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Review

Plant tissue culture as a perpetual source for production of industrially important bioactive compounds

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ABSTRACT

Plants have been used throughout the world for its medicinal powers since ancient time. The pharmacological properties of plants are based on their phytochemical components especially the secondary metabolites which are outstanding sources of value added bioactive compounds. Secondary metabolites have complex chemical composition and are produced in response to various forms of stress to perform different physiological tasks in plants. They are used in pharmaceutical industries, cosmetics, dietary supplements, fragrances, flavors, dyes, etc. Extended use of these metabolites in various industrial sectors has initiated a need to focus research on increasing the production by employing plant tissue culture (PTC) techniques and optimizing their large scale production using bioreactors. PTC techniques being independent of climatic and geographical conditions will provide an incessant, sustainable, economical and viable production of secondary metabolites. This review article intends to assess the advantages of using plant tissue culture, distribution of important secondary metabolites in plant families, strategies involved for optimal metabolite production and the industrial importance of selected secondary metabolites.

1. Introduction

Plants have always played prominent roles in traditional as well as modern medicines. Almost 80% of the world's population relies on plant derived components for their crucial health and wellness [1,2]. They are rich in phytochemicals which have the miraculous capability to cure diseases and may be used in numerous sectors such as pharmaceuticals, cosmetics, nutraceuticals, etc. [3]. They are gaining more attention among the growing population because of their affordability, accessibility, eco-friendly and promising efficacy comparable to high cost synthetic drug agents. Thus, the preliminary attention of research till date on medicinal plants has been focused in the areas of pharmacognosy, phytochemistry and horticulture. The increasing demand for such products has sparked the need to find strategies to enhance their production without disturbing their natural population [4].

Plants are regarded as safe to humans. They are used to treat and prevent specific ailments and diseases in humans since time immemorial. Thus, plants which possess therapeutic metabolites with beneficial pharmacological effects are called medicinal plants [5]. The medicinal properties of these plants are because of the existence of the heterogeneous group of natural metabolic products called secondary metabolites which are divergent in their structure and metabolic pathways. These metabolites are not essential for the growth and development of plants [6] but they play many important roles as signalling molecules and as defense agents [7]. They are regarded as economically important products as they are used in drugs, flavors, fragrances, insecticides, dyes, etc. [8]. India is a reservoir of numerous high-valued medicinal plants and is one of the major medicinal plants producing Asian country.

They are produced by diverting energy-generating routes in metabolic pathways like photosynthesis, glycolysis, Krebs cycle to biosynthetic intermediates. They are classified in different categories based on their biosynthesis, structures and functions. They are biosynthesized from acetyl coenzyme A, mevalonic acid, shikimic acid, deoxyxylulose 5-phosphate or combined pathways [9]. They are classified as terpenoids, steroids, alkaloids, saponin, terpenes, lipids and enzyme cofactors [10,11]. The different classes of secondary metabolites produced by *in vitro* callus and cell suspension cultures have been tabulated in Table 1.

Plant tissue culture (PTC) due to its various benefits has been used as a major platform for secondary metabolites production [12,13]. Thus, the review article aims to focus the merits of using PTC techniques for secondary metabolites production as well as the distribution of different classes of plant secondary metabolites in plant families, important medicinal uses and strategies to improve their production to increase yield. Few important medicinal plants along with their secondary metabolites of commercial importance have been summarized in Table 2.

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Table 1
Classes of secondary metabolites produced by plant tissue culture.

S. No.	Alkaloids	Terpenoids	Steroids	Quinones	Phenylpropanoids
1.	Acridines	Artemisinin	Brassinolide	Aloe-emodin	Anthocyanins
2.	Betalaines	Cucurbitacins	Bufadienolides	Anthraquinones	Caffeic acid
3.	Furoquinolines	Diterpenes	Catasterone	Benzoquinones	Coumarins
4.	Galanthamine	Ginsenosides	Digoxin	Chrysophanol	Eugenol
5.	Harringtonines Isoquinolines	Meroterpenes	Digitoxin	Emodin	Ferulic acid
6.	Lobeline	Monoterpenes	Digitoxigenin	β-Lapachol	Flavonoids
7.	Quinolizidines	Paclitaxel	Ecdysteroids	Naphthoquinones	Hydroxycinnamoyl derivatives
8.	Indole alkaloids	Sesquiterpenes	Helleborin	Phenanthrenequinone	Isoflavonoids
9.	Isoquinoline alkaloids	Sesterpenes	Physodine	Plumbagin	Lignans
10.	Piperidine	Thapsigargin	Ouabain	Rhein	Phenalinones
11.	Thebaine	Triterpenes	Scillaridine	Shikonin	Proanthocyanidins
12.	Trigonelline	Ursane triterpenoid	Steroidal glycosides	Thymoquinone	Stilbenes
13.	Tropane alkaloids	Withanolides	Steroidal lactones	Uglone	Tannins

2. Plant tissue culture and secondary metabolite(s) production

Consumers are becoming more conscientious about lifestyle choices which have spiked the demand for natural and organic products. The interest in medicinal plants increased in last two decades because of their health benefits in terms of safety and cost compared to synthetic drugs [14–16]. Recently, herbal medicine industry is one of the quickest emerging industries worldwide. The world trade in medicinal plants and their metabolites in the year 2000 was US$ 60 billion, with average yearly growth rate of 7 % and is anticipated to touch US$ 5 trillion by 2050 [17]. In the year 2002, 4 billion individuals of the global population were reported to utilize herbal medicines for their crucial health attention [18].

Plant metabolites can be isolated from naturally grown plants, but their commercial production is limited due to environmental and regional constraints [13]. Conventional methods are time consuming as plant takes several years to grow and reach the point for desired metabsolite production. An alternate method to surpass such situation is to use PTC techniques for the production of secondary metabolites efficiently within a short duration for commercial application [12,19]. It approvals the bulk propagation of plants in controlled environmental conditions without any seasonal constraints [20,21].

2.1. Types of in vitro systems for metabolite(s) production

In vitro secondary metabolites production is a two-stage procedure: (i) Aggregation of biomass, and (ii) Synthesis of secondary metabolites [13,20]. Organized structures like shoots and roots, calli, cell suspension, etc. were reportedly employed for production of secondary metabolites [8,22].

Under *in vitro* conditions, induced plant cells form unorganized mass of cells known as callus. For induction of callus, high amount of auxin concentrations or amalgamation of auxin and cytokinin is commonly used. Callus cultures have gained commercial potential for the manufacture of secondary metabolites of therapeutic significance [23,24]. Callus culture has been reported to be more reliable than collecting plant materials from wild for extracting the therapeutic metabolites [25]. They can be used for the generation of multiple clones of plants using micropropagation, and can also be used to develop single-cell suspension cultures employing either batch or continuous fermentation to produce the preferred secondary metabolites [26,27]. Subsequently, callus and suspension cultures have the ability to synthesize secondary metabolites and it enables the manipulation of secondary metabolites biosynthesis pathways. It has been reported that callus cultures used for the production of tropane alkaloids, α-tocopherol, ajmaline, serpentine, reserpine, flavonoids, scopolamine, paclitaxel, stilbene, resveratrol and anthocyanins [25].

Differentiated organ cultures like shoots or roots have been reported for metabolite production and can represent a metabolite profile similar to native plants [28]. Plant hairy root cultures are the furthermost auspicious alternative method for development of compounds synthesized in plant roots [29]. *Agrobacterium rhizogenes* arbitrated transformation has been used to trigger hairy roots in plants which has enabled *in vitro* construction of secondary metabolites synthesized in roots of plant [30,31]. They have established a beneficial biological scheme to study the biosynthesis of several bioactive compounds like nicotine and tropane alkaloids and [32,33], ginsenosides [34], anthraquinones [35] and artemisinin [36]. The advantage of using hairy roots for the development of compounds relies in their high productivity, constancy and competence [37–39].

3. Secondary metabolite(s) production strategies

Secondary metabolite production is an imperative technique having immense commercial application. Consistent production and high yields are the important factors in commercial development of secondary metabolites [40,41]. Traditional and metabolic engineering approaches have been utilized to enhance the construction and yields of secondary metabolites [42].

3.1. Traditional strategies

Secondary metabolites are produced as an outcome of primary metabolism. It is dependent on the rates at which substrates from primary metabolic pathways are re-routed to secondary biosynthetic pathways. Their synthesis depends on biotic as well as abiotic factors like growth and physiology, temperature, humidity, light intensity, etc. Metabolite productivity of *in vitro* cultures are dependent on culture media composition, pH, inoculum density, culture environment like temperature, light density, agitation, aeration, etc. [21,42]. Thus to improve the growth and metabolite productivity these factors has to be optimized. The choice of suitable culture media plays a vital role in secondary metabolites manufacture. The components of culture medium like macro- and micronutrients, vitamins, carbohydrates (sugars), and amino acids, and plant development regulators for example cytokinins, auxins, gibberellins, jasmonates and salicylates influence metabolite production [39].

Effective approaches to enhance biotechnological production of secondary metabolites are using elicitors which trigger secondary metabolic pathway to stimulate plant defense to protect the plant

Table 2
Medicinal plants, therapeutic metabolite(s) and their applications.

S. No.	Plant Name	Active Metabolite(s)	Application(s)
1.	*Catharanthus roseus*	Indole alkaloids, catharanthine	Cancer therapy
2.	*Dioscorea doryphora*	Diosgenin	Cancer therapy, hypercholesterolemia, Inflammation
3.	*Ruta* species	Quinolone alkaloids, furanocoumarins, acridone, flavonoids	Natural fungicides and pesticide, contraceptive preparations, anti-inflammatory, antipyretic, antioxidant, analgesic, antiviral, antiplasmodial
4.	*Ginkgo biloba*	Kaempferol, isorhamnetin, quercetin, bilobalide, ginkgolide A, B, C, ginsenoside	Anti-inflammatory, antidiabetes, cardiovascular diseases, cancer therapy
5.	*Arnebia euchroma*	Shikonin	Antimicrobial, anticancer, antipyretic, anti-inflammatory
6.	*Carthamus tinctorius*	Phenolics, flavonoids, alkaloids, lignans, carboxylic acids, steroids, polysaccharides, quinochalcone C-glycosides, quinone-containing chalcones	Gynecological diseases, cardiovascular diseases, cerebrovascular diseases anticoagulant, gastrointestinal, antioxidant, hypolipidemic
7.	*Datura stramonium*	Tropane alkaloids (hyoscyamine, atropine and scopolamine)	Relive pain of rheumatism and gout, hallucinogenic drug, treat epilepsy and depression, powerful mind altering drug, anti-inflammatory, antimicrobial
8.	*Ephedra*	Ephedrine alkaloids (1-ephedrine, *d*-pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine and methylpseudoephedrin), Flavonoids (eucodelphinidin, leucopelargonine, leucoanthocyanidin, lucenine, vicenin-1 and vicenin-2), proanthocyanidines	Appetite suppression, psychostimulant, prophylaxis of asthma, nasal congestion and hypotension caused by spinal anesthesia and urinary incontinence
9.	*Rauwolfia* species	Ajmaline, ajmalicine, solasodine, α-solanine, serpentine and reserpine, β-carboline, alstonine, rescinnamine, reserpiline	Natural tranquilizer, hypertension, insomnia, epilepsy, schizophrenia, antidote against snake bite
10.	*Vitis vinifera*	Stilbene, procyanidins, resveratrol, anthocyanins, quercetin, myricetin, laricitrin, kaempferol, syringetin, isorhamnetin, epicatechin, flavonols, vineferin	Laxative, purgative, diuretic, antioxidative, anti-inflammatory, antithrombotic, neuroprotective agents, antianxietic, anticancer agents
11.	*Taxus* species	Paclitaxel, essential oils, flavonoids, taxol, baccatin III, lignans	Treatment of rheumatism, aphrodisiac, epilepsy, antidote for snake bite, antiepileptic, anti-inflammatory, anticancer, antipyretic, analgesic, immunomodulatory, antimicrobial activities.
12.	*Scopolia parviflora*	Scopalamine, hyoscyamine	Anticholinergic drug, antispasmodic drug hallucinogenic agent, treat motion sickness
13.	*Rubia tinctorum*	Tropane alkaloids, phytosterols, saponins, tannins, phenolic compounds, anthraquinone glycosides, triterpenoids	Treatment of skin disease, spleen disorders, astringent, febrifuge, hepatoprotective agent, antithrombotic, antioxidants
14.	*Coleus blumei*	Rosmarinic acid, β-sitosterol, forskolin, apigenin, chavicol, cavacrol, eugenol, quercetin	Anticancer, antidepressant, antigalucomic, antimetastastic, cardiotonic, nervous system depressant, vasodialator, immunosuppressant, antioxidant
15.	*Saussurea medusa*	Sesquiterpenoids flavonoids, phytosterols, triterpenoids, lignans, phenolics, gallic acid, syringin, chlorogenic acid, ethyl gallat, rutin, isoquercitrin	Anti-inflammatory, anticancer, antitumor, hepatoprotective, anti-ulcer, cholagogic, immunosuppressive, spasmolytic, antimicrobial, CNS depressant, antioxidant
16.	*Trichosanthes*, *Cucurbita*, *Cucumis* and *Citrullus*	Cucurbitacins	Anti-inflammatory, anti-diabetic activity, anti-tumor, anti-artherosclerotic activity
17.	*Dryopteris filix-mas*	Phloroglucinols like aspidin, deaspidin, filixic acid	Anti-inflammatory, treating rheumatoid arthritis, wounds, ulcers
18.	*Thymus* species	Borneol, α-terpineol, carvacrol, thymol, geraniol, carotenoids, rosmarinic acid	Anti-inflammatory, hepatoprotective, antimicrobial, anti-HIV-1 activity, antiulcer, gastroprotective, hypoglycemic, antihyperlipidemic activity, antioxidative capacity, flavouring agent
19.	*Punica granatum*	Pelletierine, ellagic acid, phenol, flavonoid	Anti-cancer, cardiovascular disease, anti-diabetes, treat dental problems immune modulatory activity
20.	*Stevia rebaudiana*	Stevioside, steviol rebaudioside, essential oils	Anti-diabetes, cardiovascular disease, cancer, renal disease, obesity, treating inflammatory bowel disease, dental caries
21.	*Ammi visnaga*	Visnagin, khellin, γ-pyrones, scopoletin, dehydrogeijerin, furanochromones, pyranocoumarins, apigenin, luteolin, chrysoeriol, essential oils	Vasodilator agents, treatment of skin disorders like psoriasis and vitiligo, treatment of renal colic, abdominal cramps, mild anginal symptoms
22.	*Baccharis megapotamica*	Baccharine, clerodane, labdane diterpenoids, oleanane triterpenoids, cinnamic caid, coumarins, flavonoids	Reducing phlegm, relieving cough, invigorating blood circulation, inducing diuresis, antihelmintic, anti-inflammatory, anti-neoplastic, antioxidant, antiviral
23.	*Catharanthus roseus*	Serpentine, ajmalicine, vinblastine, vincristine, tabersonine, vindoline, vinceine	Anti-cancer, anti-diabetic, treatment of Hodgkin's disease, antineoplastic drug
24.	*Coptis* species	Berberine, palmatine, epiberberine, coptisine, jatrorrhizine	Antipyretic, antidote, anti-inflammatory, neuroprotective, antioxidant
25.	*Dioscorea* species	Diosgenin, discoeine, cortisone, allantoin, phytic acid, coumaric acid, myricetin	Anti-tumor, anorexiant, antifungal, antimutagenic, hypoglycemic, immunomodulatory, antidotes in inscet and snake bites
26.	*Panax ginseng*	Ginsenosides (dammarane and oleanane)	Tonic for gastro enteric disorders, diabetes, anti-stress agents, cardioprotective, antioxidant, anxiolytic, anticancer, aphrodisiac
27.	*Digitalis lanata*	Odoroside H, odorobioside G, verodoxin, glucodogifucoside, strospeside, digitoxin, digoxin, ouabain, oleandrin, proscillaridin	Treatment of congestive heart failure, cytotoxic, antidiabetic, antioxidant, insecticidal, immunological, hepato, neuro, cardioprotective properties
28.	*Beta vulgaris*	Betalains, apigenin flavonoids like vitexin, vitexin-2-O-rhamnoside, vitexin-2-O-xyloside	Antihypertensive, hypoglycaemic, antioxidant, anticancer agent, natural dye in food industry
29.	*Atropa belladona*	Tropane alkaloids (atropine, hyoscyamine and scopolamine)	Treatment of Parkinson's disease, hallucinogenic agent, treatment of ulcers, motion sickness, inflammation, antidote for snake bites
30.	*Anisodus luridus*	Tropane alkaloids(anisodamine, anisodine and hyoscyamine), piperidine, pyridine alkaloid	Anticholinergic, anaesthetic agents, treatment of septic shock
31.	*Heimia salicifolia*	Quinolozidine, alkaloids, nesodine, vertine, lyfoline, lythrine, demethylsubine I & II	Antisyphilitic, sudorific, antipyretic, laxative and diuretic activity, agents to reduce psychodysleptic activity

Table 2 (*Continued*)

S. No.	Plant Name	Active Metabolite(s)	Application(s)
32.	*Camptotheca acuminata*	Camptothecin, 10-hydroxycamptothecin, 10-methoxycamptothecin	Anti-cancer agents
33.	*Hypericum perforatum*	Hypericinn, hyperforin	Antidepressant, anti-inflammatory, antiviral, anti-cancer, antioxidant, nueroprotective, antibacterial activities
34.	*Maclura pomifera*	Prenylated isoflavones (scandenone and auriculasin), isoflavones (osajin and pomiferin)	Astringent, antioxidant and fungicidal agents, cardiovascular activity, insect repellant, natural dye
35.	*Commiphora wightii*	monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, steroids, flavonoids, guggultetrols, lignans	Treat inflammation, gout, rheumatism, obesity, disorders of lipids metabolism
36.	*Pueraria lobata*	Isoflavonoid (puerarin, daidzin and daidzein)	Antipretic, antidiarrhetic, diaphoretic, antiemetic, antioxidant agent
37.	*Chlorophytum borivilianum*	Phenols, saponins, flavonoids, alkaloids, tannins, steroids, triterpenoids, vitamins	Effective aphrodisiac and pro-erectile agent, spermatogenic, immunomodulatory, anti-stress, anti-oxidant activities
38.	*Bacopa monnieri*	Triterpenoid saponins (bacosides)	Nerve tonic, memory promoter, epilepsy, hepatoprotective, antioxidant
39.	*Citrullus colocynthis*	Saponins, steroids, alkaloids and flavonoids, curcubitacins	Abortifacient, treat constipation, oedema, bacterial infections, cancer, diabetes

cell [43]. Elicitors can be divided on the basis of their source *i.e.*, biotic and abiotic. Abiotic elicitors are constituents of non-biological sources like most of the inorganic compounds for example salts of heavy metals, metal ions, metal oxides, etc. [44,45]. Physical stresses such as cold shock, UV, osmotic, water stress, etc. have been reported to induce enzymatic activity and secondary metabolism [46].

Biotic elicitors are originate from biological source and they can be either exogenous or endogenous. Exogenous biotic elicitors are constituents of microbial cell walls like chitosan, chitin, or components of plant cell wall like polysaccharides or oligosaccharides. Polysaccharides or oligosaccharides are reported to be the furthermost premeditated signalling molecules for elicitation routes because it induces plant defense responses similar to pathogen invasion [47]. Endogenous biotic elicitors are polysaccharides (released by deterioration of the plant cell wall due to pathogens), intracellular molecules or proteins like salicylic acid or methyl jasmonate produced by the plant in reaction to various stress or pathogenic invasion [45]. Biotic elicitors are perceived by particular cell membrane receptors, and then transfer the stimulus to cell by signal transduction leading to the development of phytoalexins [43].

Phytohormones like salicylic acid and methyl jasmonate produced in plants in response to stress or pathogen invasion have been reported to be the signalling compounds in elicitation leading to an enhanced production of secondary metabolites like flavonoids, alkaloids, terpenoids and phenylpropanoids [48–52]. They initiate signal transduction pathways by targeting secondary signals in the cell nucleus which in turn lead to transcriptional stimulation of various genes thereby inducing the synthesis of an array of proteins involved in defense or resistance and secondary metabolites [53,54].

Many secondary metabolic pathways were reported to be triggered by microbes (fungi, bacteria, viruses, yeast, etc.). The use of bacterial, fungal and yeast extracts to induce secondary metabolites has been gaining attention due to improved yield and quality of metabolites. The most effective method to increase the productivity of secondary metabolites in PTC is the use of fungal elicitors [55]. It induces the expression of specific plant genes, activating secondary metabolic pathways thus increasing secondary metabolites [56]. It induces plant phytoalexins in the course of physiological progressions of plant disease resistance [57]. *Fusarium*, *Pythium*, yeast, *Aspergillus*, *Penicillium, Trichoderma* species, etc. are frequently extracted as fungal elicitors and are used to induce secondary metabolites in medical plants [58,59].

Aspergillus niger and *Saccharomyces cerevisiae* cell free extracts were employed as fungal elicitors for improved gymnemic acid production from cell suspension cultures of *Gymnema sylvestre* [60]. *Aspergills flavus* was reported to increase the production of terpenoid indole alkaloids in *Catharanthus roseus* [59]. Bacterial elicitors are also be used to enhance the production of secondary metabolites. Gram-positive and Gram-negative strains of bacteria were used as elicitors for tropane alkaloids biosynthesis in adventitious hairy root cultures of *Scopolia parviflora* [61]. Kang et al. [62] recorded the enhanced production of ginkgolide and bilobalide biosynthesis in *Gingko biloba* cell suspension cultures using *Staphylococcus aureus* extract. *Rhizobacterium* elicitor treatment leads to a slow expansion of hypericin and pseudohypericin in plantlets of *Hypericum perforatum* [63]. The phytotoxin coronatine produced by *Pseudomonas syringae* has the received considerable attention as an elicitor for enhanced the production of secondary metabolites. Taxane production was reported to be enhanced by accumulation of coronatine in *Taxus* cell cultures [64]. Similarly, coronatine triggered the synthesis of viniferins in the cell suspension culture of *Vitis vinifera* [65]. *Rhizobium leguminosarum* and *Agrobacterium tumefaciens* bacterial elicitors were described to increase the production of glycyrrhizic acid from root culture of *Taverniera cuneifolia* [66].

3.2. Metabolic engineering

Metabolic engineering is the amendment of metabolic routes in an organism using modern biological tools like genomics, proteomics, metabolomics, etc. to produce metabolites of commercial importance [67]. It thus allows manipulation of endogenous biochemical pathways. It involves over expression or down regulation of metabolic pathways by diverting common precursors, enzymes, regulatory proteins with the help of recombinant DNA technology. Plants contain numerous metabolic pathways accountable for the biosynthesis of complex metabolites. These pathways can be reconstituted in heterologous hosts for overproduction and isolation of economically important pant metabolites.

The biosynthetic routes for production of secondary metabolites in plants are arise from the shikimate, polyketide and terpenoid routes. The shikimate pathway is highly conserved and reported to be the main source of phenylpropanoids and aromatic compounds [68]. It is considered as an essential metabolic pathway in plants in relations to carbon flux, as it is expected that more than 30% of fixed carbon is fixed *via.* this pathway [69]. The phenolic secondary metabolites in plants are derived from phenylalanine *via.* shikimate biosynthesis. The intermediate p-coumaroyl-CoA forms the origin of metabolites like coumarins, flavonoids, lignans, stilbenoids, catechins, vanillin, gallic acid, betalains, etc.

The terpenoid pathway is also known as isoprenoid pathway. Terpenoid biosynthesis in plants use two autonomous pathways *i.e.* mevalonic acid pathway (MVA) and methyl-D-erythritol (MEP) pathway. The MVA pathway serves the precursors for synthesis of brassinosteroids, sesquiterpenoids, phytosterols, triterpenoids, and polyprenols. MEP pathway is used for the biosynthesis of monoterpenoids, diterpenoids, hemiterpenoids, tocopherols, plastoquinones, plant growth regulators like cytokinins, gibberellins, etc. Terpenoids accounts for more than one third of the plant secondary metabolites and execute several functions in plant. For example, volatile terpenoids (monoterpenoids) and carotenoids are used as pollinator attractants, phytoalexins (sesquiterpeniods, diterpenoids and triterpenes) used as antimicrobial agents, antifeedants and protection against abiotic stress, monoterpenes and sesquiterpenes used as resistance against several predators (killers). Besides, it acts as a source for the formation of naphthoquinones, cannabinoids, furanocoumarines, anthraquinones and terpenoid indole alkaloids [70].

The polyketide route executes a vital role in fatty acids biosynthesis. They are derived from either acetyl CoA or malonyl CoA. Secondary metabolites established from the polyketide pathway include acetogenins, jasmonates 6-methylsalicylic acid, plumbagin, coniine and anthraquinones [70].

3.2.1. Up-regulating pathways

Proteins that bind to specific sites *i.e.*, promoter or enhancer region of DNA to control the transcription or expression of target genes are called transcription factors. They decode information in genome and modulate the rate of transcription [71,72]. They have two functional regions namely an activator domain and a DNA binding domain. DNA binding domain possess amino acids which identify specific DNA base on the regulatory element while activator domain possess binding sites called activation functions and trans-activation domain for other proteins (transcription co-regulators).

Several transcription factors have been identified for secondary metabolite production. MYB transcription factors have been described to be the controllers of several biosynthetic routes in several species of plants. Transcription factors like R2R3-MYB, WD repeat (beta-transducin repeat), a elementary helix-loop-helix genes (*bHLH* genes), adenine nucleotide translocator (or adenine nucleotide translocase or Ant) type 1, APETALA2/ethylene response factor (AP2/ERF), WRKY, NAC, SQUAMOSA Promoter Binding Protein-Like (SPL) have been reported to control anthocyanin accumulation as well as flavonoids in plant species like *Arabidopsis, Petunia hybrida*, *Zea mays*, *Solanum lycopersicum*, *Ipomoea batatas*, etc. [73–75].

The MYB family proteins are involved in activating the phenylpropanoid pathway which regulates anthocyanin production. The expression of transcription factor MYB12 in developing seedlings of *Arabidopsis thaliana* resulted in an increase in total flavanoid content [73]. In *Arabidopsis* anthocyanin biosynthetic genes are activated by the formation of transcriptional regulation complex when MYB proteins communicate with bHLH proteins in the existence of a WD40 repeat [76]. In *C. roseus* three transcription elements ORCA1, ORCA2 and ORCA3 have been recognized to be involved in the expression of terpenoid indole alkaloid biosynthesis. Overexpression of *ORCA2* or *ORCA3* in *C. roseus* cell suspension/hairy roots boosted the synthesis of metabolites like ajmalicine, serpentine, tryptamine and catharanthine [77]. The addition of catharanthine, vindoline, strictosidine and ajmalicine increased significantly when *ORCA3* was overexpressed [78].

In *Artemisia annua*, two jasmonic acid responsive transcription factor AP2/ERF proteins (AaERF1 and AaERF2) bind to the promoter's genes which catalyzes artemisinin synthesis. An improved buildup of artemisinin and artemisinic acids was reported when AaERF1 or AaERF2 proteins were overexpressed [79]. AaWRKY1 transcription factor bind to activate the sesquiterpene synthase gene promoter for biosynthesis of artemisinin in *Artemisia annua* [80]. Transcription factor GaWRKY1 contracts to the promoter of (+)-δ-cadinene synthase genes and regulate gossypol biosynthesis [81]. One of the largest transcription factor families in plants is NAC domain-containing proteins. These proteins control numerous developmental pathways and are produced in response to various biotic and abiotic stresses. In *Arabidopsis* the regulator of camalexin was reported to be a NAC protein ANAC042. The challenging factor in up regulating pathways is to find a transcription factor that concert on precise pathway genes [82]. An alternative to such challenge is to produce synthetic transcription factors, which target more than single gene of significant [83].

3.2.2. Redirecting common precursors

Enzymes compete for precursors in a biosynthetic pathway. Metabolite production can be increased by altering the precursor towards the biosynthesis of the target metabolites by hindering the competitive pathway or by inducing over expression of genes in the precursor pathway [70].

In tomato mutant taxadiene production can be enhanced by increasing the expression of gene that deciphers taxadiene synthase which is a precursor in the carotenoid pathway. Jadaun et al. [84] reported the over-expression of 1-deoxy-d-xylulose-5-phosphate synthase (GrDXS) led to enhanced terpenoidal secondary metabolite accumulation in *Pelargonium* spp. (essential oil) and *Withania somnifera* (withanolides). In peppermint and lavender the over expression of genes in precursor pathways led to an increase in the monoterpene content in essential oils [85,86]. The direct over expression of genes related to the alkaloid pathway was effective in increasing the alkaloid buildup in *C. roseus* [87].

3.2.3. Targeting metabolites to specific cell compartments

The enzymes and metabolites of secondary metabolic pathways are confined in cytoplasm and organelles like peroxisomes, vesicles, vacuoles, etc. The enzymes for secondary metabolic pathways are coordinated into multifunctional enzyme complexes associated with substrate channeling effects where metabolic output of one enzyme act as a substrate for the subsequent enzyme [88,89].

Specific amino acid sequences are reported to target and retain proteins to particular organelles [90]. In *C. roseus* the enzymes associated with the biosynthesis of monoterpenoid indole alkaloids are located in roots, leaves, flowers, buds, etc. while the metabolite accumulates in special cells called idioblasts and laticifers. The enzymes and metabolites produced have to be transported not to a cell but between cells belonging to different types of tissue [91]. To protect cells from self-toxicity, cells compartmentalize the reactions that synthesis harmful metabolites. Alkaloid berberine syntheisized by *Coptis japonica* and nicotine in tobacco are sequestered in vacuoles [92,93]. Different enzymes like chalcone synthase, chalcone isomerase, and dihydroflavonol 4-reductase collaborate together for flavonoid biosynthesis in *Arabidopsis thaliana* [94]. Zhang et al [95] reported the transport of anthocyanins from petals to the central vacuole by intracellular vesicles or pre-vacuolar compartments in *Eustoma grandiflorum*. The multifunctional dimeric enzyme glutathione S-transferases and a vacuolar flavonoid/H+-antiporter is reported to mediate the vacuolar transport of anthocyanin and proanthocyanidin in *Arabidopsis thaliana* [96].

3.2.4. Down-regulating pathways (silencing)

The synthesis of some metabolites can be diminished by curtailing the level of enzyme, increasing catabolism and carbon

flux into competitive pathways [70]. Unwanted metabolites produced can be impeded by concealing genes that upregulate the metabolic pathway or increase their catabolism [97]. To phase out undesirable metabolites modern r-DNA technologies like antisense RNA, RNA interference (RNAi), co-supression can be used. RNA interference has been reportedly used to manipulate plant secondary metabolites like phenylpropanoids, alkaloids and terpenoids. Manipulating the phenylpropanoid pathway using RNAi has led to copious coloration in flowers, low seed coat pigment etc. It has been reportedly used to increase the production of codeine and morphine in the opium poppy, decrease the cyanogenic glucoside components of cassava, enhancing flavonoids and carotenoids in tomato, regulate fragrance in petunia flowers, etc. [98].

4. Nanoparticles for secondary metabolite(s)

The particles within the size range of 1–100 nm are known as nanoparticles. These have been used in plant tissue culture to promote germination efficiency, boost plant growth, ameliorate bioactive metabolites, etc. [99,100]. The effects of some important metal oxide nanoparticles like titanium oxide, zinc oxide, iron oxide and copper oxide have been reported for improved secondary metabolite production in plants [101,102]

Titanium oxide naoparticles distinctly increased gallic acid, chlorogenic acid, o-coumaric acid, tannic acid and cinnamic acid in embryonic calli of *Cicer arietinum* [102]. Silver nanoparticle treatment increased the concentration of artemisinin content in Artemisia annua L. hairy root cultures [57], diosgenin concentration in fenugreek [103], up regulation of biosynthetic genes for anthocyanin and flavonoid production in *Arabidopsis thaliana*, etc. [104]. Cadmium oxide nanoparticles resulted in an enhanced concentration of ferulic acid and isovitexin in barley plants [105].

5. Medicinally important plants and their secondary metabolites

Plant tissue culture research has resulted in the production of diverse bioactive molecules in bulk quantities for therapeutics. Secondary metabolites like alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids, amino acids, etc. are used in many pharmaceutical preparations [106]. Few medicinally important plants and their important metabolites are listed below.

5.1. Catharanthus roseus

Catharanthus roseus (L.) G. Don (Apocynaceae) is an important medicinal plant due to the presence of metabolites like monoterpene indole alkaloids or terpenoid indole alkaloids [107]. It is used in traditional medicine to control cancer, diabetes, hypertension, antimicrobial agents, etc. The alkaloid content was reported to be highest at the flowering stage [108]. The main alkaloids in root are ajmalicine, serpentine, and reserpine. Vincolidine, vincoline, vindolindine, cathindine, vinblastin, catharanthamine, vinblastine, catharicine, catharine, leurosinone, cathindine, cavincidine, apparicine, β-carboline, iochrovicine, rosicine, serpentine, etc. are the major alkaloids reported in leaves. Coronaridine, vindolidine, vindoline, catharanthine, ajmalicine, perivine, tetrahydroalstonine, tabersonine, carosine, catharicine, etc. alkaloids dominate the flowers [109,110]. *Catharanthus longifolius*, *Catharanthus trichophyllus*, and *Catharanthus lanceus* are reported to possess vindoline type alkaloids [111].

It also produces a range of phenolic compounds like 2,3-dihydroxybenzoic acid, salicylic acid; salicylic acid glucoside, benzoic acid, gallic acid, glucovanillin, vanillic acid, 2,5- dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid glucoside, etc. [112,113]. Phenylpropanoids like flavonoids, anthocyanins, cinnamic acid derivatives, are also reported in *Catharanthus* [114].

These phytochemicals are present in very low quantities in plant hence, to improve the phytochemical production *in vitro* methods are employed [115]. *In vitro* culture methods like callus culture, cell suspension, hairy root, etc. have been reported as advanced strategies over conventional breeding methods for the production of therapeutically important alkaloids [116,117].

Hairy root cultures of *C. roseus* transformed by *Agrobacterium rhizogenes* have been effectively employed for production of catharanthine, tabersonine, lochnericine, horhammericine indole alkaloids [118]. The alkaloid ajmalicine and serpentine has been obtained from calli, cell suspensions, sprouts, pilose roots; alstonine from calli; antirhine and cathindine in cell suspensions; acuamicine and lochnericine in calli and suspensions; horhammericine and vindoline in suspensions and sprouts; tabersonine in calli and suspensions; catharanthine in suspensions, sprouts and roots; 3,4-anhydrovinblastine and catharine in sprouts; vinblastine in calli, sprouts and somatic embryos; and vincristine in sprouts and embryos [107,119,120]. Liu et al. [121] reported the use of artemisinic acid as an elicitor for enhanced production of vindoline and vinblastine production in cell suspension of *C. roseus.*

5.2. Hypericum perforatum

Hypericum perforatum L. (Hypericaceae) is widely used in traditional and conventional medicine. *In vitro* and *in vivo* clinical studies report antiviral, antifungal, antibacterial, wound-healing, antidepressant, antioxidant properties, etc. [122,123]. The medicinal properties of the plant are ascribed to the presence of secondary metabolites like phloroglucinols (hyperforin, hyperfoliatin, adhyperforin), naphtodianthrones (hypericin, pseudohypericin), flavonoids (rutin, quercetin, quercitrin, isoquercitrin, hyperoside and amentoflavone), tannins (cathechin and epicatechin), proanthocyanidins (procyanidin B2) and bioflavonoids (biapigenin and amentoflavone) and small amounts of essential oil [124–127]. The active phytochemical principle in plant varies depending on various ecological factors like plant habitat, development, etc. One of the major metabolite of this plant is hypericin has gained interest due to its potential as an anticancer agent [128,129].

In vitro culture systems have been reported as a substitute for the large scale production of metabolites of pharmaceutical interest [28,130]. The overproduction of naphtodianthrones from cell suspension cultures of *H. perforatum* have been customized using phytohormones and different elicitors [47,127,131].

5.3. Coleus forskohlii

Coleus forskohlii (Lamiaceae/Labiatae) is a medicinal plant used in Indian Ayurvedic Medicine. It contains the secondary metabolite labdane diterpenoid forskolin which is reported to have several biological and pharmacological activities [132]. Forskolin acts as an activator of adenylate cyclase [133]. The solitary source of forskolin is the roots of *C. forskohlii* plants but the concentration of the metabolite from wild plants in natural habitats is very less [134]. Plant tissue culture techniques thus have a potential for industrial production of bioactive metabolite forskolin. Callus induced from young leaves of *C. forskohlii* plants reported the presence of forskolin [133]. Elicitors like salicylic acid and methyl jasmonate had an intense effect on forskolin production [135].

5.4. Taxus baccata

Taxus baccata(English yew) is an evergreen conifer abundantly seen in central and southern Europe. The complex

diterpene alkaloid taxol (plaxitaxol) isolated from bark of the *Taxus* tree is one of the most potent anticancer agent due to its mode of action on microtubules. Taxol binds to β-tubulin subunit, leading to interference with their normal breakdown during cell division with resultant stabilization of the polymer through protection from disassembly [136–138]. The ever-increasing demand for the alkaloid cannot be met by conventional extraction from the trees due to impeded growth of *Taxus* trees and the reduced taxol content in bark [41,139,140]. Hence, *in vitro* methods employing plant tissue culture are gaining more importance for commercial production of the metabolite taxol. Apart from *Taxus* species few gymnosperms, angiosperms and several endophytes have been reported for taxol production [141,142].

*In vitro*taxol production was first reported by Christen et al. [143]. Plant cell culture has been reported as a potent and sustainable means to produce paclitaxel from species like *Taxus baccata*, *Taxus yunnanensis*, *Taxus cuspidate*, *Taxus chinensis*, *Taxuscanadensis* and *Taxus globosa* [140]. Effective ways reported to increase the yield of paclitaxel involves the use of high-yielding cell lines, two-stage culture systems, carbohydrate optimization, pre-feeding strategies and fungal inducers like fungal extracts along with elicitors like salicylic acid, chitosan, squalene, methyl jasmonate, etc. [144,145] reported high taxane production in cell cultures which were deep brown and had reduced growth index.

5.5. Nothapodytes foetida

Nothapodytes foetida is an endangered species distributed in the Western Ghats of India succeeding worldwide significance owing to their pharmacological and therapeutic properties. It yields camptothecin a monoterpenoid indole alkaloid used in anticancer drug formulation [146]. It is also obtained from several plants like *Camptotheca acuminata*, *Ophiorrhiza* species, *Ervatamia heyneana, Meriilliodendron megacarpum* and *Nothapodytes nimmoniana* [147,148]. Camptothecin is formed in several parts of *Nothapodytes foetida* at adaptable amount [149] and the content of alkaloid increases with the age of the plant [150].

6. Conclusion and perspectives

Plants have been used for medical purposes since the ancient time. Increasing demand of sustainable and cost-effective natural phytochemicals from plants demands their mass cloning through plant tissue culture approaches. A huge number of medicinal plants and their metabolites have been produced by *in vitro* techniques in a short duration of time compared to conventional approaches. To meet the growing demand of these natural metabolites various strategies have been employed to produce plants with enticing features for metabolite production.

Author contributions

MM and HC:conceived the idea of the review, provided the general concept and inputs for each specific section, and drafted part of the manuscript. HC, MM, and TSB: wrote the review after collecting literature. MM: edited, compiled, and finalized the draft. MM and KS: approved the final version of the manuscript. Finally, all the authors read and approved it for publication.

Declaration of Competing Interest

The authors declare that there is no potential conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00450.

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