Chapter

Role of Endogenous and Exogenous Hormones in Bioactive Compounds Production in Medicinal Plants Via In Vitro Culture Technique

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Abstract

The natural compounds produced in plants are classified into two major groups (Primary and secondary metabolic compounds). These compounds are the precursor materials for thecompounds of the second group, which are represented by secondary metabolites, most of which produce from three main compounds: shikimic acid, acetate, and fatty acids. Primary metabolites are the basic units in the metabolism of secondary compounds. Tissue cultures of plants are used to produce large quantities of secondary metabolic products, although cultures of callus and cell suspensions often do not produce higher levels of the whole plant. Therefore, some technologies were used to increase the production of secondary metabolites by plant tissue culture techniques through the selection of high-production cells. The growth of plant cells in tissue cultures occurs when the requirements for division and growth are available for them from nutrients, growth regulators, and any other additives that all affect the metabolic activities within the cells. To achieve optimal productivity of secondary metabolites, it is preferable to produce cells in a medium that is optimal for increasing biomass. Plant growth regulators such as auxins and cytokinins affect cell division, various metabolic processes, and plant growth in tissue cultures.

Keywords: auxin, cytokinin, *in vitro* culture, plant hormones, secondary metabolites

1. Introduction

The plants can produce certain bioactive compounds that are mostly affected by the chemical and physical environments in which they develop. Some searches announced that plant growth regulators and light are important factors stimulating the growth, development (organogenesis), and production of plant compounds, including both primary and secondary products. In addition, plant growth regulators were applied for callus induction, and adjusting the metabolite content, carbon sources, suspension culture, temperature, pH, medium type and ammonium nitrate (NH_4NO_3) concentrations plays an important role in the formation of plant primary and secondary products [1–3]. This chapter elicits the role of endogenous and

exogenous hormones are active for enhancing the following stages–cellular division stage, cell enlargement stage, exponential stage, steady stage, and reduce biomass as well as secondary products content in medicinal plant.

2. Secondary products

The natural compounds produced in plants are classified into two major groups. The first group includes compounds that enter into primary reactions or primary metabolic compounds. This name refers mostly to the metabolic processes that produce simple basic carboxylic acids, amino acids, sugars, lipids, proteins, and nucleic acids. These compounds are the precursor materials for the compounds of the second group, which are represented by secondary metabolites, most of which produce from three main compounds: shikimic acid, acetate, and fatty acids. Primary metabolites are the basic units in the metabolism of secondary compounds, which are divided into several different groups and generally include terpenes, phenols, alkaloids, glycosides, tannins, resins, and others [3, 4].

2.1 Glycosides

These compounds are bioactive and important substances in the defense and metabolic system of medicinal plants. These compounds are helped by these plants to complete their life cycle by protecting them from biotic stresses (defense against infection with bacteria, viruses, fungi, nematodes, rodents, etc.). As well as its important role in the treatment of many diseases that affect humans and animals [3, 5, 6]. Glycosides consist of two molecules, one of which is a sugar called the glycon, which is monosaccharides, disaccharides, or polysaccharides. This part of the sugar works to transport the glycoside molecule across the cell membranes, so it has the properties of pharmacokinetics. As for the other part, it is called an aglycon, which may be an alcohol, an aldehyde, a ketone, or an ester, and it is attributed to this part of the physiochemical effectiveness (**Figure 1**). The glycon is attached to the aglycon part by several chemical bonds, which may be an oxygen, sulfur, or carbon bond. Glycoside compounds include steroids, anthraquinones, tannins, and saponins [4–6].

2.2 Alkaloids

Alkaloids are a group of low-molecular-weight basic organic compounds, whose molecule contains one or more nitrogen atoms linked to heterogeneous rings, so the alkaloids do not share a specific chemical composition. The human knew plants containing alkaloid compounds 3000 years ago and used their extracts to heal from

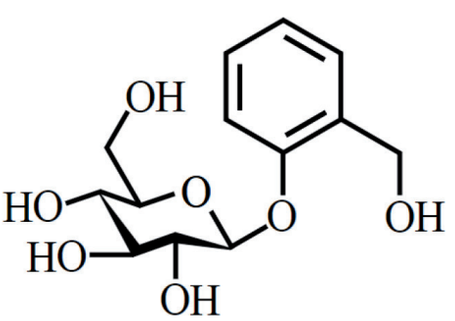

Figure 1.
Glycosides compound.

diseases, treat wounds, or make poisons used in hunting, defending him, or religious rituals. The first process of isolation of alkaloid compounds was the isolation of alkaloid morphine from the papaver plant by the German scientist Derosnein in 1803 AD. Then it was followed by the isolation of many alkaloid compounds that saved the lives of millions of people from incurable diseases or those that contributed to alleviating the pain of surgical operations [4, 6]. Alkaloids are usually found free or in the form of salts of some organic acids such as citric acid, tannic acid, and tartaric acid. Alkaloids are produced by bacteria, fungi, and higher plants and are found in all parts of the plant, such as hyoscine alkaloid in tobacco, in seeds, such as strychnine alkaloid in emetic walnut, in the roots, such as glycyrrhizin alkaloid in licorice, in the bark, such as cinchonine alkaloid in cinchona, in the fruits are like capsaicin alkaloid in the black pepper plant, or in Latex like the papaverine alkaloid in the poppy plant. Alkaloids are divided into several groups according to the chemical structure of the basic ring in the alkaloid molecule into the group of amine alkaloids, pyridine and piperidine, tropane alkaloids, quinoline alkaloids, purine alkaloids, isoquinoline alkaloids, indole alkaloids, phenolic alkaloids, tropolone alkaloids and tropolone alkaloids (**Figure 2**) [3, 4].

2.3 Phenols

Phenolic compounds are the second largest group of secondary metabolites in plants after the alkaloid group. A simple phenolic molecule contains a benzene ring to which one or more hydroxyl groups are attached. These compounds are found in both higher and lower plants (such as ferns, mosses, and many microorganisms). Phenols are also called aromatic compounds because of their distinctive smell, and they are sometimes called closed ring compounds because they contain a benzene ring (**Figure 3**). These compounds are characterized by the presence of a hydroxyl group (OH) directly attached to the aromatic ring. Sometimes several different

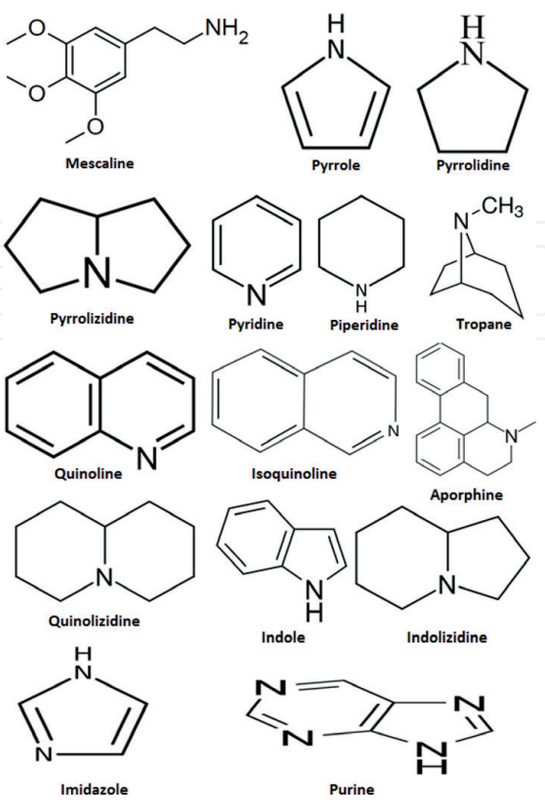

Figure 2.
Alkaloid compounds.

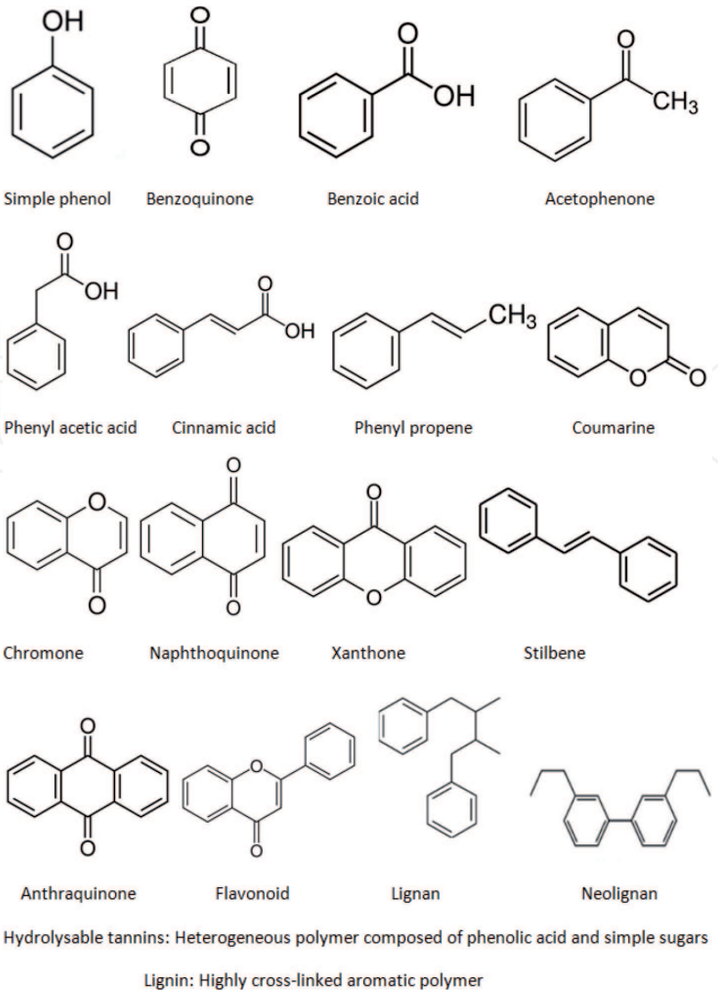

Figure 3.
Phenolic compounds.

groups are attached to the phenolic compound, such as the hydroxyl group OH, the carboxyl COOH, and the methyl CH_3 [3, 6]. The phenolic compounds may exist in the form of an open chain or aliphatic (noncyclic) compounds. Most of the phenolic compounds are not found free inside plant cells but are bound with one or several molecules of sugars to be in the form of glycosidic compounds. There are also some phenolic compounds linked with lipopolysaccharides by a glycoester with one of the OH or COOH groups to form glycolipids that are stored in the cell vacuoles. Some amino acids, such as Tryptophan, Tyrosine, and Phenylalanine, are classified as closed ring organic phenolic compounds, due to the similarity of the method of metabolism of these acids with phenolic compounds [4, 6].

2.3.1 Simple phenols

Phenols are the basic material in the biosynthesis of lignin. Phenols also play an important role in regulating plant growth and development by affecting the effectiveness of hormones and their control over the effectiveness of the formation of some enzymes. They represent one of the forms of energy compounds stored by the plant and nutrients that can be utilized when needed. It acts as an antioxidant that hinders the oxidation of chlorophyll and hormones and stabilization in the stabilization of some vital compounds. It also participates in the oxidation and respiration processes. The most important groups of phenolic compounds in higher plants are groups of cinnamic acid, coumarin, lignin, phenolic carboxylic acids, and flavonoids derivatives [4, 7].

2.3.2 Polyphenols (flavonoids)

Polyphenols or flavonoids are heterocyclic oxygen compounds of essential importance in plant life and are sometimes called Anthoxanthins. Flavonoids are distinguished by their crystalline form and yellow color, which derives from the Latin word flavus. These compounds are found in higher plants, especially some families such as Compositae, Cucurbitaceae, and Umbelliferae, in plant parts such as roots, leaves, flowers, and fruits. Flavonoids are used as an antiviral, general anti-inflammatory, anti-bacterial, and increase the level of immunomodulation. They act as antioxidants, relieve pain, swelling and bruising, stimulate blood circulation, and reduce cholesterol levels in the blood. Flavonoids are divided into several groups: Flavone, Flavonol, Flavonone, Isoflavone, Chalcone, Aurone, Anthocyanin, and Betacyanins [3, 4, 7].

2.4 Oils

2.4.1 Volatile oils

Volatile oils are organic compounds characterized by their volatility or evaporation without decomposing when exposed to heating or at room temperature. They are also called ethereal oils because of their solubility in alcohols, especially ether. Essential oils are so named for their pleasant aromas, and essential oils are so named because they are included in the basic human diet, and the cellular enzyme system in the human body cannot produce it. These oils are spread in more than 2000 plants represented by sixty families, the most important of which are Lauraceae, Labiatae, Umbelliferae, Rutaceae, Compositae, Myrtaceae, Pinaceae, and Oleaceae [3, 8]. The volatile oils in the plant act as pleasant aromas to attract insects to complete the pollination process and at the same time work to exclude other insects. Some of these oils are poisonous and others have a pungent taste that is unpalatable to insects and rodents, meaning that these oils act as an open immune system to defend the plant itself. Volatile oils have an important role in allelochemicals to reduce competition from other plants for light, water, and soil nutrients. These oils are used in the treatment of many diseases, such as eucalyptus oil, which treats inhalation shortness of breath, and bronchitis, which is Antispasmodic. Peppermint oil is used as a mouthwash and antiseptic gargle, Thymol oil is used to treat skin problems, and clove and thyme oil are used as antiseptics because they contain a high percentage of phenolic compounds. Dill oil is used as a carminative, castor oil is used as a laxative, and watercress oil is used as a cholesterol reducer. The volatile oils are divided into groups: Alcoholic, Aldehyde, Ketone, Phenolic, Nitrogenic, and Sulfuric oils [4, 8].

2.4.2 Fixed oils

Vegetable fixed oils are less dense liquids than water and do not mix with water. It is biologically built in the places of its production and is not transmitted from one plant member to another. It is often produced and stored in seeds and fruits, and a small percentage is produced in the bark and leaves. The most important fixed vegetable oils are corn, sunflower, safflower, cottonseed, sesame, soybean, linseed, archies, and olive oil. There are other fixed oils consisting of esters of unsaturated fatty acids such as linoleic acid, linolenic acid, arachidonic acid with triglyceride alcohol, and others [4, 9]. Fixed oils are distinguished by their high nutritional value, which are used in weight gain programs and in the manufacture of food and energy drinks, among others. The increasing demand for its use in every home is

due to its lack of contribution to raising the level of cholesterol and triglycerides in the blood that causes atherosclerosis and heart disease. Fixed oils are also used in the treatment of some diseases, especially spasms, muscle pain, and rheumatism, or as sterilizers or moisturizers for skin cracks resulting from infection with some fungi or bacteria, burns, or sunburn. It is also used as carriers or organic solvents for active compounds in the manufacture of some creams and ointments. These oils are also used in the manufacture of washing and cosmetics, among others. These oils are characterized by being odorless, tasteless, slightly yellowish, hydrophobic, and non-polar compounds that do not dissolve in water but dissolve in organic solvents such as chloroform, benzene, and ether. Fixed oils are divided according to the fatty acids that bind to glycerol into monoglyceride, diglyceride, and triglyceride groups [3, 4].

2.5 Resins

Resins are solid or semi-solid organic compounds of different and chemically complex compositions that result from the oxidation of volatile oils. Resins are defined as plant exudate produced by plants either naturally or when the plant is exposed to physiological damage as a result of a pathogenic condition or mechanical damage as a result of the influence of environmental factors or pest infestation. Resins can be made synthetically by freezing formaldehyde or freezing the resin after mixing it with glycerin such as Colophony resin. In general, the most common plant families that produce resins are Pinaceae, Cupressaceae, Araucariaceae, and Podocarpaceae [3, 4, 9]. The benefits of resins are their use in therapeutic recipes in eastern civilizations, especially in treating burns and superficial and deep wounds, such as Balsam resin, as well as its use in religious rituals, weddings, and astrology. Resins are also used in the manufacture of incense, such as Amber resin, soaps, and cosmetics, such as Myrrh resin. Scientific research has proven that resins have high anti-microbial, antitumor, anti-inflammatory, and anti-skin perfusion efficacy. Resins are one of the plant's defenses against insects, as some resins are formed when they are absorbed by insects to turn this formed sap into a sticky resin that prevents the insect from moving and then eliminates it, such as Shellac resin. The resins are divided into several groups; the oleo-resin group is composed of the resin and the volatile oil such as Copaiba resin, which includes in its composition diterpenes or sesquiterpenes. The second group is gum-resin which is a mixture of resin and gum-like gamboge. The third group is the oleo-gum resins, which consist of resin, gum, and volatile oil, such as asafoetida resin extracted from the rhizomes of the roots of the plant Ferula asafoetida, which consists of ferulic acid and the compound Umbelliferone and volatile oils such as sesquiterpenes such as foetidine, saradaferin, methoxy courmarin, and polysaccharides. The fourth group is glyco-resin is a mixture of resin and sugar such as jalapin and podophyllin resin. The fifth group is Balsams, which are resinous materials that contain in their composition aromatic acids such as cinnamic acid and benzoic acid or both, or esters of these acids such as Peru balsam, Tolu balsam and Storax balsam, which contain a high percentage of Aromatic balsamic acids [4, 6].

3. Plant hormones

Plant hormones are chemical runners that are created in one tissue and regulate cellular actions in another tissue by linking with certain proteins that role as receptors associate to cellular transduction pathways. The plant hormones are synthesized in one tissue and react on specific target sites in another tissue at very

low concentrations. Plant hormones that are transferred to sites of activity in tissues far away from their site of biosynthesis are indicated as endocrine hormones. Those that react on cells of tissue close by the source of biosynthesis are indicated as paracrine hormones. Plant growth and development are modulated by six major groups of hormones: auxins, cytokinins, gibberellins, abscisic acid, ethylene, and brassinosteroids [3, 10]. A diversity of other signaling compounds that play roles in impedance to pathogens and protection against herbivores have also been specified in plants, including combined and uncombined forms of jasmonic acid, salicylic acid, and small polypeptides. Another compound, strigolactone, has lately been shown to be an intendable signaling compound that regulates the growth of lateral buds [10, 11]; this compound may also be a valid plant hormone. Other groups of compounds, such as flavonoids, work as both intracellular and extracellular regulators of signal transduction pathways [12]. Indeed, the list of signaling factors and growth regulators continues to expand.

3.1 Auxins

The first signaling compound is the hormone auxin. Auxin was the first growth regulator to be calculated in plants, and a lot of the early physiological reports on the mechanism of plant cell extension were executed about auxin action. Auxin signaling has been begun to purpose in nearly every feature of plant growth and development. Moreover, auxin and cytokinin differ from the other plant growth regulators and signaling compounds in one important subject: they are desired for plant embryo viability. Whereas other plant growth regulators seem to work as regulators of separate development processes, auxin and cytokinin seem to be desired at several levels less or more continuously. The various growth and development processes that are controlled by auxin are apical dominance, stem elongation, fruit development, root initiation, oriented or topic growth, and meristem development [3, 10]. The Went's studies with gelatin and agar blocks demonstrated unequivocally that growth-promoting influence diffusing from coleoptile tip was chemical substance. The fact that it was produced at one location and transported in minute amounts to its site of action qualified it as an authentic plant hormone. In the mid-1930s it was determined that the principal natural auxin is indole-3-acetic acid (IAA), (**Figure 4**) [3, 13, 14].

3.2 Gibberellins

The second group of plant growth regulators to be recognized is the gibberellins (GAs). At least 136 natural types of GAs was produced in plants have been

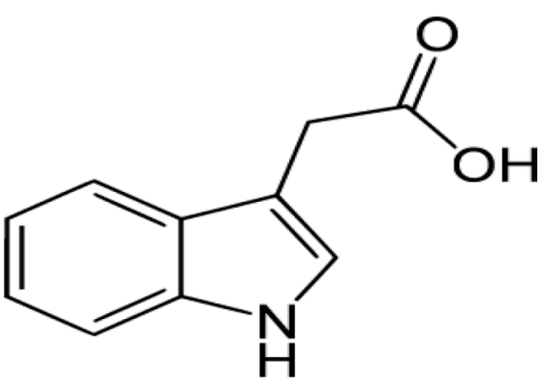

Figure 4.
Indole-3-acetic acid (IAA) structure.

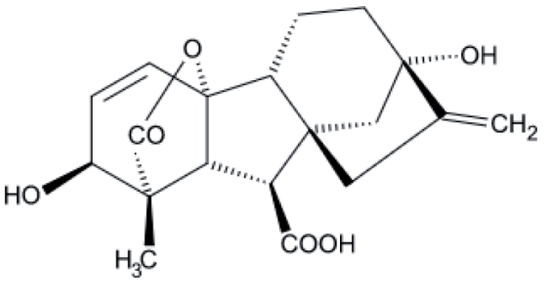

Figure 5.
Gibberellin (GA) structure.

identified [15]. Opposite of the auxins, which are identified by their biological characters, the gibberellins all share a homogenous chemical structure but relatively few of them have essential biological activity (**Figure 5**). Many of the gibberellins that do not have base biological activity are either precursor compounds of the bioactive gibberellins or their destruction products. Gibberellins also play main roles in a variety of other physiological processes, such as the transition to flowering, seed germination, and pollen growth and development. The biosynthesis of gibberellins is under rigid genetic, environmental, and developmental control [13, 14]. Gibberellins are best known for their enhancement of stem elongation, and gibberellin-deficient mutants that have dwarf phenotypes have been separated. Gibberellins first came to the observation of Western scientists in the 1950s; they had been discovered much earlier in Japan. Rice farmers had long known the fungal disease termed 'foolish seedling' that caused rice plants to grow too tall and discarded seed production. The pathologists of plants found that these symptoms of infection in rice were caused by *Gibberella fujikuroi*, which had infected the plants. The cultures of this fungus and chemical analysis in the laboratory enabled Japanese scientists in the 1930s to obtain impure substances with plant growth-enhancing activity. They named this combination of compounds gibberellin A. Gibberellins to represent a large family of tetracyclic diterpene acids biosynthesized via a terpenoid pathway. Knowledge of gibberellin synthesis and deactivation is important to understanding gibberellin homeostasis. Homeostasis depends upon the regulation of gibberellin biosynthesis, transport, and deactivation [3, 14].

3.3 Cytokinins

The cytokinins are reverses of auxins, being biosynthesized in roots but with the most spectacular effects on shoot formation. However, shoot tissues can also synthesize cytokinins, as can germinate seeds. A traditional example of cytokinins is coconut milk, the profuse liquid endosperm of the coconut fruit, which is still a common cytokinin origin in the plant cell, tissue, and organ culture media. Cytokinins were at first named for their ability to stimulate cell division, but they also purpose in the induction of shoots, retardation of senescence, and dormancy release [3, 16]. Cytokinins are imitative of adenine, one of the purine bases create in all RNA and DNA. The four main groups of natural cytokinins each have a different five-carbon side-chain linked to the N6 position. The major free cytokinin groups, dihydro-zeatin and trans-zeatin are more biologically active than the two groups found in tRNA (isopentenyl adenine and cis-zeatin) [13, 16]. The side chains of naturally revolving cytokinins are chemically related to carotenoid pigments, rubber, the plant hormones abscisic acid and gibberellin, and the plant defense substances known as phytoalexins. All of these compounds are created from isoprene units. Isoprene is alike in structure to the side chains of iP and zeatin. These cytokinin side chains are biosynthesized from isoprene imitative. Large molecules

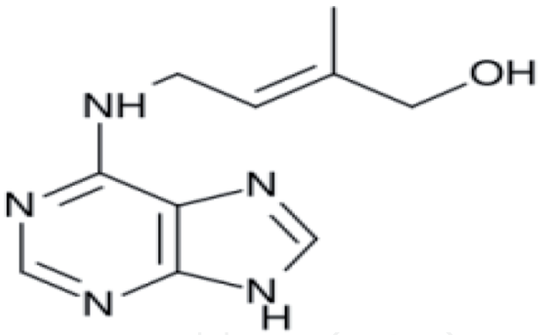

Figure 6.
Cytokininin (Zeatin) structure.

of the carotenoids and rubber are initiated by the polymerization of many isoprene units; cytokinins consist of fair one of these units. The precursor for the initiation of these isoprene units in cytokinins is dimethylallyl diphosphate (DMAPP), which is derived from either the methylerythritol phosphate (MEP) pathway (primary for DHZ, trans-zeatin, and iP) or the mevalonate pathway (primary for ciz-zeatin) (**Figure 6**) [3].

3.4 Abscisic acid

Abscisic acid (ABA) is a growth retardant name because this hormone is related to abscission layer formation (**Figure 7**). ABA does promote fruit drop, growth retardant, and closing stomata in plant leaves [17, 18]. ABA is a 15-carbon molecule and its biosynthesis occurs from the malfunction of carotenoid pigments, especially violaxanthin, a 40-carbon molecule. Formerly, mevalonic acid was believed to be the major precursor, with soon steps in similar with gibberellin biosynthesis. This other pathway may utilize in tissues such as in tomato seedlings and avocado mesocarp [19, 20]. ABA is synthesized in large quantities in water-stressed plant tissues, especially leaves and roots, but also has a role in seed ripening, senescence, and dormancy. ABA concentrations are decreased by oxidative suppression to phaseic acid or by the synthesis of glucosides [3, 13].

3.5 Ethylene

Ethylene (C2H4) is a unique gaseous hormone that diffuses rapidly out of plant tissues. Its direct precursor is 1-aminocyclopropane-1-carboxylate (ACC) which in turn produced from S-adenosyl methionine, an imitative of another common amino acid (methionine). Ethylene is synthesized in response to cell injury and other stresses such as deficient oxygen (**Figure 8**). It cumulates rapidly during fruit ripening and senescence stages, but all living cells synthesize ethylene. Oxidation and conjugation can happen, but dispersion into the atmosphere is probably the main elimination pathway [3, 13, 21].

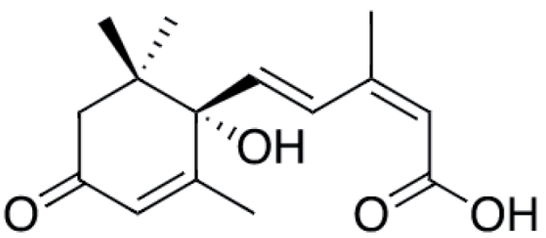

Figure 7.
Abscisic acid (ABA) structure.

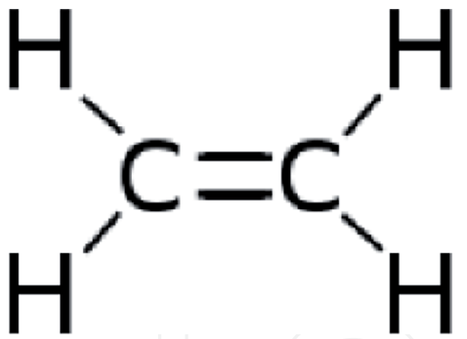

Figure 8.
Ethylene (C_2H_4) structure.

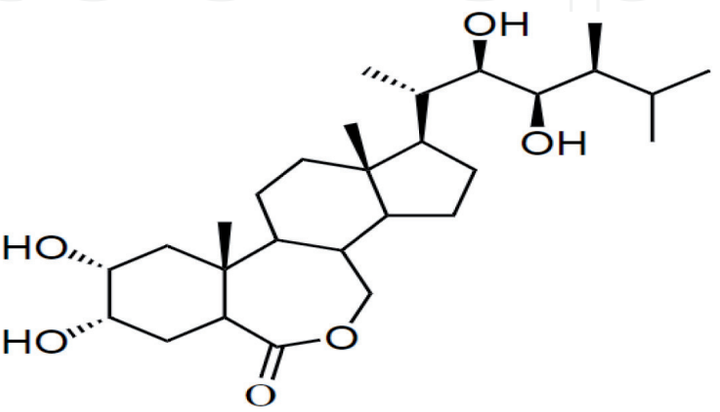

Figure 9.
Brassinosteroid (BR) structure.

3.6 Brassinosteroid

Steroid hormones have extended been recognized in animals, but they have only lately been revealed in plants. Animal steroid hormones involve the sex hormones (androgens, estrogens, and progestins) and the adrenal cortex hormones (mineralocorticoids and glucocorticoids). The brassinosteroids (BRs) are a class of steroid hormones that play more important roles in a wide domain of developmental processes in plants, including cell division and elongation in roots and stems, reproductive development, photomorphogenesis, stress responses, and leaf senescence (**Figure 9**) [22]. Studies by Mitchell et al. [23] showed that the utmost growth-promoting activity was found in the organic extract of pollen from the rape plant (*Brassica napus* L.). Such as abscisic acid and gibberellin, brassinosteroids are biosynthesized as a section of two farnesyl diphosphates to produce the C30 triterpene squalene. Squalene then succumbs to a series of ring closures to produce the pentacyclic triterpenoid (sterol) precursor cycloartenol. All steroids in plants are obtained from cycloartenol by other modifications and a series of oxidation reactions [3, 24].

4. Production of secondary metabolites in plant tissue cultures

Tissue cultures of plants are used to produce large quantities of secondary metabolic products, although cultures of callus and cell suspensions often do not produce higher levels of the whole plant. Therefore, some technologies were used to increase the production of secondary metabolites by plant tissue culture techniques through the selection of high-production cells. This is done after separating

the high-production cells from their low-production counterparts, and the latter are usually excluded by visual methods [2]. The separation process of produced cells from others is carried out using cell cloning technology, which is an easy and simple method in which single cells are taken from mostly cell suspensions that are cultured on a suitable medium. After the formation of cell masses from single cells, each cell mass is sieved separately and the types and quantities of secondary metabolites it contains are determined. The process of selecting high-producing plant cells for secondary metabolites begins with the selection of a plant with a high production for the desired secondary compound or compounds by selecting the suitable explant, it's surface sterilization, and in vitro culture on a medium prepared for the initiation of callus cells. Then the formed callus masses are culture in cell suspension cultures, from which the inoculums are transferred and spread on a solid medium [2, 25, 26].

5. The effect of the components of the medium on the production of secondary metabolites

The growth of plant cells in tissue cultures occurs when the requirements for division and growth are available for them from nutrients, growth regulators, and any other additives that all affect the metabolic activities within the cells. To achieve optimal productivity of secondary metabolites, it is preferable to produce cells in a medium that is optimal for increasing biomass. Then the cells are transferred to the production medium that achieves the highest yield of the desired compound. Note that it is not necessary for the callus medium or the perpetuation medium to be ideal for the production of secondary products. Therefore, many growth regulators and other additives are being tested to obtain an optimal medium for production. The components of the nutrient medium in general, such as carbon source, nitrogen, phosphate, growth regulators, precursors, stimulants, vitamins, additives, and others, affect the fluctuation of the production of secondary metabolites [2, 26, 27].

5.1 Effect of carbon source

The carbon source generally affects the production of secondary metabolic compounds. For example, an increase in sucrose in the production medium from 4 to 10% led to an increase in the production of alkaloids in tissue cultures of *Catharanthus roseus*. It was also found that the addition of sucrose as a carbon source was better than fructose and galactose when producing diosgenin from tissue cultures of *Dioscorea deltoidea* and *Dalanites aegyptica*. An increase in Ubiquinone-10 was also recorded in tobacco tissue cultures when low levels of sucrose were added to the production medium. In another study, it was found that adding 40 g L^{-1} of sucrose to the medium in tissue cultures in the dark led to an increase in the accumulation of the proanthocyanin compound in the plant *Hypericum perforatum*. While the accumulation of kaempferol compound when adding 50 g L^{-1} sucrose to the medium in tissue cultures of the same plant exposed to light [2].

5.2 The effect of a nitrogen source

Adding high concentrations of nitrogen sources to the media in tissue cultures stimulates cells to synthesize amino acids and proteins, including enzymes and nucleic acids. The primary products of metabolism contain nitrogen, which directly affects the formation of secondary metabolic products. In general, high

concentrations of nitrogen added to the medium lead to inhibition of the synthesis of secondary metabolites. The addition of potassium nitrate and ammonium nitrate in high concentrations to the medium prepared for tissue cultures leads to inhibition of the production of anthocyanins by 90% and alkaloids by 80% [2].

5.3 Effect of phosphate

Many secondary metabolites are produced from phosphorylated intermediates, which in turn release phosphate. Inorganic phosphates are essential in photosynthesis and respiration. Generally, high levels of phosphate stimulate cells to divide, grow, and synthesize primary metabolites. When the concentration of phosphate in the tissue cultures increases, it leads to an increase in the production of alkaloids in the plant *C. roseus*, the anthraquinone compound in the *Morinda citrifolia* plant, and the diosgenin compound in the *D. deltoidea* plant. Other studies found that decreasing the concentration of phosphate in tissue cultures led to an increase in anthocyanins and phenols in *C. roseus* and an increase in alkaloids and solasodine in *Solanum laciniatum*. While increasing or decreasing the phosphate concentration did not affect the production of the protoberberine alkaloid in the tissue cultures of *Berberis* sp. [2].

5.4 Effect of precursors

Precursors are called substrate molecules that can be incorporated into secondary metabolites and added to the medium prepared to produce the desired secondary compounds. In general, the addition of precursors stimulates the production of secondary metabolites, although it inhibits the growth of tissue cultures in several cases. For example, the addition of precursors to the medium prepared for tissue cultures of the *Datura* spp. plant led to a noticeable increase in the production of alkaloids, but this was occurred opposite by inhibition in the growth of cultures after the addition of ornithine, phenylalanine, tyrosine, or sodium phenylpyruvate. It was proven that there was a significant increase in the accumulation of ajmalicine in the cultures of the callus of *Coleus blumei* plant when the medium was enriched with the precursor tryptamine and rosmarininc acid accumulation when the medium was enriched with tryptamine compound and 50 g L^{-1} sucrose. Also, rosmarinic acid accumulated in high concentrations in the stem segment explants of the same plant when the liquid medium was included with a concentration of 10 or 20 mg L^{-1} of proline acid [2, 28].

5.5 Effect of plant growth regulators

Plant growth regulators such as auxins and cytokinins affect cell division, various metabolic processes, and plant growth in tissue cultures. Several scientific articles indicated that the type of growth regulator and its concentration affected the productivity of tissue cultures from secondary metabolites. It was found that the addition of auxin indole acetic acid, indole pyruvic acid, or naphthalene acetic acid to the medium prepared for tissue cultures of *Balanites aegyptica* increased the production of diosgenin. The addition of auxin in some cases inhibited the production of some secondary metabolites, such as inhibiting anthocyanin synthesis in carrot plant tissue cultures after enhancing the medium with naphthalene acetic acid and indole acetic acid [2, 29]. The addition of auxin 2,4-dichlorophenoxy acetic acid (2,4-D) to the tissue cultures of tobacco led to the inhibition of the production of alkaloids as well as shikonin in the tissue cultures of *Lithospermum erythrorshizon*. Another study also found that the addition of cytokinins to the cultures of

C. roseus stimulated the production of ajmalicine, and the tissue cultures of tobacco led to the production of scopolamine and scopoletin compounds, and the tissue cultures of *Ricinus* sp. led to the production of carotenoids. Cytokinins inhibited the production of secondary metabolites in some tissue cultures, such as anthroquinines in *M. citrifolia* plant cultures, nicotine in tobacco plant cultures, and Chicoine in *Lithospermum erythrorshizon* cultures. It is noted from previous studies that the addition of auxins and cytokinins to the medium separately did not give positive results in stimulating the production of secondary metabolic compounds in most cases. In general, many studies showed that the addition of combinations of auxins and cytokinins stimulated the increase in the production of secondary metabolites [2, 26].

6. Effect of some growth regulators on the production of secondary metabolites in some medicinal plants

6.1 Stevia rebaudiana

The leaves of shoots that cultured on Woody Plant Medium (WPM) supplemented with 2.27 mM thidiazuron (TDZ), 4.54 mM TDZ, 2.22 mM benzyl adenine (BA) + 2.69 mM naphthalene acetic acid (NAA), 2.22 mM BA +5.37 mM NAA, 2.32 mM kinetin (Kn) +5.71 mM indole acetic acid (IAA), or 2.32 mM Kn + 2.69 mM NAA led to stimulate steviolbioside, rubusoside, and dulcoside compounds by in vitro culture technique [30].

The 4.6 pH of the medium was the main factor for increasing concentrations of secondary metabolite compounds in stevia leaves by in vitro culture technique. The phenols and flavonoids were increased when cultured on a medium supplied with the combination of BA and GA3 or IAA compared to separately applied growth regulators appearing synergistic effects of plant growth regulators (especially of auxins and cytokinins). A positive correlation was found between the flavonoids, phenols, and the antioxidant activity in the *S. rebaudiana* extracts [31].

The highest callus-induction frequency and callus-mass increase were obtained from MS medium supplemented with 2.0 μM NAA. The leaf explants that cultured on MS medium supplemented with 2.0 μM NAA led to the highest concentration of steviol glycosides, flavonoids, and phenols, and higher antioxidant activity was determined in the secondary metabolite compounds of callus from leaf segments. Proline acid reduced the concentration of flavonoids and steviol glycosides. The callus from leaf explants that cultured on MS medium supplemented with 2.0 μM NAA and 2.0 μM proline acid recorded the highest concentration of total phenolic compounds [32].

6.2 Pimpinella alpeno

The results of one study showed that adding 200 mg L^{-1} IAA and 25 mg L^{-1} gibberellic acid (GA_3) to the medium prepared for tissue cultures of *Pimpinella alpino* leaves increased the production of saponin. While when adding 100 mg L^{-1} IAA and 25 mg L^{-1} GA_3 to the medium, it led to a decrease in the production of saponin compound in the leaves and it reached the lowest value. [33] GA_3 affects metabolism and nucleic acid which plays an important role in protein biosynthesis and enhanced the activity of enzymes for plant growth and development. Increased protein biosynthesis as crude material essential enzymes in plant metabolism and increase the production of the secondary metabolite compounds, including saponins at the final stages [34].

6.3 Crysanthemum cinerariefolium

The leaf segments of *Crysanthemum cinerariefolium* plant that cultured on MS medium supplemented with 4 mg L^{-1} 2, 4-D and 0 mg L^{-1} kinetin recorded the best-produced callus by *in vitro* culture technique. The callus contains the secondary metabolite compounds such as some of the flavonoid quercetin precursors such as tetrahydroxy chalcone and acetic acid and some other secondary products [35].

7. Conclusions

* There are factors that affect the increase in the induction and production of secondary metabolites from plants that can be applied and utilized in extracting effective compounds from medicinal plants that are used in the industry of medicines and pharmaceuticals.

* The levels of bioactive compounds in medicinal plants vary depending on the type of plant tissue.

* The possibility of using the plant tissue culture technique in the production of secondary metabolites from the explants of medicinal plants.

* Increasing the concentrations of plant growth regulators such as auxins or cytokinins or adding them in ideal combinations leads to an increase in the induction of secondary metabolites in tissue cultures of medicinal plants.

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