immobilized state that involves entrapment of cells within a gel or passive adsorption on solid support materials, thus creating a situation for cell to imitate membership in a tissue of a whole plant. The ability to immobilise plant cells for production of wide range of phytochemicals has been investigated for a number of plant cells.

The phenomenon of synthesis and accumulation of secondary compounds in response to induction of stress in plant culture in terms of specific environmental, physiological and biological conditions are called is termed as elicitation. The compounds produced under the influences of elicitors are called phytoalexin. Elicitors are the group of triggering factors that stimulate particular facets of secondary metabolism resulting in formation of substances belonging to diverse groups of compounds⁵⁵. Fungal mycella, spores, cell wall polysaccharides act as excellent elicitors stimulating secondary metabolite formation in cultured plant cell. Apart from biological elicitors, this can be induced by abiotic factors like UV rays, alkaline pH, calcium, heavy metal, osmotic pressure and nutritional stress.

Biotransformation is another most promising area in plant cell culture involving conservation of a wide range of substrate by living cell culture into a chemically different product. Stereospecific reactions *viz.*, oxidation, hydroxylation, reduction, methylation, glucosylation, acylation, amino acylation found to occur in biotransformation processes, which are of great industrial importance⁵⁴. Production of valuable plant products by biotransformation of inexpensive

precursors which cannot be transformed effectively by chemical or microbial methods is an interesting area for commercial application of plant tissue culture. Transformation of plant cell using engineered genes to yield novel plant constituents is the new trend and major challenge in modern plant biotechnology. The cultures obtained by genetic transformations of plant are endowed with rapid growth in the absence of auxins, genetic and biochemical stability and biosynthetic capacity for producing high level of secondary metabolites. Progress in genetic engineering has added further new dimensions to phytoprospecting. In fact, the modern research in this area has emerged as a result of amalgamation of the most recent branches of biology, *viz.*, molecular biology, chemical ecology and phytochemistry. Genetic diversity of plants offers invaluable materials for genetic engineering. Through well designed manipulation of genes, it is possible to engineer the biosynthetic pathways to the desired direction for the production of designer compounds of desired bioactivity.

The advantages of biotechnological tools include increased purity of the end product, conversion of inexpensive precursors to expensive end products and the potential for feeding novel compounds clearly suggests that secondary metabolites possessing significant biological activities would be the ideal compounds for production through tissue and cell culture⁵⁴. Application of biotechnological methods for large scale production of secondary metabolites is advantageous, as there are problems in the extraction of metabolites from field grown plants. In many cases, the production of secondary metabolites from cell cultures is higher in comparison to the small amount extracted from *in-vivo* grown plants⁵⁷. During the last ten years, biotechnology has largely extended its application to pharmaceutical, agricultural and forestry sectors⁵⁸. Biotechnological techniques have encroached upon the conventional methods employed in large scale production of plant secondary metabolites and can be used for the yield improvement of secondary compounds of medicinal plants.

CONCLUSION: Pharmaceutical industry is under constant pressure to discover, develop and deliver effective natural drugs for the treatment of various diseases. Modern sophisticated tools and techniques have made it possible to understand the

pharmacological properties of traditional and folk medicine in new perspectives. Versatile application of plant chemicals as pharmaceuticals has attracted the attention of scientists all over the world. Scientists have tried to synthesize the vital metabolites in laboratories but achieved a little success only. Biotechnological principles have now emerged as the frontier areas for the development of secondary metabolites production technologies. Interest in the drug of natural origin has become revived because the drug obtained through chemical synthesis is either less effective as compared to the same drug of natural origin or has some side effect.

Search for the drug effective against cancer, aging, AIDS, hypercholesterolemia, hyperlipidemia is has become the focal point of research throughout the world. Development of more and more sophisticated techniques for plant drug analysis or for studying their effects on mammalian system has opened the new avenues of re-investigating a large number of already known plants and their active compounds. This led to the discovery of some new products earlier or new pharmacological action of known products. Many drugs have also been evolved on the basis of their use in traditional or folk medicine after a thorough scientific investigation. All these reasons contributed significantly in creating a great demand for the plant based products. In near future, biotechnological intervention will go a long way in development new therapeutic products from plant kingdom thereby providing effective and affordable remedies for tackling challenges of fatal diseases.

Of the approximately 2,50,000 species of higher plants, about 1%, or roughly 3000 has been utilized for food. On the other hand, approximately 10000 of the world's plants have documented use. This is still a very small percentage of all higher plants. Thus, there are potentially many more important discoveries in the plant kingdom to be exploited for various applications. India has a number of alternative medicines, like Ayurveda, Unani, Siddha and Homeopathic systems which are predominantly based on plant. Herbal preparations for pharmaceutical and cosmetic purposes form part of the traditional biodiversity uses in India. India's richness in plant resources and related indigenous knowledge is well recognized. India, claiming prime position among the 12 mega biodiversity centres across the world and holding

splendid forest floral resources, presents enormous opportunity for exploration and invention of value added plant products. Furthermore, plant resources are becoming unavailable and extinct due to the population growth and extensive usages stressing the need to find out solution to this problem. If the rich phytodiversity of India is conserved and its potential is systematically tapped, the country can provide lead to future plant-based industries.

The medicinal plant sector has the potential to raise local peoples' incomes through collection/ harvesting, processing and trade. These activities can become an integral component of country's forest and biodiversity resource management. Through value adding processing at the local level and efficient marketing, the sector can create rural income, employment and livelihood opportunities. Development of small and micro-enterprises, especially for processing and marketing of medicinal plants seem to have the potential to generate income opportunities for the poor. In this way safety, efficacy and quality of the herbal products can be maintained and would capitalize billions of dollars for emerging herbal markets for the country.

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