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PHARMACEUTICAL USAGE OF SECONDARY METABOLITES EXTRACTED THROUGH PLANT TISSUE CULTURE

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ABSTRACT

With medicinal plants serving as raw material for herbal drugs, natural health products, and secondary metabolites, their demand and use is growing rapidly throughout the world. The secondary metabolites produced are beneficial to mankind as foods, medicines, and industrial raw materials. Many medicinal plants are on the brink of extinction as a result of overharvesting and habitat destruction. Many plants produce high-value secondary metabolites but are difficult to cultivate. Tissue culture has helped in healthy and rapid production of plants maintaining the characteristics and enhancing the production of secondary metabolites. Being independent of climatic and geographical conditions, tissue culture techniques will provide an incessant, economical and viable production of secondary metabolites and also prevent the collection of endangered wild species. In this review we discussed some important secondary metabolites like- Digitoxin, Vinblastine, Vincristine, Quinine, Ginsenoside, and Reserpine, their plant source, usage and their extraction through plant tissue culture.

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INTRODUCTION

Medicinal plants have many pharmacological effects that are dependent on their phytochemical constituents. Based on their role in the basic metabolic processes, these fall into two categories namely primary and secondary metabolites. Primary plant metabolites are involved in basic life functions, they are responsible for growth of plants and utilized as a source of food. On the other hand, secondary metabolites are not instantly concerned in the typical growth, development, or reproduction of an organism. They most often play a principal position in plant safety towards herbivory and other interspecies defenses. Secondary metabolites are often used in medical treatments, food flavorings, and recreational medicinal drugs. They are products of subsidiary pathways such as the shikimic acid pathway. Secondary plant metabolites played an important role in alleviating several ailments in the traditional medicine and indegenious uses. The compounds derived from them are used for the preparation of medications for the treatment of a vast range of ailments, from migraine up to cancer. The secondary metabolites are of great pharmaceutical significance because of their diverse pharmacological activities, and till date, their number with described structures exceeds 100,000. Secondary plant metabolites are classified according to their chemical structures into various classes (Hussein and El-Anssary, 2019; Srivastava *et al.*, 2020).

Pharmaceutically significant secondary metabolites, including alkaloids, glycosides, flavonoids, volatile oils, tannins, and resins, can be uniquely sourced from medicinal plants. Demands of these secondary metabolites are not readily accomplished because of their low yield in intact plants, unfavourable environment, geographical locations and/or governmental restrictions. Chemical synthesis or semi-synthesis of these metabolites are either extremely difficult or economically infeasible because of their highly complex structures and stereospecific chemical nature. Plant cell culture is an alternative, but to date it witnessed limited commercial success. Precursor feeding has been a successful approach for enhanced production of secondary metabolites from Plant cells grown in vitro (Namdeo *et al.*, 2007). 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 cosmetics, pharmaceutical industries, dietary supplements, flavor additives, fragrances, 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 techniques and optimizing their large-scale production using bioreactors. Plant tissue culture techniques being independent of climatic and geographical conditions will provide an incessant, sustainable, economical and viable production of secondary metabolites (Tiwari and Rana, 2015). The secondary metabolites, being commercially important, have aroused great interest in its industrial production. Researchers are exploring the possibilities of enhancing its production by tissue culture techniques. Plants cell culture technologies were introduced at the end of 1960'S as a possible tool for both studying and producing plant secondary metabolites. The focus of the present review is the application of tissue culture technology for the production of some other important plant secondary metabolites. Most polyphenol nutraceuticals from plant origin ought to endure intestinal transformations, by microbiota and enterocyte enzymes, as a way to be absorbed at enterocyte and colonocyte levels. This gives upward thrust to diverse priceless effects within the customer, including a mammoth array of protecting effects against viruses, bacteria, and protozoan parasites (Hakkinen et al., 2012).

The first documented use of plants of medicinal importance dates back to 1800 BC Papyrus Ebers. It is only less than 200 years ago the isolation of the first active chemical constituent (secondary metabolite) responsible for its pharmacological effect occurred. Many plants containing high-value secondary metabolites are difficult to cultivate or are becoming endangered because of overharvesting (Shakya, 2016). The global value of medicinal plants as sources of new drugs has increased with almost 80% of the population adapting herbal medicines over synthetic medicines due to their potent therapeutic efficacy and economic viability with no side effects. This property of the medicinal plants is attributed to the presence of metabolites in them. Every part of these plants has medicinal properties including flower, root, and stem, leaves, fruits, seed and whole plants. Herbal medicines, derived directly or indirectly are mostly isolated from wild species. With the increasing demand for herbal drugs, natural health products, and secondary metabolites of medicinal plants, these wild plants are facing extinction due to over exploitation (Chen *et al*., 2016; Taarit *et al*., 2009). Traditional methods of propagation using seeds and stem cuttings have not been able to keep up with the increased demand and overexploitation due to limitations such as seasonal barriers, pests and diseases which slows down production (Arikat *et al*., 2004). Environmental conditions, such as temperature, day length, and light, influence the quantity and quality of secondary metabolites. Tissue culture, at an industrial level, has helped in maintaining the characteristics consistent along with rapid production. It has provided suitable platforms for the production of healthy plant material. Being independent of climatic and geographical conditions, tissue culture techniques promise to provide an incessant, sustainable, economical and viable production of secondary metabolites. Beneficial uses of tissue culture for the purpose of extraction of secondary metabolites include avoidance of - collection of endangered wild species, production of secondary metabolites irrespective of seasonal and climatic conditions, and rapid production of secondary metabolites due to rapid growth of cultures in vitro (Chandran et al., 2020; Chevallier, 1996).

SOME IMPORTANT SECONDARY METABOLITES AND THEIR PHARMACEUTICAL USAGE

DIGITOXIN: Digitoxin is a cardiac glycoside.

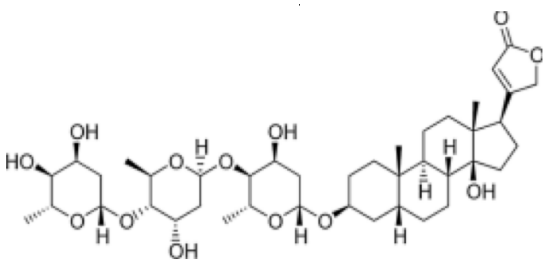
Plant Source: *Digitalis* L. genus (family Plantaginaceae) is a representative of several medicinal ornamental plants that are widely used in the production of herbal medicines (Bown, 1995). It includes two most important species –*Digitalis purpurea* L. (commonly known as Foxglove) (Figure 1) and *Digitalis lantana* (commonly known as Grecian Foxglove) (Figure 2).

Figure 1: *Digitalis purpurea L.* (Source: https://www.picfair.com/pics/07925012-purple-

foxglove-digitalis-purpurea, accessed on 2 December 2020.)

Figure 2: *Digitalis lantana* (Source: 10.1016/j.indcrop.2016.08.031)

Chemical Structure:



$C_{41}H_{64}O_{13}$

Uses: It was used earlier for the treatment of ulcers, boils, abscesses, headaches and paralysis. It was also externally used for the granulation of poorly healing wounds and to cure ulcers. After William Withering work, the digoxin is isolated from *Digitalis* species as a life-saving cardiac drug. It has a profound tonic effect upon heart disease, enabling the heart to beat more slowly, powerfully and regularly without requiring more oxygen. At the same time, it stimulates the flow of urine which lowers the volume of blood and lessens the load on the heart (Al-Snafi, 2017; Verma *et al.*, 2016). It is eliminated hepatically, making it useful in patients with poor or erratic kidney function. It is the commercial source of digoxin. But excessive use can lead to nausea, vomiting, slow pulse, visual disturbance, anorexia and fainting (Bhusare *et al.*, 2018).

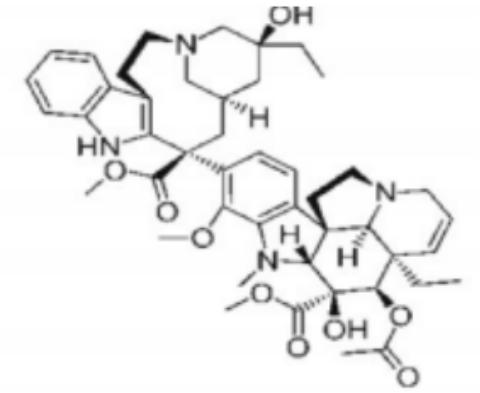
Production through tissue culture: Cardenolides are known to have an important role in tumor therapy. The chemical synthesis of cardenolides is arduous due to its structural complexity. To prevent the exhaustion of the natural *Digitalis* population and to improve the plant quality and genetic preservation of the superior seeds for future use, *In vitro* techniques can be used for the large-scale production of cardenolides. The production of useful secondary metabolites depends on the overall wellness of the plant from which extraction is to be made (Bota and Deliu, 2015). Direct regeneration without callus formation will produce true to type plantlets preventing somaclonal variants. Leaf and petiole have been used as explants for direct organogenesis to achieve rapid and large-scale clonal propagation, production and extraction of secondary metabolites, ex situ conservation (Barrales-Cureno *et al.*, 2019). Cell suspension cultures of *Digitalis lanata* supplemented with BA and IAA improved the flavonoids production. Therefore in vitro techniques can be successfully used for large scale propagation of *Digitalis lanata*.

VINBLASTINE & VINCRISTINE: Vinblastine and vincristine are vinca alkaloid anti-neoplastic agents.

Plant Source: Vinblastine and vincristine were originally found in Madagascar periwinkle, *Catharanthus roseus* (formally known as Vinca rosea, family Apocynaceae) (Figure 3).

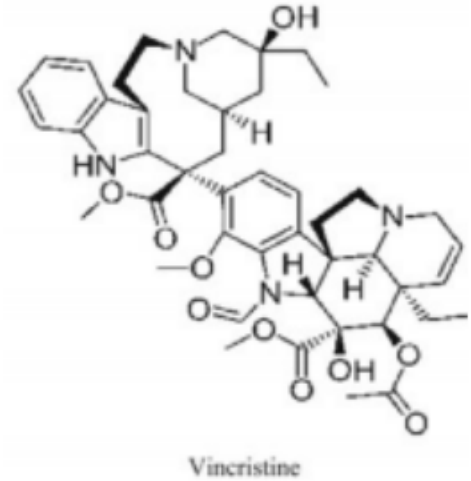
Figure 3: *Catharanthus roseus* (Source: Davis, A., https://www.nature.com/articles/d41586-018-05106-z)

Chemical Structure:



Vinblastine

Formula: $C_{46}H_{58}N_4O_9$



Vincristine

Formula: $C_{46}H_{56}N_4O_{10}$

Uses: The plant was traditionally used to treat diabetes by the natives of Madagascar, in India it has been used to treat ailments from wasp stings, in the Caribbean it has been used to treat eye infection. Vinblastine and vincristine were reported to lower the count of White Blood Cells. A high number of WBCs indicates leukemia. Vinblastine is used for treating Hodgkin's disease, advanced breast cancer and advanced testicular cancer. It can be used for the treatment of Kaposi's sarcoma, choriocarcinoma, Letterer-Siwe disease, head, neck and bladder cancers, melanoma, soft tissue sarcoma, mycosis fungoides (T-cell lymphoma), fibromatosis, germ cell tumor, and certain blood disorders such as histiocytes.

Vincristine has been reported to be used for the treatment of acute lymphocytic leukemia, lymphosarcoma, lymphogranulomatosis and solid infant tumors (Khashan and Husain, 2015; Mekky *et al.*, 2018).

Production through tissue culture: The accumulation of vincristine and vinblastine were low in *ex vitro* grown plants due to environmental conditions, physiological and developmental stages of the plant. Low yield of secondary metabolites and high costs for its isolation are the reasons for the use of plant tissue culture to increase the metabolite content (Maqsood and Abdul, 2017). The growth hormone kinetin and indole acetic acid had an enhancing effect on vincristine, vinblastine (Hanafy *et al.*, 2016). Induction of biotic stress using microbes that act as elicitors was found successful in increasing the secondary metabolite concentration in *Catharanthus roseus* (Maqsood and Abdul, 2017). Embryogenic callus of *C. roseus* was exposed to *Aspergillus flavus* which induced extracellular stress leading to increased synthesis of vincristine and vinblastine. Yeast extract as an elicitor had an enhancing effect on vincristine and vinblastine obtained from protoplast culture (Hanafy *et al.*, 2016).

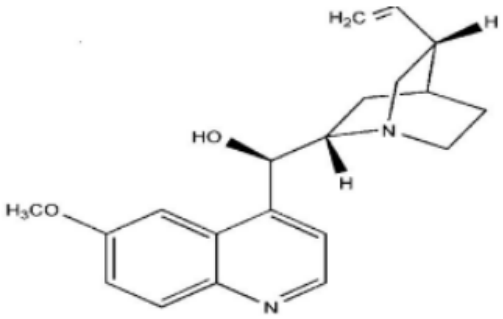
Agrobacterium rhizogenes -mediated transformation of *Catharanthus roseus* was used to increase the yield of vincristine and vinblastine. Maximum accumulation of vinblastine, vincristine and catharanthine were observed in transgenic hairy roots (Achan *et al.*, 2011; Hacker, 2009).

QUININE: Quinine is a cinchona alkaloid that belongs to the aryl amino alcohol group of drugs (Armijos-Gonzalez and Perez-Ruiz, 2016).

Plant Source: *Cinchona officinalis* (family Rubiaceae) is well known for its medicinal value, as the bark yields Quinine, an alkaloid compound that is effective treatment against malaria (Figure 4).

Figure 4: *Cinchona officinalis* (Source: https://www.healthbenefitstimes.com/cinchona/amp/)

Chemical Structure:



Formula: $C_{20}H_{24}N_2O_2$

Uses: Quinine, is derived from the bark of the *Chincona* (quina-quina) tree. It was used successfully to treat Malaria from as early as the 1600s. It was then referred to as the "Jesuits' bark," "cardinal's bark," or "sacred bark." as it was used in 1630 by Jesuit missionaries in South America. The occurrence of this disease is common in areas such as Africa, South America, and Southern Asia. Quinine is used alone or with medications to treat malaria caused by parasites. Parasites that cause malaria typically enter the body through mosquito bites. Quinine can cause serious side effects on heart, kidneys or blood cells (Pratiwi *et al.*, 2018).

Production through tissue culture: Over-exploitation of *Cinchona* for its secondary metabolites and its slow regeneration in its natural habitat has left this plant threatened. Moreover, they have limited distribution ranges and require specific conditions for their growth (Ratnadewi *et al.*, 2016). This problem has been overcome by plant cell culture which has been successful in producing secondary plant metabolites when exposed to stress (Peng *et al.*, 2012). Studies showed that the percentage of secondary metabolites were higher in in vitro propagated cinchona than in conventionally propagated cinchona (Zheng *et al.*, 2018).

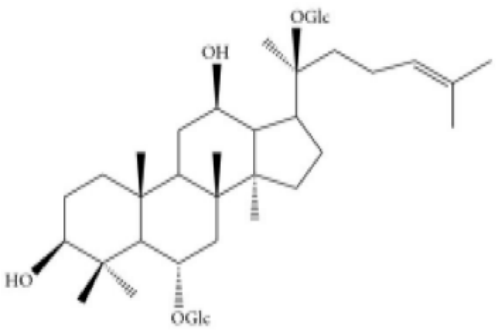
GINSENOSIDE:

Ginsenosides are a class of natural product steroid glycosides and triterpene saponins.

Plant Source: Ginsenoside is the bioactive component of the genus *Panax* (Araliaceae family) cultivated in China, Japan, Korea and Russia, as well as in the US and Canada. *Panax ginseng* has been most widely studied due to its use in traditional Chinese medicine (it is also known as Asian, Chinese or Korean Ginseng) (Figure 5). There are Ginsenosides unique to American Ginseng (*Panax quinquefolius*) and Japanese Ginseng (*Panax japonicus*) (Zheng *et al.*, 2018).

Figure 5: *Panax ginseng* -plants and roots (Source:
Grant, A., (2019)
https://www.gardeningknowhow.com/;
https://killcliff.com/)

Chemical Structure:



Formula: $C_{30}H_{52}O_2$

Uses: Ginseng root has been used as an oriental folk medicine for several thousand years. In the past 20 years it is a highly valued medicinal plant in the Far East and also popular in the West. About 40 ginsenosides have been identified from the root of *Panax ginseng* (Zhang *et al.*, 2014). Ginsenosides have unique biological activity and medicinal value, such as antitumor, anti-inflammatory, antioxidation, and inhibition of cell apoptosis. In recent years, some studies have shown that ginsenosides have a certain role in the prevention and treatment of neurological diseases. However, the research is still in its infancy, and the relevant mechanisms are complex (Song *et al.*, 2017).

Production through tissue culture: Conventional method of propagation has failed to meet the increasing demand for ginseng, leading to overexploitation of natural sources of wild ginseng. Several methods like classical tissue culture, bioreactor culture, *Agrobacterium*-mediated hairy root production, using elicitors in cell cultures, and mutation breeding by γ-irradiation have been used to increase the production of ginseng. Mutation breeding by γ-irradiation is currently being used for the genetic transformation of this species (Kim *et al.*, 2012). In in vitro propagation, root cultures exhibited faster growth rate, however, the content of secondary metabolites were low. Elicitors like jasmonic acid and methyl jasmonate (MeJA) were successful in increasing the content of ginsenoside though the biomass was reduced. *Bacillus stratosphericus*, an endophytic bacterium was found to be an effective elicitor increasing both the biomass and secondary metabolite content. Therefore, this bacterium helped in increasing metabolite content in root cultures which is generally low in secondary metabolites (https://www.britannica.com/science/reserpine, accessed on December 10, 2020). Genetic improvement and transformation in *Panax ginseng* were achieved

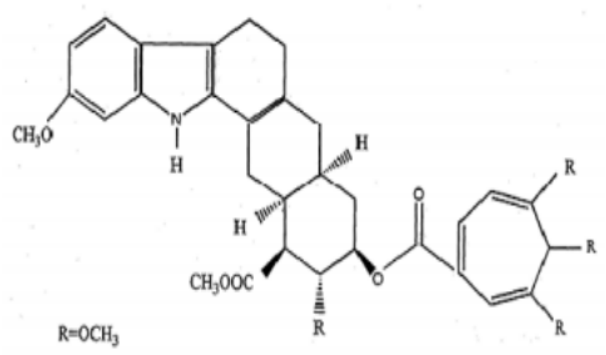
Genetic improvement and transformation in *Panax ginseng* were achieved by high proliferation and regeneration rate of somatic embryos.

RESERPINE:

Reserpine is a Rauwolfia alkaloid.

Plant Source: Reserpine is a naturally occurring alkaloid originally isolated from *Rauwolfia serpentina*, in 1952. It is also found in *Rauwolfia vomitoria* (Figure 6) *Rauwolfia* is indigenous to India, Burma, Malaysia, Thailand, Nepal, and Indonesia.

Chemical Structure:



Formula: $C_{33}H_{40}N_2O_9$

Uses: The powdered root of *Rauwolfia serpentina* historically has been used to treat snakebites, insomnia, hypertension (high blood pressure) and insanity. It was also used to treat schizophrenia (Rohela *et al.*, 2019). By lowering high blood pressure, Reserpine helps to prevent strokes, heart attacks, and kidney problems. It works by decreasing certain substances in the body (such as norepinephrine). This causes the blood vessels to relax so that blood can flow more easily and also slows the heart rate. These effects help to lower blood pressure (Kaur *et al.*, 2018).

Figure 6: *Rauwolfia serpentina* - plant and roots (Source: Bandyopadhya, D., (2019) https://www.boldsky.com/; Muller, M., (2015) https://www.homeremediess.com/)

Production through tissue culture: *Rauwolfia serpentina*, an important medicinal herb, is being over exploited due to its increased use in pharmaceutical industries (Alatar, 2015).Due to the presence of a strong seed inhibitor, cinnamic acid derivatives, the percentage of seed germination is low. Therefore in vitro techniques have been used for successful rapid propagation of this plant (Kad *et al.*, 2017). Micropropagation of Rauwolfia tetraphylla, using leaf and stem-based callus, was successful in producing plantlets of true to type genotype of the plant -

along with its mass propagation (Alatar, 2015). Thidiazuron was used for ex vitro conservation and improvement of secondary metabolites in *Rauwolfia serpentina* (Mahadik *et al.*, 2020). Suspension cultures were found to be successful in producing *Rauwolfia serpentina* plantlets within a short time interval (Ahmad *et al.*, 2019). The callus culture of *Rauwolfia serpentina* produced secondary metabolites of improved quality than those produced by its root extract (Isah, 2019).

EXTRACTION OF SECONDARY METABOLITES THROUGH PLANT TISSUE CULTURE

Plants are an incredible source of drugs and other products of diverse applications. Leaves, stems, roots, meristems, etc. can be used as explants for rapid multiplication and production of secondary metabolites. High producing cell lines and optimum mediums, transformation methods have led to improvement in secondary metabolite production and recombinant bioactive products. Production of secondary metabolites through the hairy root system is also an efficient method of secondary metabolite production in the plant roots. E.g., *Agrobacterium rhizogenes* inoculation (Jones and Kinghorn, 2012).

In the growth condition(s) of plants, numerous secondary metabolites (SMs) are produced by them to serve a variety of cellular functions essential for physiological processes, and recent increasing evidence has implicated stress and defense response signaling in their production. The type and concentration(s) of secondary molecule(s) produced by a plant are determined by the species, genotype, physiology, developmental stage and environmental factors during growth. This suggests the physiological adaptive responses employed by various plant - taxonomic groups in coping with the stress and defensive stimuli. The past decades had witnessed renewed interest to study abiotic factors that influence secondary metabolism during in vitro and *in vivo* growth of plants. Application of molecular biology tools and techniques are facilitating understanding the signaling processes and pathways involved in the SMs production at subcellular, cellular, organ and whole plant systems during in vivo and in vitro growth, with application in metabolic engineering of biosynthetic pathways intermediates (Sasidharan *et al.*, 2011). Apart from general methods of extraction, selective methods are used to isolate specific classes of phytochemicals and interfering compounds. Successful extraction begins with careful selection and preparation of plant samples. It is also important to minimize interference compounds from co-extracting with the target compounds to avoid contamination of the extract, as well as to prevent decomposition of important metabolites (Mohiuddin, 2018). General methods of isolation of bioactive compounds are sonification, heating under reflux, soxhlet extraction and maceration. However, modern extraction techniques include solid-phase micro-extraction, supercritical-fluid extraction, chromatography, pressurized-liquid extraction, microwave assisted extraction, solid-phase extraction, and surfactant-mediated techniques are preferred due their ease of automation and other advantages like isolation of pure compounds, reduction in organic solvent consumption and in sample degradation, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement in extraction efficiency, selectivity, and/ kinetics of extraction (Gorlenko *et al.*, 2020).

CONCLUSION

Biotechnology is a vital alternative in the production of pharmaceutical plant secondary metabolites to support industrial production and mitigate over-exploitation of natural sources. Plants have been used for medicinal purposes since ancient times. Increasing demand of sustainable and cost-effective natural phytochemicals from plants demands their mass cloning through plant tissue culture approaches. High-value pharmaceuticals that include alkaloids, flavonoids, terpenes, steroids, among others, are biosynthesized as a defensive strategy by plants in response to perturbations under natural environmental conditions. However, they can also be produced using plant cell, tissue, and organ culture techniques through the application of various in vitro approaches and strategies. 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.

Most of the herbal drugs produced currently in the majority of the developing countries lack proper quality specification and standards. Herbal drugs used in traditional medicine may contain a single herb or combinations of several different herbs. Both the raw drugs and the finished herbal products manufactured contain complex mixtures of organic compounds, such as fatty acids, sterols, alkaloids, flavonoids, polyphenols, glycosides, saponins, tannins, terpenes etc. Bacterial drug resistance has grown in the last decades, but the rate of discovery of new antibiotics has steadily decreased. Therefore, the search for new effective antibacterial agents has become a top priority. Plants can be considered as a source of antibacterial agents as they are readily available and cheap, extracts or compounds from plant sources often demonstrate high-level activity against pathogens, and they rarely have severe side effects. The huge variety of plant-derived compounds provides very diverse chemical structures that may supply both the novel mechanisms of antimicrobial action and provide us with new targets within the bacterial cell. The rapid development of modern biotechnologies has opened up many ways for obtaining enhanced bioactive compounds of consistent quality in environmentally friendly and low-toxic conditions at a faster rate.

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REFERENCES

Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N., ... and D'Alessandro, U. (2011). Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malaria journal, 10(1), 1-12. https://doi.org/10.1186/1475-2875-10-144

Ahmad, B., Raina, A., and Khan, S. (2019). Secondary Metabolite Production in Medicinal Plants Using Tissue Cultures. In Medically Important Plant Biomes: Source of Secondary Metabolites (pp. 133-152). Springer, Singapore. https://doi.org/10.1007/978-981-13-9566-6_7

Al-Snafi, A. E. (2017). Phytochemical constituents and medicinal properties of Digitalis lanata and Digitalis purpurea-A review. Indo American Journal of Pharmaceutical Sciences, 4(2), 225-234.http://doi.org/10.5281/zenodo.344926.

Alatar, A.A. (2015). Thidiazuron Induce deficient in vitro multiplication and ex vitro conservation of Rauvolfia serpentina – A Potent Antihypertensive Drug Producing Plant. Biotechnology & Biotechnological Equipment, 29(3), 489-497. http://dx.doi.org/10.1080/13102818.2015.1017535

Arikat, N. A., Jawad, F. M., Karam, N. S., and Shibli, R. A. (2004). Micropropagation and

accumulation of essential oils in wild sage (Salvia fruticosa Mill.). Scientia Horticulturae, 100(1-4), 193-202. https://doi.org/10.1016/j.scienta.2003.07.006

Armijos-González, R., and Pérez-Ruiz, C. (2016). In vitro germination and shoot proliferation of the threatened species Cinchona officinalis L (Rubiaceae). Journal of forestry research, 27(6), 1229-1236. https://doi.org/10.1007/s11676-016-0272-8

Barrales-Cureño, H. J., Reyes, C. R., García, I. V., Valdez, L. G. L., De Jesús, A. G., Ruíz, J. A. C., and Montoya, J. M. (2019). Alkaloids of Pharmacological Importance in Catharanthus roseus. In Alkaloids-Their Importance in Nature and Human Life. IntechOpen. https://doi.org/10.5772/intechopen.82006

Bhusare, B. P., John, C. K., Bhatt, V. P., and Nikam, T. D. (2018). In vitro propagation of Digitalis lanata Ehrh. through direct shoot regeneration–A source of cardiotonic glycosides. Industrial Crops and Products, 121, 313-319.https://doi.org/10.1016/j.indcrop.2018.05.019

Bota, C., and Deliu, C. (2015). Effect of plant growth regulators on the production of flavonoids by cell suspension cultures of Digitalis lanata. Farmacia, 63(5), 716-719. URL: http://www.revistafarmacia.ro/201505/art-15-Bota_Deliu_716-719.pdf

Bown, D. (1995). Encyclopaedia of Herbs and Their Uses. Dorling Kindersley, London 1995, ISBN 0-7513-020-31.

Chandran, H., Meena, M., Barupal, T., and Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotechnology Reports, e00450. https://doi.org/10.1016/j.btre.2020.e00450

Chen, S. L., Yu, H., Luo, H. M., Wu, Q., Li, C. F., and Steinmetz, A. (2016). Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chinese medicine, 11(1), 1-10. 10.1186/s13020- 016-0108-7

Chevallier, A. (1996). The Encyclopedia of Medicinal Plants. Dorling Kindersley. London. ISBN 9-780751-303148.

Gorlenko, C. L., Kiselev, H. Y., Budanova, E. V., Zamyatnin, A. A., and Ikryannikova, L. N. (2020). Plant Secondary Metabolites in the Battle of Drugs and Drug-Resistant Bacteria: New Heroes or Worse Clones of Antibiotics?. Antibiotics, 9(4), 170. https://doi.org/10.3390/antibiotics9040170

Hacker, M. (2009). History of pharmacology-From antiquity to the twentieth century. In Pharmacology. 1-7, Academic Press. https://doi.org/10.1016/B978-0-12-369521-5.00001-4

Häkkinen, S. T., Ritala, A., Rischer, H., and Oksman-Caldentey, K. M. (2012). Medicinal plants, engineering of secondary metabolites in cell cultures. In Encyclopedia of Sustainability Science and Technology (pp. 6519-6538). Springer.

Hanafy, M.S., Matter, M.A., Asker, M.S. and Rady, M.R. (2016). Production of Indole alkaloids in Hairy Root Cultures of Catharanthus roseus L. and their Antimicrobial Activity. South African Journal of Botany, 105. 9-16. https://doi.org/10.1016/j.sajb.2016.01.004

Hussein, R. A., and El-Anssary, A. A. (2019). Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. Herbal Medicine, 1, 13. https://doi.org/10.5772/intechopen.76139

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. Biological research, 52. http://dx.doi.org/10.1186/s40659-019-0246-3

Jones, W. P., and Kinghorn, A. D. (2012). Extraction of plant secondary metabolites. Natural products isolation, 341-366. https://doi.org/10.1007/978-1-61779-624-1_13

Kad, A., Pundir, A., Sharma, S., and Sood, H. (2017). Development of suspension cultures and ex-vitro rooting in Rauwolfia serpentina for rapid and large scale multiplication. International Journal of Innovative Research in Science & Engineering, 3, 135-143.

Kaur, S. (2018). In vitro callus Induction in Inflorescence Segments of Medicinally important

endangered plant Rauwolfia Serpentina (L.) Benth. Ex Kurz – A Step Towards Ex Situ conservation. Annals of Plant Sciences, 7.2, 1986-1991. http://dx.doi.org/10.21746/aps.2018.7.2

Khashan, K. T., and Husain, M. A. (2015). Effect of biotic factors stresses on vinblastine and vincristine production from callus of *Catharanthus roseus*. Euphrates J Agric Sci, 7(2), 25-41.

Kim, Y. J., Lee, O. R., Kim, K. T., and Yang, D. C. (2012). High frequency of plant regeneration through cyclic secondary somatic embryogenesis in *Panax ginseng*. Journal of ginseng research, 36(4), 442. https://doi.org/10.5142/jgr.2012.36.4.442

Mahadik, S.M., Sawardekar, S.V., Kelkar, V.G and Gokhale, N.B. (2020). In vitro regenerationtechnique in Rauwolfia serpentina and quantification of Reserpine. International Journal Of Chemical Studies, 8(6), 520-525. https://doi.org/10.22271/chemi.2020.v8.i6h.10827

Maqsood, M. and Abdul, M. (2017). Yeast Extract Elicitation Increases Vinblastine And Vincristine Yield in Protoplast Derived Tissues and Plantlets in *Catharanthus roseus*. *Revista Brasileirade* Farmacognosia, 27(5), 549-556. https://doi.org/10.1016/j.bjp.2017.05.008

Mekky, H., Al- Sabahi, J and Abdel Kreem, M.F.M. (2018). Potentiating biosynthesis ofthe Anticancer alkaloid's Vincristine and Vinblastine in callus cultures of Catharanthus roseus. South African Journal of Botany 114, 29-31. https://doi.org/10.1016/j.sajb.2017.10.008

Mohiuddin, A. K. (2018). Secondary metabolism and therapeutic efficacy of medicinal plants. Journal of Pharmaceutical and Biological Sciences, 6(4), 104-108. https://doi.org/10.18231/2320-1924.2018.0016

Namdeo, A. G., Jadhav, T. S., Rai, P. K., Gavali, S., and Mahadik, K. R. (2007). Phcog Rev.: Review Article Precursor feeding for enhanced production of Secondary metabolites: A review. Pharmacognosy reviews, 1(2).

Peng, D., Wang, H., Qu, C., Xie, L., Wicks, S. M., and Xie, J. (2012). Ginsenoside Re: its chemistry, metabolism and pharmacokinetics. Chinese Medicine, 7(1), 1-6. https://doi.org/10.1186/1749-8546-7-2

Pratiwi, D. R., Sari, P. T., and Ratnadewi, D. (2018). Cinchona cells performance in in vitro culture: quinine alkaloid production with application of different elicitors. In IOP Conference Series: Earth and Environmental Science (Vol. 185, 1, 012029). IOP Publishing. https://doi.org/10.1088/1755-1315/185/1/012029

Ratnadewi, D., Satriawan, D., and Pratiwi, D. R. (2016). Alkaloid quinine in cell suspension culture of Cinchona ledgeriana Moens: biological study towards industrial application. International Proceedings of Chemical, Biological and Environmental Engineering (IPCBEE), 97, 57-62. https://doi.org/10.7763/IPCBEE

Rohela, G.K.,Jogam, P.,Bylla, P and Reuben, C. (2019). Indirect Regeneration and Assessment of Genetic Fidelity of Acclimated Plantlets by SCOT, ISSR, and RAPD Markers In Rauwolfia tetraphylla L.: An Endangered Medicinal Plant. Biomed Research International, Volume 2019. https://doi.org/10.1155/2019/3698742

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., and Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines, 8(1). https://doi.org/10.4314/ajtcam.v8i1.60483

Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. International Journal of Herbal Medicine, 4(4), 59-64. 10.13140/RG.2.1.1395.6085

Song, X., Wu, H., Yin, Z., Lian, M. and Yin, C. (2017). Endophytic Bacteria Isolated from Panax Ginseng Improves Ginsenoside Accumulation in Adventitious Ginseng Root Culture. Molecules, 22(6), 837.

Srivastav, V. K., Egbuna, C., and Tiwari, M. (2020). Plant secondary metabolites as lead compounds for the production of potent drugs. In Phytochemicals as lead compounds for new drug discovery (pp. 3-14). Elsevier

https://doi.org/10.1016/B978-0-12-817890-4.00001-9

Taarit, M. B., Msaada, K., Hosni, K., Hammami, M., Kchouk, M. E., and Marzouk, B. (2009). Plant growth, essential oil yield and composition of sage (Salvia officinalis L.) fruits cultivated under salt stress conditions. Industrial Crops and Products, 30(3), 333-337. https://doi.org/10.1016/j.indcrop.2009.06.001

Tiwari, R., and Rana, C. S. (2015). Plant secondary metabolites: a review. International Journal of Engineering Research and General Science, 3(5), 661-670.

Verma, S.K., Das, A.K., Cingoz, G.S. and Gurel, E. (2016). In Vitro Culture of Digitalis L. (Foxglove) and the Production of Cardenolides: An Up-To-Date Review. Industrial Crops And Products, 94, 20-51. https://doi.org/10.1016/j.indcrop.2016.08.031

Zhang, J. Y., Sun, H. J., Song, I. J., Bae, T. W., *et al*. (2014). Plant regeneration of Korean wild ginseng (Panax ginseng Meyer) mutant lines induced by γ-irradiation (60Co) of adventitious roots. Journal of ginseng research, 38(3), 220-225. https://doi.org/10.1016/j.jgr.2014.04.001

Zheng, M., Xin, Y., Li, Y., Xu, F., Xi, X., Guo, H., and Han, C. (2018). Ginsenosides: a potential neuroprotective agent. BioMed research international. Article ID 8174345. https://doi.org/10.1155/2018/8174345