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Review

Advancements in the Production of Secondary Metabolites

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ABSTRACT

Plant cell culture systems represent a potential renewable source of valuable medicinal compounds, flavours, fragrances, and colorants, which cannot be produced by microbial cells or chemical synthesis. The evolving commercial importance of the secondary metabolites has in recent years resulted in a great interest, in secondary metabolism, and particularly in the possibility to alter the production of bioactive metabolites by means of cell culture technology. The principle advantage of this technology is that it may provide continuous, reliable source of plant pharmaceuticals and could be used for the large scale culture of plant cells from which these metabolite can be extracted. Plant cell and tissue cultures hold great promise for controlled production of myriad of useful secondary metabolites on demand. The current yield and productivity cannot fulfill the commercial goal of plant cell-based bioprocess for the production of most secondary metabolites. In order to stretch the boundary, recent advances, new directions and opportunities in plant cell based processes are being critically examined.

Keywords: Secondary metabolites; Cell culture; Plant pharmaceuticals.

INTRODUCTION

Medicinal plants are the most exclusive source of life saving drugs for majority of the world's population. The utilization of plant cells for the production of natural or recombinant compounds of commercial interest has gained increasing attention over past decades (Canter, et al., 2005). Bioactive compounds currently extracted from plants are used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides. A number of plant species have been used for generation and propagation of cell-suspension cultures, ranging from model systems like *Arabidopsis*, *Catharanthus* and *Taxus*, to important monocotyledon or dicotyledonous crop plants like rice, Soya bean, alfalfa and tobacco. The secondary metabolites are known to play a major role in the adaptation of plants to their environment, but also represent an important source of pharmaceuticals (Ramachandra Rao and Ravishankar, 2002). Cell

suspension cultures systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantages of this method are that it can ultimately provide a continuous reliable source of natural products. In recent years, traditional system of medicine has become a topic of global importance. Although modern medicine may be available in developed countries, herbal medicines [phytopharmaceuticals] have often maintained popularity for historical and cultural reasons.

Discoveries of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last few years. The scheme of production of some important plant pharmaceuticals produced in cell cultures (Vanisree and Tsay, 2004) has been presented in Table 1. New physiologically active substances of medicinal interest have been found by bioassay. It has been demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformation of natural compounds has been demonstrated (Ravishankar and Ramachandra Rao, 2000). Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations.

The major advantages of a cell culture system over the conventional cultivation of whole plants are: (1) Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions; (2) Cultured cells would be free of microbes and insects; (3) The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites; (4) Automated control of cell growth and rational regulation of metabolite processes would reduce of labor costs and improve productivity; (5) Organic substances are extractable from callus cultures.

In order to obtain high yields suitable for commercial exploitation, efforts have been focused on isolating the biosynthetic activities of cultured cells, achieved by optimizing the cultural conditions, selecting high producing strains and employing precursor feeding, transformation methods, and immobilization techniques (Dicosmo and Misawa, 1995). Transgenic hairy root cultures have revolutionized the role of plant tissue culture in secondary metabolite production. They are unique in their genetic and biosynthetic stability, faster in growth, and more easily maintained. Using this methodology a wide range of chemical compounds have been synthesized (Giri and Narasu, 2000). Advances in tissue culture, combined with improvement in genetic engineering of pharmaceuticals, nutraceuticals and other beneficial substances (Hansen and Wright, 1999). Recent advances in the molecular biology, enzymology and fermentation technology of plant cell cultures suggest that these systems will become a viable source of important secondary metabolites (Abdin, 2007). Genome manipulation is resulting in relatively large amounts of desired compounds produced by plants infected with an engineered virus, whereas transgenic plants can maintain constant levels of production of proteins without additional intervention (Abdin and Kamaluddin, 2006). Large-scale plant tissue culture is found to be an attractive alternative approach to traditional methods of plantation as it offers controlled supply of biochemical's independent of plant availability (Sajc, et al., 2000).

Current developments in tissue culture technology indicate that transcription factors are efficient new molecular tools for plant metabolic engineering to increase the production of valuable compounds (Gantet and Memelink, 2002). In vitro cell culture offers an intrinsic advantage for foreign protein synthesis in certain situations since they can be designed to produce therapeutic proteins, including monoclonal antibodies, antigenic proteins that act as immunogenes, human serum albumin, interferon, immuno-contraceptive protein, ribosome unactivator trichosantin, antihypersensitive drug angiotensin, leu-enkephalin neuropeptide, and human hemoglobin (Doran, 2000). The appeal of using natural products for medicinal purposes is

increasing, and metabolic engineering can alter the production of pharmaceuticals and help to design new therapies. At present, researchers aim to produce substances with antitumor, antiviral, hypoglycaemic, anti-inflammatory, antiparasitic, antimicrobial, tranquilizer and immunomodulating activities through tissue culture technology.

Exploration of the biosynthetic capabilities of various cell cultures has been carried out by a group of plant scientists and microbiologists in several countries during the last decade. Most applications of plant-cell-suspension cultures in biotechnology are aimed at the production of naturally occurring secondary metabolites. This has included production of shikonin, anthocyanins, and ajmalicine and, recently, important anti-tumor agents like taxol, vinblastine and vincristine (Oksman-Caldentey and Inze, 2004). In the last few years promising findings have been reported for a variety of medicinally valuable substances, some of which may be produced on an industrial scale in the near future. Today, the expression of recombinant anti bodies and antibody fragments in plants is a well-established technique, and the advantages of plants over bacterial or mammalian production systems have been reviewed (Hiatt and Mostov, 1993). The aim of the present review is to focus on the importance of tissue culture technology in production of some of the plant pharmaceuticals.

Tissue Culture Producing Pharmaceutical Products of Interest Case Studies

Research in the area of plant tissue culture technology has resulted in the production of many pharmaceutical substances for new therapeutics. Advances in the area of cell cultures for the production of medicinal compounds has made possible the production of a wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids, and amino acids. Successful attempts to produce some of these valuable pharmaceuticals in relatively large quantities by cell cultures are illustrated.

Taxol: Taxol (plaxitaxol), a complex diterpene alkaloid found in the bark of the *Taxus* tree, is one of the most promising anticancer agents known due to its unique mode of action on the micro tubular cell system (Oksman-Caldentey and Inze, 2004). At present, production of taxol by various *Taxus* species cells in cultures has been one of the most extensively explored areas of plant cell cultures in recent years owing to the enormous commercial values of taxol, the scarcity of the *Taxus* tree, and the costly synthetic process (Jennewein, et al., 2005). In 1989, Christen, et al., reported for the first time the production of taxol (paclitaxel) by *Taxus* cell cultures. Fett-Neto, et al., (1995) have studied the effect of nutrients and other factors on paclitaxel production by *T.suspidata* cell cultures (0.02% yield on dry weight basis). Srinivasan, et al., (1995) have studied the kinetics of biomass accumulation and paclitaxel production by *T.baccata* cell suspension cultures. Paclitaxel was found to accumulate at high yields (1.5 mg/l) exclusively in the second phase of growth. Kim, et al., (1995) established a similar level of paclitaxel from *T. brevifolia* cell suspension cultures following 10 days in culture with optimized medium containing 6% fructose. Ketchum, et al., (2003) reported that addition of carbohydrate during the growth cycle increased the production rate of paclitaxel, which accumulated in the culture medium (14.78mg/l). In addition to paclitaxel, several other toxoids have been identified in both cell and culture medium of *taxus* cultures. Parc, et al., (2002) reported production of toxoids by callus cultures from selected Taxus genotypes. In order to increase the toxoid production in these cultures, the addition of different amino acids to the culture medium were studied, and phenylalanine was found to assist in maximum taxol production in *T.cuspidata* cultures (Long and Croteau, 2005). The influence of biotic and abiotic elicitors was also studied to improve the production and accumulation of taxol through tissue cultures. The production of taxol from nodule cultures containing cohesive multicultural units displaying a high degree of differentiation has been achieved from cultured needles of seven *Taxus* cultivars (Wu, et al., 2001). Factors influencing stability and recovery of paclitaxel from suspension cultures and the media have been studied in detail by (Nguyen, et al., 2001). The effects of rare earth elements and gas concentrations on taxol production have been reported.

Morphine and codeine: Latex from the opium poppy, *Papaver somniferum* is a commercial source of the analgesics, morphine and codeine. Callus and suspension cultures of *P.somniferum* are being investigated as an alternative means for production of these compounds. Production of morphine and codeine in morphologically undifferentiated culture has been reported (Yu, et al., 2002). Removal of exogenous hormones from large-scale systems could be implemented using a two-stage process strategy by Siah and Doran (1991). Without exogenous hormones, maximum codeine and morphine concentrations were 3.0 mg/g dry weight and 2.5 mg/g dry weight, respectively, up to three times higher than in cultures supplied with hormones. Biotransformation of codeinone to codeine with immobilized cells of *P.somniferum* has been reported by Furuya, (1988). The conversion yield was 70.4%, and about 88% of the codeine converted was excreted into the medium (Palazon, et al., 2006).

Ginsenosides: The root of *Panax ginseng* C.A.Mayer, so-called ginseng, has been widely used as a tonic and highly prized medicine since ancient times (Srivastava and Srivastava, 2007). Ginseng has been recognized as a miraculous promoter of health and longevity. The primary bioactive constituents of ginseng were identified as ginsenosides, a group of triterpenoid saponins. Among them, ginsenoside Rg 1 is one of the major active molecules from *Panax ginseng*. Chang and Hsing (1980a and 1980b) obtained repeatable precocious flowering in the embryos derived from mature ginseng root callus cultured on a chemically defined medium. Also, plant regeneration through somatic embryogenesis in root-derived callus of ginseng has been reported (Chang and Hsing, 1980b). In recent years ginseng cell culture has been explored as a potentially more efficient method of producing ginsenosides. The effect of medium components like carbon, nitrogen, phosphate concentrations and plant growth hormones (Furuya, 1988) were thoroughly studied to increase the production of ginsenosides. Influence of potassium ion was also studied. Large-scale suspension culture of ginseng cells was first reported by Yasuda, et al., (1972). Later on an industrial scale culture process was initiated by Nitto Denko Corporation (Ibaraki, Osaka, Japan) in the 1980's using 2000 and 20000-l stirred tank fermenters to achieve productivities of 500-700 mg/l per day (Hu, et al., 2001). This process is considered an important landmark in the commercialization of plant tissue and cell culture on a large scale. In addition to this, *Agrobacterium tumefaciens* infected root cultures were introduced, productivity of which was found to exceed the callus of normal roots threefold (Hu, et al., 2001). Other types of tissue cultures, such as embryonic tissues and hairy roots transformed by *Agrobacteria* (Mousumi, et al., 2006) have been examined. Yu, et al., (2002) reported ginsenoside production using elicitor treatment. These developments indicate that ginseng cell culture process is still an attractive area for commercial development around the world and it possesses great potential for mass industrialization (Sarin, 2005). Concentration of plant growth regulators in the medium influences the cell growth and ginsenoside production in the suspension cultures (Zhang, et al., 2005).

Recent studies have shown that addition of methyljasmonate or dihydro-methyl jasmonate to suspension cultures increases the production of ginsenosides (Wang and Zhong, 2002). Also, jasmonic acid improves the accumulation of ginsenosides in the root cultures of ginseng.

L-DOPA: L-3,4-dihydroxyphenylalanine, is an important intermediate of secondary metabolism in higher plants and is known as a precursor of alkaloids, betalain, and melanine, isolated from *Vinca faba, Mucuna, Baptisia* and *Lupinus* (Srivastava and Srivastava, 2007). It is also a precursor of catecholamines in animals and is being used as a potent drug for Parkinson's disease, a progressive disabling disorder associated with a deficiency of dopamine in the brain (Huang, et al., 2002). The widespread application of this therapy created a demand for large quantities of L-DOPA at an economical price level, and this led to the introduction of cell cultures as an alternative means for enriched production (Canter, et al., 2005). The callus tissue of *Mucuna pruriense* accumulated 25 mg/1 DOPA in the medium containing relatively high concentrations of 2, 4-D. Teramoto and Komamine (1988) induced callus tissues of *Mucuna hassjoo*, *M. Pruriense*, and *M. deeringiana* and optimized the culture conditions. The highest concentration of

DOPA was obtained when *M. hassjoo* cells were cultivated in MS medium with 0.025 mg/l 2, 4 – D and 10mg/l kinetin. The level of DOPA in the cells was about 80 m mol/g-f.w (Vaniserce, et al., 2004).

Berberine: Berberine is an isoquinoline alkaloid found in the roots of *Coptis japonica* and cortex of *Phellondendron amurense*. This antibacterial alkaloid has been identified from a number of cell cultures, notably those of *Coptis japonica*, *Thalictrum* spp, and *Berberis* spp (Dubey, et al., 2004). The productivity of berberine was increased in cell cultures by optimizing the nutrients in the growth medium and the levels of phytohormones. By selecting high yielding cell lines, Mitsui group produced berberine on a large scale with a productivity of 1.4 g/l over 2 weeks. Other methods for increasing yields include elicitation of cultures with a yeast polysaccharide elicitor, which has been successful with a relatively low producing *T. rugosum* culture (Sarin, 2005). The influence of spermidine on berberine production in *Thalictrum minus* cell cultures has been reported by Hara, et al., (1991).

Diosgenin: Diosgenin is a precursor for the chemical synthesis of steroidal drugs and is tremendously important to the pharmaceutical industry (Eibl and Eibl, 2006). In 1983, Tal, et al., reported on the use of cell cultures of *Dioscorea deltoidea* for production of diosgenin. They found that carbon and nitrogen levels greatly influenced diosgenin accumulation in one cell line. Ishida (1988) established *Dioscorea* immobilized cell cultures, in which reticulated polyurethane foam was shown to stimulate diosgenin production, increasing the cellular concentration by 40% and total yield by 25%. Several other groups have also attempted cell cultures for diosgenin production (Sarin, 2005). Kaul, et al., (1969) studies the fluency of various factors on diosgenin production by *Dioscorea deltoidea* callus and suspension cultures. The search for high-producing cell lines coupled to recent developments in immobilized cultures and the use of extraction procedures, which convert furostanol saponins to spirostanes such as diosgenin, should prove useful in increasing productivity in the years to come (Sarin, 2005).

Capsaicin: Capsaicin, an alkaloid, is used mainly as a pungent food additive in formulated foods (Ravishankar, et al., 2003). It is obtained from fruits of green pepper (*Capsicum* spp,). Capsaicin is also used in pharmaceutical preparations as a digestive stimulant and for rheumatic disorders (Sharma, et al., 2008). Suspension cultures of *Capsicum frutescens* produce low levels of capsaicin, but immobilizing the cells in reticulated polyurethane foam can increase production approximately 100-fold. Further improvements in productivity can be brought about by supplying precursors such as isocapric acid. Lindsey (1985) reported that treatments, which suppress cell growth and primary metabolism, seem to improve capsaicin synthesis. A biotechnological process has been developed for the production of capsaicin from *C. frutescens* cells. Holden, et al., (1988) have reported elicitation of capsaicin in cell cultured of *C. frutescens* by spores of *Gliccladium deliquescens*. The effects of nutritional stress on capsaicin production in immobilized cell cultures of *Capsicum annum* were studied thoroughly by Ravishankar and Ramachandra Rao (2000). Biotransformation of externally fed protocatechuic aldehyde and caffeic acid to capsaicin in freely suspended cells and immobilized cells cultures of *Capsicum frutescens* has also been reported (Sanatombi and Sharma, 2007).

Camptothecin: Campothecin, a potent antitumor alkaloid was isolated from *Camptotheca acuminate* (Padmanabha, et al., 2006). Sakato and Misawa (1974) induced *C. acuminata* callus on MS medium containing 0.2mg/l 2,4-D and l mg/l kinetin and developed liquid cultures in the presence of gibberellin, L-tryptophan, and conditioned medium, which yielded camptothecin at about 0.0025% on a dry weight basis. When the cultures were grown on MS medium containing 4 mg/l NAA, accumulation of camptothecin reached 0.998 mg/l (Thengane, et al., 2003). 10-Hydroxycamptothecin, a promising derivative of camptothecin is in clinical trials in the US.

Vinblastine and Vincristine: The dimeric indole alkaloids vincristine and vinblastine have become valuable drugs in cancer chemotherapy due to their potent antitumor activity against various leukemias and solid tumors. These compounds are extracted commercially from large quantities of *Catharanthus roseus*. Since the intact plant contains low concentrations (0.0005%),

plant cell cultures have been employed as an alternative to produce large amounts of these alkaloids (Oksman-Caldentey and Inze, 2004). Vinblastine is composed of catharanthine and vindoline. Since vindoline is more abundant than catharanthin in intact plants, it is less expensive (Aslam, et al., 2009). Misawa, et al., (1988) established an economically feasible process consisting of production of catharanthine by plant cell fermentation and a simple chemical or an enzymatic coupling. The significant influence of various compounds, like vanadyl sulphate, abscisic acid, and sodium chloride on catharanthin production have been described by Smith, et al., (1987). Endo, et al., (1988) attempted synthesis of anhydrovinblastine (AVLB from catharanthine and vindoline through enzymatic coupling followed by sodium borohydride reduction). A crude preparation of 70% ammonium sulphate precipitated protein form the cultured cells of *C. roseus* were used as an enzyme source. The reaction mixture contained catharanthine, vindoline, Tris buffer, pH 7.0, and the crude enzyme; the mixture was incubated at 300^0C and for 3 h. The products of the reaction were various dimeric alkaloids including vinamidine, 3(R) -hydroxyvinamidine, and 3, 4-anhydrovinblastine. Dimerization using ferric ion catalyst in the absence of enzyme resulted in anhydrovinblastine and vinblastine in 52.8% and 12.3% yield, respectively. The yield of vinblastine via chemical coupling was improved in the presence of ferric chloride, oxalate, maleate, and sodium borohydride (Verma, et al., 2007). Influence of various parameters like stress, addition of bioregulators, elicitors and synthetic precursors on indole alkaloids production were studied in detail by Zhao, et al., (2001 a and 2001b). Also, metabolic rate limitations through precursor feeding (Morgahn and Shanks, 2000) and effect of elicitor dosage on biosynthesis of indole alkaloids in *Catharanthus roseus* hairy root cultures have been reported.

Tanshinones: Tanshinones are a group of quinoid diterpenoids believed to be active principles of Danshen (*Salvia miltiorrhiza*), a well-known traditional Chinese medicine (Wang, et al., 2007). Tanshinone I and cryptotanshinone prevent complications of myocardial ischemia; tanshinone II A has undergone successful clinical trials for the treatment of angina pectoris in China ((Jian Yong, et al., 2007). Plant cell and organ culture technology provide an alternative means of producing these active ingredients. Nakanishi, et al., (1983) established a cell line containing abundant amounts of cryptotanshinone from *S. miltiorrhiza*. Adventitious root cultures of *S. miltiorrhiza* and the culture condition for high yield production of tanshinones in the adventitious roots were reported by shimomura, et al., (1991). Diterpenoid production in Ti-transformed root or hairy root cultures of *S. miltiorrhiza* production has also been established by Hu and Alfermann (1993). In these cultures, although relatively high tanshinone production was achieved, the morphological characteristics of the hairy roots require special bioreactors for the cultivation, which has hindered the scale-up of such processes (Ge and Wu, 2005).

Podophyllotoxin: Podophyllotoxin is an antitumor aryltetralin lignin found in *Podophyllum peltatum* and *Podophyllum hexandrum*. It also serves as a starting material for the preparation of its semisynthetic derivatives, etoposide and teniposide, widely used in anti-tumor therapy (Chattopadhyay, et al., 2002). These plants, which grow very slowly, are collected from the wild and are thus increasingly rare. This limits the supply of podophyllotoxin and necessitates the search for alternative production methods (Palazon, et al., 2006). Cell cultures of *P.peltatum* for production of podophyllotoxin were first attempted by Kadkade (1982). To increase the yield of podophyllotxin, Woerdenberg, et al., (1990) used a complex of a precursor, conifery1 alcohol, and b-cyclodextrin to *P.hexandrum* cell suspension cultures. The addition of 3mM conifery1 alcohol complex yielded 0.013% podophyllotoxin on a dry weight basis, but the cultures without the precursor produced only 0.0035% (Koulman, et al., 2003). Smollny, et al., (1992) reported that callus tissues and suspension culture cells of *Lilium album* produced 0.3% podophyllotoxin. Several other tissue culture approaches have been studied to increase the yields. Since 5-methoxypodophyllotoxin, an analogue of podophyllotoxin, has strong cytostatic activity, many researchers have tried to improve its yield through tissues cultures.

Bioprocess technology for production of plant secondary metabolites: In literature plant cells are described as extremely sensitive for shear forces, necessitating the use of special low-shear bioreactors, e.g. airlift bioreactors. However, in industry such bioreactors are not common; most processes are runned in stirred-tank. As a consequence, such a bioreactor is preferable for plant cell culture; it is the lowest cost process-unit (Martin, et al., 2008). More recent studies on the shear sensitivity of plant cells, among others in laboratories, have shown that in fact plant cells in general are quite shear-stress tolerant (Srivastava and Srivastava, 2007). This is supported by the fact that a series of large-scale processes have been reported with plant cell cultures, e.g. shikonin production. Plant cells have even been cultured in a 60m3 stirred tanks (Min, et al., 2007). The technology being, feasible, how about the economy? A number of papers have appeared on this (Hadacek, 2002). Assuming a yearly production of 3000 kg/year of compound produced by a cell culture at a level of 0.3g/l, resulted in a calculated price of 1500 US$/kg. An increase of productivity with a factor 10 (i.e, 3 g/l) results in a price of 430$/kg (Fischer, et al., 2003). In both cases a fed-batch type of process was applied. These prices are high, but a number of natural products have even much higher prices (e.g taxol, vinblasine and vincristine) (Lamboursain and Jolicoeur, 2003). However, most of the high-value specialty chemicals are produced at too low levels in the plant cell cultures (Chattopadhyay, et al., 2002). Their production must thus be increased to make an industrial process possible.

CONCLUSION

In vitro propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds. To improve yields metabolic engineering offers promising perspectives, but requires the understanding of the regulation of the secondary metabolite pathways involved on the levels of products, enzymes and genes, including aspects as transport and compartmentation.

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Table-1: Bioactive secondary metabolites from plant cell culture (Vanisree and Tsay, 2004).

Plant name	Active ingredient	Culture type	Reference
Agave amaniensis	Saponins	callus	Andrijany et al., 1999
Ailanthus altissima	Alkaloids	suspension	Anderson et al., 1987
Ailanthus altissima	Canthinone alkaloids	suspension	Anderson et al., 1986
Allium sativum L.	Allicin	callus	Malpathak and David, 1986
Aloe saponaria	Tetrahydroanthracene glucosides	suspension	Yagi et al., 1983
Ambrosia tenuifolia	Altamisine	callus	Goleniowski and Trippi, 1999
Anchusa officinalis	Rosmarinic acid	suspension	De Eknamkul and Ellis,1985
Brucea javanica (L.) Merr.	Canthinone alkaloide	suspension	Liu et al., 1990
Bupleurum falcatum	Saikosaponins	callus	Wang and Huang, 1982
Camellia sinensis	Theamine,γ-glutamylderivatives	suspension	Orihara and Furuya, 1990
Canavalia ensiformis	L-canavanine	callus	Ramirez et al., 1992
Capsicum annuum L.	Capsaicin	suspension	Johnson et al., 1990
Cassia acutifolia	Anthraquinones	suspension	Nazif et al., 2000
Catharanthus roseus	Indole alkaloids	suspension	Moreno et al., 1993
Catharanthus roseus	Catharanthine	suspension	Zhao et al., 2001
Chrysanthemum cinerarifolium	Pyrethrins	callus	Rajasekharan et al., 1991
Chrysanthemum cinerarifolium	Chrysanthemic acid and pyrethrins	suspension	Kueh et al., 1985
Cinchona L.	Alkaloids	suspension	Koblitz et al., 1983
Cinchona robusta	Robustaquinones	suspension	Schripsema et al., 1999
Cinchona spec.	Anthraquinones	suspension	Wijnsma et al., 1985
Cinchona succirubra	Anthraquinones	suspension	Khouri et al., 1986
Citrus sp.	Narigin, Limonin	callus	Barthe et al.,1987
Coffea arabica L.	Caffeine	callus	Waller et al., 1983
Corydalis ophiocarpa	Isoquiniline alkaloids	callus	Iwasa and Takao, 1982
Croton sublyratus kurz	Plaunotol	callus	Morimoto and Murai, 1989
Cruciata glabra	Anthraquinones	suspension	Dorenberg and Knorr, 1996
Cryptolepis buchanani	Cryptosin	callus	Venkateswara et al., 1987
Digitalis purpurea L.	Cardenolides	suspension	Hagimori et al., 1982
Dioscorea deltoidea	Diosgenin	suspension	Heble and Staba, 1980
Dioscorea doryophora Hance	Diosgenin	suspension	Huang et al., 1993
Duboisia leichhardtii	Tropane alkaloids	callus	Yamada and Endo, 1984
Ephedra spp.	L-ephedrine,D- pseudoephedrine	suspension	O'Dowd et al., 1993
Eriobotrya japonica	Triterpenes	callus	Taniguchi et al., 2002
Eucalyptus tereticornis SM.	Sterols and phenolic compounds	callus	Venkateswara et al., 1986
Eucommia ulmoides	Chlorogenic acid	Suspension	Wang et al., 2003
Fumaria capreolata	Isoquinoline alkaloids	suspension	Tanahashi and Zenk, 1985
Gentiana sp.	Secoiridoid glucosides	callus	Skrzypczak et al., 1993
Ginkgo biloba	Ginkgolide A	suspension	Carrier et al., 1991
Glehnia littoralis	Furanocoumarin	suspension	Kitamura et al., 1998
Glycirrhiza echinata	Flavanoids	callus	Ayabe et al., 1986
Glycirrhiza glabra var.glandulifera	Triterpenes	callus	Ayabe et al., 1990
Hyoscymus niger	Tropane alkaloids	callus	Yamada and Hashimoto, 1982
Isoplexis isabellina	Anthraquinones	suspension	Arrebola et al.,1999
Linum flavum L.	5-Methoxypodophyllotoxin	suspension	Uden et al., 1990

Table-1: Continue

Lithospermum erythrorhizon	Shikonin derivatives	suspension	Fujita et al., 1981
Lithospermum erythrorhizon	Shikonin derivatives	suspension	Fukui et al., 1990
Lycium chinense	Cerebroside	suspension	Jang et al., 1998
Morinda citrifolia	anthraquinones	suspension	Zenk et al., 1975
Morinda citrifolia	anthraquinones	suspension	Basseti et al., 1995
Mucuna pruriens	L-DOPA	suspension	Wichers et al., 1993
Mucuna pruriens	L-DOPA	callus	Brain et al., 1976
Nandina domestica	Alkaloids	callus	Ikuta and Itokawa, 1988.
Nicotiana rustica	Alkaloids	callus	Tabata and Hiraoka, 1976.
Nicotiana tobacum L.	Nicotine	suspension	Mantell et al., 1983
Nothapodytes foetida	Camptothecin	callus	Thengane et al., 2003
Ophirrhiza pumila	Camptothecin related alkaloids	callus	Kitajima et al., 1998
Panax ginseng	Saponins and sapogenins	callus	Furuya et al., 1973
Panax notoginseng	Ginsenosides	suspension	Zhong and Zhu, 1995
Papaver bracteatum	Thebaine	callus	Day et al., 1986
Papaver somniferum L.	Alkaloids	callus	Furuya et al., 1972
Papaver somniferum	Morphine,codeine	suspension	Siah and Doran, 1991
Peganum harmala L.	β-Carboline alkaloids	suspension	Sasse et al., 1982
Phytolacca americana	Betacyanin	suspension	Sakuta et al., 1987
Picrasma quassioides Bennett	Quassin	suspension	Scragg and Allan, 1986
Podophyllum hexadrum royle	Podophyllotoxin	suspension	Chattopadhyay et al., 2002
Polygala amarella	Saponins	callus	Desbene et al., 1999
Polygonum hydropiper	Flavanoids	suspension	Nakao et al., 1999
Portulaca grandiflora	Betacyanin	callus	Schroder and Bohm, 1984
Ptelea trifoliata L.	Dihydrofuro[2,3-β] quinolinium alkaloids	callus	Petitpaly et al., 1987
Rauwolfia sellowii	Alkaloids	suspension	Rech et al., 1998
Rauwolfia serpentina Benth.	Reserpine	suspension	Yamamoto and Yamada, 1986
Ruta sp.	Acridone and Furoquinoline	callus	Baumert et al., 1992
Salvia fruticosa	Rosmarinic acid	callus & suspension	Karam et al., 2003
Scopolia parviflora	Alkaloids	callus	Tabata et al., 1972
Scutellaria columnae	Phenolics	callus	Stojakowska and Kisiel, 1999
Solanum chrysotrichum (Schldl)	Spirostanol saponin	suspension	Villarreal et al., 1997
Solanum laciniatum	Solasodine	suspension	Chandler and Dodds, 1983
Solanum paludosum	Solamargine	suspension	Baddaoui et al., 1996
Stizolobium hassjoo	L-DOPA	suspension	Huang et al., 2002
Taxus spp.	Taxol	suspension	Wu et al., 2001
Taxus baccata	Taxol, Baccatin III	suspension	Cusido et al., 1999
Taxus cuspidata	Taxoids	suspension	Ketchum et al., 2003
Thalictrum minus	Berberin	suspension	Kobayashi et al., 1987
Thalictrum minus	Berberin	suspension	Nakagawa et al., 1986
Torreya nucifera var. radicans	Diterpenoids	suspension	Orihara et al., 2002
Trigonella foenum graecum	Saponins	suspension	Brain and Williams, 1983