

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

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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_{95%}: 89.78-95.86), 91.98 (CI_{95%}: 89.13-95.25), 95.69 (CI_{95%}: 92.84-98.68) and 93.86% (CI_{95%}: 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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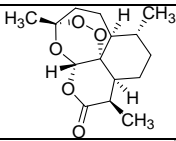
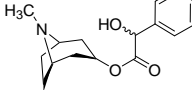
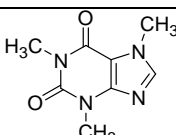
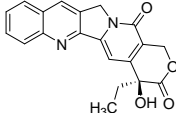
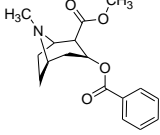
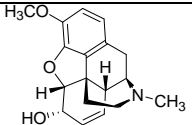
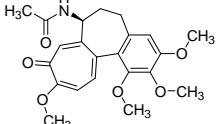
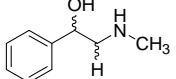
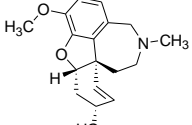
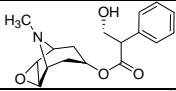
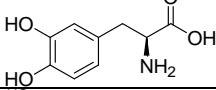
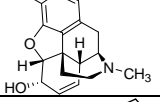
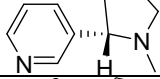
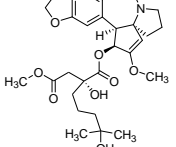
Introduction

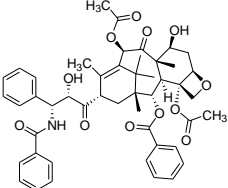
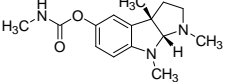
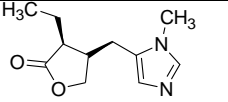
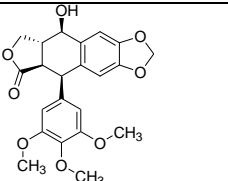
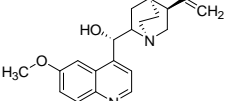
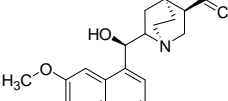
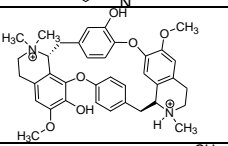
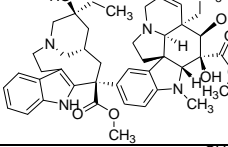
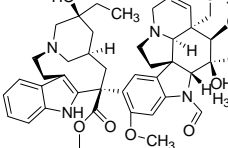
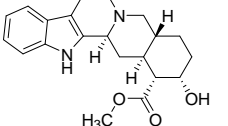
Natural-based drugs are the important bioactive substances, which have been used for prevention and treatment of diseases. Moreover, several diseases are need to develop the novel drugs with the higher efficacies and low side effects. Natural sources play important roles in production of drugs. Surprisingly, 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	μ -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 carniolica</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	μ - δ -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 ⁺ /K ⁺ -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 α-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

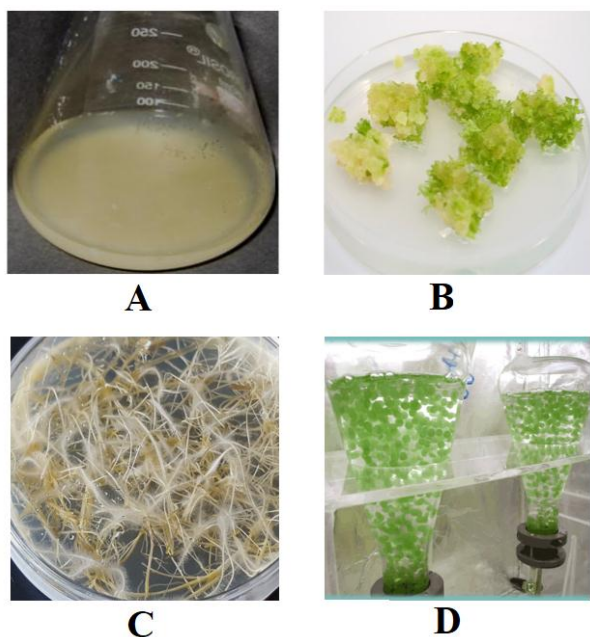


Figure 1 The main methods for production of plant-based drugs (A: Cell suspension, B: Callus, C: Hairy root, D: Immobilized plant cell).

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, CO₂ and O₂

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 ¹HNMR, ¹³C 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.

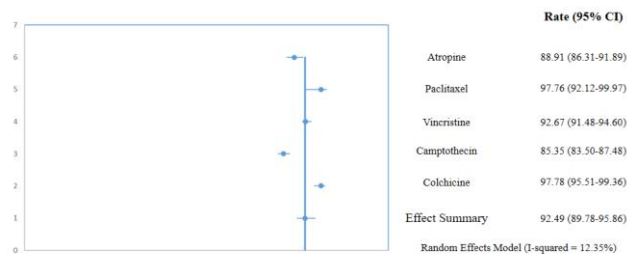


Figure 2 Meta-analysis of the efficacy of cell suspension method in selected natural-based drugs (CI: Confidence Interval).

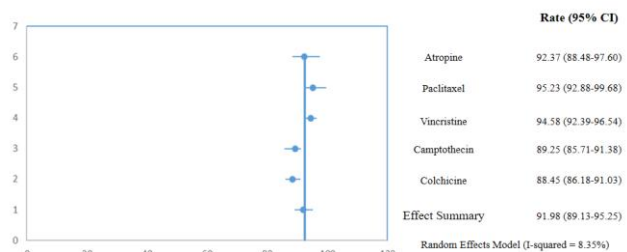


Figure 3 Meta-analysis of the efficacy of callus method in selected natural-based drugs (CI: Confidence Interval).

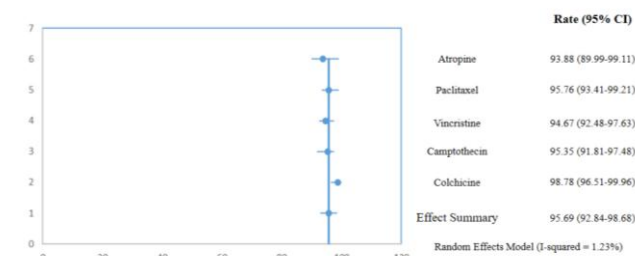


Figure 4 Meta-analysis of the efficacy of hairy root method in selected natural-based drugs (CI: Confidence Interval).

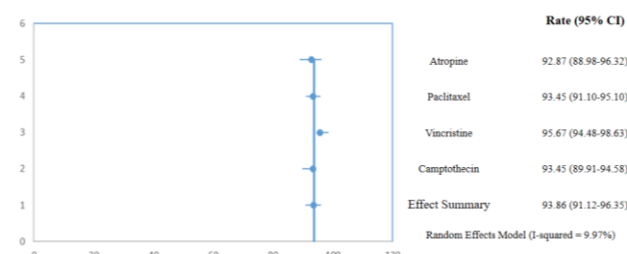


Figure 5 Meta-analysis of the efficacy of immobilized plant cell method in selected natural-based drugs (CI: Confidence Interval).

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.

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Conflict of interest

The authors have no conflict of interest.

References

1. Fakhri M, Farhadi R, Mousavinasab N, Hosseinimehr SJ, Yousefi SS, Davoodi A, et al. Preventive effect of purgative manna on neonatal jaundice: A double blind randomized controlled clinical trial. *J Ethnopharmacol* 2019;236:240-9.
2. Hosseini A, Mirzaee F, Davoodi A, Bakhshi Jouybari H, Azadbakht M. The traditional medicine aspects, biological activity and phytochemistry of *Arnebia* spp. *Med Glas* 2018;15:1-9.
3. Azadbakht M, Hoseinzadeh S. Quantitative analysis of total alkaloids by titration and Hyoscyamine by HPLC in indigenous *Datura* species in north of Iran. *J Mazand Univ Med Sci* 2005;15:32-7.
4. Shokrzadeh M, Azadbakht M, Ahangar N, Hashemi A, Saedi Saravi SS. Cytotoxicity of hydro-alcoholic extracts of

- Cucurbita pepo and Solanum nigrum on HepG2 and CT26 cancer cell lines. *Pharmacog Mag* 2010;6:176-9.
5. Chen SH, Hong J. Novel tubulin-interacting agents: a tale of *Taxus brevifolia* and *Catharanthus roseus*-based drug discovery. *Drug Fut* 2006;31:123-50.
 6. Mohamed AE, El-Sayed MA, Hegazy ME, Helaly SE, Esmail AM, Mohamed NS. Chemical constituents and biological activities of *Artemisia herba-alba*. *Rec Nat Prod* 2010;4:1-25.
 7. Kaur R, Arora S. Alkaloids-important therapeutic secondary metabolites of plant origin. *J Cri Rev* 2015;2:1-8.
 8. Hong JH, Jang S Heo YM, Min M, Lee H, Lee YM, Lee H, Kim JJ. Investigation of marine-derived fungal diversity and their exploitable biological activities. *Marine Drug* 2015;13:4137-55.
 9. Gribble G. Biological activity of recently discovered halogenated marine natural products. *Marine Drug* 2015;13:4044-136.
 10. Khosroushahi AY, Valizadeh M, Ghasempour A, Khosrowshahli M, Naghdibadi H, Dadpour MR, et al. Improved Taxol production by combination of inducing factors in suspension cell culture of *Taxus baccata*. *Cell Biol Int* 2006;30:262-9.
 11. Montazeri M, Azadbakht M, Najafi Zarrini H, NematZadeh GH, Pakdin Parizi A, Davoodi A. Hairy Root Cultures of *Hypericum perforatum* L.; A Promising Method for The High Scale Production of Hypericin. *J Med Plant* 2018;3:55-67.
 12. Newell CA. Plant transformation technology. *Mol Biotech* 2000;16:53-65.
 13. Brodelius P. Immobilized plant cells, in *Immobilized Cells and Organelles*. 2018, CRC Press. 27-56.
 14. Yue W, Ming QL, Lin B, Rahman K, Zheng CJ, Han T, et al. Medicinal plant cell suspension cultures: pharmaceutical applications and high-yielding strategies for the desired secondary metabolites. *Crit Rev Biotech* 2016;36:215-32.
 15. Caponetti JD, Gray DJ, Trigiano RN. *History of plant tissue and cell culture in Plant Tissue Culture Concepts and Laboratory Exercises*, Second Edition. 2018, Routledge. 11-17.
 16. Patra N, Srivastava AK. Artemisinin production by plant hairy root cultures in gas-and liquid-phase bioreactors. *Plant Cell Rep* 2016;35:143-53.
 17. Sivakumar G, Alba K, Phillips GC. Biorhizome: A Biosynthetic Platform for Colchicine Biomanufacturing. *Front Plant Sci* 2017;8:1-9.
 18. Khan T, Khan T, Hanob C, Abbasi BH. Effects of chitosan and salicylic acid on the production of pharmacologically attractive secondary metabolites in callus cultures of *Fagonia indica*. *Ind Crop Prod* 2019;129:525-35.
 19. Amiri S, Kazemitabar SK, Ranjbar GA, Azadbakht M. In vitro propagation and whole plant regeneration from callus in *Datura* (*Datura stramonium* L.). *African J Biotech* 2011;10:442-8.
 20. Amiri S, Kazemitabar SK, Ranjbar GA, Azadbakht M. The Effect of Different Carbon Sources and Concentrations and Growth Regulators on Anther Culture and Embryogenesis of *Datura stramonium* L. *J Plant Prod* 2011;18:77-91.
 21. Wei X, Chen J, Zhang C, Wang Z. In vitro shoot culture of *Rhododendron fortunei*: An important plant for bioactive phytochemicals. *Ind Crop Prod* 2018;126:459-65.
 22. Jiao J, Gai QY, Wang W, Zang YP, Niu LL, Fu YJ, et al. Remarkable enhancement of flavonoid production in a co-cultivation system of *Isatis tinctoria* L. hairy root cultures and immobilized *Aspergillus niger*. *Ind Crop Prod* 2018;112:252-61.
 23. Adenubi OT, Ahmed AS, Fasina FO, McGaw LJ, Eloff JN, Naidoo V. Pesticidal plants as a possible alternative to synthetic acaricides in tick control: A systematic review and meta-analysis. *Ind Crop Prod* 2018;123:779-806.
 24. Neyeloff JL, Fuchs SC, Moreira LB. Meta-analyses and Forest plots using a microsoft excel spreadsheet: step-by-step guide focusing on descriptive data analysis. *BMC Res Not* 2012;5:52-7.
 25. Ghosh B, Mukherjee S, Jha TB, Jha S. Enhanced colchicine production in root cultures of *Gloriosa superba* by direct and indirect precursors of the biosynthetic pathway. *Biotech Lett* 2002;24:231-4.
 26. Leek H, Thunberg L, Jonson AC, Öhlén K, Klarqvist M. Strategy for large-scale isolation of enantiomers in drug discovery. *Drug Dis Tod* 2017;22:133-9.
 27. Tierney T, Bodnár K, Rasmuson Å, Hudson S. Carrier particle design for stabilization and isolation of drug nanoparticles. *Int J Pharmaceut* 2017;518:111-8.
 28. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer lett* 2016;371:48-61.
 29. Shi L. Bioactivities, isolation and purification methods of polysaccharides from natural products: A review. *Int J Biolog Macromol* 2016;92:37-48.
 30. Blunt JW, Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR. Marine natural products. *Nat Prod Rep* 2017;34:235-94.
 31. Kim DW, Weon KY, Hong EP, Chung EK, Lee KT. Comparative physicochemical and pharmacokinetic properties of quetiapine and its active metabolite norquetiapine. *Chem Pharmaceut Bul* 2016;64:1546-54.
 32. Ochi M, Kimura K, Kanda A, Kawachi T, Matsuda A, Yuminoki K, et al. Physicochemical and pharmacokinetic characterization of amorphous solid dispersion of meloxicam with enhanced dissolution property and storage stability. *AAPS Pharm Sci Tech* 2016;17:932-9.
 33. Shakeran Z, Keyhanfar M, Asghari Gh, Ghanadian M. Improvement of atropine production by different biotic and abiotic elicitors in hairy root cultures of *Datura metel*. *Turk J Biol* 2015;39:111-8.
 34. Mohamed M, Scragg A. A comparison of the growth and alkaloids production by suspension and immobilized *Catharanthus roseus* cells in 61 and 71 bioreactors. *Immobil cell* 1990;1:335-42.
 35. Werner S, Maschke R, Eibl D, Eibl R. *Bioreactor Technology for Sustainable Production of Plant Cell-Derived Products*, in *Bioprocessing of Plant In Vitro Systems*, A. Pavlov and T. Bley, Editors. 2018, Springer International Publishing: Cham. 413-432.
 36. Price RN, Nosten F, Luxemburger C, ter Kuile FO, Paiphun L, Chongsuphajaisiddhi T, et al. Effects of artemisinin derivatives on malaria transmissibility. *Lancet* 1996;347:1654-8.
 37. Das A. Anticancer effect of antimalarial artemisinin compounds. *Annal Med Heal Sci Res* 2015;5:93-102.
 38. Kim WS, Choi WJ, Lee S, Kim WJ, Lee DC, Sohn UD, Shin HS, Kim W. Anti-inflammatory, Antioxidant and Antimicrobial Effects of Artemisinin Extracts from *Artemisia annua* L. *Kor J Physiol Pharmacol* 2015;19:21-7.
 39. Šramska P, Maciejka A, Topolewska A, Stepnowski P, Haliński ŁP. Isolation of atropine and scopolamine from plant material using liquid-liquid extraction and EXTrelut® columns. *J Chromat B* 2017;1043:202-8.
 40. Naveen P, Lingaraju HB, Deepak M, Medhini B, Prasad KS. Method Development and Validation for the Determination of Caffeine: An Alkaloid from *Coffea arabica* by High-performance Liquid Chromatography Method. *Pharmacog Res* 2018;10:88-91.
 41. Pohanka M. The perspective of caffeine and caffeine derived compounds in therapy. *Brat Lek List* 2015;116:520-30.
 42. Zhang F, Zhu G, Jacobson O, Liu Y, Chen K, Yu G, et al. Transformative nanomedicine of an amphiphilic camptothecin prodrug for long circulation and high tumor uptake in cancer therapy. *ACS Nano* 2017;11:8838-48.
 43. Su H, Zhang P, Cheetham AG, Koo JM, Lin R, Masood A, et al. Supramolecular crafting of self-assembling camptothecin

- prodrugs with enhanced efficacy against primary cancer cells. *Theranost* 2016;6:1065-74.
44. Mayer FP, Luf A, Nagy C, Holy M, Schmid R, Freissmuth M, et al. Application of a combined approach to identify new psychoactive street drugs and decipher their mechanisms at monoamine transporters, in *Neuropharmacology of New Psychoactive Substances (NPS)*. 2016, Springer. 333-50.
 45. Gu H, Han D. Use of Knock-In Mice to Investigate the Molecular Mechanism of Cocaine Action, in *The Neuroscience of Cocaine*. 2017, Elsevier. 135-42.
 46. Milano G, Maria Natta W, Bello A, Martelli A, Mattioli F. Codeine precipitating serotonin syndrome in a patient in therapy with antidepressant and triptan. *Clinic Psychopharmacol Neurosci* 2017;15:292-5.
 47. Gardiner SJ, Chang AB, Marchant JM, Petsky HL. Codeine versus placebo for chronic cough in children. *Coch Data Sys Rev* 2016;7:1-22.
 48. Angelidis C, Kotsialou Z, Kossyvakis C, Vrettou AR, Zacharoulis A, Kolokathis F, et al. Colchicine pharmacokinetics and mechanism of action. *Curr Pharmaceut Des* 2018;24:659-63.
 49. Dubey KK, Kumar P, Labrou NE, Shukla P. Biotherapeutic potential and mechanisms of action of colchicine. *Crit Rev Biotech* 2017;37:1038-47.
 50. Lipka AF, Vrinten C, Van Zwet EW, Schimmel KJ, Cornel MC, Kuijpers MR, et al. Ephedrine treatment for autoimmune myasthenia gravis. *Neuromuscul Dis* 2017;27:259-65.
 51. Saad B, Zaid H, Shanak S, Kadan S. Herbal-Derived Anti-obesity Compounds and Their Action Mechanisms, in *Anti-diabetes and Anti-obesity Medicinal Plants and Phytochemicals*. 2017, Springer. 129-144.
 52. Nikiforuk A, Potasiewicz A, Kos T, Popik P. The combination of memantine and galantamine improves cognition in rats: the synergistic role of the $\alpha 7$ nicotinic acetylcholine and NMDA receptors. *Behav Br Res* 2016;313:214-8.
 53. Golime R, Palit M, Acharya J, Dubey DK. Neuroprotective Effects of Galantamine on Nerve Agent-Induced Neuroglial and Biochemical Changes. *Neurotox Res* 201;33:738-48.
 54. Murray-Brown F, Davies IL. Oesophageal spasm, vomiting and hyoscine hydrobromide patch. *BMJ Supp Pall Care* 2016;6:125-7.
 55. Golding JF, Wesnes KA, Leaker BR. The effects of the selective muscarinic M3 receptor antagonist darifenacin, and of hyoscine (scopolamine), on motion sickness, skin conductance and cognitive function. *British J Clin Pharmacol* 2018;84:1535-43.
 56. Schintu N, Zhang X, Alvarsson A, Marongiu R, Kaplitt MG, Greengard P, et al. p11 modulates L-DOPA therapeutic effects and dyskinesia via distinct cell types in experimental Parkinsonism. *Proc Nat Acad Sci* 2016;113:1429-34.
 57. Valldeoriola F, Santacruz P, Ríos J, Compta Y, Rumià J, Muñoz JE, et al. L-Dopa/carbidopa intestinal gel and subthalamic nucleus stimulation: Effects on cognition and behavior. *Br Beh* 2017;7:848-854.
 58. Powers D, Erickson S, Swortwood MJ. Quantification of Morphine, Codeine, and Thebaine in Home-Brewed Poppy Seed Tea by LC-MS/MS. *J For Sci* 2018;63:1229-1235.
 59. As-Sanie S, Till SR, Mowers EL, Lim CS, Skinner BD, Fritsch L, et al. Opioid Prescribing Patterns, Patient Use, and Postoperative Pain After Hysterectomy for Benign Indications. *Obs Gyn* 2017;130:1261-8.
 60. Tseng TY, Ostroff JS, Campo A, Gerard M, Kirchner T, Rotrosen J, et al. A randomized trial comparing the effect of nicotine versus placebo electronic cigarettes on smoking reduction among young adult smokers. *Nicotine Tobacco Res* 2016;18:1937-43.
 61. Hong W, Hao BW, Peng F, Peng GY, Huang LM, Xu J, et al. The mechanism of nicotine on human bronchial smooth muscle cell contraction. *Chinese J Tubercul Respirat Dis* 2018;41:333-9.
 62. Rosshandler Y, Shen AQ, Cortes J, Khoury HJ. Omacetaxine mepesuccinate for chronic myeloid leukemia. *Exp Rev Hematol* 2016;9:419-24.
 63. Novotny L, Al-Tannak L, Hunakova L. Protein synthesis inhibitors of natural origin for CML therapy: semisynthetic homoharringtonine (Omacetaxine mepesuccinate). *Neoplasma* 2016;63:495-503.
 64. Chou YS, Yen CC, Chen WM, Lin YC, Wen YS, Ke WT, et al. Cytotoxic mechanism of PLK1 inhibitor GSK461364 against osteosarcoma: mitotic arrest, apoptosis, cellular senescence, and synergistic effect with paclitaxel. *Int J Oncol* 2016;48:1187-94.
 65. Khanna C, Rosenberg M, Vail D. A review of paclitaxel and novel formulations including those suitable for use in dogs. *J Veter Inter Med* 2015;29:1006-12.
 66. Park JH, Lee JY, Kim KT, Joe HE, Cho HJ, Shin YK, et al. Physostigmine-loaded liposomes for extended prophylaxis against nerve agent poisoning. *Int J Pharmaceut* 2018;553:467-73.
 67. Effenberger-Neidnicht K, Jägers J, Verhaegh R, Kirsch M. Therapeutic effects of physostigmine during systemic inflammation. *J Inflamm Res* 2018;11:465-75.
 68. Skaat A, Rosman MS, Chien JL, Mogil RS, Ren R, Liebmann JM, et al. Effect of pilocarpine hydrochloride on the Schlemm canal in healthy eyes and eyes with open-angle glaucoma. *JAMA Ophthalmol* 2016;134:976-81.
 69. Franco MC, Schimpl FC, Mazzafera P. Reference genes for quantitative RT-PCR of the pilocarpine producer *Pilocarpus microphyllus* and two other *Pilocarpus* species. *Theo Exper Plant Physiol* 2017;29:51-9.
 70. Kumari A, Singh D, Kumar S. Biotechnological interventions for harnessing podophyllotoxin from plant and fungal species: current status, challenges, and opportunities for its commercialization. *Crit Rev Biotech* 2017;37:739-53.
 71. Safarpour M, Yousefinejad M, Javadian H, Asfaram A, Ghasemi Z, Jaberli H, et al. Podophyllotoxin extraction from *Linum usitatissimum* plant and its anticancer activity against HT-29, A-549 and MDA-MB-231 cell lines with and without the presence of gold nanoparticles. *App Organomet Chem* 2018;32:4024-36.
 72. Bozic B, Uzelac TV, Kezic A, Bajcetic M. The Role of Quinidine in the Pharmacological Therapy of Ventricular Arrhythmias 'Quinidine'. *Mini Rev Med Chem* 2018;18:468-75.
 73. Bharti N, Mohapatra PP, Singh F. Plant-Based Antiamoebic Natural Products: Literature Review and Recent Developments. *Fro Nat Prod Chem* 2018;4:1-40.
 74. Jäger H. Quinine tree invasion and control in Galapagos: a case study, in *Understanding Invasive Species in the Galapagos Islands*. 2018, Springer. 69-76.
 75. Böttger A, Vothknecht U, Bolle C, Wolf A. Plant-Derived Drugs Affecting Ion Channels, in *Lessons on Caffeine, Cannabis & Co*. 2018, Springer. 121-40.
 76. Lunko O, Grushkovska IV, Lun'ko OO, Marchenko SM. Effect of tubocurarine on large-conductance cationic channels in the inner nuclear membrane of Purkinje neurons of the rat cerebellum. *Neurophysiol* 2016;48:332-5.
 77. Caputi L, Franke J, Farrow SC, Chung K, Payne RME, Nguyen TD, et al. Missing enzymes in the biosynthesis of the anticancer drug vinblastine in Madagascar periwinkle. *Sci* 2018;360:1235-9.
 78. Alam MM, M. Naem, Khan MAM, Uddin M. Vincristine and vinblastine anticancer catharanthus alkaloids: Pharmacological applications and strategies for yield improvement, in *Catharanthus roseus*. 2017, Springer. 277-307.
 79. Aboubakr EM, Taye A, Aly OM, Gamal-Eldeen AM, El-Moselhy MA. Enhanced anticancer effect of Combretastatin A-4 phosphate when combined with vincristine in the treatment of hepatocellular carcinoma. *Biomed Pharmacotherap* 2017;89:36-46.

80. Shimokawa T, Tsutsui H, Miura T, Nishinaka T, Terada T, Takama M, et al. Renoprotective effect of yohimbine on ischaemia/reperfusion-induced acute kidney injury through α 2C-adrenoceptors in rats. *Europ J Pharmacol* 2016;781:36-44.
81. Liu Y, Yu HY, Xu HZ, Liu JJ, Meng XG, Zhou M, et al. Alkaloids with Immunosuppressive Activity from the Bark of *Pausinystalia yohimbe*. *J Natl Prod* 2018;81:1841-9.
82. Schmitz C, Fritsch L, Fischer R, Schillberg S, Rasche S. Statistical experimental designs for the production of secondary metabolites in plant cell suspension cultures. *Biotech Lett* 2016;38:2007-14.
83. Jan T, Qadri R, Naqvi B, Adhikari A, Nadeem S, Muhammad A. A novel Salviolactamine from the callus culture of *Salvia santolinifolia* Boiss. *Nat Prod Res* 2018;32:749-54.
84. Grąbkowska R, Matkowski A, Grzegorzczak-Karolak I, Wysokińska H. Callus cultures of *Harpagophytum procumbens* (Burch.) DC. ex Meisn.; production of secondary metabolites and antioxidant activity. *S Afr J Bot* 2016;103:41-8.
85. Chung IM, Rekha K, Rajakumar G, Thiruvengadam M. Production of bioactive compounds and gene expression alterations in hairy root cultures of chinese cabbage elicited by copper oxide nanoparticles. *Plant Cell Tis Org Cul* 2018;134:95-106.
86. Hashemi SM, Naghavi MR. Production and gene expression of morphinan alkaloids in hairy root culture of *Papaver orientale* L. using abiotic elicitors. *Plant Cell Tis Org Cul* 2016;125:31-41.
87. Ochoa-Villarreal M, Howat S, Hong S, Jang MO, Jin YW, Lee EK, et al. Plant cell culture strategies for the production of natural products. *BMB Rep* 2016;49:149-158.
88. Moharrami F, Hosseini B, Sharafi A, Farjaminezhad M. Enhanced production of hyoscyamine and scopolamine from genetically transformed root culture of *Hyoscyamus reticulatus* L. elicited by iron oxide nanoparticles. *In Vitro Cell Develop Biol Plant* 2017;53:104-111.
89. Malik S, Cusidó RM, Mirjalili MH, Moyano E, Palazón J, Bonfill M. Production of the anticancer drug taxol in *Taxus baccata* suspension cultures: a review. *Proc Biochem* 2011;46:23-34.
90. Ni X, Wen S, Wang W, Wang X, Xu H, Kai G. Enhancement of camptothecin production in *Camptotheca acuminata* hairy roots by overexpressing ORCA3 gene. *J Appl Pharmaceut Sci* 2011;1:85-8.