

# Plant cells technology as an effective biotechnological approach for high scale production of pharmaceutical natural compounds: A meta-analysis study

Ali Davoodi<sup>1</sup>, Elnaz Khoshvishkaie<sup>2</sup>, Mohammad Azadbakht<sup>1\*</sup>

<sup>1</sup>Department of Pharmacognosy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

<sup>2</sup>Pharmaceutical Cares Department, Ayatollah Khamenei Hospital, Mazandaran University of Medical Sciences, Abbas Abad, Iran

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\*Corresponding author:

[azadbakht110@gmail.com](mailto:azadbakht110@gmail.com)

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## ABSTRACT

Natural-based drugs are the important bioactive substances that have been used for prevention and treatment of diseases. Natural products are prepared in commercial scale from relevant medicinal plants. Hence, large amounts of the plants are needed for extraction and isolation of naturally occurring compounds. Plant cells technology is the best strategy for the production of the plant-derived drugs, which have difficulty process in large scale production. This study was conducted for types, frequencies and efficacies of production methods for natural-based drugs in plant cell technology as an alternative method to preparation from whole herb. Pharmaceutical and biomedical databases including PubMed/Medline, Scopus, Web of Science, Embase, ProQuest and Google Scholar were searched in this study. Moreover, keywords words were "secondary metabolite production", "pharmaceutical natural compounds", "high scale production", "cell suspension", "immobilized plant cell", "hairy root", "elicitor", "substrate", "plant cell", "callus", "medicinal plants", "isolation and purification". The correlations have been investigated by random effect model in an Excel program. Findings of this meta-analysis study showed all production methods had high efficacies and percentages of high scale production from 90 to 100%, which were comparable with conventional direct extractions. In addition to, median efficacy values for cell suspension, callus, hairy root and immobilized plant cell methods in production of selected drugs (atropine, paclitaxel, vincristine, camptothecin and colchicine) with 1124, 257, 797 and 969 events were 92.49 (CI<sub>95%</sub>: 89.78-95.86), 91.98 (CI<sub>95%</sub>: 89.13-95.25), 95.69 (CI<sub>95%</sub>: 92.84-98.68) and 93.86% (CI<sub>95%</sub>: 91.12-96.35), respectively. The plant cell technology for production of secondary metabolites has various advantages including high accuracy, repeatability and productivity, that is a best strategy for production of natural-based drugs.

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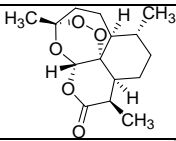
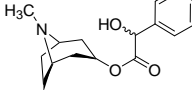
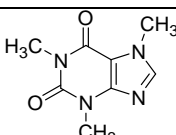
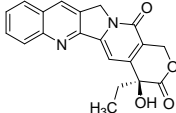
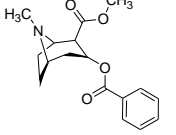
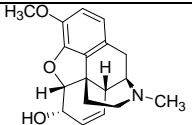
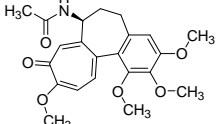
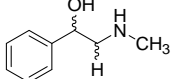
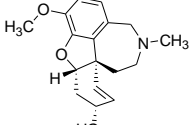
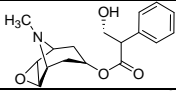
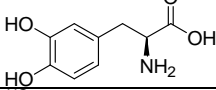
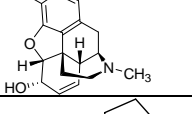
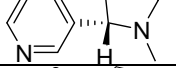
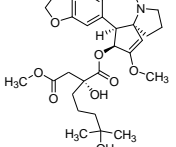
## Introduction

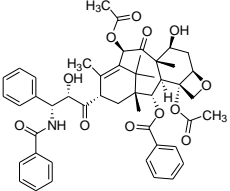
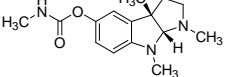
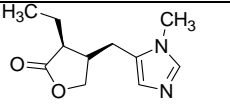
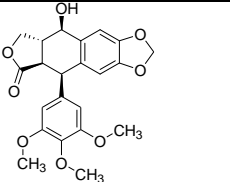
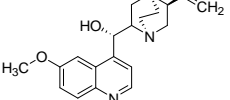
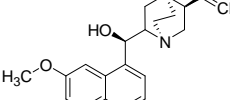
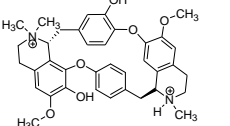
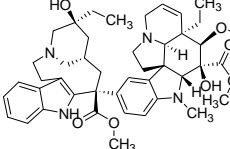
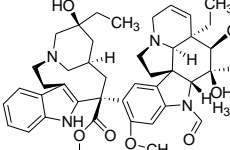
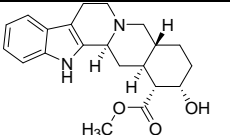
Natural-based drugs are the important bioactive substances, which have been used for prevention and treatment of diseases. Moreover, several diseases are need to develop the novel drugs with the higher efficacies and low side effects. Natural sources play important roles in production of drugs. Surprisingly, some natural-based drugs are the only choices for the prevention and treatment of the relevant diseases. In addition to, natural-based compounds especially plant-isolated substances are the main important patterns for the effective drug design in drug discovery studies (1, 2). Several classes of phytochemical compounds especially alkaloids, saponins, terpenoids and flavonoids have been used in the medicinal purposes. These compounds have been isolated from the important plant families. For instance, *Atropa*, *Scopolia*, *Duboisia*, *Datura*, *Hyoscyamus* and *Mandragora* plants from Solanaceae family have anticholinergic alkaloids, *Catharanthus* plant from Apocynaceae family and *Taxus* plant from Taxaceae family have the anticancer alkaloids, *Artemisia* plant from Asteraceae family contains antimalarial sesuiterpene lactones, and *Papaver* plants from Papaveraceae family contains antinociceptive and antitussive alkaloids. Moreover,

fungi and marine sources contain some similar compounds (3-9). Table 1 shows the important plant-based drugs, occurring plants, structural formula, medicinal uses and mechanisms of action. These natural-based drugs should be produced in commercial scale using relevant medicinal plants, which are cultivated or existed in the nature. Hence, large amounts of the plants are needed for extraction and isolation of the mentioned compounds that threatening the environment and living organisms in most cases. Extraction of paclitaxel from the bark of *Taxus brevifolia* is one of the examples, which is damage to the plant. Then, the alternative methods should be used for the production of natural-based drugs especially the plant-derived compounds (10, 11).

Plant cells technology is the best strategy for the production of the plant-derived drugs. This technology is proceeded using two cell types, generally, genetic-modified cells and/or genetic unmodified cells. Type 1 cells were produced by DNA modifications for production of more yielding cells. Unlike that, type 2 cells have been used with effective substrates and elicitors for high scale production of plants secondary metabolites especially natural-based drugs (12, 13). Several methods have been used for high scale

**Table 1** The main important pharmaceutical natural compounds.

Plant-based drugs	Structural classification	Structural formula	Main source(s)	Mechanism(s) of action	Medicinal use (s)	References
Artemisinin*	Sesquiterpene lactone terpenoid		<i>Artemisia annua</i> L., Asteraceae family	Production of free radicals and damage to susceptible cells	Parasitic diseases such as malaria, cancer diseases, Autoimmune diseases	[36-38]
Atropine	Tropane alkaloid		<i>Atropa belladonna</i> L., <i>Datura stramonium</i> L., Solanaceae Family	Peripheral anticholinergic with antimuscarinic activity	Poisoning diseases, Cardiovascular diseases, Secretion glands, Hyperactivity diseases	[33,39]
Caffeine	Methylxanthine alkaloid		<i>Camellia sinensis</i> (L.) Kuntze, Theaceae Family, <i>Coffea arabica</i> L., Rubiaceae family	Adenosine receptor antagonist, Monoamine neurotransmitter releaser	Pulmonary diseases, Cardiovascular diseases	[40,41]
Camptothecin	Quinoline alkaloid		<i>Camptotheca acuminata</i> Decne., Nyssaceae family	Topoisomerase Enzyme Inhibitor	Cancer diseases	[42,43]
Cocaine	Tropane alkaloid		<i>Erythroxylum coca</i> Lam., Erythroxylaceae family	Sodium Channel Blocker, Monoamine Neurotransmitters Reuptake Inhibitor, Monoamine Neurotransmitters Receptor agonist	Local anesthetics	[44,45]
Codeine	Modified benzyltetrahydroisoquinoline alkaloids		<i>Papaver somniferum</i> L., Papaveraceae family	$\mu$ -Opioid receptor agonist	Cough, Pain	[46,47]
Colchicine	Tropolone alkaloid		<i>Colchicum autumnale</i> L., <i>Gloriosa superba</i> L., Colchicaceae family	Antitubulin agent, Proinflammatory inhibitor	Gout disease, Cancer diseases	[48,49]
Ephedrine	Phenethylamine alkaloid		<i>Ephedra sinica</i> Stapf, Ephedraceae family	Sympathomimetic agent	Motion sickness, Pulmonary diseases,	[50,51]
Galantamine	Benzazepine alkaloid		<i>Galanthus caucasicus</i> (Baker) Grossh., <i>Galanthus woronowii</i> Losinsk., Amaryllidaceae family	Brain acetylcholinesterase enzyme inhibitor	Alzheimer's disease	[52,53]
Hyoscyine	Tropane alkaloid		<i>Scopolia carnioica</i> Jacq., <i>Hyoscyamus niger</i> L., Solanaceae family	Peripheral anticholinergic with antimuscarinic activity	Motion sickness, Gastro-intestinal diseases, uro-genital diseases	[54,55]
L-Dopa	Dihydroxyphenylalanin alkaloid		<i>Mucuna pruriens</i> (L.) DC., <i>Vicia faba</i> L., Fabaceae Family	Dopaminergic Agent	Parkinson's Disease	[56,57]
Morphine	Modified benzyltetrahydroisoquinoline alkaloids		<i>Papaver somniferum</i> L., Papaveraceae Family	$\mu$ - $\delta$ -Opioid Receptor Agonist	Severe Pains	[58,59]
Nicotine	Pyrrolidine alkaloid		<i>Nicotiana tabacum</i> L., Solanaceae family	Nicotinic acetylcholine receptors agonist	Smoke diseases	[60,61]
Omacetaxine Mepesuccinate*	Benzazepine dioxin alkaloid		<i>Cephalotaxus harringtonii</i> (Knight ex J. Forbes) K.Koch, Cephalotaxaceae family	Protein translation inhibitor	Cancer diseases	[62,63]

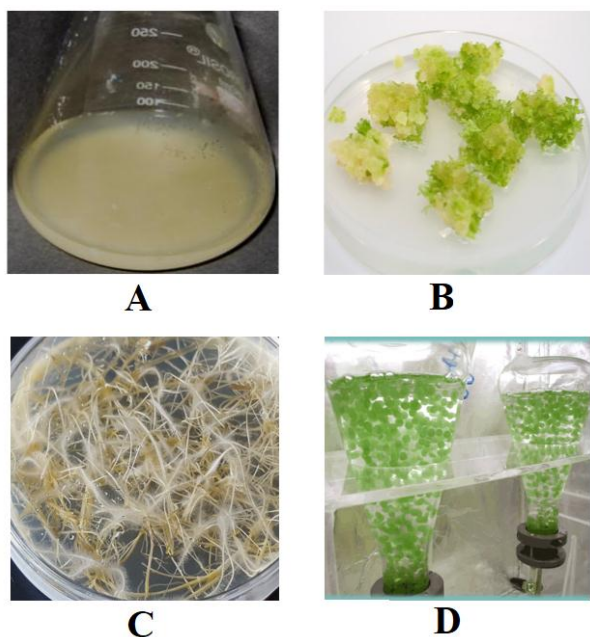
Paclitaxel	Taxol alkaloid		<i>Taxus brevifolia</i> Nutt., Taxaceae family	Antitubulin agent	Cancer diseases	[64, 65]
Physostigmine	Pyrroloindole alkaloids		<i>Physostigma venenosum</i> Balf., Fabaceae family	Acetylcholinesterase enzyme inhibitor	Eye diseases, Gastro-intestinal diseases	[66, 67]
Pilocarpine	Imidazole alkaloids		<i>Pilocarpus jaborandi</i> Holmes, Rutaceae family	Direct cholinergic agent	Gastro-Intestinal Diseases Especially Xerostomia, Eye Diseases	[68, 69]
Podophyllotoxin	Aryl tetralin lignan		<i>Podophyllum peltatum</i> L., Berberidaceae family	Antitubulin agent, DNA deactivator	Cancer diseases, Viral diseases	[70, 71]
Quinidine	Quinoline alkaloids		<i>Cinchona officinalis</i> L., Rubiaceae family	Na <sup>+</sup> /K <sup>+</sup> -ATPase enzyme transporter blocker	Cardio-vascular diseases especially arrhythmia	[72, 73]
Quinine	Quinoline alkaloids		<i>Cinchona officinalis</i> L., Rubiaceae family	Hemozoin biocrystallization inhibitor, Purine nucleoside phosphorylase enzyme inhibitor	Parasitic diseases especially malaria	[73, 74]
Tubocurarine	Benzyl tetrahydroisoquinoline alkaloids		<i>Chondrodendron tomentosum</i> Ruiz & Pav., Menispermaceae family	Neuromuscular blocker with neuromuscular non-depolarizing activity	Anesthesia	[75, 76]
Vinblastine	Terpenoid indole alkaloids		<i>Catharanthus roseus</i> (L.) G. Don, Apocynaceae family	Antitubulin agent	Cancer diseases	[77, 78]
Vincristine	Terpenoid indole alkaloids		<i>Catharanthus roseus</i> (L.) G. Don, Apocynaceae family	Antitubulin agent	Cancer diseases	[78, 79]
Yohimbine	Indoloquinolizidine alkaloid		<i>Pausinystalia johimbe</i> (K. Schum.) Pierre ex Beille, Rubiaceae family	Sympatholytic agent with $\alpha$ -receptors antagonism activity	Uro-genital diseases	[80, 81]

\*An important semisynthetic drug.

production of plant secondary metabolites with two mentioned cell types including cell suspensions, cell masses such as callus, hairy roots and immobilized plant cells (Fig. 1).

In addition to, the cells are need to optimize cultural conditions (apparatus, temperature, pH, airflow,

medium, substrates, elicitor, etc.) for high-yielding production (13-18). Table 2 shows the production methods with their preparation procedures, advantages and disadvantages. In cell suspension method, productive cells have been directly used. Hairy root and callus methods are used using differentiated



**Figure 1** The main methods for production of plant-based drugs (A: Cell suspension, B: Callus, C: Hairy root, D: Immobilized plant cell).

and undifferentiated cell masses, respectively. Unlike those, immobilized plant cells have been prepared by immobilization of cells on some matrices including macromolecules such as calcium alginate, agar, carrageenan and some polymers such as polyethylene and polystyrene. This immobilization can help to decrease cell damages, increase the productivity of cells and increase production of the secondary metabolites (5, 11, 19-22).

This meta-analysis study provides the data from selected relevant studies about types, frequencies and efficacies of production methods of selected natural-based drugs and important naturally occurring drugs. Moreover, we analyzed the results of these studies with regards to efficacy of secondary metabolite production especially the approved drugs by cell technologies as alternative methods to herbs. Finally, the main methods for extraction, isolation, structure elucidation and physicochemical evaluation of produced drugs have been presented.

## Methodology

### Search strategy

In this meta-analysis study, pharmaceutical and biomedical databases as PubMed/Medline, Scopus, Web of Science, Science Direct, Embase, ProQuest and Google Scholar were selected from January 1, 1950, to February 29, 2019. The keywords were "secondary metabolite production", "pharmaceutical natural compounds", "high scale production", "cell suspension", "immobilized plant cell", "hairy root", "elicitor", "substrate", "plant cell", "callus", "medicinal plants" and "isolation and purification" (23).

### Inclusion criteria

The selected articles from above mentioned databases were evaluated to have all topics listed below:

- Secondary metabolite production with at least two fold higher than primary conditions
- Secondary metabolite production with optimized conditions
- The selection of the best plant species for production of pharmaceutical natural compounds
- The selection of the best plant cell lines

### Meta-analysis

All data for high scale production of secondary metabolites and five selected drugs (atropine, paclitaxel, vincristine, camptothecin and colchicine) were extracted and collected from peer-reviewed original articles that have been obtained from scientific journals. Moreover, data have been filtered and removed the duplicate cases and arranged by Microsoft Excel® software. The final data were coded including: the percentages of methods and effective methods.

In addition to, number of cases, amounts of all methods and outcomes have been calculated based on the obtained data and all cases have been analyzed by Neyeloff2012 method (Random-effect model) (24).

## Results

### Methods for production

In the study, four main methods for production of five high important plant-based drugs including atropine, paclitaxel, vincristine, camptothecin and colchicine, have been selected and evaluated. Table 3 shows the main methods and plant sources for production of mentioned drugs. According to the results, the frequency of cell suspension, callus, and hairy root and immobilized plant cell methods for production of the drugs were 35.72, 8.17, 25.33 and 30.79%, respectively.

### Meta-analyses

According to the meta-analyses results, all production methods had high efficacy and percentage of high scale production that were more than 90% and comparable with conventional direct extraction. In addition to, median efficacy values for cell suspension, callus, hairy root and immobilized plant cell methods in production of selected drugs (Atropine, Paclitaxel, Vincristine, Camptothecin and Colchicine) 1124, 257, 797 and 969 events were 92.49 (CI95%: 89.78-95.86; Fig. 2), 91.98 (CI95%: 89.13-95.25; Fig. 3), 95.69 (CI95%: 92.84-98.68; Fig. 4) and 93.86% (CI95%: 91.12-96.35; Fig. 5), respectively.

## Discussion

Various parameters determine the amount of secondary metabolite production including medium of culture, temperature, airflow, CO<sub>2</sub> and O<sub>2</sub>

**Table 2** The main methods for production of secondary metabolites in high scale with their preparation procedures, advantages and disadvantages

Production methods	Preparation procedures	Advantages	Disadvantages	References
Cell Suspension	Cells isolated from plant organs and cultivated with suspension form in medium.	1. High scale production in optimized condition 2. Usable in many plants	1. Low stability 2. Low commerciality 3. Low productivity	[14, 82]
Callus	Cells isolated from plant organs and cultivated to forms a biomass in medium.	1. High scale production in optimized condition. 2. Usable in many plants 3. Higher stability than cell suspension	1. Low commerciality. 2. Moderate productivity 3. Moderate affordability	[83, 84]
Hairy Root	Cells isolated from plant organ and differentiated to hairy roots by bacterial inoculation in medium.	1. High scale production in optimized condition 2. Higher stability than cell suspension 3. High affordable 4. High commerciality	1. Difficult stages for preparation. 2. Do not usable for all plants	[85, 86]
Immobilized Plant Cell	Cells isolated from plant organs and cultivated with suspension form in medium. Finally, cells were immobilized on a matrix.	1. High scale production in optimized condition 2. Higher stability than cell suspension 3. Usable in many plants 4. High Affordable 5. High commerciality	1. Difficult stages for preparation	[13, 87]

**Table 3** The main methods for production of selected natural-based drugs and selected plants for high scale production.

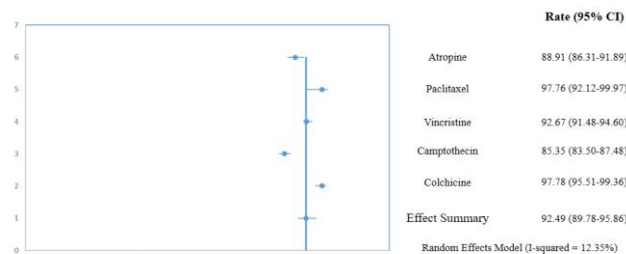
Plant-based drug	Main production method	Plant and cell line	References
Atropine	Hairy root	<i>Hyoscyamus reticulatus</i> L.	[88]
Paclitaxel	Cell suspension	<i>Taxus baccata</i> L.	[89]
Vincristine	Immobilized plant cell	<i>Catharanthus roseus</i> (L.) G. Don	[78]
Camptothecin	Hairy root	<i>Camptotheca acuminata</i> Decne.	[90]
Colchicine	Hairy root	<i>Gloriosa superba</i> L.	[25]

concentrations, salts, Ca, P and N concentrations and existence of substrates. In addition to, some drugs are needed specific condition for high scale production (25). In addition to, plant-based drugs after production using cell technology should be isolated and purified from culture. Several methods have been used for isolation and purification of the drugs such as crystallization, precipitation and centrifugation (26-28). The chemical structure of the drugs have been identified using analytical methods such as <sup>1</sup>HNMR, <sup>13</sup>C NMR, DEPT, COSY, NOESY, HMBC, IR, UV and MASS (29, 30). Physicochemical and pharmacokinetic properties of all produced drugs should be analyzed using pharmacopeial methods including macroscopic and crystal properties, purity, moisture contents, foreign matter, ash values, assay, bioavailability, body distribution, metabolism and body excretion. These parameters determine the quality of the drugs for

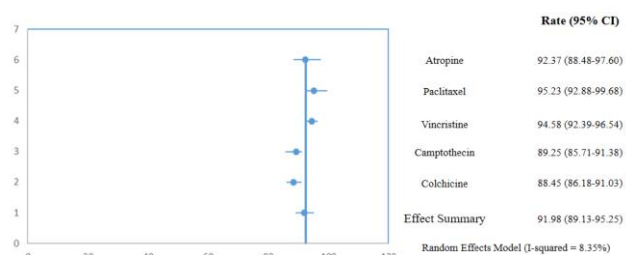
future clinical usage (31, 32). Based on the meta-analyses results, the efficacy values of more important methods for production of the plant-based drugs were approximately 90% to 100%. Many attempts have been done for optimization of production of the plant-based drugs by alternative methods, but no extensive studies have been done for determination of efficacy of these methods.

Paclitaxel as an important anticancer drug that has been produced by cell suspension methods with cells of bark of *Taxus brevifolia* plant from Taxaceae family (10). In that study, the suspension of cells from the plant bark has been prepared and cultured in MS (Murashige and Skoog) medium for a few days and paclitaxel is extracted from final cell suspension. Moreover, Khosroushahi *et al.* demonstrated that salicylic acid and fungal elicitors increased the

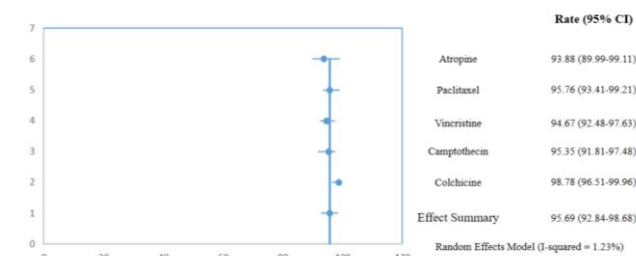
production of paclitaxel 1000-fold higher than other elicitors. This process was more effective than extraction of paclitaxel from the relevant plant. Shakeran *et al.* produced the high scale of atropine by *Datura metel* hairy roots. The biotic and abiotic elicitors as calcium phosphate and yeasts were used for production of atropine for optimization. According to the results, atropine was produced 2-fold higher than control group.



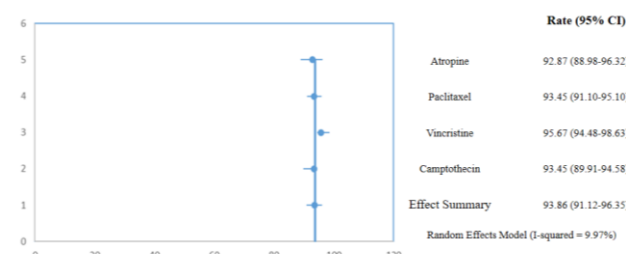
**Figure 2** Meta-analysis of the efficacy of cell suspension method in selected natural-based drugs (CI: Confidence Interval).



**Figure 3** Meta-analysis of the efficacy of callus method in selected natural-based drugs (CI: Confidence Interval).



**Figure 4** Meta-analysis of the efficacy of hairy root method in selected natural-based drugs (CI: Confidence Interval).



**Figure 5** Meta-analysis of the efficacy of immobilized plant cell method in selected natural-based drugs (CI: Confidence Interval).

In addition to, the use of the mentioned method can be Affordable in pharmaceutical manufacturing of active pharmaceutical ingredients (33). Mohamed *et al.* produced alkaloids especially vincristine using immobilized *Catharanthus roseus* cells from Apocynaceae family. The alkaloids were produced in high scale by variation of the culture conditions (34). Hypericin is a naphthodianthrone compound isolated from *Hypericum perforatum* plant from Hypericaceae family has been used for many diseases such as cancer. This natural product was produced using *H. perforatum* hairy root with about 75-fold higher than primary conditions (11). In a study that published by Werner *et al.*, bioreactor technologies were introduced as the effective methods for sustainable production of valuable plant cell-derived products. In this review study different aspects especially economically justifiability have been evaluated (35). All alternative methods for production of secondary metabolites can be helps to prepare the naturally occurring drugs, especially compounds with difficult synthesis process. The plant cells technology protects plants from their extinction, it is unnecessary to cut high amounts of plants for production of drugs at high scale. Therefore, secondary metabolite production technologies are very applicable in pharmaceutical industries.

## Conclusion

The plant cell technology is an important strategy for production of plant-based drugs. It has several advantages such as high accuracy, repeatability and productivity, then, this technology can be use instead of whole herbs. These methods should be optimized and commercialized for each natural compound.

## Acknowledgement

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## Conflict of interest

The authors have no conflict of interest.

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