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Review Article

Plant Bioactive Compounds: Crucial Pharmacological Properties and Role of Elicitors in Enhancing Production

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ABSTRACT

Plant secondary metabolites play a vital role in developing numerous industrial products. Nutritive products developed from natural sources have better acceptance and demand than chemically synthesized products. Biologically active secondary metabolites play an important role in synthesizing various pharmaceutical drugs, and demand for its industrial manufacture is on the rise. *In vitro* cell suspension cultures using elicitors are a means of increasing the production of secondary metabolites in plant cells. Plant cells subjected to stress can be regulated to produce secondary metabolites using biotic and abiotic elicitors leading to either increase in cell volume or an increase in the rate of cell division, or both. The review aims to present the primary role of bioactive compounds and the potential of regulated *in vitro* cell suspension cultures in elevating the yield of pharmacologically crucial plant secondary metabolites via the active participation of various elicitors.

Keywords: Culture, Secondary metabolites, Elicitors, Bioactive compounds, Pharmacological properties.

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INTRODUCTION

Plants are an indispensable source of food, nutrition, and medicine and are entwined with our well-being. Despite various synthetic compounds and products being established by the industries, the world is highly promoting natural and organic products. Plant metabolites have a recognizable critical role in profuse plant mechanisms viz., formulating the plant structure, stimulatory and inhibitory action towards enzyme productions, cofactor for improved catalytic activity, defense mechanism, and fuel production. Primary metabolites are actively engaged in the growth and maturation of plants; on the contrary, secondary metabolites are less involved in plant fundamentals that comprise a varied array of physiologically bioactive compounds that originate biosynthetically from plant primary metabolites and often employed for plant defense mechanisms and color characterization.[1] In particular, higher plants develop secondary metabolites for their protection against pests and insects and have an indispensable metabolic activity.[1] Furthermore, the lack of secondary metabolites has long-term consequences on an organism's capacity for survival, reproduction, and aesthetic preservation rather than instantaneous death. Within a phylogenetic cluster, these traits are frequently limited to a few species and exhibit differences in the nature and quantity of secondary metabolites among plant species that thrive in distinct geographical locations.[2] An extensive variety of secondary metabolites, including alkaloids, flavonoids, anthocyanins, lignans, quinones, peptides, phenolics, terpenoids, and amines, are primarily employed in agricultural fertilizers, nutritional supplements, and resonate either directly or indirectly with the aroma, color, and flavor of the plants.[1,2]

From an evolutionary pharmacological standpoint, secondary metabolites constitute an intriguing collection of bioactive substances that have been employed by humans as herbal drugs, toxins, arrow poisons, and insecticides.[1] Consequently, bioactive compounds hold a potential background and have attracted investments in natural by-products with a hike in market prices.[2,3] The current issue in pharmacology is to characterize and comprehend the diversity of secondary metabolites, and their ways of action either individually or in naturally occurring combinations as seen in plants. Another fascinating challenge is to investigate the type of plants that have been employed in different traditional medical systems across the world, analyze their phytochemistry, and describe whether or not their secondary metabolites might have contributed to the documented pharmacological activity and to improve the synthesis of therapeutically crucial secondary metabolites.[4] The prevailing threat environment is the main factor influencing

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the occurrence of specific potential bioactive compounds. For instance, leaves typically produce phytoalexins under stress conditions, which act as antimicrobial stress-suppressor metabolites.[4] In this context, secondary metabolites have been extracted only in smaller amounts and hence several techniques have emerged to mass-produce secondary metabolites. Plant tissue culture technique is considerably practiced for several factors such as (i) for the cultivation of indigenous plants, (ii) for high product yield, (iii) for automated cell growth control, (iv) for cost-effectiveness, and (v) for eliciting bioactive compounds.[5] In contrast to plants grown in the field, tissue, and organ cultures have greater growth proliferation with a relatively short biosynthesis cycle.[6] Besides, these tissue or organ cultures are prone to proliferate at their optimum growth rates in a secure environment, unlike field plants subjected to climate, ecology, and environment.[6] Recent research experiments have demonstrated that elicitation considerably affects the increased production of bioactive compounds by understanding their metabolic pathway. Although deciphering the metabolism of secondary metabolites is a demanding process, it is essential to understand the mechanism for improving metabolite production. The central theme of this study is to highlight alternative approaches for enhancing the production of pharmacologically critical bioactive compounds in high demand by therapeutic industries that are achieved through the utilization of various elicitors employing diverse culture systems. This resourceful review will help investigators derive potential frameworks to carry out research, related to, enhancing plant metabolite production, through cell suspension cultures, the means of quantifying and testing their significance for medicinal grounds.

Supreme Contribution and Classification of Bioactive Compounds

The predominant contributions of bioactive compounds are as follows. They (a) act as a supreme weapon that is highly employed against microorganisms, (b) act as an agent for transporting metal ions, (c) act against herbivores, arthropods, and vertebrates by releasing harmful substances like glycol cyanide which leads to a perilous impact (d) act as hormones for plant reproduction and (e) ultimately serve as an effector for differentiation.[7] A wide range of therapeutic and pharmacological benefits have been discovered as a result of comprehensive biological analyses into the role of bioactive compounds. Some prominent plant secondary metabolites of industrial significance are enumerated as follows. Codeine from *Papaver somniferum* is frequently prescribed by medical professionals to alleviate intense coughing and is structurally akin to morphine, which shares a close resemblance and acts as a controlled drug;[8] to treat taxol-resistant chronic ovarian cancer, the therapeutic approach involves the administration of camptothecin (quinoline-alkaloid) from *Camptotheca accuminata*;[9] malaxin, an alkaloid containing dihydroartemisinin extracted from *Vanda hindsii*, has been applied in diverse regions of Africa and Korea for the precise management of *Plasmodium falciparum* malaria;[10] inhibition of angiogenesis and halting the migration or spreading of stomach and breast malignancies, denbinobin (1,4-phenanthrenequinone) isolated from *Ephermerantha lonchophylla* reduces the emergence of tumors.[11] Based on whether a nitrogen molecule is present or absent, secondary metabolites are divided into two classes as nitrogen-containing metabolites which include alkaloids, and nitrogen-free metabolites including terpenoids and phenolics.[4]

Nitrogen-Containing Secondary Metabolites

Alkaloids

Alkaloids are one of the most potent classes of secondary metabolites and are prevalent in a vast range of plants (angiosperms).[4,12,13] In general, alkaloids are renowned for being animal toxins and they do indeed primarily exist as chemicals of defense against animals. These secondary metabolites are vital for both pharmacological and therapeutic treatments (Table 1). For example, coupling a chemotherapy treatment with miscellaneous lipophilic alkaloids and polymers of ABC transporters could be an endeavor to combat multi-resistant cancer.[14]

Nitrogen-Free Secondary Metabolites

Terpenoids

Terpenoids represent the major part of secondary plant metabolites that confer essential functions related to crop productivity and plant defense.[20-22] They are made up of the most significant class of biologically active substances found in plants and have around 23,000 different structural variants which originate from acetate through the mevalonic acid channel. They are isoprene derivatives of polymeric nature distinguished by the number of isoprene units they contain.[23,24] Terpenoids are slightly more efficient against a variety of pathogens, notably membrane-embedded viruses, and are being used extensively in herbal therapy to treat infections (Table 1).

Phenolics

Phenolics are one of the ultimate subsets of plant secondary metabolites encompassing more than 8000 identified components, making up a comprehensive class of bioactive compounds.[24] Traditionally, phenolics are found as glycosides or esters coupled to other naturally occurring substances including alcohols, sterols, and flavonoids, and are biosynthesized through the Shikimic acid pathway (phenylpropanoid pathway) which also builds the primary metabolism by facilitating the production of aromatic amino acids.[26,27] The majority of phenolic compounds get polymerized into more substantial molecules known as polyphenols, which are critical among several phenolic compounds because of their ample distribution throughout nature, diversity, and their potential contribution to

Table 1: Pharmacological effects of bioactive compounds belonging to major secondary metabolite groups from various plant species.

Type of secondary metabolite		Plant source	Bioactive compound	Pharmacological and therapeutic value
Alkaloids	*Amaryllidaceae* alkaloids	*Galanthus woronowii.*	Galanthamine	To treat Alzheimer's disease by inhibiting cholinesterase.[14,15]
	Tryptamines	*Psilocybe mexicana.*	Psilocin	Utilized as a neurotransmitter (5-hydroxy tryptamine) drug provides psychedelic illusions and euphoric emotions.[14,15]
	Colchicine	Genera of *Colchicum.*	Colchicine	Used as a drug to treat acute gout by limiting the migration of macrophages toward inflammatory joints.[14,15]
	Diterpene alkaloids	*Aconitum* species.	Aconitine	Utilized to alleviate neurological pain, such as that brought on by trigeminus nerve inflammation.[14-16]
		Veratrum species	Protoveratrine B	
	Ergot alkaloids	*Rivea corymbosa.*	Ergotamine	To alleviate migraine in obstetrics.[14,15]
			Ergometrine	Employed to control bleeding, following delivery, and abortion.[14,15]
	Indole alkaloids	*Rauvolfia serpentina.*	Ajmaline	Act as antiarrhythmic and antihypertensive.[14,15]
		Physostigma venenosum.	Physostigmine	Practiced for the treatment of Alzheimer's disease as well as a miotic in eye treatment.[14,15]
		Camptotheca acuminate.	Camptothecin	Augmented as a cancer treatment drug that inhibits DNA topoisomerase.[14,15]
		Rauvolfia serpentina.	Reserpine	Functions as a tranquilizer and antihypertensive drug by preventing neurotransmitter transporters.[14,15]
		Sceletium species.	Mesembrine	Function as an antidepressant.[14,15]
	Isoquinoline alkaloids	*Chelidonium majus.*	Chelidonine	Employed as a spasmolytic, cholagogue, diuretic, and analgesic medication in ethnomedicine, as well as to cure warts, asthma, abdominal pain, and spasms.[14,17]
		Peumus boldo	Aporphine	To alleviate cholelithiasis and hepatic dysfunction.[14]
		Psychotria ipecacuanha.	Emetine, cephaeline	Used as an anti-amoebic, emetic, and expectorant.[14]
		Stephania species.	Cepheranthine	To treat leprosy and tuberculosis.[14,15]
	Phenylpropylamines	*Ephedra* species.	Ephedrine	To treat sinusitis, rhinitis, and asthma.[14,15]
	Piperidine alkaloids	*Lobelia* species.	Lobeline	Employed for the development of anti-smoking drugs through inhibition of ABC transporter.[14,15,18]
		Punica granatum.	Pelletierine	Utilized to combat gastrointestinal parasitic tapeworms.[14,15]

	Purine alkaloids	*Camellia sinensis, Coffea arabica, Cola acuminata, Cola nitida, Ilex paraguarensis, Paullinia cupana, Theobroma cacao.*	Caffeine, theophylline, and theobromine	Serve as central nervous system agonists which promote intensifying mental activity and alertness while inhibiting adenosine receptors and cAMP phosphodiesterase.[14,15]
	Pyrollidine alkaloids	*Nicotiana tabacum.*	Nicotine	Prolifically employed in agriculture as a natural pesticide.[14]
	Pyrrolizidine alkaloids	*Crotalaria, Heliotropium, Senecio* species.	Senecionine, heliotrine	Used to treat diabetes.[14,15]
	Quinolizidine alkaloids	*Cytisus scoparius.*	Sparteine	Utilized medically during pregnancy (causes contractions in the uterus); To alleviate cardiac arrhythmia as sodium channel blockers.[14,15]
	Quinolone alkaloids	*Acanthaceae, Rubiaceae, Rutaceae* species.	Quinine, quinidine, cinchonidine	To treat malaria. Shows antiarrhythmic characteristics via inhibiting sodium channels.[4,19]
	Steroid alkaloids	*Solanum* species.	Solanine and chaconine	Employed as a drug to treat inflammation. Acts as a natural insecticide in agriculture.[14,15]
	Tropane alkaloids	*Atropa* species.	Atropine	Utilized medicinally to alleviate smooth muscle spasms of the bronchia, gastrointestinal, urinary tract, and gall ducts. Employed for the treatment of hyperhidrosis and bradycardic arrhythmia. Used locally as mydriatic and cycloplegic drug to improve eye diagnosis.[14,15]
		Scopolia species.	Scopolamine	Used to alleviate travel illness as transdermal bandages.[14,15]
		Hyoscyamus species.	Hyoscyamine	Used as a drug to prevent narcosis due to its sedative qualities.[14,15]
Terpenoids	Monoterpenes	*Cymbopogan citratus.*	Citral	Employed in aromatherapy and utilized to ameliorate rheumatism and microbial infections.[25]
	Iridoid glucosides	*Gentianaceae* and *Menyanthaceae.*	Gentiopicrosides	Possess a bitter taste and are carried out to enable better digestion and increase their appetite.[14,15]
	Diterpenes	*Daphne mezereum*	Mezerein	Employed as a laxative drug.[14]
	Steroidal glycosides	*Cucurbitaceae* species.	Cucurbitacins	Prevent tumor growth *in vivo* and *in vitro*; Applied to treat nasopharyngeal cancer.[14]
	Saponins	*Glycyrrhiza glabra.*	Glycyrrhizic acid	It possesses anti-inflammatory properties.[14]
	Sesquiterpene	*Artemisia annua.*	Artemisin	Artesunate is a recently generated strong antimalarial medication that combats the potentially fatal *Plasmodium falciparum* parasites.[14,15]

Phenolics	Phenylpropanoids	*Lamiaceae* species.	Rosmarinic acid	Possess anti-viral and anti-inflammatory characteristics.[14,15]
		Psoralea bituminosa.	Furanocoumarins	Utilized to alleviate vitiligo and psoriasis as they can inhibit multiplying keratocytes in the epidermis when exposed to ultraviolet rays.[14,15,29]
		Aronia, Euterpe, Punica, Vaccinium, Vitis species.	Anthocyanins	Employed in nutraceuticals and phytomedicine as potent antioxidants to treat diseases caused by ROS.[14,15,29]
		Silybum marianum.	Stilbenes (silybin, silychristin, silandrin)	It possesses anti-hepatotoxic characteristics that are utilized to cure liver cirrhosis and *Amanita* species toxicity.[14,15,29]
		Glycine max, Trifolium pratensis.	Isoflavones	Employed as phytoestrogens (mimics of the female sex hormone, estradiol) to prevent several malignancies and menopause or osteoporosis difficulties by inhibiting tyrosine kinases as it possess potent antioxidant activities.[14,15,29]
	Phenolic hydroxyl group	*Agrimonia, Alchemilla, Krameria* species.	Tannins	Utilized as an herbal drug due to its antioxidant, antiparasitic, antimicrobial, and cytotoxic properties in both orthodox medicine and contemporary phytotherapy.[14,15]

the prevention of several disorders such as carcinoma, cardiac disease and neurological disorders (Table 1).[28]

Efficiency of Elicitors: Bolster to Discern the Pathway of Secondary Metabolites

Elicitors in plants are likely exposed to a combination of chemical polymers, which can provoke alterations in morphology and physiology.[30-32] A vast assortment of secondary metabolites and plant defense signaling molecules have evolved when cultures have been inoculated with elicitors or afflicted by antipathetic microorganisms.[31,33]

Despite the cycle of elicitation being incredibly challenging because of its hundreds of entangled phenomena, empirical studies have outlined the mechanism of elicitation and detailed the synthesis of secondary metabolites through occurrences such as stimulation of transcription factors and excitation of cell signaling pathways.[34-37] Besides, various events oscillate with specificity, origin, plant nutrient absorption, physiochemical interactions, plant growth cycle, and elicitors' intensity.[31,37] Aside from this fact, there are some primary elements instigated in almost all of the plant cells, and elicitor interactions like ROS burst, MAPK phosphorylation, and Ca^{2+} flux have been assessed.[37] The identification of cell signal on the plasma membrane has been facilitated through both receptors and elicitor binding sites, which trigger the subsequent series of actions such as Ca^{2+} burst, fluxes of ions, ROS burst, acidification of the cytoplasm, activation of NADPH oxidase, phosphorylation of MAPK and stimulation of G-proteins.[38] Ion exchange has led to acquiring the preliminary response to elicitors, for example, H^+/Ca^{2+} influxes and Cl^-/K^+ effluxes.[38] Ca^{2+} influx is recognized as a principal occurrence due to its extensive interventions in both cellular and physiological function.[39] Secondary messengers, for instance, DAG and IP3 and along with conformational alterations in proteins that bind to Ca^{2+} like phospholipases, CDPKs, calmodulin, and calmodulin-like proteins have influenced Ca^{2+} signals.[40] The stimulation of numerous plant physiological expressions has been attributed to Ca^{2+} and calmodulin-induced pathways.[38] CDPKs have performed multiple roles in phosphorylating proteins to synchronize metabolic functions such as signaling via hormones, expression of genes, and oxidative burst regulation.[41] ROS are generated by the existence of NADPH oxidase and Ca^{2+} spiking, which in turn leads to another notable event in plant defense mechanism functions.[39] G-proteins have also taken part in the generation of ROS, plant cell death, and enhancing enzymes like phospholipase A, phospholipase C, phospholipase D, and ion channels.[34] Eventually, target kinases such as PKC and PKA are induced when active G-proteins raise the intensity of DAG, IP3, and cAMP.[38,39] Triggered protein kinases have enabled MAPK phosphorylation, resulting in enzyme activities and expression of genes and consequently reconfiguring the cascade of synthesis of secondary metabolites that are exhibited in Figure 1.[39,40] Furthermore, Tables 2 and 3 stress on the utilization of different biotic and abiotic elicitors for the enhancement of bioactive compounds with a remarkable fold from the existing one.

Recent Insights on Bioactive Compounds

Enhancing product yields without concurrent commercial-process implementation does not inherently constitute research

Table 2: Variation in bioactive compound enhancement induced by biotic elicitors across different culture types and their pharmacological effects.

Plant	Biotic elicitor	Bioactive compound	Change in concentration of bioactive compound on elicitation			Culture type	Pharmacological effect
			Before elicitation (control)	After elicitation	Fold increase		
Solanum khasianum	*Aspergillus niger*	Solasodine	1.172 mg/g	1.930 mg/g	1.6	Hairy root culture	Possess neuroprotective, anticancer, and hepatoprotective activity.[42,43]
Solanum khasianum	*Aspergillus niger*	α- solanine	3.157 mg/g	5.213 mg/g	1.6	Hairy root culture	Exhibit anti-cancer effects on breast and pancreatic malignancies.[43-45]
Rauwolfia serpentina	*Saccharomyces cerevisiae*	Ajmaline	0.328 mg/g	0.975 mg/g	2.9	Hairy root culture	Act as a sodium channel inhibitor for determining distinct subgroups of Brugada Syndrome.[43,46]
Rauwolfia serpentina	*Saccharomyces cerevisiae*	Ajmalicine	0.007 mg/g	0.014 mg/g	2	Hairy root culture	Helps to avert strokes and lowers blood pressure.[43,47]
Gymnema Sylvestre	*Polyancora globosa & Xylaria* species	Gymnemic acid	13.4 mg/g	139.98 mg/g	10.45	Suspension culture	Exhibits hypoglycemic characteristics and serves as the base for diabetic therapies.[48,49]
Echinacea purpurea	Yeast extract	Caffeic acid	7.1 µg/g	10.8 µg/g	1.5	Suspension culture	Utilized for the treatment of Alzheimer's disease.[50,51]
Echinacea purpurea	Yeast extract	Alkamide	24.2 µg/g	55.7 µg/g	2.3	Suspension culture	Used as a new anti-depressant drug.[50,52]
Echinacea purpurea	Chitosan	Caffeic acid	7.4 µg/g	30.2 µg/g	4.08	Suspension culture	Utilized for the treatment of Alzheimer's disease.[50,51]
Echinacea purpurea	Chitosan	Alkamide	24.1 µg/g	60.2 µg/g	2.49	Suspension culture	Used as a new anti-depressant drug.[50,52]
Panax ginseng	*Mesorhizobium amorphae*	Ginsenoside Rb2	0.05 mg/g	0.97 mg/g	19.4	Adventitious root culture	Used for the treatment of cardiovascular disorders.[53, 54]
		Ginsenoside Rb3	0.05 mg/g	0.94 mg/g	18.8		

and development. Several bioactive compounds have been subjected to empirical testing to ascertain their pharmacological properties. The evaluation of solasodine (a steroidal glycoalkaloid compound) extracts derived from *in vitro* cultured specimens of four *Solanum* species *viz., S. incanum, S. nigrum, S. surattense*, and *S. villosum* was conducted on two distinct cancer cell

Table 3: Variation in bioactive compound enhancement induced by abiotic elicitors across different culture types and their pharmacological effects.

Plant	Abiotic elicitor	Bioactive compound	Change in concentration of bioactive compound on elicitation			Culture type	Pharmacological effect
			Before elicitation (control)	After elicitation	Fold increase		
Solanum khasianum	NaCl	Solasodine	1.023 mg/g	7.161 mg/g	7	Hairy root culture	Possess neuroprotective and hepatoprotective activity.[42,43]
Rauwolfia serpentina	NaCl	Ajmaline	0.328 mg/g	0.510 mg/g	1.5	Hairy root culture	Act as a sodium channel inhibitor for determining distinct subgroups of Brugada Syndrome.[43,46]
Rauwolfia serpentina	NaCl	Ajmalicine	0.003 mg/g	0.058 mg/g	19.33	Hairy root culture	Helps to avert strokes and lowers blood pressure.[43,47]
Lonicera japonica Thunb.	MeJA	Chlorogenic acid	20.47±1.40 mg/g	69.90±1.40 mg/g	3.4	Cell suspension	Possesses anti-cardiovascular, anti-mutagenic, and immunomodulatory properties.[55]
Echinacea purpurea	Sorbitol	Caffeic acid	7.4 µg/g	25.5 µg/g	3.4	Suspension culture	Utilized for the treatment of Alzheimer's disease.[50,51]
Echinacea purpurea	Sorbitol	Alkamide	23.4 µg/g	41.9 µg/g	1.79	Suspension culture	Used as a new anti-depressant drug.[50,52]
Fagopyrum tataricum	UV-B	Rutin	3.19 mg/g	29.79 mg/g	9.33	Hairy root culture	Possesses, anti-diabetic, and anti-allergic properties.[56,57]
Rubia tinctorum L.	Salicylic acid	Purpurin	0.47 mg/g	0.73 mg/g	1.55	Hairy root culture	Act as an anti-depressant drug.[58,59]
Rubia tinctorum L.	Salicylic acid	Alizarin	2.18 mg/g	4.42 mg/g	2	Hairy root culture	Employed for the treatment of Parkinson's disease.[59,60]

lines, HT-29 human colorectal adenocarcinoma cell line and MG-63 osteosarcoma cell line which indicated a cytotoxic effect, as determined by the MTT assay, exhibiting a greater inhibition percentage of 56.7% in HT-29 and 59.2% in MG-63, highlighting its robust anti-proliferative property, suggesting the potential utility of solasodine extracts as innovative targeted therapeutic agents for bone and colon cancers.[61] Ginsenoside Rd (a triterpenoid compound) demonstrated a mitigating effect on AFB1-induced apoptosis by regulating antioxidative mechanisms in both H9c2 cells and 3D heart spheroids, which attenuated superoxide dismutase activity in heart spheroids, presenting a potential avenue for the innovation of new antidotes targeting toxins and countering AFB1-induced cardiac injury.[62] Cytotoxicity assessments have reported that the incorporation of rutin (a glycoside flavonol) into PCL-PEG nanoparticles presented a strategic avenue for drug development at the targeted site, demonstrating a discernible time- and dose-dependent manifestation, elucidating the inhibitory effect on Skov3 cell proliferation, and emphasizing the anti-cancer efficacy of rutin-packed PCL-PEG against the Skov3 human ovarian cancer cell line.[63] Alizarin (a dihydroxyanthraquinone) stated to impede the proliferation of pancreatic cancer cells through the negation of NF-κB stimulation, and CCK8 assays were used to assess its inhibitory efficacy on two pancreatic cancer cell lines, PANC-1 and MIA PaCa-2, which exhibited a critical antitumor efficacy of alizarin in a time- and dose-dependent fashion.[64] The co-administration of chlorogenic acid and cinnamaldehyde exhibited potent anti-breast cancer effects in HCC1419, MDA-MB-231, and MCF-7 breast cancer cells, which resulted in cell death concurrent with a decrease in the potential of the mitochondrial membrane, enhanced mitochondrial levels of superoxide, diminished ATP synthesis and altered mitochondrial and cellular morphology, demonstrating realistic efficacy in mitigating breast cancer.[65]

The adoption of elicitors has become an extensible method among vast techniques to markedly maximize the concentration of bioactive secondary metabolites of interest.[66] In particular, the finest quantity of desired bioactive compounds has been achieved at a marginal level of elicitors within a comparatively brief period.[38] Modern research studies have highlighted the evolution of nanoparticles as elicitors in the discipline of plant tissue or organ culture for boosting the number of secondary metabolites.[31] The

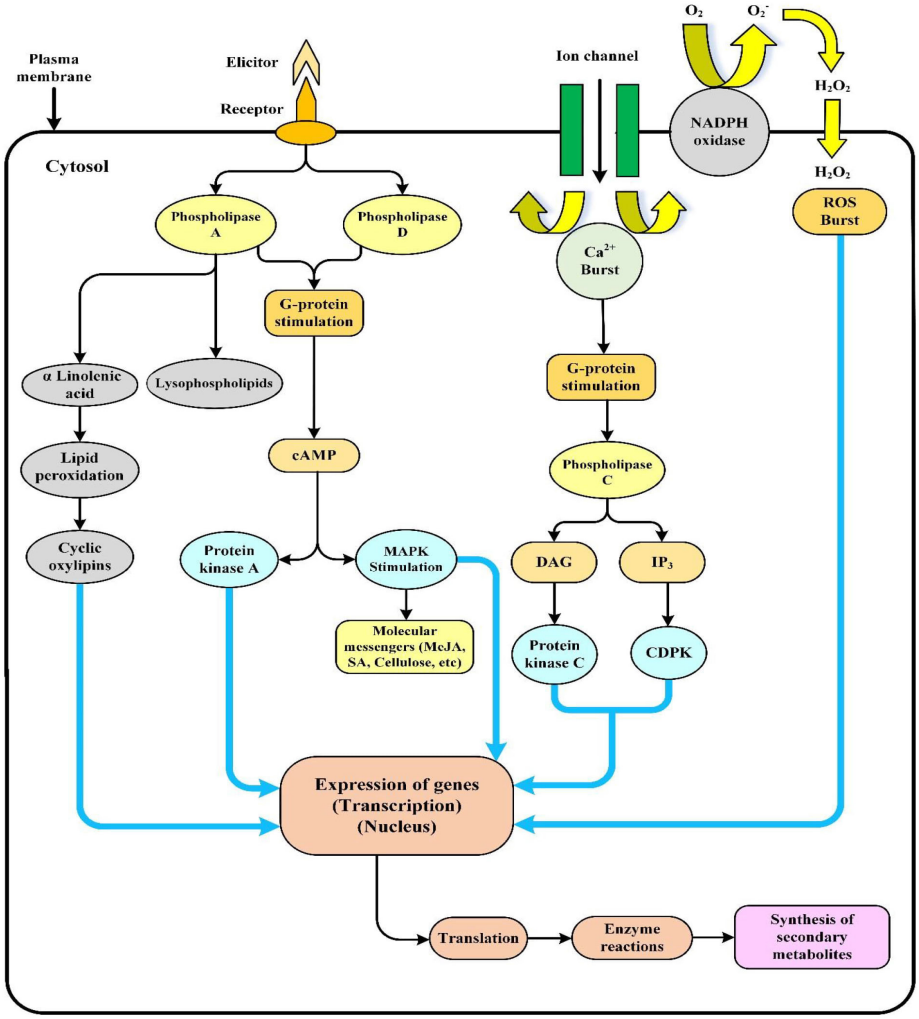

Figure 1: Comprehensive insight into the metabolic pathway governing plant secondary metabolite synthesis.

practice of Nano-elicitors has contributed to promising outcomes in plant biotechnology, which is chiefly intensifying metabolic pathways of bioactive compounds.[67] The implementation of nanomaterials has convincingly endorsed different perspectives on enhancing medicinally crucial metabolites.[67] For instance, when coupled with plant hormones in Murashige Skoog media and at a concentration of 60 µg L-1 with a size of 40 nm AgNP were found to boost callus biomass in cultures of the critically endangered medicinal plant *Caralluma tuberculate*. The callus cultures developed more phenolics of about 3.0 mg TPC (Total phenolic content), flavonoids of around 1.8 mg TFC (Total flavonoid content), 90% anti-oxidant activity at a composition of 90 µg L-1 AgNP and also showed elevated anti-oxidant enzymes like ascorbate peroxidase, superoxide dismutase, catalase, and peroxidase, even in the absence of plant growth hormones.[68] Artemisinin, a biologically active anti-malarial compound from *Artemisia annua* was greatly increased by 2.25 fold when cobalt nanoparticles at a concentration of 5 mg L-1 with a size of 10nm were introduced into suspension cultures.[69] However, the interconnection between biological framework and nanoscale materials is still obscure.[66] Even so, comprehensive research reviews and concrete suggestions are indeed to vividly highlight the influence of nanoscale elicitors and ultimately understand the mechanism of elicitors on secondary metabolite pathways in both aromatic and medicinal plants.[66] The emergence of metabolic engineering approaches is another contemporary focus of research on the synthesis of potential plant secondary metabolites. By intentionally and precisely altering metabolic pathways, transgenic crops have been engineered to enhance the production of bioactive compounds through targeted modifications of energy distribution, supramolecular construction, and chemical transformation processes. For example, Agrobacterium-mediated genetic transformation using transcription factors of myeloblastosis derived from *Arabidopsis thaliana* incorporated into *Humulus lupulus* yielded a discernible augmentation in flavonoid biosynthesis.[70,71] The hairy roots of *Catharanthus roseus*, cloned under the influence of *Agrobacterium rhizogenes* isolated within the geographical boundaries of Vietnam, demonstrated the ability to synthesize both vinblastine

and vincristine (an alkaloid compound) upon exposure to L-tyrosine and L-phenylalanine precursor compounds.[72]

CONCLUSION

In recent years, the findings of promising medicinally valuable and economically critical bioactive compounds from plants have evolved. Even though extensive tissue or organ culture advancements have been established in the past few years to circumvent constraints, the generation of desired bioactive secondary metabolites in ample amounts remains a critical challenge. Another cause for limited production is a paucity of insight into secondary metabolite pathways. In light of an extensive body of research on the influence of biotechnological practices, this review substantiates the pivotal role of elicitors in orchestrating a requisite function within the intricate framework of plant secondary metabolite synthesis. Comprehensive data encompassing the utilization of both biotic and abiotic elicitors across diverse culture types culminate in the augmented production of biologically active compounds, demonstrating pharmacological and economic significance. In lieu of the traditional knowledge reliant on field-cultivated medicinal plants, industries can utilize the strategic potential to acquire bioactive compounds using *in vitro* culture techniques. Empirical evidence from the literature underscores a noteworthy increase in yield, which is approximately 19-fold greater than the extant levels derived from conventional plant sources. Advances in biotechnology, particularly within plant tissue culture techniques, have unveiled novel avenues for the industrial processing of endangered or extinct plant species. The principal advantage conferred by plant tissue culture is its substantial advancement in improving our understanding of plant physiology.

This technique serves as a sustainable reservoir to produce naturally occurring bioactive substances synthesized within a regulated environment independent of soil and climatic variables. The progression of molecular biology techniques aimed at generating transgenic cultures is poised to constitute a considerable stride in broadening the universal applicability of cell cultures for industrial-scale synthesis of secondary metabolites, which are pivotal in instigating extensive research on the regulation and expression of biosynthetic metabolic pathways. This review delineates the involvement of elicitors and their consequential reactions in the biosynthesis of secondary metabolites, thereby contributing to the elucidation of metabolic engineering methodologies that emphasize possible alternative production modalities associated with the restricted production of desired plant metabolites in the future. Consequently, there is a need to develop technical approaches targeted at garnering information at the molecular and cellular levels, given the rudimentary state of understanding biosynthetic metabolic pathways and culture techniques for desired plant metabolites. Sustained and heightened endeavors in this discipline will culminate in the proficient and regulated biotechnological production of distinctive, essential, and undiscovered phytochemical compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ROS: Reactive oxygen species; **MAPK:** Mitogen-activated protein kinase; **NADPH:** Nicotinamide adenine dinucleotide phosphate; **DAG:** Diacyl glycerol; **CDPK:** Calcium-dependent protein kinase; **IP_3:** Inositol triphosphate; **cAMP:** Cyclic adenosine monophosphate; **AgNP:** Silver nanoparticle; **SMT:** (S)-scoulerine 9-O-methyltransferase; **PMT:** putrescine N-methyltransferase; **UV-B:** Ultra Violet-B rays; **MeJA:** Methy jasmonate; **SA:** Salicylic acid; **AFB1:** Aflatoxin B1; **H9c2 cells:** embryonic BD1X rat heart tissue; **PCL-PEG:** poly(ε-caprolactone)-poly(ethylene glycol) copolymers; **NF-κB:** Nuclear factor kappa B.

SUMMARY

The primary aim of this review paper is to present an extensive analysis of the pivotal function of elicitors in promoting the accumulation of pharmacologically critical bioactive compounds within *in vitro* plant tissue culture systems. To accomplish this goal, meticulous and collective retrieval of pertinent literature was performed through a systematic search of diverse academic and scientific electronic databases. The therapeutic effects of diverse bioactive constituents, stratified by their respective classes, along with the experimental data from the reviewed literature, were compiled systematically and structured in a tabular form. All studies analyzed in this review highlighted the substantial therapeutic and pharmacodynamic potential of bioactive metabolites, demonstrating a marked enhancement in their accumulation following exposure to both biotic and abiotic elicitors, in contrast to untreated cell cultures. Augmentation of bioactive metabolite yields via elicitation constitutes a crucial *in vitro* culture methodology for scalable production in the pharmaceutical sector, underscoring the potential application of elicitors as key modulators for the discovery and commercial production of novel and essential phytochemical entities. Nonetheless, the authors advocate further investigations to elucidate how this elicitation approach may reduce reliance on harvesting field plants, thereby mitigating the associated environmental impacts of industrial exploitation.

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