"NATURE'S HIDDEN CHEMISTRY: EXPLORING THE WORLD OF SECONDARY METABOLITES"

SERDAR KARAKURT

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"NATURE'S HIDDEN CHEMISTRY: EXPLORING THE WORLD OF SECONDARY METABOLITES"

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"Nature's Hidden Chemistry: Exploring the World of Secondary Metabolites"
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Dedicated to my Familiy…

PREFACE
EXPLORING THE REALM OF SECONDARY METABOLITES: NATURE'S HIDDEN PHARMACEUTICALS

In the pages of "Exploring the Realm of Secondary Metabolites: Nature's Hidden Pharmaceuticals," readers embark on a captivating journey into the world of secondary metabolites, unveiling the intricate secrets and profound significance of these compounds within the realm of biology, ecology, medicine, and beyond.

"Exploring the Realm of Secondary Metabolites" is an eye-opening narrative that celebrates the wonders of nature's chemical diversity. Through captivating anecdotes, cutting-edge research insights, and thought-provoking reflections, this book bridges the gap between scientific discovery and the awe-inspiring intricacies of the natural world. Whether you're a scientist, a student, or an enthusiast of nature's mysteries, this book promises an enlightening expedition into the hidden world of secondary metabolites.

1. INTRODUCTION: UNVEILING NATURE'S HIDDEN CHEMICAL ARTISTRY

In the intricate tapestry of life, secondary metabolites stand as nature's uncelebrated masterpieces, often residing in the shadows cast by their primary counterparts. These compounds, though lesser-known, weave a captivating tale of chemical diversity intricately crafted by the hand of nature herself. As you embark on the journey through the pages of this book, you are about to uncover the profound world of secondary metabolites, gaining insights into their origins, functions, ecological significance, and their extraordinary potential as wellsprings of pharmaceutical treasures and invaluable substances. Secondary metabolites, the unsung heroes of the organic realm, are produced by a variety of organisecondary metabolites including plants, fungi, and bacteria. Unlike primary metabolites essential for growth and development, these compounds venture into uncharted territories, revealing a wide spectrum of biological activities. From fortifying defenses against predators and parasites to orchestrating the harmonious dance of pollinators and seed dispersers, and even regulating the intricate symphony of plant growth and development, secondary metabolites command a myriad of roles that often go unnoticed by casual observers. The production of secondary metabolites is a symphony conducted by myriad factors. A plant's environment, its genetic makeup, and its developmental stage interplay to determine the chemical ensemble it will perform. Stress, that universal force, acts as a catalyst, prompting the creation of secondary metabolites in response to challenges such as infections or injuries. These compounds emerge as nature's solution to the perpetual struggle for survival, revealing the elegant dance between organisecondary metabolites and their surroundings. Secondary metabolites extend a helping hand to science and medicine, hinting at untold possibilities. Within their molecular structures lie promising potential to combat diseases and conditions that plague humanity. They wield anti-cancer, anti-inflammatory, and anti-microbial properties, showcasing their versatility in addressing a range of ailments. Furthermore, investigations delve into the realm of neurodegenerative diseases, where these compounds exhibit potential as treatments for conditions like Alzheimer's and Parkinson's disease. While the research journey is still in its nascent stages, the prospects that secondary metabolites present are nothing short of awe-inspiring.

As you turn these pages, you will traverse the realms of science, ecology, and medicine, exploring the depths of secondary metabolites' influence on

life as we know it. Prepare to be captivated by their hidden roles, inspired by their potential, and awed by the intricate chemical tales they spin. Let the journey commence, unraveling the enigma of secondary metabolites and revealing the secrets that nature has woven into their very existence.

2. MANUFACTURING MARVELS: UNVEILING THE BIRTHPLACES OF SECONDARY METABOLITES

The therapeutic portions of plants can be divided into four separate categories based on the patterns of secondary metabolite synthesis, accumulation, and distribution: Roots and stems are the first, followed by leaves, flowers, fruits, and seeds. Moreover, different secondary metabolites may synthesize through unique regulatory pathways and unique transport routes in certain organs, tissues, and cells due to the complexity and diversity of secondary metabolites in various medicinal plant parts. As a result, the manufacture and accumulation of Secondary metabolites reveal the specialization of an organ or tissue. In the following sections, we'll go into great detail about the recent development of secondary metabolites biosynthesis and accumulation in various parts of medicinal plants.

3. PLANTS ORGANS SECRETED SECONDARY METABOLITES

3.1. Root and stem

The pivotal repositories of potent therapeutic compounds within plants are their roots and stems. The accumulation of these dynamic substances in root and stem herbs is intricately shaped by growth phases, seasons, and years. Among the array of medicinal flora, certain plants strategically gather secondary metabolites primarily during their reproductive growth stage. Such as, in the fruiting phase, roots and rhizomes of Echinacea purpurea aged two years yield heightened levels of cichoric acid. Similarly, Astragalus compactus Lam. from the Fabaceae family exhibits a greater total phenolic content during fruiting than in its vegetative and flowering stages. Before the bloom of Scutellaria baicalensis Georgi, its roots undergo a swift accumulation of principal flavonoids. Nature's artistic hand reveals itself in the nuanced variations within a single plant's components. For instance, Scutellaria baicalensis Georgi's root sustains a consistent level of total flavonoids throughout its growth cycle, while the presence of baicalin follows a distinct trajectory of increase and gradual decline.

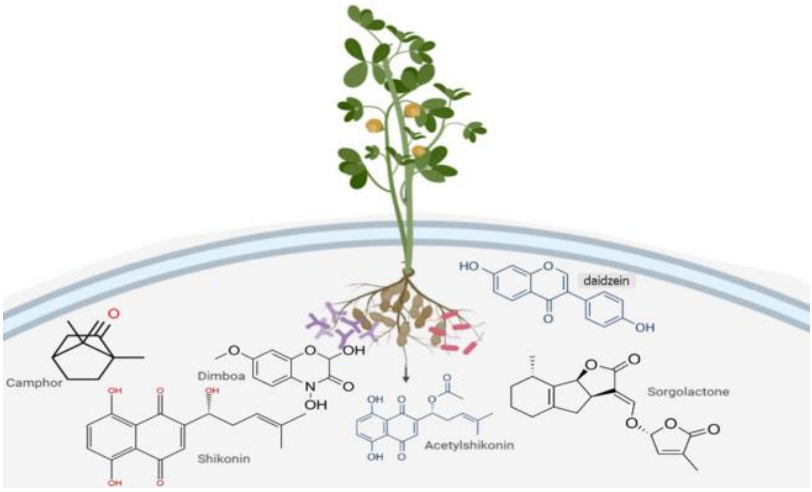

Figure 1. Secondary metabolites involved in plant interactions in the near root(Adedeji and Babalola 2020)

The life cycles of diverse plants unveil a captivating choreography of secondary metabolite evolution. Perennial herbs, in general, follow a pattern where the content and yield of secondary metabolites escalate alongside their growth. An illustrative example lies in the roots of a three-year-old Panax notoginseng, boasting the highest concentration of saponins (Hong, Lau et al.

2005). This pattern extends to Panax ginseng, where saponin content parallels the plant's growth years (Shi, Liu et al. 2007). In contrast, the aging process of Codonopsis pilosula sees a decline in triterpene levels (He, Zhu et al. 2014). The canvas of nature's narrative is rich with insights into plant development and secondary metabolite dynamics. From roots to stems, these organs reveal a tapestry of life cycles, where healing potential is interwoven with the ebb and flow of growth stages.

3.2. Leaf

Among the intricate machinery of plants, leaves emerge as the primary sites for photosynthesis, a vital process that sustains their existence. Beyond their photosynthetic role, leaves also serve as reservoirs for the synthesis and storage of secondary metabolites, the potent chemical compounds that hold significant therapeutic potential. This chapter unveils the intricate tale of leaves, where the synthesis and accumulation of secondary metabolites become a nuanced interplay influenced by an array of factors.

Figure 2. Leaves play a pivotal role in capturing solar energy for plants and producing various secondary metabolites essential for their survival.

The abundance of secondary metabolites in medicinal plant leaves is a symphony orchestrated by diverse factors. Leaf age emerges as a critical determinant, with studies by (Vázquez-León, Páramo-Calderón et al. 2017)

showcasing its significant influence. The harvesting season also leaves its imprint (Gomes, Almeida et al. 2019). Furthermore, the growth stage of leaves, a pivotal parameter, comes into play as demonstrated (Li, Kong et al. 2016). Nature's chemistry unfolds intriguingly early in some cases. Certain monoterpenes and sesquiterpenoids, like pinene, commence their biosynthesis as early as the first cotyledon stage of Melaleuca alternifolia (Southwell and Russell 2002). Notably, the youthful leaves of one-year-old Cinnamomum verum stand as a hotspot for the synthesis and accumulation of essential oil's key component, eugenol (Li, Kong et al. 2016). However, the symphony of chemical production extends throughout the leaf's life cycle. For instance, substances tied to the sabinene hydrate-terpinen-4-ol-terpinene pathways manifest later in the growth process. Leaves, whether outside or within, harbor enigmatic secretory structures such as nectaries, resin ducts, secretory vesicles, salt glands, oil cells, and secretory trichomes (Fahn 1988). These secretory structures often emerge as primary sites for the synthesis and accumulation of secondary metabolites (Figueiredo, Barroso et al. 2008). However, the secretions from these structures' manifest unique characteristics, intricately tied to the developmental stage of the leaf, impacting the production and effectiveness of therapeutic compounds (Verma and Shukla 2015). Oil cells emerge as key players in the synthesis and storage of essential oils within leaves. Variations in essential oil yields stem from alterations in oil cell distribution density and growth extent with leaf age. Notably, the density and development of oil cells share a close connection with essential oil yield. The leaves of a two-year-old branch, characterized by the highest oil cell density (6.91 n/mm^2), the highest percentage of oil cells at the oil saturation stage (48.05%), and the highest oil production (2.12%), provide a striking example. Conversely, lower oil cell percentages at oil saturation stages in leaves of annual branches (6.72% and 33.71%) lead to diminished oil yields (1.01% and 0.54%). This correlation underscores the profound influence of oil cell density and development on essential oil yield.

3.3. Flower

The majority of plants' flowers have an aromatic scent that is mostly made of terpenes and aromatic chemicals, whose synthesis and accumulation dynamics are primarily controlled by various developmental phases, circadian rhythms, biological, and abiotic factors (Figueiredo, Barroso et al. 2008). Several growth phases of *Magnolia zenii* flower buds appeared to have variable amounts of volatile oils. As the development of flower buds, volatile oil production first grew and then declined. From the buds, the maximum oil yield

was obtained in October (Hu, Yuan et al. 2012). In contrast, the finest months for gathering premium raw materials were January and February, when *Magnolia biondii* flowers had higher dry weights, volatile oil yields, and total contents of therapeutic compounds. However, there are also substantial variations in the many components that occur while plants grow. With the growth of *Achillea millefolium's* blooms, the levels of azulene declined while those of camphor and 1,8-cineole grew (Figueiredo, Barroso et al. 2008). After flowering, the concentrations of elemene and ocimene grew quickly on the second day and peaked on the sixth day in *Antirrhinum majus* (Dudareva, Martin et al. 2003).

Figure 3. Flowers, as the reproductive structures of plants, engage in intricate biochemical processes involving secondary metabolites to determine their color, fragrance, and nutritive features, which in turn influence pollination mechanisms and reproductive success within the plant's ecological context.

These variations might be controlled by how floral organs grow, how volatile chemical composition biosynthesis-regulating genes and their encoded proteins express themselves spatially and temporally, and other things (Lepelley, Cheminade et al. 2007). Due to the specialized expression of regulatory enzymes and associated genes for Secondary metabolites in plant tissues and cells, Secondary metabolites in plants are frequently produced and released in certain plant tissues and organs at particular times (Belkheir, Gaid et

al. 2016). In the *Antirrhinum majus* flower, for instance, myrcene and ocimene synthase mRNA first appeared in mature flower buds, grew slowly, and peaked on the fourth day of flowering. Latest observations on the dynamics of luteolin and chlorogenic acid accumulation at various growth stages in *Lonicera japonica Thunb*. suggest that during the growth of L. japonica, the major component accumulation first grew and then dropped. Additional investigation demonstrates that a critical regulatory gene (HQT) and the enzymatic protein it encodes directly regulate the kinetics of chlorogenic acid buildup during the development of the flower organ in *L. japonica* (Li, Kong et al. 2019). During the development of flower organs, the expression features of luteolin's regulating enzyme, CHI, are intimately correlated with each other (Kong, Li et al. 2017).

3.4. Fruits and seed

Many plants include valuable therapeutic elements in their fruits and seeds, and the stages of development also have a big impact on the components' content and makeup. Volatile oils, the primary active component in citrus fruits, are reportedly impacted by the maturing secretory cavity of the fruit (Li, Lian et al. 2006). The volatile oil concentration is often maximum when the fruit is pale yellow, and this can be utilized as a morphological indication for harvesting. In Citrus medica L. var. sarcodactylis, (Wu, Li et al. 2013) discovered that essential oil outputs increased dramatically during the maturity process and varied greatly during the maturation phases. Likewise, capsules had the highest concentrations of morphine, codeine, and thebain. When Papaver somniferum L roots achieve maturity, the maximum amount of morphine is present in the capsule (Shukla and Singh 2001). Once more, the plant's developmental stages have an impact on the content and composition of Secondary metabolites (Verma and Shukla 2015). Similar to this, there was a strong correlation between the synthesis and accumulation of Secondary metabolites and the stage of development of the seeds of medicinal plants. Coffee's quinic acid content is rather steady, however the amount of dicaffee quinic acids declines with the stage at which the seeds are developing. Quinic acid, a precursor chemical for the manufacture of chlorogenic acid, is present in high concentrations during the early stages of seed formation and noticeably declines as development progresses.

Figure 4. Secondary metabolites in fruits and seeds influence their development, protection, and dispersal by serving as attractants for seed dispersers, chemical defenses against herbivores, and regulators of germination and early seedling growth.

Table 1. Developmental stages change on the content of various plant Secondary metabolites.

Metabolite Class	Metabolite Name	Concentration Change	Developmental stages	Plant Species	Parts
Phenols	Cichoric acid	Higher	Fruiting stage	*Echinacea purpurea*	Root
	Total phenolic	Higher	Fruiting stage	*Astragalus compactus*	Root
	Chlorogenic acid,	Highest	13-year-old	*Magnolia officinalis*	Bark
	Magnolol	Highest	10-year-old	*Magnolia officinalis*	Bark
	Eugenol	Highest	1-year-old	*Cinnamomum verum*	Leaf
	Chlorogenic acid	Increasing first and then decreasing	Whole growth stage	*Lonicera japonica*	Flower
	Coffee quinic	Stable	Whole growth	coffee	Seed

Metabolite Class	Metabolite Name	Concentration Change	Developmental stages	Plant Species	Parts
	acids		stage		
	Dicoffee quinic acids	Decrease	with the developmental stage	coffee	Seed
	Quinic acid	High	Early developmental stage	coffee	Seed
Flavonoids compounds	Flavonoids	Strong increase	Before the full-bloom stage	*Scutellaria baicalensis*	Root
	Total flavonoids	Stable	Whole growth stage	*Scutellaria baicalensis*	Root
	Baicalin	Increases and then gradually decreases	Whole growth stage	*Scutellaria baicalensis*	Root
	Hyperin and quercetin	Highest	13-year-old	*Magnolia officinalis*	Bark
	Rutin, Quercitrin	Highest	7-year-old	*Magnolia officinalis*	Bark
	Luteolin	Increasing first and then decreasing	Whole growth stage	*Lonicera japonica*	Flower
Terpenoids/ Essential Oils	Triterpene	Low	Older tree	*Codonopsis pilosula*	Root
	Essential oils	Increase	Increased years	*Cinnamomum cassia*	Stem bark
	Essential oils	Highest	2-year-old branch	*Cinnamomum cassia; Cinnamomum verum*	Leaf
	Essential oils	Highest	October	*Magnolia zenii*	Flower bud
	Camphor, 1,8-cineole	Increase	With the development of the flowers	*Achillea millefolium*	Flower
	Myrcene, Ocimene	Highest	4th day of flowering	*Antirrhinum majus*	Flower
	Essential oils	Highest	Fruit is light yellow	*Citrus*	Fruit
	Essential oils	Significant	Maturation	*Citrus medica*	Fruit

Metabolite Class	Metabolite Name	Concentration Change	Developmental stages	Plant Species	Parts
		increase	process		
Others	*Trans*-cinnamaldehyde	Increasing first and then decreasing	1-12 year-old	*Cinnamomum cassia*	Stem bark
	Saponins	Highest	3 year old	*Panax notoginseng*	Root
		Increase	1–5 year old	*Panax ginseng*	Root
	Oleanolic acid, Ecdysterone	High	Vegetative growth period	*Achyranthes bidentata*	Root
	Azulene	Decrease	With the development of the flowers	*Achillea millefolium*	Flower

4. CLASIFICATION OF SECONDARY METABOLITES

Secondary metabolites known as plant polyphenols are produced by many higher plant species. The following general criteria help to distinguish them: a) Water solubility; b) Molecular weight; c) Structure and polyphenolic character; d) Intermolecular complexation; and e) Structural properties. Three distinct general properties that all polyphenols share to varying degrees allow them to play specific roles. These include their capacity to form complexes with other molecules, including macromolecules like proteins and polysaccharides, their antioxidant and radical-scavenging properties, and their complexation with metal ions (such as iron, copper, aluminum, calcium, etc) (Haslam 1996). Secondary plant metabolites are divided into many classes based on their chemical compositions. The nature of secondary plant metabolites will be covered in this chapter as a foundation for a study of the primary constituent groups thought to be of therapeutic value. Each section begins with a broad description of the structure, botanical distribution, and pharmacological principles of a particular class of secondary plant metabolites, followed by illustrations of representative molecules. Secondary plant metabolites can be divided into several categories, such as phenols, alkaloids, saponins, terpenes, lipids, and carbohydrates.

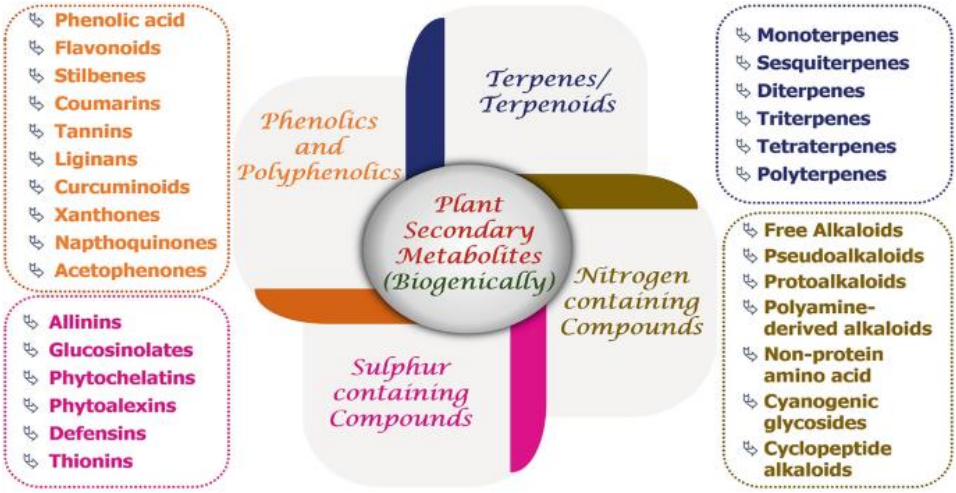

Figure 5. Classification of secondary metabolites

4.1. Phenolics

The greatest group of secondary metabolites found in plants are likely phenols. They range in complexity from simple compounds with one aromatic ring to highly complex polymeric substances, but they all have one thing in

common: the presence of one or more phenol groups**.** They are abundant in plants and significantly affect the color, flavor, and aroma of many herbs, foods, and beverages. Pharmacologically, some phenolics are prized for their anti-inflammatory effects, such as quercetin, or for their antihepatotoxic effects, such as silybin. Others have phytoestrogenic properties like genistein and daidzein, while others like naringenin are insecticidal. Many phenolic compounds, particularly flavonoids, are powerful antioxidants and free radical scavengers. Phenolics can be categorized based on their structure or place of biosynthesis. The following phenolic compounds can be categorized as a result of their structural differences: simple phenolics, tannins, flavonoids, alkaloids and xanthones, stilbenes, and lignans.

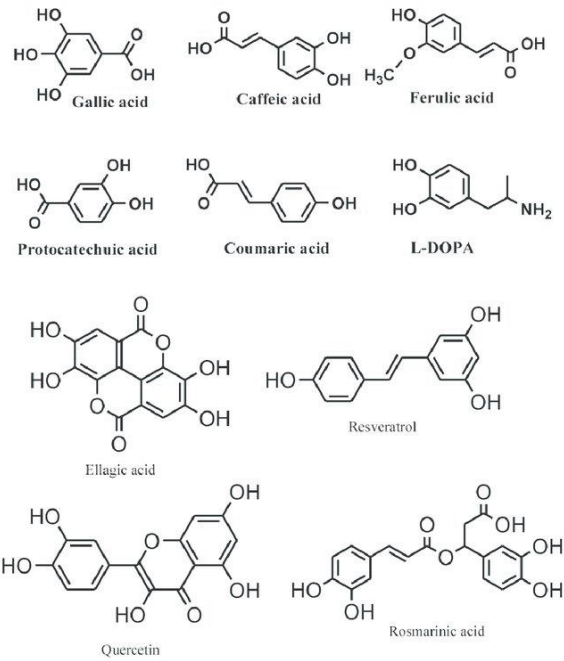

Figure 6. Structure of phenolics and flavanoids

4.2. Simple Phenolics

Plants produce phenolic acids in abundance; whereas free phenols are uncommon, gallic acid, the parent substance of the gallotannins, is quite common. Gallic acid is well recognized for its astringent effects, but it also exhibits a wide range of additional activities in vitro, including bronchodilatory, choleretic, antibacterial, antiviral, antifungal, anti-inflammatory, anticancer, antianaphylactic, and antimutagenic effects. Additionally, it inhibits insulin breakdown and encourages relaxation of smooth muscles. Eugenol, a phenolic

phenylpropane, vanillin, salicylic, ferulic, and caffeic acids are among the phenolic compounds in this group, which vary depending on their functional group, which might be a hydroxyl, aldehydic, or carboxylic group (phenolic acids).

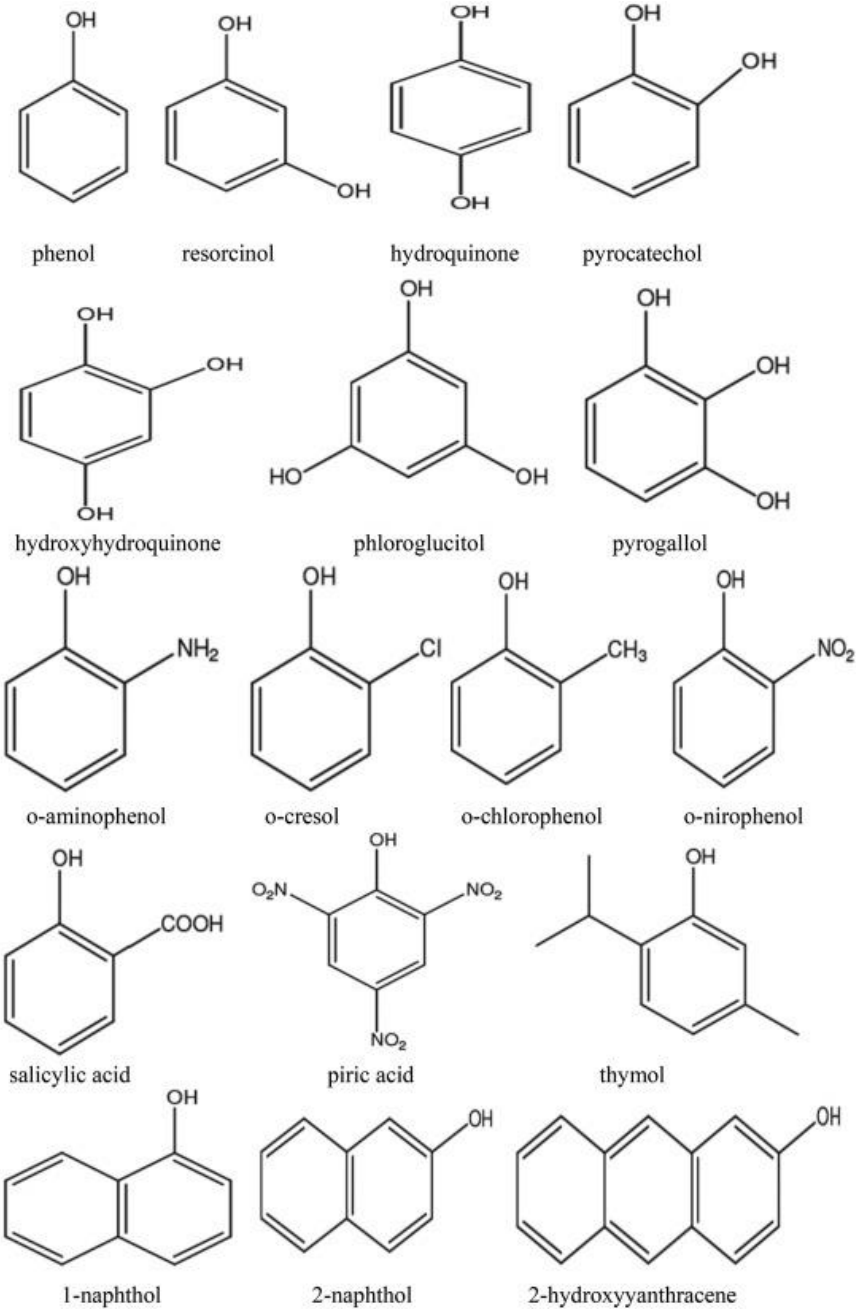

Figure 7. Structure of simple phenolics

4.3. Tannins

Polyphenols called tannins have the capacity to precipitate protein. For many years, these substances have been utilized to turn unprocessed animal hides into leather. Tannin molecules crosslink the protein during this step, increasing its resistance to bacterial and fungal attack. However, several chemicals that are currently classified as tannins due to their structure and biosynthetic origin have little to no capacity to produce leather. The systematic classification of tannins is based on their unique structural features and complex chemistry due to their complex chemistry. Tannins can be separated into two basic categories: hydrolysable and non-hydrolysable (condensed tannins), based on their chemical and structural characteristics (Hough, Briggs et al. 1982). Gallotannins and ellagitannins, two types of hydrolyzable tannins, are found in plants. When handled with tannases or hot water, hydrolyzable tannins can be separated hydrolytically into their constituent parts. Additionally, the fundamental core of tannins is made up of hydroxyl groups and polyhydric alcohols like glucose. Gallic acid (gallotannins), in which galloyl units are attached to various polyol-, catechin-, or triterpenoid units, esterifies them either completely or partially. Additionally, hexahydroxydiphenic acids (ellagitannins) lack a glycosidically linked catechin unit and are made up of at least two galloyl units that are C-C coupled to one another (Khanbabaee and van Ree 2001). Gallotannins and ellagitannins, two types of hydrolyzable tannins, are found in plants. While corilagin, chebulinic acid, and chebulagic acid are significant ellagitannins, Chinese tannin (tannic acid), Turkish tannin, Tara tannin, Acer tannin, and Hamamelis tannin are significant examples of gallotannins.

Gallotannins are hydrolyzed to produce glucose and gallic acids by enzymes, bases, or acids. Condensed tannins, on the other hand, are oligomeric and polymeric proanthocyanidins that cannot be hydrolyzed. Condensed tannins have a far more complicated structure than hydrolysable tannins. All oligomeric and polymeric proanthocyanidins known as condensed tannins are created when the C-4 of one catechin is linked to the C-8 or C-6 of the following monomeric catechin (Khanbabaee and van Ree 2001).

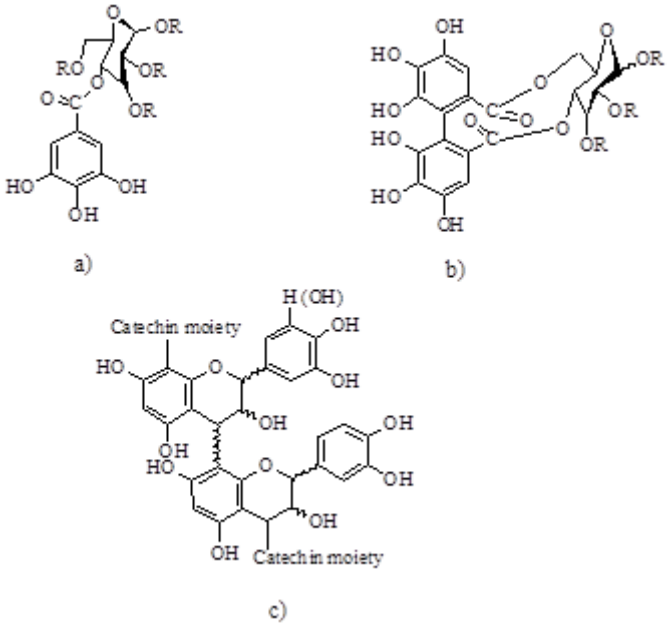

Figure 8. Gallotannic, elagitannin, and non-hydrolyzable tannins are examples of hydrolyzable tannins' structures (condensed tannins) Catechin-(48)-epicatechin, or (c) (procyanidin B4).

Tannic acid is a hydrolyzable polyphenol created by plants' secondary metabolism. Tannic acid enters the body when fruits, vegetables, drinks, and barks high in polyphenols are consumed. A typical human diet may contain 1-2 g of these ingredients per day, according to estimates (Markham 1989). Tannic acid-rich plants include tea, cocoa, beans, grapes, strawberries, and especially the bark of several oak species, sumac, and myrobalan (Gnittke and Kunze 1976, Nepka, Asprodini et al. 1999).

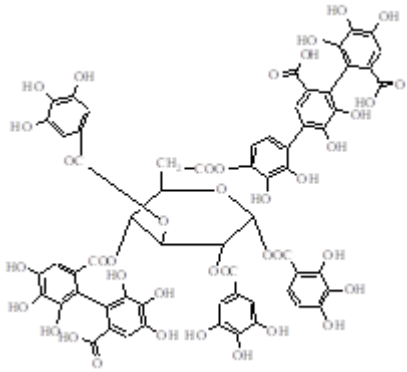

Figure 9. Structure of tannic acid.

4.4. Flavonoids

The majority of phenols that occur in nature are flavonoids. Nearly 500 of these compounds—out of the more than 2000 that are already known—occur in the free state. Flavonoids have chroman rings with an aromatic ring in position 2, 3, or 4 as part of their structural skeleton. According to the degree of oxidation of the core ring, flavonoids can be categorized into several classes (ring C). The most prevalent of them are flavones, flavonols, and anthocyanins. The Latin word for yellow, flavus, refers to the flavones and their near relatives. Although they are found throughout nature, they are more prevalent in higher plants and in juvenile tissues where they are found in the cell sap. Flavonoids are regarded as dietary supplements that promote health and fight disease. Flavonoids may have preventive effects against a number of disease conditions, including cancer and cardiovascular disease, according to epidemiological, clinical, and animal studies. Additionally, flavonoids have antiviral, antibacterial, and anti-inflammatory properties. Consumption of flavonoids is negatively connected with cardiovascular disease mortality, according to population research. According to studies, flavonoids have a positive effect on atherosclerosis-related indicators like blood platelet aggregation, lipoprotein oxidation, and vascular reactivity. It is demonstrated that the anti-inflammatory, antithrombotic, antioxidant, and hypolipidemic characteristics of flavonoids contribute significantly to the lower cardiovascular mortality seen with increasing flavonoid intake. As a result, flavonoids are currently the subject of considerable attention in both nutrition and medicine. Depending on the carbon of the C ring that the B ring is linked to as well as the level of unsaturation and oxidation of the C ring, flavonoids can be split into many subgroups. Isoflavones are flavonoids with a B ring attached to the third position of the C ring. Neoflavonoids are those in which the B ring is joined in position 4; those in which the B ring is linked in position 2 can be further split into a number of subgroups based on the structural characteristics of the C ring. These subgroups include chalcones, anthocyanins, flavones, flavonols, flavanones, and flavanonols.

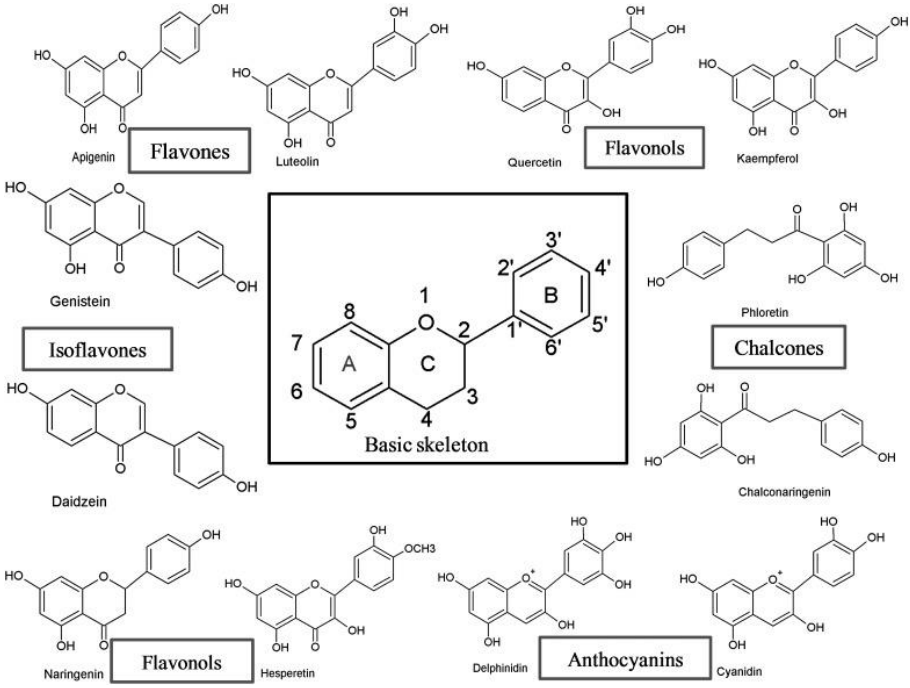

Figure 10. Basic skeleton structure of flavonoids and their classes.

4.5. Alkaloids

Alkaloids, which are typically extracted from plants, are primarily biosynthesized from amino acids and result in a range of chemical configurations. Alkaloids are often derivatives of the chemical quinolizidine that are bicyclic, tricyclic, or tetracyclic in nature. About 20% of plant species contain modest amounts of alkaloids, and research and development continue to be heavily focused on their production (including in biotechnology), extraction, and processing. For example, genetic manipulation of alkaloid biosynthesis pathways can be used to increase alkaloid output levels. Both human treatment and an organism's natural defense depend heavily on lkaoids. About 20% of the known secondary metabolites discovered in plants are alkaloids. Alkaloids in plants guard against predators and control growth. Alkaloids are particularly well known for their therapeutic uses as anesthetics, cardioprotectants, and anti-inflammatory drugs. Among the well-known alkaloids used in clinical settings are nicotine, ephedrine, strychnine, quinine, and quinine. Recently, there has been a renaissance of interest in bioactive natural compounds, motivated by both their potential for drug discovery and a very pro-active development in the area of traditional remedies. In the Dictionary of Natural Products as of 2020,

there were 27683 alkaloids listed, and between 2014 and 2020, 990 new or reexamined alkaloids from nature were reported.

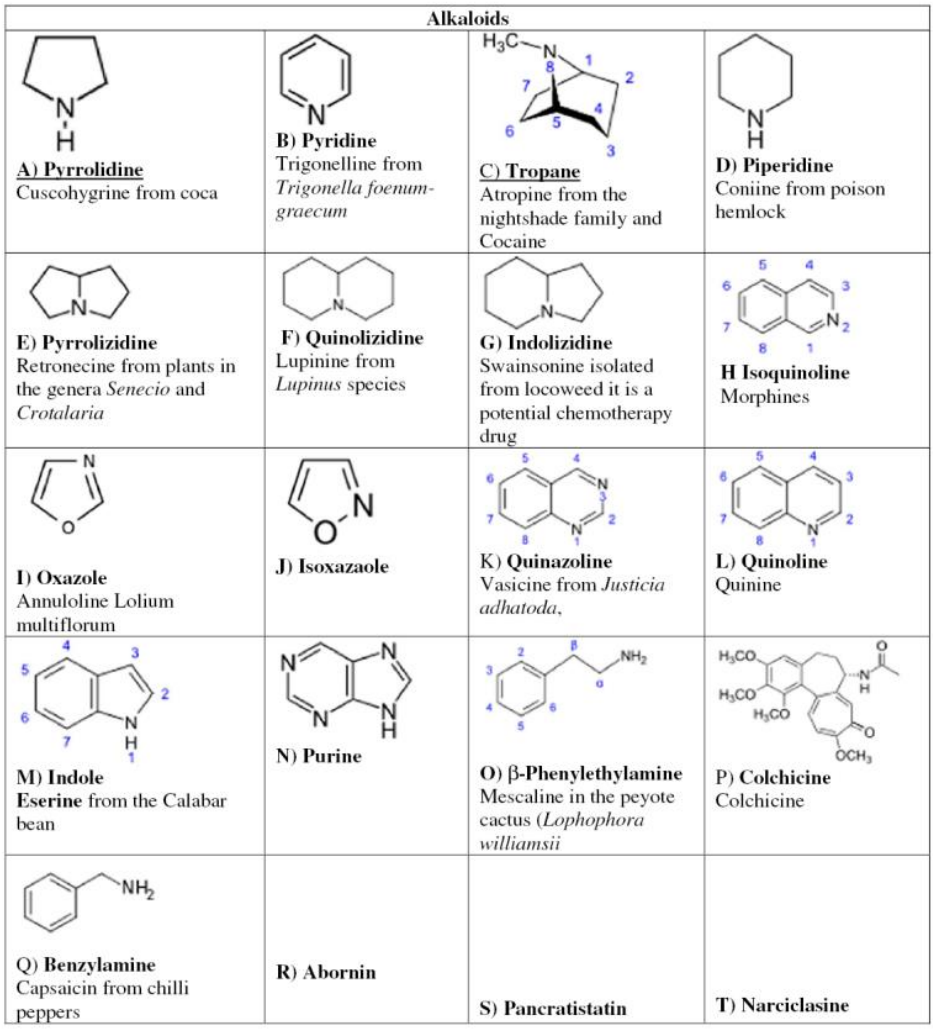

Figure 11. Different families of plant alkaloid chemicals (A–Q) are present in plants. (R) Uapaca togoensis arborinin is cytotoxic to a variety of cancer cell lines (Amer et al., 2019).

4.6. Stilbenes

A large collection of natural defense polyphenols that are present in many plant species includes stilbene chemicals. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a well-known polyphenol phytoalexin that is primarily found in the skin of grapes. Due to its potential health benefits related to its cardiovascular (French paradox), chemopreventive, antiobesity, antidiabetic, and

neuroprotective properties, it has attracted significant scientific attention. Recent research has shown that alternative stilbene chemicals, including pterostilbene, a 3,5-dimethyl ether derivative of resveratrol, may have higher bioavailability and better neuroprotective properties against AD than resveratrol alone. Numerous plant species, such as those that produce grape wine (Vitis vinifera), peanuts (Arachis hypogaea), sorghum (Sorghum bicolor), and numerous tree species, contain stilbene chemicals (Pinus and Picea). Pterostilbene is primarily found in bilberries (Vaccinium myrtillus), blueberries (several Vaccinium species), and some other berries as well as in grapes and juice residues which are an important source of this stilbene when it is used in nutraceuticals. Additionally, commercial sources of stilbenes include many plants cultivated in Asia as folk medicines, such as Polygonum cuspidatum, Rhodomyrtus tomentosa, Rheum. The primary enzyme that catalyzes the manufacture of stilbenic chemicals is called stilbene synthase (STS). In plants that make stilbenes, STS has reportedly undergone multiple independent evolutionary transitions from the chalcone synthases (CHSs). It's interesting to note that different STS genes express differently depending on the tissue and stage of development. Accordingly, it has been noted that STS genes showed lower expression levels in young than in older grape leaves, whereas the transcript levels of eight STS genes dramatically increased in the berry skin of the Cabernet Sauvignon and Norton grape cultivars post véraison, peaking at the time of harvest. Pinosylvin is abundant in pine trees' heartwood, but it is also accumulated in large concentrations in early seedlings in response to fungus or UV light stress.

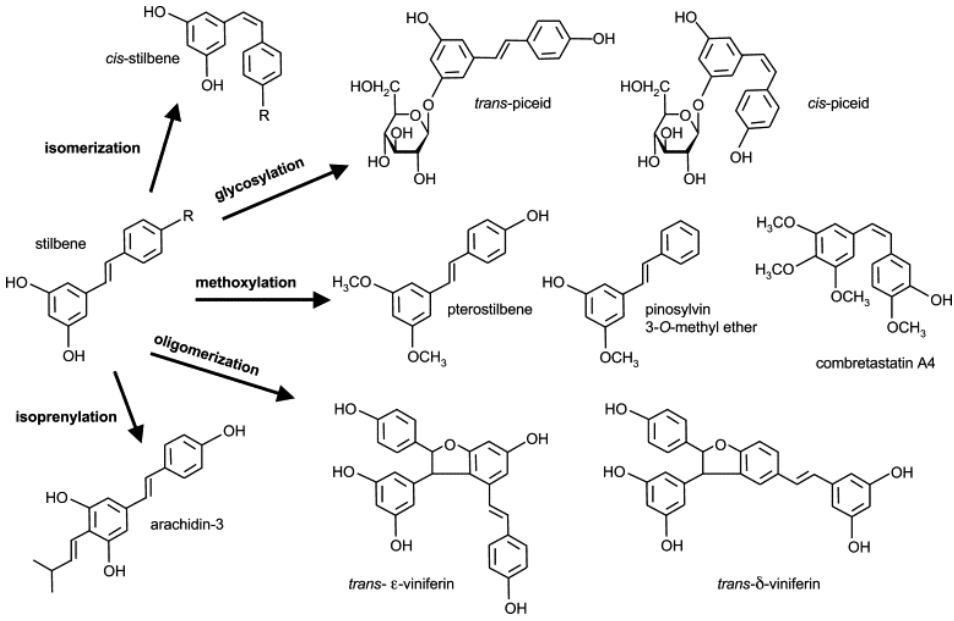

Figure 12. Most typical plant stilbene alterations.

5. SYNTHESIS OF SECONDARY METABOLITES

The synthesis of secondary metabolites in plants, fungi, and microorganisecondary metabolites involves complex biosynthetic pathways. These pathways are regulated by the expression of specific genes and the action of enzymes that catalyze various chemical reactions. While the synthesis mechanisecondary metabolites of secondary metabolites can vary depending on the specific compound and organism, there are common principles and steps involved.

The synthesis of secondary metabolites begins with the availability of precursor molecules. These precursors are often derived from primary metabolic pathways, such as the metabolism of sugars, amino acids, or fatty acids. Primary metabolites are converted into intermediate compounds, which serve as building blocks for secondary metabolite synthesis. The expression of specific genes plays a crucial role in the synthesis of secondary metabolites. The genes responsible for encoding enzymes involved in the biosynthetic pathways of secondary metabolites are often organized in gene clusters within the organism's genome. The expression of these genes is regulated by various factors, including environmental cues, developmental stages, and signaling molecules. Enzymes play a central role in the synthesis of secondary metabolites. They catalyze specific chemical reactions, including condensation, cyclization, oxidation, reduction, glycosylation, and methylation, among others. These enzymatic reactions modify the precursor molecules, leading to the formation of various intermediate compounds and the final secondary metabolite product. The intermediates produced through enzymatic reactions undergo further assembly and modification steps. These processes involve the action of additional enzymes that catalyze reactions such as glycosylation, acylation, prenylation, or other chemical modifications. These modifications can alter the chemical structure and properties of the intermediates, leading to the formation of diverse secondary metabolites. In many cases, the synthesis of secondary metabolites occurs within specific cellular compartments, such as organelles or specialized structures. Compartmentalization allows for the organization of enzymatic reactions and the sequestration of potentially toxic or reactive intermediates. The synthesis of secondary metabolites is tightly regulated to ensure proper timing, quantity, and quality of the final products. Feedback inhibition mechanisecondary metabolites are often present, where the end products of the pathway can inhibit or regulate the enzymes involved in their own synthesis. This regulation helps maintain homeostasis and prevent the overproduction or depletion of secondary metabolites.

It is important to note that the synthesis mechanisecondary metabolites of secondary metabolites can be highly complex and specific to individual compounds and organisecondary metabolites. Advances in genetic and biochemical research have significantly contributed to our understanding of these mechanisecondary metabolites. However, the complete elucidation of all the steps and regulation involved in the synthesis of secondary metabolites remains an ongoing scientific endeavor.

5.1. Significance of Studying Synthesis Mechanisecondary metabolites: Rationale for investigating the synthesis mechanisecondary metabolites of secondary metabolites and their potential applications.

The study of synthesis mechanisecondary metabolites of secondary metabolites holds significant importance in several ways. Understanding these mechanisecondary metabolites provides valuable insights into the natural world's molecular intricacies and opens up new avenues for various applications. Here are some key significances of studying synthesis mechanisecondary metabolites:

1. **Unraveling Nature's Molecular Artistry:** Secondary metabolites represent a diverse array of chemical compounds produced by plants, fungi, and microorganisecondary metabolites. Exploring the synthesis mechanisecondary metabolites of secondary metabolites allows us to appreciate the fascinating complexity and sophistication of nature's molecular artistry. By deciphering the intricate pathways and reactions involved, we gain a deeper understanding of the chemical diversity and evolutionary significance of these compounds.
2. **Discovery of Novel Bioactive Compounds:** Secondary metabolites have long been recognized for their pharmacological properties and potential therapeutic applications. Investigating the synthesis mechanisecondary metabolites provides a pathway to discover new bioactive compounds with medicinal, antimicrobial, or other beneficial properties. By understanding the biosynthetic pathways, scientists can identify the genes, enzymes, and regulatory elements responsible for secondary metabolite production. This knowledge can be harnessed to manipulate and optimize the synthesis of specific compounds, leading to the discovery of novel drugs or bioactive molecules.
3. **Drug Development and Natural Product-Based Therapies:** Many important drugs in modern medicine are derived from secondary metabolites or inspired by their structures. Studying the synthesis

mechanisecondary metabolites helps in understanding the production and regulation of these compounds, enabling the development of new drug candidates or the optimization of existing natural products. By elucidating the biosynthetic pathways, scientists can identify potential targets for drug development, enhance production yields, or engineer organisecondary metabolites for the sustainable production of secondary metabolites.

4. **Agricultural Applications:** Secondary metabolites also play a vital role in plant defense against pathogens, herbivores, and environmental stresses. Investigating the synthesis mechanisecondary metabolites helps us understand how plants produce compounds that confer resistance and protection. This knowledge can be utilized in agriculture to enhance crop resistance against pests and diseases, reduce reliance on chemical pesticides, and improve overall plant health.
5. **Biotechnological Applications:** The synthesis mechanisecondary metabolites of secondary metabolites provide a foundation for biotechnological applications. By understanding and manipulating these pathways, scientists can optimize the production of valuable secondary metabolites through genetic engineering, metabolic engineering, and synthetic biology approaches. This has implications in industries such as pharmaceuticals, flavors and fragrances, cosmetics, and agrochemicals, where secondary metabolites are used as key ingredients.
6. **Ecological and Environmental Significance:** Secondary metabolites play crucial roles in ecological interactions, such as plant-herbivore interactions, allelopathy, and microbial interactions. Understanding their synthesis mechanisecondary metabolites helps us comprehend the ecological functions and impacts of these compounds in ecosystems. This knowledge can contribute to the development of sustainable agricultural practices, conservation strategies, and environmental management approaches.
7. **Evolutionary Insights:** Studying the synthesis mechanisecondary metabolites of secondary metabolites provides insights into their evolutionary origins and diversification. By comparing biosynthetic pathways across species and tracing their evolutionary history, scientists can unravel the evolutionary forces that have shaped secondary metabolite production. This understanding enhances our knowledge of species interactions, adaptation, and the evolution of chemical defense strategies.

In conclusion, investigating the synthesis mechanisecondary metabolites of secondary metabolites is of great significance due to the broad range of potential applications and benefits it offers. Understanding these mechanical secondary metabolites provides insights into nature's molecular complexity and opens up opportunities for innovation and sustainable exploitation of secondary metabolites for different sectors, including drug development, agriculture, biotechnology, ecology, and evolutionary biology.

5.2. Primary Metabolic Pathways: Exploration of the precursor molecules derived from primary metabolic pathways, such as sugars, amino acids, and fatty acids.

Primary metabolic pathways play a fundamental role in the synthesis of precursor molecules that serve as building blocks for the production of secondary metabolites. These pathways involve the metabolism of essential macromolecules, such as sugars, amino acids, and fatty acids, which are then utilized for the biosynthesis of a wide range of secondary metabolites. Here is an exploration of the precursor molecules derived from primary metabolic pathways:

1. **Sugars:** Sugars, derived from photosynthesis in plants or the breakdown of complex carbohydrates in organisecondary metabolites, serve as essential precursors for secondary metabolite synthesis. Monosaccharides like glucose, fructose, and ribose are central to many biosynthetic pathways. They can be converted into various sugar derivatives, such as nucleotide sugars, activated glycosyl donors, or sugar phosphates, which are utilized in glycosylation reactions during secondary metabolite synthesis. Sugars also contribute to the synthesis of aromatic compounds through the shikimate pathway.
2. **Amino Acids:** Amino acids, derived from protein breakdown or de novo synthesis, are crucial precursors for the synthesis of secondary metabolites. Several amino acids serve as building blocks for the production of diverse secondary metabolites. For example, phenylalanine and tyrosine are utilized in the biosynthesis of phenolic compounds and alkaloids. Tryptophan is a precursor for the synthesis of indole derivatives, while leucine and valine contribute to the production of branched-chain secondary metabolites.
3. **Fatty Acids:** Fatty acids, derived from lipid metabolism, are important precursors for the synthesis of various secondary metabolites. They serve as building blocks for the production of lipids, which are essential

components of cell membranes and play roles in signaling and energy storage. Fatty acids are also utilized for the synthesis of complex lipophilic secondary metabolites, including polyketides, terpenoids, and steroids. These compounds are formed through iterative condensation reactions of acetyl-CoA or malonyl-CoA units, leading to the formation of diverse structures and functional groups.

4. **Nucleotides:** Nucleotides, derived from nucleotide metabolism, are involved in the synthesis of specialized secondary metabolites, such as alkaloids, aminoglycosides, and nucleoside antibiotics. Nucleotides serve as donors of nucleotide moieties, which are incorporated into the structures of these metabolites. For example, nucleotides like adenosine triphosphate (ATP) and S-adenosylmethionine (SAM) contribute to the transfer of methyl groups during methylation reactions, a common modification in secondary metabolite biosynthesis.
5. **Organic Acids:** Organic acids, derived from various metabolic pathways, provide precursor molecules for the synthesis of secondary metabolites. For example, malonate and acetyl-CoA serve as building blocks for the biosynthesis of polyketides. Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), derived from the mevalonate or non-mevalonate pathways, act as precursors for the synthesis of terpenoids, including monoterpenes, sesquiterpenes, and diterpenes.

These precursor molecules derived from primary metabolic pathways provide the essential building blocks for the synthesis of a diverse array of secondary metabolites. They undergo further modifications and reactions through specific biosynthetic pathways, ultimately leading to the production of unique chemical compounds with various biological activities. Understanding the interplay between primary and secondary metabolic pathways is crucial for unraveling the complex biosynthetic processes that generate the rich diversity of secondary metabolites found in nature.

5.3. Intermediate Formation: Discussion of the conversion of primary metabolites into intermediate compounds, serving as building blocks for secondary metabolite synthesis.

Intermediate formation is a critical step in the synthesis of secondary metabolites, where primary metabolites are converted into intermediate compounds that serve as building blocks for further biosynthetic reactions. These intermediates play a key role in the diversification and modification of

precursor molecules, leading to the production of a wide range of secondary metabolites.

1. **Formation of Building Blocks:** Primary metabolites, such as sugars, amino acids, fatty acids, and organic acids, serve as the starting materials for the biosynthesis of secondary metabolites. These primary metabolites undergo enzymatic reactions to form intermediate compounds that act as building blocks for secondary metabolite synthesis.
2. **Sugar Derivatives:** Sugars, derived from primary metabolism, can be converted into various sugar derivatives, such as nucleotide sugars, activated glycosyl donors, or sugar phosphates. These sugar derivatives serve as building blocks for glycosylation reactions, where sugars are attached to other molecules, such as aromatic compounds or lipids, during secondary metabolite biosynthesis.
3. **Amino Acid Derivatives:** Amino acids are modified through enzymatic reactions to produce intermediate compounds that act as precursors for secondary metabolites. For instance, phenylalanine and tyrosine can be converted into phenolic compounds through reactions involving hydroxylation and decarboxylation. Tryptophan can undergo various enzymatic transformations to generate indole derivatives, which serve as building blocks for alkaloid synthesis.
4. **Fatty Acid Derivatives:** Fatty acids can be modified to produce intermediate compounds that serve as building blocks for the synthesis of complex lipophilic secondary metabolites. These modifications involve enzymatic reactions, such as elongation, desaturation, or cyclization, leading to the formation of diverse fatty acid derivatives. These derivatives can then be incorporated into polyketide, terpenoid, or steroid structures.
5. **Organic Acid Derivatives:** Organic acids derived from primary metabolism can be further modified to generate intermediate compounds. For example, malonate and acetyl-CoA undergo iterative condensation reactions to form polyketide intermediates. Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), derived from organic acids in the mevalonate or non-mevalonate pathways, serve as building blocks for terpenoid synthesis.
6. **Enzymatic Reactions:** The conversion of primary metabolites into intermediate compounds is facilitated by specific enzymes. These enzymes catalyze a range of reactions, including hydroxylation, decarboxylation, acylation, prenylation, methylation, cyclization, and condensation, among others. Each enzymatic reaction introduces specific

functional groups or structural modifications, leading to the diversification and modification of the intermediate compounds.

7. **Specificity and Selectivity:** The enzymes involved in intermediate formation exhibit specificity and selectivity for particular substrates and reactions. Different enzymes or enzyme families are responsible for converting primary metabolites into specific intermediate compounds, ensuring the controlled and precise generation of building blocks for secondary metabolite synthesis. The specificity and selectivity of enzymes contribute to the structural diversity and functional complexity of secondary metabolites.
8. **Pathway Branching and Cross-Talk:** Intermediate formation can involve pathway branching and cross-talk, where precursor molecules are channeled into different biosynthetic pathways, leading to the production of multiple secondary metabolites. Enzymes and regulatory factors determine the fate of intermediates, directing them towards specific biosynthetic pathways and facilitating the synthesis of different classes of secondary metabolites.
9. **Regulation and Feedback Inhibition:** The formation of intermediates is subject to regulation and feedback inhibition to maintain homeostasis and control the synthesis of secondary metabolites. Regulatory factors and signaling molecules influence the expression and activity of enzymes involved in intermediate formation, ensuring the appropriate production and balance of building blocks for secondary metabolite biosynthesis.

The conversion of primary metabolites into intermediate compounds is a crucial step in secondary metabolite synthesis. Enzymatic reactions catalyzed by specific enzymes result in the formation of diverse intermediate molecules that act as building blocks for further biosynthetic reactions. The specificity and selectivity of enzymes, as well as the regulation and feedback inhibition mechanisecondary metabolites, contribute to the controlled and precise generation of intermediates, allowing for the synthesis of a wide range of secondary metabolites with unique structures and biological activities.

5.4. Priming Processes: Examination of priming mechanisecondary metabolites that prepare the precursor molecules for further modifications and reactions

Priming processes are essential steps in secondary metabolite synthesis that prepare precursor molecules for further modifications and reactions. These processes involve various molecular and enzymatic events that modify or

activate the precursor molecules, enabling them to undergo subsequent biosynthetic reactions.

1. **Activation of Precursors:** In some cases, precursor molecules require activation or modification to become reactive or suitable for subsequent biosynthetic reactions. This activation step may involve enzymatic reactions or chemical modifications. For example:
    a) **Amino Acid Activation:** Some amino acids, such as phenylalanine and tryptophan, undergo activation through enzymatic reactions. These reactions, such as hydroxylation or decarboxylation, introduce functional groups or modify the structure of the amino acids, making them suitable for further incorporation into secondary metabolites.
    b) **Sugar Activation:** Sugars can undergo activation through phosphorylation or nucleotide conjugation. Enzymes such as kinases or nucleotidyltransferases catalyze these reactions, resulting in the attachment of phosphate groups or nucleotide moieties to the sugars. This activation step prepares the sugars for subsequent glycosylation reactions in secondary metabolite biosynthesis.
    c) **Fatty Acid Activation:** Fatty acids may require activation before they can participate in the biosynthesis of complex lipophilic secondary metabolites. Activation occurs through the attachment of coenzyme A (CoA) to the fatty acid, catalyzed by enzymes called acyl-CoA synthetases. This activation step allows the fatty acid to be utilized in subsequent biosynthetic reactions, such as polyketide or terpenoid synthesis.

2. **Modification of Precursors:**Priming processes can involve modification of precursor molecules to generate intermediate compounds with altered properties or reactivity. These modifications may occur through enzymatic reactions, chemical transformations, or the action of specific enzymes or enzymes families. For instance:
    a) **Hydroxylation:** Hydroxylation reactions introduce hydroxyl groups (-OH) into precursor molecules, increasing their polarity and altering their chemical properties. Hydroxylation can be catalyzed by enzymes such as oxygenases or cytochrome P450 enzymes. Hydroxylated intermediates serve as building blocks for the synthesis of various secondary metabolites, including flavonoids, alkaloids, and phenolic compounds.

b) **Methylation:** Methylation reactions involve the addition of a methyl group (-CH3) to precursor molecules. These reactions are catalyzed by enzymes called methyltransferases. Methylation can influence the biological activity, stability, or solubility of the precursor molecule, preparing it for subsequent reactions. Methylation reactions commonly occur in the synthesis of alkaloids, flavonoids, and terpenoids.

c) **Acylation:** Acylation reactions involve the addition of an acyl group (-COCH3) or fatty acid chain to precursor molecules. These reactions can be catalyzed by acyltransferases or acyl-CoA ligases. Acylation modifies the hydrophobicity, stability, or reactivity of the precursor molecule, allowing it to participate in subsequent biosynthetic steps.

3. **Co-factor Activation:**Priming processes can involve the activation of co-factors necessary for subsequent biosynthetic reactions. Co-factors, such as coenzymes or metal ions, play essential roles in the catalytic activity of enzymes involved in secondary metabolite synthesis. Activation of co-factors can occur through enzymatic reactions or enzymatic conversions. Examples include:

   a) **Coenzyme Activation**: Coenzymes, such as S-adenosylmethionine (SAM) or adenosine triphosphate (ATP), are often involved in methylation or phosphorylation reactions, respectively. These coenzymes may require enzymatic activation or conversion to their active forms before participating in subsequent biosynthetic steps.

   b) **Metal Ion Activation:** Some enzymes involved in secondary metabolite synthesis require metal ions, such as iron (Figueiredo, Barroso et al.), zinc (Zn), or magnesium (Spitaler, Schlorhaufer et al.) , for their catalytic activity. Activation of these metal ions may involve their binding to specific proteins or chelation with other molecules to form bioactive complexes.

Priming processes are crucial for preparing precursor molecules to undergo subsequent modifications and reactions in the synthesis of secondary metabolites. Activation, modification, and co-factor activation steps enhance the reactivity, stability, or specificity of the precursor molecules, ensuring their suitability for incorporation into complex secondary metabolite structures. These priming mechanisecondary metabolites, along with the subsequent

biosynthetic reactions, contribute to the formation of the diverse array of secondary metabolites found in nature.

5.5. Gene Clusters: Description of gene clusters responsible for encoding enzymes involved in secondary metabolite synthesis.

Gene clusters play a significant role in the biosynthesis of secondary metabolites. These clusters consist of a group of genes that are physically linked together on the genome and are responsible for encoding enzymes and regulatory elements involved in the synthesis of specific secondary metabolites. The presence of gene clusters allows for coordinated expression and regulation of the biosynthetic pathway, ensuring the efficient production of secondary metabolites.

1. **Gene Cluster Structure:** Gene clusters are characterized by a contiguous arrangement of genes on the genome. These clusters can range in size from a few genes to several dozen genes. Typically, gene clusters include not only the genes encoding enzymes involved in the biosynthetic pathway but also regulatory elements, transporters, and other accessory genes necessary for the production of the secondary metabolite.
2. **Coordinated Gene Expression:** The physical proximity of genes within a cluster facilitates their coordinated expression. The genes within the cluster are often transcribed as a single polycistronic mRNA, allowing for synchronized regulation. Coordinated gene expression ensures that the enzymes and regulatory elements required for secondary metabolite synthesis are produced simultaneously, enhancing the efficiency of the biosynthetic pathway.
3. **Enzyme Complementation:** Gene clusters often encode a series of enzymes that catalyze sequential reactions in the biosynthetic pathway. The close proximity of these genes within the cluster enables efficient enzyme complementation. Intermediate metabolites produced by one enzyme can be quickly passed to the next enzyme in the pathway, minimizing loss or diffusion of intermediates and facilitating the overall flow of the biosynthetic pathway.
4. **Regulation and Control:** Gene clusters contain regulatory elements that control the expression of the genes within the cluster. These regulatory elements can include promoters, enhancers, transcription factors, and other regulatory motifs. The coordinated regulation of gene expression within the cluster allows for precise control of secondary metabolite synthesis in response to various signals, such as environmental cues, developmental stages, or stress conditions.

5. **Evolutionary Conservation:** Gene clusters involved in secondary metabolite synthesis are often evolutionarily conserved across related organisecondary metabolites. This conservation suggests that the acquisition and maintenance of gene clusters have provided selective advantages for the production of specific secondary metabolites. The presence of conserved gene clusters enables the transfer of biosynthetic pathways between organisecondary metabolites through horizontal gene transfer or other genetic mechanisecondary metabolites.
6. **Facilitating Genetic Manipulation:** The clustering of genes involved in secondary metabolite synthesis provides practical advantages for genetic manipulation. The presence of a gene cluster makes it easier to identify and isolate the genes of interest, enabling the study of individual enzymes or the entire biosynthetic pathway. Gene cluster organization also facilitates the engineering of organisecondary metabolites for enhanced secondary metabolite production through genetic modification or synthetic biology approaches.
7. **Predictive Power:** The presence of gene clusters associated with specific secondary metabolites provides valuable information for predicting the biosynthetic potential of an organism. By analyzing the genome and identifying gene clusters, researchers can gain insights into the secondary metabolites an organism is capable of producing. This predictive power aids in the discovery of novel secondary metabolites or the optimization of existing pathways for desired compound production.

Gene clusters play a crucial role in the biosynthesis of secondary metabolites. They provide a means for coordinating gene expression, facilitating enzyme complementation, and ensuring efficient production of secondary metabolites. Gene clusters also contribute to the regulation, control, and evolutionary conservation of secondary metabolite synthesis. Their presence aids in genetic manipulation and provides predictive power for understanding an organism's secondary metabolite potential. Studying gene clusters is essential for unraveling the biosynthesis of secondary metabolites and exploring their diverse functions and applications.

5.6. Regulatory Factors: Overview of environmental cues, developmental stages, and signaling molecules that regulate gene expression and influence secondary metabolite production.

Regulatory factors play a vital role in controlling gene expression and influencing secondary metabolite production. These factors respond to various

environmental cues, developmental stages, and signaling molecules to modulate the expression of genes involved in secondary metabolite biosynthesis. Understanding these regulatory mechanisecondary metabolites is crucial for manipulating secondary metabolite production and optimizing their yield. Here is an overview of the regulatory factors that influence secondary metabolite production:

Environmental cues such as light, temperature, nutrient availability, and biotic or abiotic stressors can trigger changes in secondary metabolite production. Light quality, intensity, and photoperiod can influence the expression of genes involved in secondary metabolite biosynthesis. For instance, plants' ability to produce flavonoids can be controlled by light. Temperature fluctuations can impact secondary metabolite production. Some plants and microorganisecondary metabolites produce secondary metabolites in response to specific temperature ranges. The availability of essential nutrients can affect secondary metabolite biosynthesis. Nutrient limitation or excess can induce or suppress the production of certain secondary metabolites. Plants and microorganisecondary metabolites often produce secondary metabolites in response to biotic stresses such as pathogen attack or herbivory, as well as abiotic stresses such as drought, salinity, or heavy metal exposure. Secondary metabolite production is often developmentally regulated. Different stages of growth and development, including seed germination, vegetative growth, flowering, and senescence, can influence the expression of genes involved in secondary metabolite biosynthesis. For instance, Floral Development: Secondary metabolites such as volatile compounds or pigments are often produced during flower development to attract pollinators or protect reproductive structures. Secondary metabolites can accumulate during senescence, which is the aging process in plants and microorganisecondary metabolites. These metabolites may have roles in nutrient recycling or defense mechanisecondary metabolites.

Signaling molecules, such as hormones and signaling pathways, can modulate secondary metabolite production. They act as molecular messengers, transmitting information and triggering specific cellular responses. Phytohormones, including auxins, gibberellins, cytokinins, abscisic acid, and ethylene, can influence secondary metabolite production in plants. These hormones regulate gene expression and metabolic pathways involved in secondary metabolite biosynthesis. Signaling pathways associated with defense responses, such as jasmonic acid (Lepelley, Cheminade et al. 2007) and salicylic acid (Rai, Gaur et al.) pathways, can induce the production of secondary metabolites with antimicrobial or anti-herbivory properties.

Intracellular signaling pathways, such as protein kinases and transcription factors, can regulate secondary metabolite biosynthesis by activating or repressing the expression of relevant genes. Epigenetic mechanisecondary metabolites, such as DNA methylation, histone modifications, and small RNA-mediated gene silencing, can influence secondary metabolite production. These modifications can alter chromatin structure and gene expression, thereby impacting the synthesis of secondary metabolites. Secondary metabolite biosynthesis is often subject to feedback inhibition, where the end products or intermediate metabolites regulate the expression or activity of enzymes involved in their own biosynthesis. Feedback inhibition ensures the maintenance of appropriate levels of secondary metabolites and prevents their overproduction or depletion.

Understanding and manipulating these regulatory factors can provide strategies for optimizing secondary metabolite production. By modifying environmental conditions, developmental stages, or signaling pathways, it is possible to enhance or suppress the production of specific secondary metabolites with desired properties. Harnessing the regulatory factors that influence secondary metabolite production holds significant potential for various applications, including medicine, agriculture, and biotechnology.

5.7. Epigenetic Regulation: Discussion of epigenetic modifications that can modulate gene expression and impact secondary metabolite synthesis.

Epigenetic regulation plays a crucial role in modulating gene expression and influencing secondary metabolite synthesis. Epigenetic modifications are heritable changes in gene function that do not involve alterations in the DNA sequence. These modifications can impact the accessibility of genes to transcriptional machinery, leading to changes in gene expression levels.

1. **DNA Methylation:** DNA methylation is one of the most well-studied epigenetic modifications. It involves the addition of a methyl group (-CH3) to the cytosine residue of DNA, typically occurring at CpG dinucleotides. DNA methylation patterns can vary across the genome and can influence gene expression by affecting transcription factor binding, chromatin structure, and the recruitment of chromatin remodeling complexes.
2. **Gene Silencing:** DNA methylation can lead to gene silencing, resulting in the downregulation of genes involved in secondary metabolite biosynthesis. Methylation of promoter regions or gene bodies can inhibit the binding of transcription factors, RNA polymerase, or other regulatory proteins, thereby reducing gene expression levels.

3. **Differential Methylation:** Changes in DNA methylation patterns can occur in response to environmental cues, developmental stages, or stress conditions. These changes can modulate the expression of genes involved in secondary metabolite biosynthesis, leading to altered secondary metabolite profiles.
4. **Histone Modifications:** Histone proteins, which make up the core of nucleosomes, can undergo various post-translational modifications that impact chromatin structure and gene expression. Histone modifications can be dynamic and reversible, playing a role in regulating gene expression states. Some histone modifications relevant to secondary metabolite synthesis include:
5. **Histone Acetylation:** Acetylation of histone proteins, catalyzed by histone acetyltransferases (HATs), leads to a more open chromatin structure and increased gene expression. Acetylation of histones near genes involved in secondary metabolite biosynthesis can enhance their expression.
6. **Histone Methylation:** Methylation of histone proteins can have different effects depending on the specific residue and the degree of methylation. For example, trimethylation of histone H3 lysine 4 (H3K4me3) is associated with active gene transcription, while trimethylation of histone H3 lysine 27 (H3K27me3) is generally associated with gene repression. Histone methylation can influence the expression of genes involved in secondary metabolite biosynthesis.
7. **Small RNA-Mediated Gene Silencing:** Small RNAs, including microRNAs (miRNAs) and small interfering RNAs (siRNAs), can modulate gene expression through sequence-specific interactions with target mRNAs. These small RNAs guide the RNA-induced silencing complex (RISC) to degrade target mRNAs or inhibit their translation. Small RNA-mediated gene silencing can impact secondary metabolite biosynthesis by repressing the expression of genes involved in the pathway or targeting key regulatory genes.
8. **Transposable Elements:** Transposable elements are repetitive DNA sequences that can move or transpose within the genome. These elements can impact gene expression by inserting near or within genes, affecting their regulation. Transposable elements can introduce epigenetic modifications and alter chromatin structure, leading to changes in the expression of genes involved in secondary metabolite biosynthesis.
9. **Crosstalk with Other Epigenetic Mechanisecondary metabolites:** Epigenetic modifications do not act independently but often crosstalk

with each other, forming a complex regulatory network. For example, DNA methylation can recruit proteins that recognize methylated DNA and further recruit histone-modifying enzymes. This crosstalk between different epigenetic mechanisecondary metabolites can modulate gene expression and impact secondary metabolite synthesis.

Understanding the role of epigenetic regulation in secondary metabolite synthesis is crucial for manipulating and optimizing their production. By modulating the epigenetic landscape through genetic or environmental interventions, it is possible to enhance the expression of key genes involved in secondary metabolite biosynthesis and achieve desired secondary metabolite profiles. Epigenetic regulation provides a powerful avenue for fine-tuning secondary metabolite production and exploring the potential of these compounds in various applications.

5.8. Catalytic Enzymes: Examination of specific enzymes involved in the biosynthesis of secondary metabolites, including condensation, cyclization, oxidation, reduction, glycosylation, and methylation.

Catalytic enzymes play a crucial role in the biosynthesis of secondary metabolites, facilitating a wide range of reactions such as condensation, cyclization, oxidation, reduction, glycosylation, and methylation. These enzymes are responsible for the specific modifications and transformations of precursor molecules, leading to the production of structurally diverse and biologically active secondary metabolites. Here is an examination of specific enzymes involved in the biosynthesis of secondary metabolites:

1. **Condensation Enzymes:** Condensation enzymes catalyze the joining of two or more precursor molecules, often through the formation of carbon-carbon or carbon-nitrogen bonds. Examples include:
    a) **Polyketide Synthases (PKSs):** PKSs are responsible for the assembly of polyketides, a large class of secondary metabolites. They catalyze iterative condensation reactions between acyl-CoA or malonyl-CoA building blocks, leading to the formation of diverse polyketide structures.
    b) **Non-Ribosomal Peptide Synthetases (NRPSs):** NRPSs are involved in the biosynthesis of peptides, including many antibiotics and cyclic peptides. NRPSs assemble amino acids into peptide chains through a series of condensation reactions, with each amino acid added in a specific order.

2. **Cyclization Enzymes:** Cyclization enzymes catalyze the formation of cyclic structures within secondary metabolites. These enzymes bring reactive groups in the precursor molecules into proximity, facilitating intramolecular reactions. Examples include:
    a) **Terpene Cyclases:** Terpene cyclases are responsible for the cyclization of linear isoprenoid precursors, leading to the formation of diverse terpenoid structures. They catalyze cyclization reactions that involve rearrangement of carbon-carbon bonds and formation of new carbon-carbon or carbon-oxygen bonds.
    b) **Polyketide Cyclases:** Polyketide cyclases are involved in the formation of cyclic structures in polyketides. They catalyze intramolecular cyclization reactions, determining the position and stereochemistry of the cyclization events within the polyketide chain.

3. **Oxidation and Reduction Enzymes:** Oxidation and reduction enzymes modify precursor molecules by adding or removing electrons, altering the oxidation state of atoms within the molecule. Examples include:
    a) **Cytochrome P450 Monooxygenases:** Cytochrome P450 enzymes are involved in the oxidation of various substrates in secondary metabolite biosynthesis. They introduce oxygen atoms into precursor molecules, leading to the formation of hydroxyl groups or other oxidized functional groups.
    b) **Flavin-Dependent Monooxygenases:** Flavin-dependent monooxygenases catalyze oxidation reactions by utilizing flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) as cofactors. They introduce oxygen into the substrate, leading to the formation of various oxidized products.
    c) **Reductases:** Reductases catalyze reduction reactions, involving the addition of electrons to precursor molecules. They can reduce functional groups such as carbonyl groups to hydroxyl groups or facilitate the formation of specific stereochemical configurations.

4. **Glycosylation Enzymes:** Glycosylation enzymes catalyze the addition of sugar moieties to precursor molecules, forming glycosidic bonds. Glycosyltransferases transfer activated sugar moieties from nucleotide sugars to acceptor molecules, such as phenols, alkaloids, or lipids. These enzymes play a key role in the diversification and modification of secondary metabolites through glycosylation reactions.

5. **Methylation Enzymes:** Methylation enzymes add methyl groups (-CH3) to precursor molecules, often through the transfer of a methyl group from a methyl donor. Methyltransferases transfer a methyl group from a methyl donor, such as S-adenosylmethionine (SAM), to specific acceptor molecules. Methylation reactions can occur on various functional groups, including hydroxyl, amino, or carboxyl groups.

These are just a few examples of the diverse array of enzymes involved in the biosynthesis of secondary metabolites. Each class of secondary metabolites may have specific enzymes tailored to their unique biosynthetic pathways. The activity and specificity of these enzymes contribute to the structural diversity and complexity of secondary metabolites, allowing for the production of compounds with a wide range of biological activities.

5.9. Enzymatic Cascade Reactions: Description of sequential enzymatic reactions that convert precursor molecules into intermediate compounds.

Enzymatic cascade reactions involve a series of sequential enzymatic reactions that convert precursor molecules into intermediate compounds. These cascades play a crucial role in the biosynthesis of complex molecules, including secondary metabolites. The reactions occur in a specific order, with each enzyme catalyzing a specific transformation, leading to the stepwise conversion of the precursor molecule into the desired intermediate. Here is a description of the process of enzymatic cascade reactions:

1. **Recognition and Activation:** The enzymatic cascade begins with the recognition of the precursor molecule by the first enzyme in the pathway. This enzyme recognizes the specific substrate and binds to it, facilitating the activation of the precursor through various mechanisecondary metabolites. Activation can involve the modification of functional groups, such as phosphorylation, acetylation, or oxidation, to create a reactive intermediate that can undergo further transformations.
2. **Sequential Enzymatic Transformations:** Once the precursor molecule is activated, it undergoes a series of sequential enzymatic transformations. Each enzymatic step is catalyzed by a specific enzyme that recognizes the activated intermediate and performs a specific chemical transformation. The reactions can include functional group modifications, bond formation or cleavage, or stereochemical changes. Each enzymatic step is tightly regulated to ensure the proper order and coordination of reactions.

3. **Intermediate Formation:** As the enzymatic cascade progresses, intermediate compounds are formed. These intermediates are the products of each individual enzymatic step and serve as substrates for the subsequent enzymatic reactions. The intermediates can be chemically modified versions of the precursor molecule, containing additional functional groups or altered structures. The intermediates may also have increased or decreased reactivity compared to the precursor molecule, making them suitable for further enzymatic transformations.
4. **Substrate Channeling:** In some cases, enzymatic cascade reactions involve substrate channeling, where the intermediate product of one enzyme is directly transferred to the active site of the next enzyme in the pathway without being released into the bulk solvent. Substrate channeling can enhance the efficiency of the cascade by minimizing diffusion and reducing the loss of intermediates. It allows for efficient transfer of reactive intermediates and minimizes side reactions or competition with other molecules.
5. **Regulation and Control:** Enzymatic cascade reactions are tightly regulated to ensure the proper timing and efficiency of the pathway. Regulatory factors, such as co-factors, signaling molecules, or transcriptional control, can influence the expression, activity, or localization of the enzymes involved in the cascade. Feedback inhibition, allosteric regulation, or substrate availability can also modulate the enzymatic reactions, maintaining the balance between precursor molecule availability and intermediate formation.
6. **Final Product Formation:** The enzymatic cascade culminates in the production of the final product, which is the result of the sequential enzymatic transformations of the precursor molecule. The final product can be a complex secondary metabolite with unique structures and biological activities. The product can then undergo further modifications or be exported from the cell, contributing to the organism's physiology or interaction with the environment.

Enzymatic cascade reactions are a powerful strategy for the stepwise transformation of precursor molecules into intermediate compounds and final products. The sequential nature of these reactions allows for precise control over the biosynthetic pathway, ensuring the production of complex molecules with high specificity and efficiency. Understanding the enzymatic cascade reactions involved in the biosynthesis of secondary metabolites provides

insights into the complexity and diversity of these natural products and opens avenues for their manipulation and optimization.

5.10. Co-factor Involvement: Discussion of co-factors, such as cofactors or coenzymes, required for enzymatic reactions and their role in secondary metabolite synthesis.

Co-factors, including cofactors and coenzymes, play a crucial role in enzymatic reactions involved in secondary metabolite synthesis. These small molecules are required by enzymes to catalyze specific reactions and facilitate the biosynthesis of complex secondary metabolites. Co-factors often act as carriers of chemical groups or electrons, provide structural support, or serve as co-substrates for enzymatic transformations.

1. **Coenzymes:** Coenzymes are small organic molecules that associate with enzymes and assist in catalytic reactions. They often act as carriers of chemical groups, such as functional moieties or electrons, during the enzymatic transformations. Coenzymes are derived from vitamins and are essential for the proper functioning of enzymes involved in secondary metabolite synthesis. Examples of coenzymes involved in secondary metabolite biosynthesis include:
2. **S-Adenosylmethionine (SAM):** SAM is a coenzyme derived from the amino acid methionine. It serves as a methyl group donor in methylation reactions, contributing to the modification of secondary metabolite precursors.
3. **Adenosine Triphosphate (ATP):** ATP is a universal energy carrier and coenzyme involved in various enzymatic reactions. ATP provides energy for biosynthetic reactions, including those involved in the formation of covalent bonds during secondary metabolite synthesis.
4. **Coenzyme A (CoA):** CoA is derived from pantothenic acid (vitamin B5) and plays a critical role in acylation reactions. CoA functions as an acyl group carrier, facilitating the transfer of acyl groups to precursor molecules during secondary metabolite biosynthesis.
5. **Metal Ions:** Metal ions, such as iron (Figueiredo, Barroso et al. 2008), zinc (Zn), magnesium (Spitaler, Schlorhaufer et al.), or copper (Behrens, Smith et al.), often serve as co-factors for enzymes involved in secondary metabolite synthesis. Metal ions can coordinate with certain amino acid residues within the enzyme's active site, enabling catalytic activity. Metal ions are particularly important for redox reactions or for stabilizing reaction intermediates. For example:

a) **Iron-Sulfur Clusters:** Iron-sulfur clusters, composed of iron and sulfur atoms, are involved in electron transfer reactions. They participate in redox reactions, such as oxidation or reduction, during secondary metabolite biosynthesis.
b) **Non-Heme Iron:** Non-heme iron co-factors are essential for various oxygenation reactions, including hydroxylation or epoxidation reactions. These reactions are common in the biosynthesis of secondary metabolites, such as flavonoids or alkaloids.

6. **NAD(P) and FAD:** Nicotinamide adenine dinucleotide (Lepelley, Cheminade et al. 2007) and flavin adenine dinucleotide (FAD) are coenzymes involved in redox reactions. They can accept or donate electrons during enzymatic reactions, participating in oxidation or reduction reactions in secondary metabolite biosynthesis. These coenzymes are crucial for reactions that involve the transfer of hydride ions (H-) or single electrons.
7. **Tetrahydrofolate (THF):** Tetrahydrofolate (THF) is derived from folic acid (vitamin B9) and serves as a co-factor in one-carbon transfer reactions. THF plays a role in the transfer of one-carbon units, such as methyl groups or formyl groups, during the biosynthesis of secondary metabolites. It participates in the synthesis of amino acids, nucleotides, and other important intermediates in secondary metabolite pathways.

Co-factors are essential for the proper functioning of enzymes involved in secondary metabolite synthesis. They provide the necessary chemical groups, energy, or redox potential required for enzymatic reactions. Co-factors play a critical role in the biosynthesis of complex secondary metabolites by facilitating the diverse array of transformations and modifications necessary for their production. Understanding the involvement of co-factors in enzymatic reactions provides insights into the metabolic pathways and regulatory mechanisecondary metabolites underlying secondary metabolite biosynthesis.

5.11. Enzymatic Modifications: Exploration of enzymes catalyzing assembly and modification reactions, including glycosylation, acylation, prenylation, and other chemical modifications.

Enzymatic modifications play a crucial role in the assembly and modification of secondary metabolites. These modifications are catalyzed by

specific enzymes that introduce functional groups, attach chemical moieties, or perform other transformations on precursor molecules. Enzymatic modifications contribute to the structural diversity and biological activities of secondary metabolites.

1. **Glycosylation:** Glycosylation involves the attachment of sugar moieties to precursor molecules, forming glycosidic bonds. This modification can impact the solubility, stability, and bioactivity of secondary metabolites. Glycosylation enzymes, such as glycosyltransferases, catalyze the transfer of activated sugar molecules from nucleotide sugars to acceptor molecules. Examples of glycosylation in secondary metabolite biosynthesis include the addition of glucose, rhamnose, or glucuronic acid to secondary metabolites.
2. **Acylation:** Acylation involves the addition of an acyl group (-COCH3) or fatty acid chain to precursor molecules. Acylation can modulate the hydrophobicity, stability, or biological activity of secondary metabolites. Acylation enzymes, including acyltransferases or acyl-CoA ligases, catalyze the transfer of acyl groups to acceptor molecules. Acylation reactions are involved in the biosynthesis of various secondary metabolites, such as fatty acid-derived compounds, polyketides, or flavonoids.
3. **Prenylation:** Prenylation is the addition of prenyl groups (isoprenoid units) to precursor molecules. Prenylation reactions can influence the biological activity, subcellular localization, or stability of secondary metabolites. Prenylation enzymes, such as prenyltransferases, catalyze the transfer of prenyl groups derived from isopentenyl pyrophosphate (IPP) or its derivatives to acceptor molecules. Prenylation reactions are common in the biosynthesis of terpenoids, where the addition of prenyl groups leads to the formation of diverse terpenoid structures.
4. **Methylation:** Methylation involves the addition of a methyl group (-CH3) to precursor molecules. Methylation reactions can impact the biological activity, stability, or solubility of secondary metabolites. Methylation enzymes, such as methyltransferases, transfer a methyl group from a methyl donor, often S-adenosylmethionine (SAM), to specific acceptor molecules. Methylation reactions occur on various functional groups, including hydroxyl, amino, or carboxyl groups. Methylation plays a significant role in the biosynthesis of alkaloids, flavonoids, and other secondary metabolites.
5. **Hydroxylation:** Hydroxylation involves the addition of a hydroxyl group (-OH) to precursor molecules. Hydroxylation reactions can alter the

polarity, stability, or reactivity of secondary metabolites. Hydroxylation enzymes, including oxygenases or cytochrome P450 enzymes, catalyze the introduction of hydroxyl groups. Hydroxylation is a common modification in the biosynthesis of phenolic compounds, alkaloids, and flavonoids.

6. **Oxidation and Reduction:** Oxidation and reduction reactions involve the addition or removal of electrons from precursor molecules. These reactions can lead to the formation of new functional groups or changes in oxidation states. Oxidation and reduction enzymes, such as cytochrome P450 monooxygenases or reductases, catalyze these transformations. These enzymatic reactions are involved in the biosynthesis of various secondary metabolites, including polyketides, alkaloids, and phenolic compounds.
7. **Other Chemical Modifications:** Secondary metabolites can undergo various other chemical modifications catalyzed by specific enzymes. Examples include ring cleavage reactions, rearrangements, or cyclization reactions. These modifications contribute to the structural diversity and complexity of secondary metabolites and are essential for their biological activities.

Enzymatic modifications are crucial for the assembly and modification of secondary metabolites. The specific enzymes involved in these modifications catalyze the introduction of functional groups, attachment of chemical moieties, or other transformations, leading to the production of structurally diverse and biologically active secondary metabolites. Understanding the enzymes involved in these modifications provides insights into the biosynthetic pathways and regulatory mechanisecondary metabolites underlying the production of secondary metabolites.

5.12. Chemical Diversity Generation: Analysis of the modifications that lead to the formation of diverse secondary metabolites with unique chemical structures and properties.

Chemical diversity generation is a key aspect of secondary metabolite biosynthesis, leading to the formation of a vast array of compounds with unique chemical structures and properties. These modifications occur through a series of enzymatic reactions that introduce diverse functional groups, alter stereochemistry, create cyclic structures, or form complex scaffolds.

1. **Functional Group Modifications:** Secondary metabolites often undergo modifications that introduce or modify functional groups.

a) **Hydroxylation:** The addition of hydroxyl groups (-OH) can increase polarity, alter reactivity, or introduce sites for further modifications.
b) **Methylation:** Methylation involves the addition of a methyl group (-CH3) and can impact solubility, stability, or biological activity.
c) **Glycosylation:** Glycosylation adds sugar moieties to secondary metabolites, affecting solubility, bioactivity, and cellular recognition.
d) **Acylation:** Acylation involves the addition of acyl groups (-COCH3) or fatty acid chains, influencing lipophilicity and biological activity.
e) **Oxidation/Reduction:** The addition or removal of electrons can lead to the formation of functional groups, such as aldehydes, ketones, or alcohols, altering reactivity and properties.

2. Stereochemical Modifications:

Stereochemical modifications contribute to the structural diversity and biological activity of secondary metabolites. Enzymes can introduce stereochemical changes by:

a) **Cyclization:** Enzymatic cyclization reactions generate complex ring structures, leading to diverse scaffolds and stereocenters.
b) **Epimerization:** Epimerization involves the reversible interconversion of stereoisomers, leading to changes in the configuration of specific carbon atoms.
a) **Reduction/Oxidation:** These reactions can change the oxidation state of carbon atoms, leading to stereoselective modifications.
b) **Cyclization and Ring Formation:** Cyclization reactions play a significant role in generating structural diversity. Enzymes can catalyze cyclization reactions that result in the formation of various ring structures, such as:
c) **Intramolecular Reactions:** Enzymes catalyze intramolecular reactions, bringing reactive groups in the precursor molecule into proximity to form cyclic structures.
d) **Enzymatic Cascade Reactions:** Sequential enzymatic transformations can lead to the stepwise assembly of complex ring systems.
e) **Cyclases:** Cyclases are enzymes that specifically catalyze the formation of cyclic structures in secondary metabolites, resulting in diverse scaffolds and ring systems.
f) **Rearrangements and Cleavage Reactions:** Rearrangements and cleavage reactions contribute to the chemical diversity of secondary metabolites by introducing structural changes or generating new functional groups. Examples include:

g) **Rearrangement Reactions:** Enzymes can catalyze rearrangement reactions, involving the migration of functional groups or the formation of new bonds within the molecule.
h) **Ring Cleavage:** Enzymatic ring-opening reactions can lead to the formation of new functional groups or the generation of smaller fragments with distinct chemical properties.

3. Non-Enzymatic Modifications:

In addition to enzymatic modifications, secondary metabolites can undergo non-enzymatic modifications that contribute to their chemical diversity. These modifications can be triggered by environmental factors, such as pH, temperature, or light, and include processes like oxidation, photochemical reactions, or spontaneous rearrangements.

The combination of these modifications, along with enzymatic transformations and non-enzymatic reactions, contributes to the extraordinary chemical diversity observed in secondary metabolites. This diversity enables secondary metabolites to exhibit a broad range of biological activities, including antimicrobial, antioxidant, anticancer, and immunomodulatory properties. Understanding the modifications that lead to chemical diversity is crucial for exploring the potential applications of secondary metabolites in fields such as medicine, agriculture, and biotechnology.

5.13. Compartmentalization: Examination of the role of cellular compartments in facilitating specific enzymatic reactions and sequestering intermediates.

Compartmentalization plays a crucial role in facilitating specific enzymatic reactions and sequestering intermediates within distinct cellular compartments. The organization of enzymes and metabolites into specialized compartments enables the efficient synthesis, modification, and regulation of secondary metabolites. The following describes the function of cellular compartments in the synthesis of secondary metabolites:

1. **Membrane-Bound Compartments:**
   a. **Endoplasmic Reticulum (ER):** The ER is an essential membrane-bound compartment involved in the synthesis and modification of secondary metabolites. It houses enzymes responsible for lipid biosynthesis, oxidation reactions, and the formation of complex molecules such as alkaloids and terpenoids.
   b. **Golgi Apparatus:** The Golgi apparatus is involved in post-translational modifications, including glycosylation and sulfation, of

secondary metabolites. It receives molecules from the ER and facilitates their processing and trafficking to specific destinations.

2. **Cytoplasm:** The cytoplasm is a dynamic compartment that houses numerous enzymatic reactions involved in secondary metabolite biosynthesis. It provides a diverse array of enzymes and co-factors required for various biosynthetic pathways. Cytoplasmic enzymes can participate in reactions such as condensation, cyclization, oxidation, reduction, and other modifications.
3. **Chloroplasts:** Chloroplasts are specialized organelles found in plant cells that are involved in photosynthesis. They play a critical role in the biosynthesis of secondary metabolites such as flavonoids, carotenoids, and isoprenoids. Chloroplasts provide a controlled environment for enzymatic reactions, including light-dependent and light-independent processes, that generate precursor molecules for secondary metabolites.
4. **Mitochondria:** Mitochondria, known as the powerhouses of the cell, have essential roles in energy production and metabolism. They are involved in several enzymatic reactions that contribute to secondary metabolite biosynthesis. For example, mitochondria are involved in the biosynthesis of coenzyme Q, a key component of the electron transport chain, and certain amino acids that serve as precursors for alkaloids.
5. **Vacuoles:** Vacuoles are large membrane-bound compartments found in plant cells. They serve as storage sites for secondary metabolites, including pigments, alkaloids, and flavonoids. Vacuoles sequester and accumulate these metabolites, protecting the cell from potential toxicity and providing a reservoir for physiological responses and plant defense.
6. **Extracellular Compartments:**

In some cases, secondary metabolites are transported and accumulated in extracellular compartments. These include:

   a. Cell Walls: Certain secondary metabolites, such as lignin, tannins, or pectins, are deposited in the cell walls of plants. These metabolites provide structural support, defense against pathogens, or regulate cell-cell communication.

   b. Apoplast: The apoplast refers to the extracellular space outside the plasma membrane. It serves as a transport pathway for secondary metabolites, allowing them to move between cells or into the surrounding environment.

Compartmentalization in cells allows for spatial and temporal regulation of enzymatic reactions and the sequestration of intermediates. It enables the segregation of incompatible reactions, protects sensitive intermediates, and

provides a specialized environment for specific enzymatic activities. The distinct composition and conditions within each compartment influence the activity, localization, and interaction of enzymes involved in secondary metabolite biosynthesis. Understanding the role of cellular compartments in secondary metabolite biosynthesis is essential for unraveling the complexity of these pathways and optimizing their production in various applications.

6. TRANSPORT OF PLANT SECONDARY METABOLITES

At different degrees of gene expression, the secondary metabolite biosynthesis process and accumulation are closely regulated. These control systems specify the spatiotemporal pattern of secondary metabolite accumulation and enable the plant to react to various ecological conditions, such as external biotic and abiotic pressures. In order to fine-tune the overall biosynthetic apparatus optimally, the inter- and intracellular movement of secondary metabolites and their precursors should be strictly regulated. In this respect, highly selective and tightly regulated transport of tiny organic molecules is made possible by transporter-mediated transport of secondary metabolites.

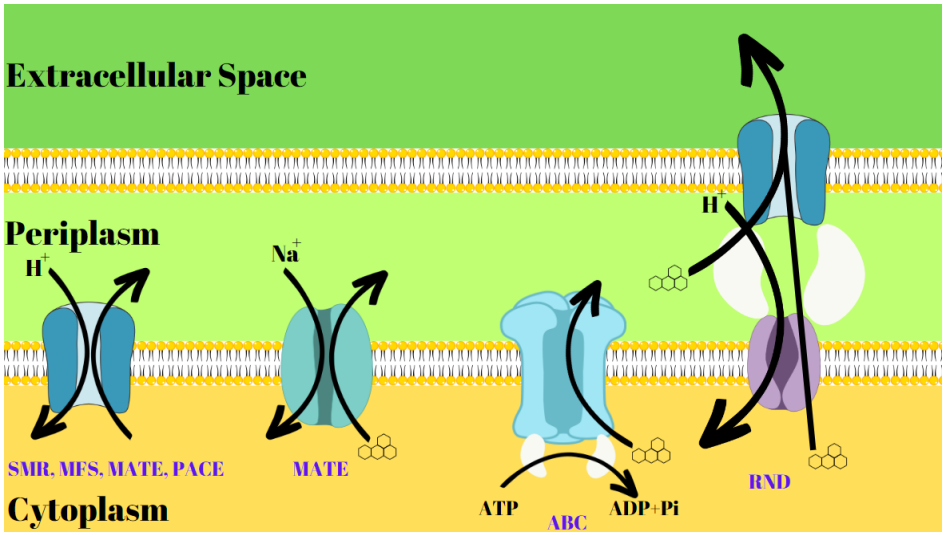

Figure 13. Regulation of secondary metabolite biosynthesis and accumulation allows plants to respond to environmental changes, with precise control of molecule movement achieved through transporter-mediated transport.

6.1. ABC transporters

Throughout all three domains of life, from bacteria to higher plants, there is a huge, diversified, and widespread family of proteins known as ATP-binding cassette (ABC) transporters (Vasiliou, Vasiliou et al. 2009). The nucleotide-binding domain (NBD), also known as the ATP binding cassette, is present in these proteins and contains multiple highly conserved motifs, including the Walker A and Walker B motifs as well as the ABC signature motif ([LIVMFY]-S-[SG]-G-X3-[RKA]-[LIVMYA]-X-[LIVMF]-[AG]) (Higgins and Linton 2004, Rai, Gaur et al. 2006). They also have transmembrane domains (TMDs),

which are collections of hydrophobic-helices, in addition to NBDs. There are four significant domains in a typical functional ABC transporter: two NBDs and two TMDs. The TMDs are important in substrate recognition, and the two NBDs cooperate to bind and hydrolyze ATP, giving the energy for transport (Martinoia, Klein et al. 2002). Full-size ABC transporters are those that include all four components—two TMDs and two NBDs—in one polypeptide chain. Half-size members only have one TMD and one NBD, which can be found in homo- or heterodimers. There are three subfamilies of ABC transporters:

1. The ABCB subfamily of p-glycoproteins (PGP) or multidrug resistance (MDR), which has domains arranged in a forward direction (TMD1—NBD1—TMD2—NBD2).
2. The pleiotropic drug resistance (PDR)/ABCG subfamily has the domain organization (NBD1—TMD1—NBD2—TMD2) in the reverse orientation.
3. The domains of the Multidrug Resistance-Associated Protein (MRP)/ABCC subfamily are also oriented forward, but some of them have five -helices at the N terminus (Nuruzzaman, Zhang et al. 2014).

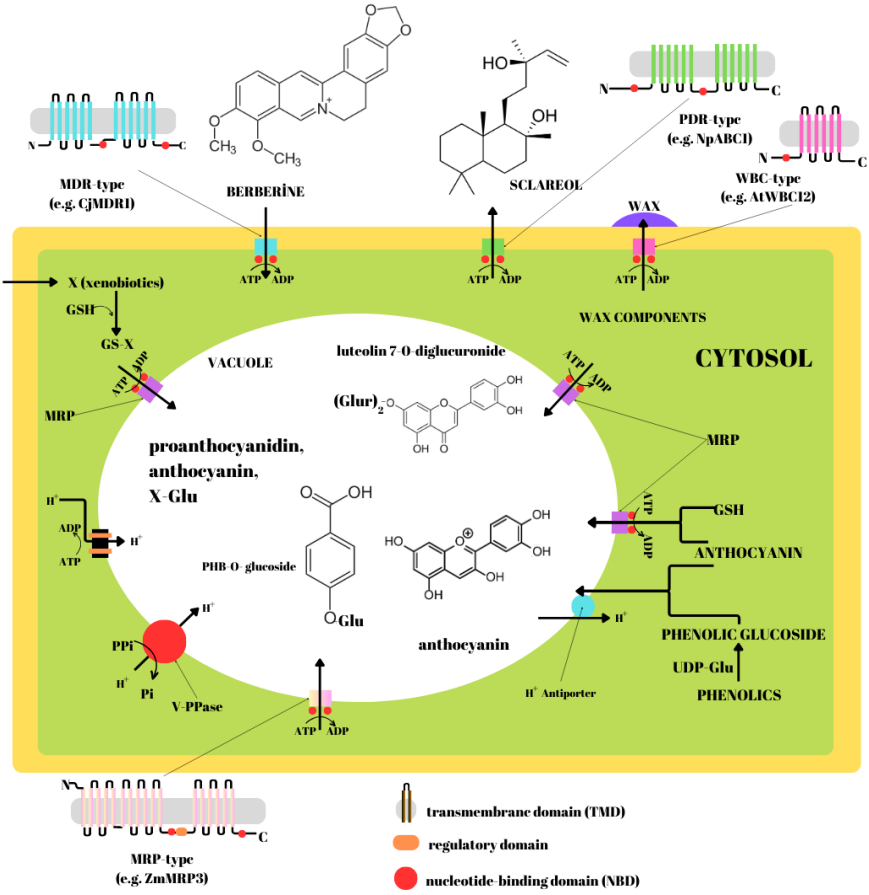

Figure 14. ABC transport systems

6.2. MATE transporters

Formerly included in the major facilitator (MFS) family, MATE (multidrug and toxic chemical extrusion or Multi-antimicrobial extrusion) transporters are now considered to be a separate family due to their distinct structure and widespread prevalence in practically all domains of life. The topology of MATE transporters typically consists of two bundles of 12 putative transmembrane helices (TMs), each of which has extended N- and C-terminal extensions (Hvorup, Winnen et al. 2003, Moriyama, Hiasa et al. 2008). The absence of "signature sequences" unique to other multidrug transporter subfamilies can be seen in these proteins. Nevertheless, EDS5 proteins only contain 9 to 11 TMs in Arabidopsis, while FRD3 proteins contain 14 TMs as an exception (Nawrath, Heck et al. 2002, Green and Rogers 2004). MATE transporters are found to be remarkably prevalent in plants, demonstrating their

significance in the kingdom. In general, MATE transporters are important for the detoxification of metabolic products like cationic ions, organic acids, and are actively involved in the transport of secondary metabolites (Wang, Hou et al. 2017, Chen, Ludwiczuk et al. 2018). Now that several members have been linked to the transfer of plant hormones, the regulation of cell and organ growth, as well as senescence, the functional importance of MATE transporters has expanded (Qin, Zhang et al. 2014, Kobayashi, Suzuki et al. 2015, Jia, Xiong et al. 2019). By acting as antiporters, the MATE proteins relate the movement of the substrate with electrochemical gradients of either H+ or Na+ produced by proton pumps (Omote, Hiasa et al. 2006)

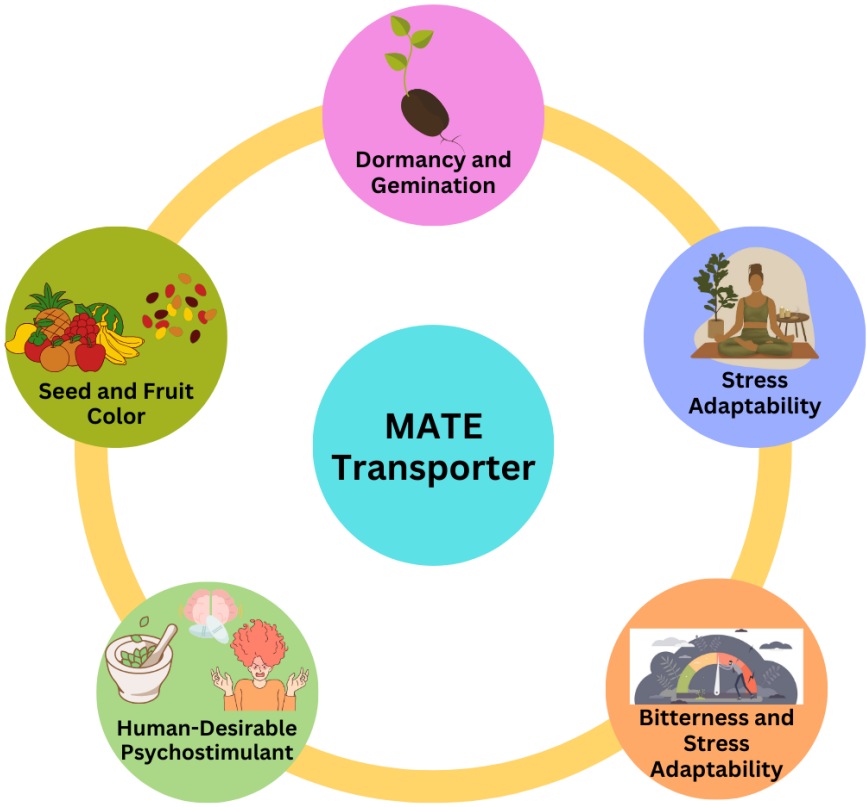

Figure 15. Mate transporters in plant

6.3. Nitrate-peptide transporter

Peptide transporters (PTR), also known as nitrate-peptide transporters (NRT), are a big family that includes the members NRT1/PTR, NRT2, and

NRT3. The proteins in question were first identified as a group of transporters with specific roles in nitrate absorption and transport in plants (Masclaux-Daubresse, Daniel-Vedele et al. 2010). Plant nitrate transporter 1 (NRT1) gene family has been renamed the NPF family (Léran, Varala et al. 2014).The NPF gene family, which includes the gene NPF6.3 (also known as NRT1.1 or CHL1), has a large number of genes that can be divided into 8–10 subfamilies. The 53-member NRT1 family, which includes NRT1.1 or CHL1, was the first to be identified in an Arabidopsis chlorate-resistant mutant.

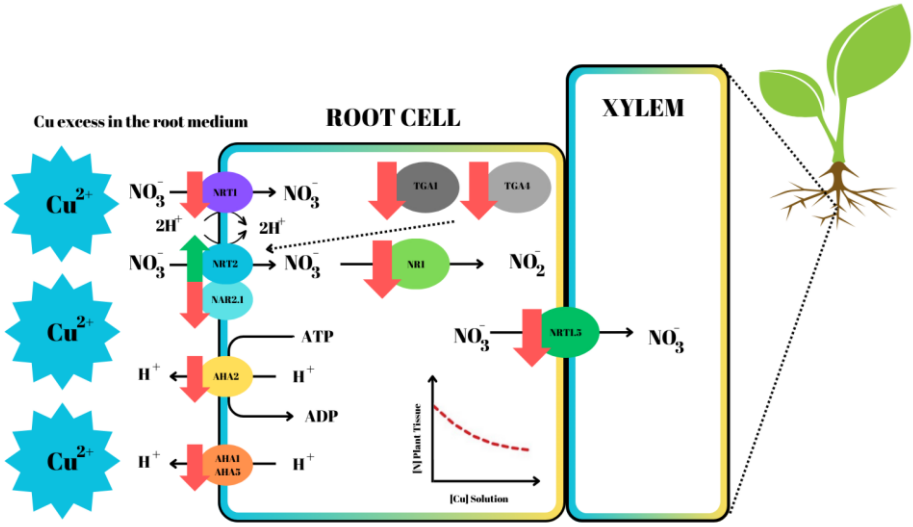

Figure 16. Nitrate – peptide transporter system

6.4. PUP (purine uptake permease)

It was previously thought that the purine uptake permease (PUP) transporter family in plants was mainly responsible for moving purine nucleobase substrates. The moderately sized PUP-like gene family in Arabidopsis consists of 21 genes, one of which is a pseudogene. A PUP-like transporter typically has 10 projected transmembrane spanning domains and is thought to work as proton symporters or other substrate influx mechanisecondary metabolites. Adenine, cytosine, and cytokinins are examples of molecules with purine rings that PUP transporters primarily move, as are compounds with pyridine rings, such as pyridoxine and pyridoxal. First founding member of the PUP-like transporter family to be discovered, AtPUP1 from Arabidopsis exhibited high-affinity adenine absorption activity in yeast (Gillissen, Bürkle et al. 2000). Moreover, a PUP-like homolog has been investigated in tobacco, where it exhibits nicotine uptake permease activity, influences nicotine metabolism, and stimulates root cell proliferation (Hildreth, Gehman et al. 2011).The physiological function of

PUP-like transporter proteins in plant secondary metabolism has also been expanded as a result of these results.

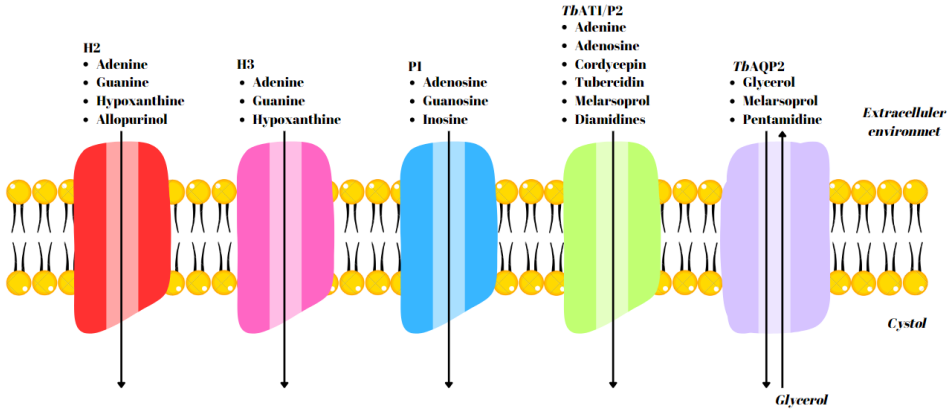

Figure 17. PUP transporter system

Table 2. Developmental stages change on the content of various plant Secondary metabolites.

Name of transporter	Plant species	Transporter class	Sub-cellular localization	System(s) used for identification/transport study	Function(s)	References
Transporters of alkaloids						
Nt-JAT1	*Nicotiana tabacum*	MATE	Tonoplast	Yeast cells, proteoliposome based system	vacuolar nicotine transport in the leaf	(Shitan, Morita et al. 2009, Shitan, Minami et al. 2014)
Nt-JAT2	*N. tabacum*	MATE	Tonoplast	Yeast cells	vacuolar nicotine transport in the leaf	(Shitan, Minami et al. 2014)
NtMATE1	*N. tabacum*	MATE	Tonoplast	Transgenic plants, yeast cells	vacuolar nicotine transport in the root	(Shoji, Inai et al. 2008)

Name of transporter	Plant species	Transporter class	Sub-cellular localization	System(s) used for identification/transport study	Function(s)	References
NtMATE2	*N. tabacum*	MATE	Tonoplast	Transgenic plants, yeast cells	vacuolar nicotine transport in the root	(Shoji, Inai et al. 2008)
NtNUP1	*N. tabacum*	PUP	Plasma membrane	Transgenic plants, yeast cells	Mediates the influx of nicotine at the plasma membrane of the root cells	(Hildreth, Gehman et al. 2011, Kato, Shitan et al. 2015)
CrTPT2	*Catharanthus roseus*	ABCG	Plasma membrane	Transgenic plants, yeast cells	Efflux transporter exporting catharanthine to the surface of the leaf	(Yu and De Luca 2013)
CrNPF2.9	*C. roseus*	NRT	Tonoplast	Transient system, *Xenopus laevis* oocytes	vacuolar export of strictosidine	(Payne, Xu et al. 2017)
CrNPF2.4, CrNPF2.5, CrNPF2.6	*C. roseus*	NRT	Plasma membrane	*X. laevis* oocytes	Transports iridoid glucosides 7-deoxyloganic acid, loganic acid, loganin and secologanin	(Larsen, Fuller et al. 2017)
CjABCB2	*Catharanthus. japonica*	ABCB	Plasma membrane	Yeast cells	Transports berberine from the root tissue (source organ) to the rhizome (sink organ)	(Shitan, Dalmas et al. 2013)
CjABCB3	*C. japonica*	ABCB	Plasma membrane	Yeast cells	Transports berberine from the root tissue (source organ) to the rhizome (sink	(Shitan, Dalmas et al. 2013)

Name of transporter	Plant species	Transporter class	Sub-cellular localization	System(s) used for identification/transport study	Function(s)	References
					organ)	
CjMATE1	*C. japonica*	MATE	Tonoplast	Yeast cells	Facilitates the berberine accumulation in the vacuoles	(Takanashi, Yamada et al. 2017)
BUP1	*Papaver sominferum*	PUP	Plasma membrane	Yeast cells	Benzylisoquinoline alkaloid transporter	(Dastmalchi, Chang et al. 2019)
Transporters of phenylpropanoids						
ZmMRP3	*Zea mays*	ABCC	Tonoplast	Mutant analysis, transgenic plants	Vacuolar anthocyanin transporter[a]	(Goodman, Casati et al. 2004)
VvABCC1	*Vitis vinifera*	ABCC	Tonoplast	Yeast cells	Transports anthocyanidin 3-O-glucosides	(Francisco, Regalado et al. 2013)
AtABCC2	*A. thaliana*	ABCC	Tonoplast	Yeast cells	Mediates the vacuolar transport of anthocyanins and other flavonoids in the vegetative tissues	(Behrens, Smith et al. 2019)
MtABCG10	*Medicago truncatula*	ABCG	Plasma membrane	Transgenic plants, *N. tabacum* BY-2 cells	Mediates the flavonoid secretion and modulates the isoflavonoids levels in roots, transporter of 4-coumarate and liquiritigenin	(Banasiak, Biała et al. 2013, Biała, Banasiak et al. 2017)

Name of transporter	Plant species	Transporter class	Sub-cellular localization	System(s) used for identification/transport study	Function(s)	References
AtABCG37	*A. thaliana*	ABCG	Plasma membrane	Mutant analysis	Efflux transporter of scopoletin into soil[a]	(Fourcroy, Sisó-Terraza et al. 2014)
AtTT12	*A. thaliana*	MATE	Tonoplast	Yeast cells	Vacuolar proanthocyanidin transporter	(Debeaujon, Peeters et al. 2001, Marinova, Pourcel et al. 2007)
AtFFT	*A. thaliana*	MATE	Tonoplast	Mutant analysis	Transport of flavonoids[a]	(Kitamura 2006, Thompson, Davies et al. 2010)
MdMATE1	*Malus* x *domestica* Borkh	MATE	Not determined	Complementation of Arabidopsis *tt12* mutant	Acts as a flavonoid transporter in proanthocyanidin accumulation[a]	(Frank, Keck et al. 2011)
MdMATE2	*M. domestica* Borkh	MATE	Not determined	Complementation of Arabidopsis *tt12* mutant	Active flavonoid transporter in proanthocyanidin accumulation[a]	(Frank, Keck et al. 2011)
MtMATE1	*M. truncatula*	MATE	Tonoplast	Transgenic plants, yeast cells	Proanthocyanidin transporter mediating the transport of epicatechin 3´-O-	(Zhao and Dixon 2009)

Name of transporter	Plant species	Transporter class	Sub-cellular localization	System(s) used for identification/transport study	Function(s)	References
					glucoside	
MtMATE2	*M. truncatula*	MATE	Tonoplast	Transgenic plants, yeast cells	Flavonoid transporter displaying preferential transport of malonylated flavonoids	(Zhao, Huhman et al. 2011)
SlMTP77	*Solanum lycopersicum*	MATE	Not Determined	Transgenic plants	Functions as an anthocyanin transporter in the leaf[a]	(Mathews, Clendennen et al. 2003)
VvAM1	*V. vinifera*	MATE	Tonoplast	Transgenic plants	Anthocyanin transporter	(Gomez, Conejero et al. 2011)
VvAM3	*V. vinifera*	MATE	Tonoplast	Transgenic plants	Anthocyanin transporter	(Gomez, Conejero et al. 2011)
VvMATE1	*V. vinifera*	MATE	Tonoplast	Not available	Proanthocyanidin transporter in the vacuoles[a]	(Pérez-Díaz, Ryngajllo et al. 2014)
VvMATE2	*V. vinifera*	MATE	Golgi complex	Not available	Proanthocyanidin transporter in the golgi complex[a]	(Pérez-Díaz, Ryngajllo et al. 2014)
FaTT12-1	*Strawberry*	MATE	Tonoplast	Transgenic plants	Mediates the accumulation of proanthocyanidin	(Chen, Tang et al. 2018)
Transporters of terpenoids						
AtPDR12/AtABCG40	*A. thaliana*	ABCG	Plasma membra	Mutant analysis, *N. tabacum* BY-2	Imparts resistance to	(Campbell,

Name of transporter	Plant species	Transporter class	Sub-cellular localization	System(s) used for identification/transport study	Function(s)	References
			ne	cells, yeast cells	sclareol, functions as a phytohormone abscisic acid (ABA) transporter	Schenk et al. 2003, Kang, Hwang et al. 2010)
NpPDR1/ NpABC1	*Nicotiana plumbaginifolia*	ABCG	Plasma membrane	Transgenic plants	Efflux transporter of sclareol, functions in defense against several fungal pathogens	(Jasiński, Stukkens et al. 2001, Stukkens, Bultreys et al. 2005, Bultreys, Trombik et al. 2009)
NtPDR1	*N. tabacum*	ABCG	Plasma membrane	*N. tabacum* BY-2 cells	Mediates the efflux of antifungal diterpenes and functions in chemical defense of plants	(Crouzet, Roland et al. 2013)
SpTR2	*Spirodela polyrrhiza*	ABCG	Plasma membrane	Transgenic plants in heterologous system (*A. thaliana*)	Transport of sclareol[a]	(Van Den Brûle, Müller et al. 2002)
AaPDR3	*Artemisia annua*	ABCG	Plasma membrane	Transgenic plants, yeast cells	Transports sesquiterpene β-caryophyllene	(Fu, Shi et al. 2017)
CsABCC4	*Crocus sativus*	ABCC	Tonoplast	Yeast cells, transient assay using *Nicotiana*	Functions as a vacuolar transport of	(Demurtas, de Brito

Name of transporter	Plant species	Transporter class	Sub-cellular localization	System(s) used for identification/transport study	Function(s)	References
				benthamiana leaves	crocins	Francisco et al. 2019)
Transporters of glucosinolates						
AtGTR1	*A. thaliana*	NRT	Plasma membrane	Mutant analysis, *X. laevis* oocytes	Glucosinolate transporter, positively regulates stamen development by mediating the transport of gibberellins and jasmonoyl-isoleucine	-(Nour-Eldin, Andersen et al. 2012, Andersen, Nour-Eldin et al. 2013, Saito, Oikawa et al. 2015)
AtGTR2	*A. thaliana*	NRT	Plasma membrane	Mutant analysis, *X. laevis* oocytes	Glucosinolate transport	(Nour-Eldin, Andersen et al. 2012, Andersen, Nour-Eldin et al. 2013)
Transporters of volatile compounds						
PhABCG1	*Petunia hybrida*	ABCG	Plasma membrane	Transgenic plants, *N. tabacum* BY-2 cells	Mediates the emission of volatile compounds from the flower	(Adebesin, Widhalm et al. 2017)

7. TRANSPORTATION OF SECONDARY METABOLITES

7.1. Transport of Alkaloids

Alkaloids are nitrogen-containing, low-molecular-weight molecules with a variety of chemical structures that help plants chemically defend themselves from pests, insects, bacteria, and other herbivores (Steppuhn, Gase et al. 2004). These substances make up a sizable class of plant natural products with various substances that have potential pharmaceutical applications due to their powerful biological activity. Exceptional examples include morphine, which serves as an analgesic, and the prescription anticancer medications taxol, vincristine, and vinblastine (VLB), which are advised for use in chemotherapies. Studies on certain significant alkaloid biosynthesis routes at the molecular and cellular levels have shown that alkaloids and the biosynthetic intermediates they produce are transported within plant tissues in a controlled and dynamic manner (Shitan, Dalmas et al. 2013, Verma and Shukla 2015). Inter-organ, intercellular, and intracellular transport are known to play diverse roles in alkaloid production and accumulation (Shitan and Yazaki 2007). In-depth research has been done on the alkaloid transport, especially in the model system Nicotiana tabacum. To protect itself from herbivory, this plant primarily manufactures nicotine, a pyridine alkaloid that functions as a neurotoxic (Shoji, Inai et al. 2009, Dewey and Xie 2013). The central vacuole of the leaf is where the nicotine accumulates after being biosynthesized in the roots and acting in the defense response after being transferred to the aerial plant parts via xylem (Steppuhn, Gase et al. 2004). While two vacuolar membrane-localized MATE transporters, namely NtMATE1 and NtMATE2, have been suggested to potentially play a role in nicotine transport into root vacuoles, experimental studies have shown that JAT1 and JAT2 transporters are involved in the transfer of nicotine into leaf vacuoles (Shoji, Inai et al. 2009). The in vitro tests revealed that these transporters aren't just limited to transporting nicotine; they can also move other structurally similar alkaloids like scopolamine and anabasine. A PUP family member named Nicotine uptake permease1 (NUP1) has been shown to be involved in the influx of nicotine at the cell membrane of the root tip in addition to the previously stated tonoplast-localized transporters (Hildreth, Gehman et al. 2011, Kato, Shitan et al. 2015).

Through the dimerization of catharanthine and vindoline (monoterpenoid indole alkaloids), which is catalyzed by class III basic peroxidase (Prx1), two significant leaf-derived bisindole alkaloids, vinblastine and vincristine, are produced in the medicinal plant Catharanthus roseus (Kumar, Dutta et al. 2007, Costa, Hilliou et al. 2008); Sottomayor and Bar Inter- and intracellular transport

of intermediate metabolites involving many organelles and cells is seen during alkaloid production in C. roseus. Recent research has shown that the efflux of catharanthine to the leaf surface is mediated by CrTPT2, an ABCG transporter that is localized to the plasma membrane (Yu and De Luca 2013). Another study discovered that particular proton antiport systems cause significant monoterpenoid indole alkaloids (MIAs) from C. roseus to accumulate in the mesophyll cell vacuoles, including vindoline. A clear explanation for the low concentration of dimeric MIAs accumulated in plants can be found in the location of the two monomeric MIAs (vindoline and catharanthine) in various cells. Catharanthine levels at the leaf surface were seen to be reduced following the knockdown of the CrTPT2 transporter gene using the VIGS (virus-induced gene silencing) technique, whereas higher catharanthine levels were seen in the internal leaf cells, which led to a rise in catharanthine-vindoline dimers within the leaf. Metabolomic and cell-specific localization investigations with imaging mass spectrometry demonstrated that some major TIAs, such serpentine and vindoline, are localized in the idioblast cells while TIA precursors like iridoids are localized in the epidermal cells.

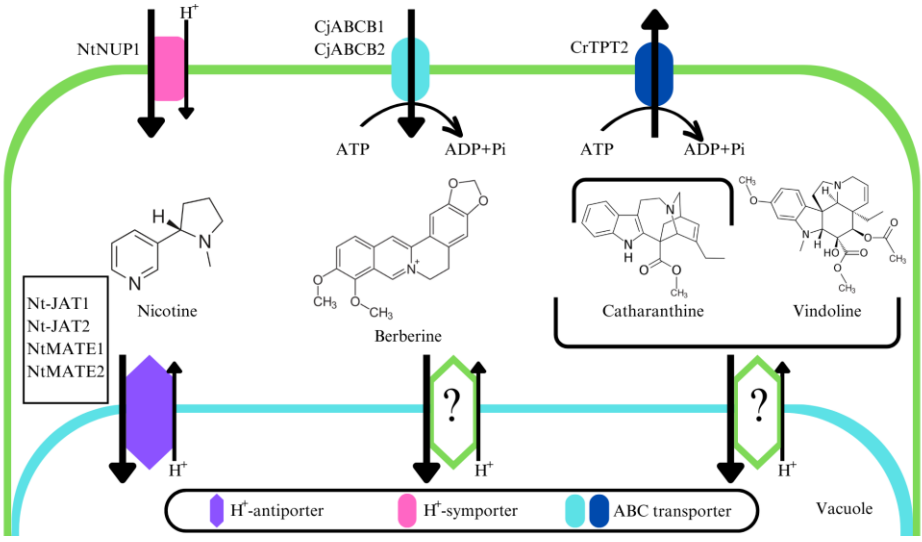

Figure 18 Transport of Alkoloids

7.2. Transport of phenylpropanoids

Often known as plant phenolics, phenylpropanoids have an aromatic ring with one or more hydroxyl substituents and various subgroups, including flavonoids and lignans. A fascinating class of plant natural chemicals known as flavonoids share a common three-membered ring structure chemically (C6–C3–

C6). These substances have been the subject of in-depth research due to their widespread distribution, immense structural variety, and several biological roles in plants, including the regulation of auxin transport, UV protection, interactions with microbes, and the variance of flower color (Falcone Ferreyra, Rius et al. 2012). The majority of flavonoids in plants exist in the cell in their glycosylated form, and glycosylation is crucial for their effective vacuolar transport. According to (Peer, Brown et al. 2001, Saslowsky and Winkel-Shirley 2001, Buer and Muday 2004) the production of flavonoids takes place in the cell's cytoplasm, where they concentrate in the vacuole and exhibit long-distance transport. Many transporter proteins, particularly from the ABC and MATE families, have been discovered as the flavonoid transporters over the past 20 years. For instance, it has been demonstrated that members of the ABC family of transporters are involved in the uptake of flavone glucoside in Arabidopsis vacuoles as well as the vacuolar transport of flavone glucuronide conjugates in rye mesophyll cells (Klein, Martinoia et al. 2000, Frangne, Eggmann et al. 2002). Genetic research has shown that ZmMRP3 is a C-type ABC transporter protein that mediates the accumulation of anthocyanin in the vacuoles of maize crops (Goodman, Casati et al. 2004). According to studies, ZmMRP3 is involved in the transport of anthocyanin since its expression is considerably reduced when ZmMRP3 is suppressed. During the ripening stage of the grape berry, the ABC transporter VvABCC1 that is localized to the tonoplast plays a role in the vacuolar accumulation of anthocyanins. According to biochemical research, the VvABCC1 transporter makes it possible for glucosylated anthocyanidin and malvidin 3-O-glucoside to be transported alongside glutathione (GSH). As a result, it serves as an anthocyanin transporter (Francisco, Regalado et al. 2013). Also, it has recently been proposed that the Arabidopsis ABC transporter AtABCC2 as well as other ABCC transporters play a role in the vacuolar transport of anthocyanins and a select number of other flavonoids inside the vegetative tissue (Behrens, Smith et al. 2019). In order to establish a symbiotic relationship with soil rhizobia, legumes manufacture and secrete a small number of flavonoid aglycons from their roots, suggesting the possibility of flavonoid exudation in the rhizosphere (Smit, Swart et al. 1992).

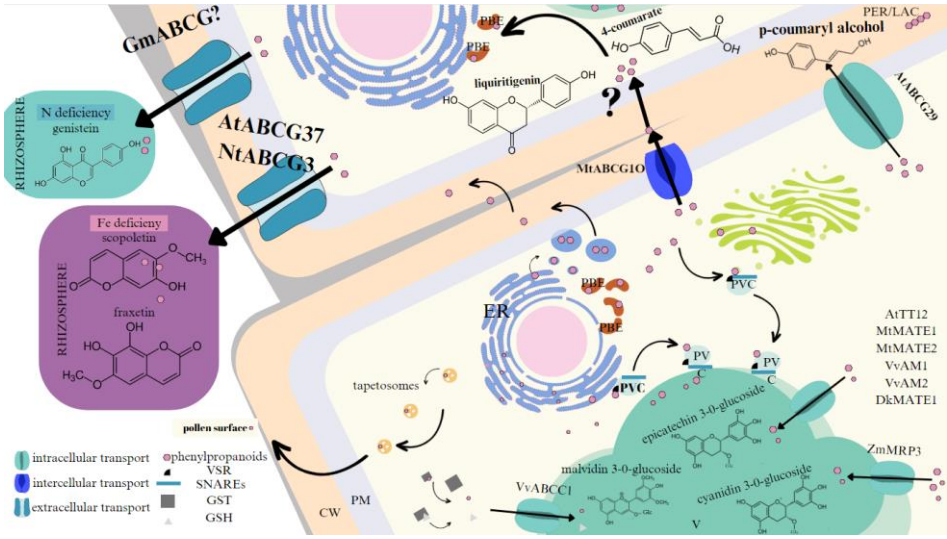

Figure 19 Transport of phenylpropanoids

7.3. Transport of Terpenoids

The class of plant natural products known as terpenoids is large and structurally diverse. Certain substances, such as monoterpenes, sesquiterpenes, diterpenes, and triterpenes, may serve as secondary metabolites and play significant roles in plant-environment interactions depending on the amount of isoprene units they contain (Trapp and Croteau 2001, Pichersky and Gershenzon 2002, Aharoni, Jongsma et al. 2005, Staniek, Bouwmeester et al. 2013). Moreover, it has been suggested that several terpenoid backbones, including cytokinins, gibberellic acid, abscisic acid, brassinosteroid, and strigolactones, operate as phytohormones. The Dictionary of Natural Products contains a list of more than 30,000 isoprenoids, the majority of which are of plant origin (Brand, Buckingham et al. 2004). There is potential value in many terpenoids as plant medicines.

Several ABCG subfamily transporters that have been assigned functional functions in the transport of a few distinct terpenes have been reported thus far. The first ABC G sub-family transporter, which was proposed to serve in sclareol transport at its leaf surface (Marchand-Brynaert and Boutrya), is a pleiotropic drug resistance1 (NpPDR1/NpAB1) transporter from Nicotiana plumbaginifolia that preferentially expresses in the leaf glandular trichomes, flower petals, and roots. Because NpPDR1 promotes the movement of the antifungal diterpene sclareol onto the plant surface, it plays a significant role in disease resistance. Silencing NpPDR1 in N. plumbaginifolia increased susceptibility to several fungal and oomycete pathogens (Bultreys, Trombik et

al. 2009). Furthermore, it has been reported that the ABCG transporter NtPDR1 in N. tabacum mediates the transport of anti-fungal diterpenes (manool, sclareol, and macrocyclic cembrene) to the apoplast and serves in plant chemical defense, but it was not discovered to transport eucalyptol, a monoterpene, in tobacco BY2 cells (Crouzet, Roland et al. 2013). It is clear that artemisinin itself or its immediate precursors are exported out of the apical cells in A. annua L., or annual wormwood, where it is generated largely in glandular trichomes and accumulates in the subcuticular space of the glandular secretory trichomes (Duke, Paul et al. 1994, Tellez, Canel et al. 1999)

Apocarotenoids can be moved and gathered in many places inside or outside the plant cell. There is currently little known about how apocarotenoids are transported across membranes. Using a transportomic method, it has recently been discovered that an ABCC subfamily of transporters is involved in the vacuolar transport of crocins in the stigmas of saffron (Crocus sativus). According to reports, the ABC transporter CsABCC4 is found on tonoplasts, and heterologous expression in Nicotiana benthamiana leaves led to increased crocin accumulation (Demurtas, de Brito Francisco et al. 2019).

7.4. Transport of glucosinolates

Glucosinolates and thioglucosidases (myrosinases) accumulate in the vacuoles of both root cortex cells and epidermal leaf edges, but they are often kept in separate subcellular sites. It has been discovered that these active substances are transmitted between nearby cells or are given to distant organs. GTR1 and GTR2, two high-affinity H+-dependent transporters from the nitrate/peptide (NRT/PTR) subfamily, have been shown to serve in Arabidopsis as glucosinolate-specific transporters. In source tissues, such as leaves, glucosinolates were found to be over-accumulated by many folds, demonstrating their critical function in the long-distance mode of glucosinolate transport, according to the analysis of gtr1 and gtr2 double mutants (Nour-Eldin, Andersen et al. 2012). Consequently, it has been suggested that the two transporters GTR1 and GTR2 control how glucosinolates are transported in leaves from the apoplasm to the phloem. Studies revealed that the GTR1 transporter not only transports glucosinolate but also gibberellins and jasmonoyl-isoleucine when expressed heterologously in oocytes. It may also play a role in Arabidopsis stamen formation by regulating the supply of gibberellins (Saito, Oikawa et al. 2015). These investigations show that the well-known glucosinolate transporter GTR1 is versatile and transports ligands with various structural characteristics.

8. ABIOTIC FACTORS INFLUENCING SECONDARY METABOLITES

The plants could be harmed by a variety of environmental conditions, including pathogen infection, UV damage, high and low temperatures, alkalinity, and drought. Elicitation has frequently been employed in in vitro plant cell cultures to boost output or promote de novo synthesis of secondary metabolites. Several researchers have used a variety of elicitors to increase the synthesis of secondary metabolites in cultures of plant cell, tissue, and organ. Phenylpropanoids are frequently accumulated more as a result of environmental challenges like pathogen attack, UV radiation, high light, wounding, nutritional deficits, temperature, and herbicide treatment. Phenolic levels in plant tissues are significantly impacted by nutrient stress. The metabolic processes that lead to the accumulation of related natural products are influenced by the concentrations of various secondary plant products, which in turn are greatly influenced by the growth conditions. Experiencing salt stress or drought in plants results in a variety of typical responses. Cellular dehydration, which is caused by these stresses, results in osmotic stress and the evacuation of water from the cytoplasm to vacuoles. The buildup of phenylpropanoids is directly influenced by nitrogen and phosphate deficiency It has also been suggested that low levels of potassium, sulfur, and magnesium enhance phenolic concentrations. Increased root release of phe-nolic acids can result from low iron levels. Many abiotic stressors, such as cold, drought, and salinity, have been linked to calcium levels in plants. It has been observed that the expression of several genes rises in response to osmotic stress, reactive oxygen species, low temperature, and high temperature. One of the main pressures, particularly in arid and semi-arid settings, is salt stress in soil or water, which can severely restrict plant development and output. When plants are under stress, one of two things can happen: either carbon is converted to biomass or defensive secondary chemicals are formed.

8.1. Salt Stress

High-salt soils cause nutrient imbalances, hyperosmotic stress, and a reduction in photosynthesis, growth, and nutrient uptake in plants (Banerjee and Roychoudhury 2017). In response to salinity-induced osmotic stress or particular ion toxicity, plant SM concentrations may go up or down.

Salt Stress When cells are exposed to a salt environment, they get dehydrated, which results in osmotic stress and the evacuation of water from the cytoplasm, which lowers the cytosolic and vacuolar volumes. Both ionic and

osmotic stress are frequently caused by salt stress in plants, which causes an accumulation or decrease in a number of secondary metabolites. Plants exposed to salinity stress produce more alkaloids, tannins, phenolics, saponins, flavonoids, proline, and saponins (Abd EL-Azim and Ahmed 2009, Haghighi, Karimi et al. 2012, Verma and Shukla 2015). Recent studies found that under salt stress, oil production decreased (Ali, Athar et al. 2008). According to reports, anthocyanin levels rise in response to salt stress. 17 Contrarily, salt stress caused the anthocyanin level in salt-sensitive species to drop. It was noted that, in contrast to salt-sensitive plants, salt-tolerant alfalfa plants rapidly increased the amount of pro-line in their roots. Proline accumulation and salt tolerance were found to be correlated in Lycopersicon esculens and Aegiceras corniculatum, respectively.

Table 3. Metabolite Changes in Response to Salinity and Plant Species

Metabolite Class	Metabolite Name	Concentration Change	Environment Factor	Plant Species	Parts
Phenolics	Tannin	Increase	Salinity	*Achillea fragratissima*	Whole plant
Alkaloids	Recinine alkaloids	Increase	NaCl	*Ricinus communis*	Shoot
	Alkaloid	Increase	Salinity	*Achillea fragratissima*	Whole plant
Flavonoids compounds	Flavonoids	Increase	NaCl	*Plantago ovata*	Root and shoot
Monoterpenes/Essential Oils	Oil contents	Increase	NaCl	*Ricinus communis*	Shoot
			25 and 50 mM NaCl	Coriandrum sativum	Leaf
	Oil contents	Decrease	High salinity	Coriandrum sativum	Leaf
			100 Mm NaCl	Origanum majorana	Shoots
	Octanal; Borneol; (E)-2-Nonenal	Increase	Salinity	Coriandrum sativum	Leaf

Metabolite Class	Metabolite Name	Concentration Change	Environment Factor	Plant Species	Parts
	α-Pinene; (Z)-Myroxide	Decrease	Salinity	Coriandrum sativum	Leaf
	Trans-sabinene Hydrate; γ-Terpinene	Decrease	NaCl	Origanum majorana	Aerial part
	cis-Sabinene Hydrate; Linalyl acetate; Terpinene-4-ol	Increase	NaCl	Origanum majorana	Aerial part
Others	Saponins, Proline	Increase	NaCl	*Plantago ovata*	Root and shoot

8.2. Drought Stress

One of the most important abiotic stresses that affects plant growth and development is drought stress. Drought stress decreases water absorption and water potentials in plants, which thereby negatively influence various physiological processes and can alter SM biosynthesis. When soil water availability falls to dangerously low levels and ongoing water loss is exacerbated by meteorological conditions, drought stress results. All plants can withstand drought stress, however, to varying degrees depending on the species. The lack of water causes the drought stress, which is typically accompanied by high temperatures and sun radiation. In order to secure the survival of agricultural crops and sustained food production, water shortage and salt stress are major global challenges. Drought frequently results in oxidative stress, and willow leaves have been found to contain higher levels of flavonoids and phenolic acids. The balance between sources and sinks of carbohydrates has a significant impact on the elevation of phenolic and flavonoid molecules. According to a paper, the reduced transit of soluble sugars during water stress has an impact on the buildup of soluble carbohydrates in plant cells. Plants undergo variations in gene expression in response to stressors (Kilian, Whitehead et al. 2007). The expression of many genes involved in the synthesis

pathway of phenolic compounds, such as phenylalanine ammonialyase (PAL), is increased in low to moderate drought stress conditions and requires high energy inputs, whereas these energy-intensive processes are less restricted in moderate to severe stress conditions (Król, Amarowicz et al. 2014). In some licorice genotypes, dryness raised the root concentrations of glycyrrhizin, which is supported by increases in the expression of the glycyrrhizin biosynthetic pathway genes SQS1, SQS2, bAS, CYP88D6, CYP72A154, and UGT73 (Hosseini, Ebrahimi et al. 2022) Changes in the ratio of chlorophylls "a" and "b" and carotenoids were altered by drought stress. Under drought stress and Catharanthus roseus, cotton was shown to have less chlorophyll. In Chenopodium quinoa, drought reduced the amount of saponins from 0.46% dry weight (Haslam 1996) in plants that were growing in moderate water deficit settings to 0.38% in plants that were grown in severe water deficit situations. According to reports, anthocyanins accumulate in cold and drought-stressed environments. Anthocyanin-containing plant tissues typically exhibit a high level of drought resistance.

Table 4. Metabolite Changes in Response to Drought stress

Metabolite Class	Metabolite Name	Plant Species	Parts
Phenols	Total phenolics	Hypericum brasiliense	Shoots and roots
		Trachyspermum ammi	Leaf
		Labisia pumila	Leaf
	Baicalin	Scutellaria baicalensis	Whole plant
	Rutin, Quercetin,	*Hypericum brasiliense*	
	Anthocyanins	*Labisia pumila*	Leaf
Pentacyclic triterpenoid	Betulinic acid	*Hypericum brasiliense*	
Sesquiterpene lactone	Artemisinin	*Artemisia*	Whole plant

8.3. Influence of Heavy Metal Stress on Secondary Metabolites

Secondary metabolite production is also regulated by metal ions (lanthanum, europium, silver, and cadmium), as well as oxa-late. The urease enzyme, which is necessary for plant development, contains the trace metal nickel (Li, Kong et al. 2020), which is also required. High Ni concentrations, however, inhibit plant growth. Ni stress has been observed to cause a considerable drop in antho-cyanin levels. Furthermore, Ni has been found to prevent anthocyanin buildup.

By decreasing the activity of l-phenylalanine ammonia-lyase, trace metals clearly impede the biosynthesis of anthocyanins (PAL). Brassica juncea's oil content increased by up to 35% as a result of the efficient accumulation of metals (Cr, Fe, Zn, and Mn). It has been demonstrated that Cu^{2+} and Cd^{2+} increase the yields of secondary metabolites such shikonin38 and have a positive impact on the production of digitalin. In Beta vulgaris, Cu^{2+} increased the growth of bet-alains as well. The formation of secondary metabolites is stimulated by Co^{2+} and Cu^{2+}. The hairy roots were treated to metal ions in an effort to increase the formation of betalaines. Cu^{2+} has been shown to have stimulatory effects on the accumulation of betacyanins in Amaranthus caudatus callus cultures. Lepidium sativum cultures produced more lepidine when Zn^{2+} (900 M) was added. Cu, however, was more successful than Zn in increasing yield. In hairy root cultures of Brugmansia candida, $AgNO_3$ or $CdCl_2$ caused the overproduction of two tropane alkaloids, scopolamine and hyoscyamine.

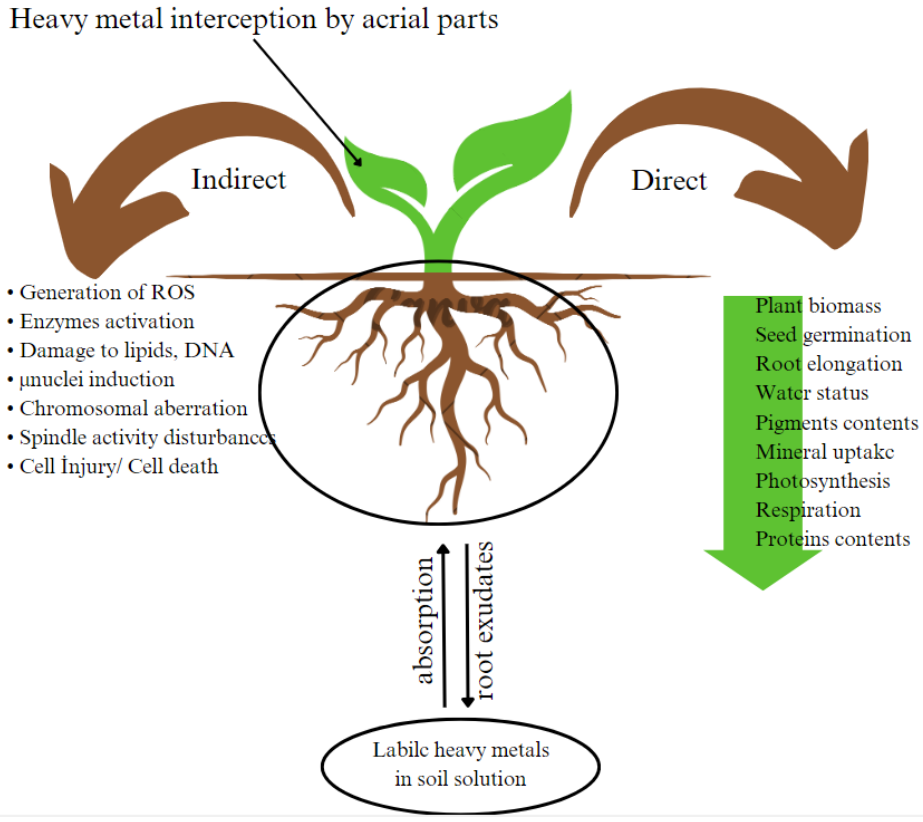

Figure 20. Heavy metals uptake by plant roots and possible direct and indirect toxic effects resulting in reduced crop production. ↓ indicates decrease

8.4. Influence of Cold Stress on Secondary Metabolites

One of the most damaging abiotic stresses on temperate plants is low temperature. These species have adjusted their metabolism during the fall to increase their content of a variety of cryo-protective chemicals to enhance their cold tolerance. This has allowed them to adapt to variations in temperature. Plants may experience a number of challenges during the cryopreservation process due to environmental changes such as osmotic damage, desiccation, and low temperature. The metabolism of temperate plants is switched over to the manufacture of cryoprotectant chemicals such sugar alcohols (sorbitol, ribitol, inositol), soluble sugars (saccharose, raffinose, stachyose, trehalose), and low-molecular weight nitrogenous compounds during overwintering (proline, glycine betaine). Cold stress enhances phenolic synthesis and their subsequent incorporation, either as suberin or lignin, into the cell wall. Also, it was discovered that a high quantity of chlorogenic acid was linked to apple tree adaptability to cold climates. It has also been demonstrated that lignification and suberin deposition increase tolerance to cold temperatures. a process via which lignin and suberin may shield plants from frost damage. According to Christie et al., anthocyanins can reproduce under cold stress. According to Pedranzani et al., Pinus pinaster endog-enous jasmonates are altered by cold and water stressors. According to Lei et al., mela-tonin protects carrot suspension cells against cold-induced apoptosis via upregulating polyamines (putrescine and spermine). Moreover, Melatonin helps cucumber (Cucumis sativus L.) seed germination under chilling stress.

8.5. Temperature Variations Influence Plant Growth and Secondary Metabolite Production

Temperature has a significant impact on plant ontology and metabolic activity, and extreme heat can hasten the senescence of leaves. Temperature ranges at which plants are present have a direct impact on their growth and development. Plant development and productivity may be negatively impacted by extremes in temperature (Yadav 2010). The photochemical efficiency of photosystem II declines in plants growing in hot environments, indicating greater stress (Maxwell and Johnson 2000). High temperatures in plants are also connected with the manufacture of Secondary metabolites (Verma and Shukla 2015). Although some research suggested that plants under high-temperature stress produced less Secondary metabolites than those under non-stress conditions, high-temperature stress typically boosted the synthesis of Secondary metabolites (Naghiloo, Movafeghi et al. 2012). After thermal treatments, it was discovered that carotenoids in the Brassicaceae, particularly -carotene, had

somewhat lower levels. In the herb Panax quinquefolius, elevated temperatures increase the quantities of both root secondary metabolites and leaf senescence. P. quinquefolius would produce less biomass and less photosynthesis at 5°C higher temperatures, however storage of ginsenoside is said to be improved at these higher temperatures. Increased temperatures have been the subject of numerous research looking at how they affect plant production of secondary metabolites. Steroid furostanol and spirostanol saponin levels rose in response to cooler soil temperatures. In plant cell cultures, temperature fluctuations have a variety of effects on the metabolic control, permeability, and rate of intracellular reactions. The physiology and metabolism of cultured cells can be altered by altering the culture temperature, which can then have an impact on growth and the synthesis of secondary metabolites. Typically, callus tissues are induced and cultured cells proliferate at temperatures between 17 and 25 °C. According to Chan et al., Melastoma malabathricum cell cultures cultivated at a lower temperature range (20 ±2°C) produced more anthocyanin and grew more successfully than those grown at 26 2°C and 29 2°C. As shown in cell cultures of Perilla frutescens and strawberry, an optimal temperature of 25°C optimizes the anthocyanin output. Anthocyanin accumulation is encouraged at lower temperatures, but cell development is inhibited.

Table 5. Metabolite Changes in Response to Temperature Changes

Metabolite Class	Metabolite Name	Environment Factor	Concentration Change	Plant Species	Parts
Sesquiterpene lactone	Artemisinin	A transient pre-chilling treatment	Increase	*Artemisia annua*	Whole plant
Phenols	Phenolics	High-temperature	Increase	*Astragalus compactus*	Roots, leaf and flowers
	Anthocyanins	High temperature	Decrease	*Chrysanthemum*	Whole plant
Fatty acid	α-linolenic acid, Jasmonic acid	Low temperature	Increase	*Camellia japonica*	Leaf

8.6. Influence of Light on Secondary Metabolite Production

The generation of metabolites can be impacted by light, a physical component that is well understood. In medicinal plants, the impact of ultraviolet (UV) light exposure on Secondary metabolites is typical. In the culture of Z. officinale callus, light can induce the formation of such secondary metabolites as gingerol and zingiberene. The amounts of phenolics have been found to increase in direct proportion to light intensity. Increased UV radiation from increased solar radiation received by the plants may be the cause of an increase in total phenolic content (Naghiloo, Movafeghi et al. 2012). In genetically homogenous populations grown over an altitudinal gradient, (Spitaler, Schlorhaufer et al. 2006) showed that the induction of phenolics is a major role in the reactive oxygen species scavenging mechanism, and is most likely connected with enhanced UV-B light. Foliar tannin and phenolic glycoside levels in the foliage of shaded willows decreased, according to Larsson et al. In his research, Arakawa examined how anthocyanin synthesis in light-colored sweet cherries is affected by UV light. Anthocyanins, carotenoids, flavonoids, lignin, phytosterols, saponins, and tannins are examples of UV-B absorbing alkaloids, anthocyanins, carotenoids, flavonoids, and tannins that are produced as a result of UV solar radiation. When paired with red light, UV light from 280 to 320 nm in apples synergistically stimulates anthocyanin production. Perilla frutescens cell suspension cultures were used to study the impact of light irradiation on the anthocyanin synthesis. The cultures exposed to 10-d continuous darkness showed the lowest pigment level, whereas the cultures exposed to 10-d continuous irradiance showed the highest pigment content. Moderate light intensity (301-600 lx) increased anthocyanin accumulation. Flavonoids found in barley and polyamines found in cucumber have been observed to rise in response to UV-B. Hagimori et al. described how the formation of digitoxin in the plant Digitalis purpurea L. was affected by light and plant growth regulators. Moreover, the impact of light irradiation affected the manufacture of artemisinin in Artemisia annua's hairy roots. With a reduction in chlorophyll concentration, UV-B radiation may lead to an increase in flavonoid content and phenylalanine ammonia-lyase (PAL) activity. Flavonoids were elevated in the roots of pea plants by UV light (300–400 nm). It has also been demonstrated that silver birch and grape leaf flavonol synthesis is stimulated by UV-B.

Table 6. Metabolite Responses to Light

Metabolite Class	Metabolite Name	Environment Factor	Concentration Change	Plant Species	Parts
Phenols	Anthocyanins, lignin, tannins	UV-B	Increase	*Withania somnifera*	Root and leaf
	Phenolic acids	UV-B	Increase	*Chrysanthemum*	Flower
			Increase	*Astragalus compactus*	Leaf
			Increase	*Arnica montana*	Flowering heads
			No effect	Tarbush	Leaf
				Chrysanthemum	Flower
				Nasturtium officinale	Leaf
	Scutellarin	Full sunlight	Increase	*Erigeron breviscapus*	Leaf
	Flavonol quercetin-4′-	UV-B	Increase	*Asparagus officinalis*	Whole plant
Alkaloids	Alkaloids	UV-B	Increase	*Withania somnifera*	Root and leaf
	Alkaloids	30 and 50% Full sunlight	Increase	*Mahonia bodinieri*	Whole plant.
				Mahonia breviracema	Root, stem
	Sabinene, b-pinene.	50% shade	Increase	*Flourensia cernua*	Leaf
	Essential oil	Full sunlight	Increase	*Mahonia breviracema*	Leaf
Others	Saponins,	UV-B	Increase	*Withania somnifera*	Root and leaf
	Phytosterols	UV-B	Increase	*Withania somnifera*	Root and leaf
	Hexadecanoic acid	50% Full sunlight	Increase	*Mahonia bodinieri*	Leaf
	Glucosinolate	UV	Increase	*Nasturtium officinale*	Leaf

8.7. Influence of Polyamines on Secondary Metabolites

Several types of species, including bacteria, plants, and animals, include polyamines such as putrescine, spermine, and spermidine. Polyamines play a role in several physiological processes in plants, including growth, aging, and stress reactions. Plant resistance to a variety of environmental stressors is correlated with high cellular levels of polyamines. Furthermore, stress-tolerant plants typically have a greater potential to increase polyamine production in response to abiotic stress than susceptible plants do. Contrarily, using exogenous polyamines at the same time as polyamine biosynthesis inhibitor therapies reduces the body's ability to withstand stress. In Coffea canephora, the impact of polyamines on in vitro morphogenetic response and caffeine biosynthesis was described. Certain polyamines have secondary metabolic roles that include acting as elicitors when fed externally. In red beet hairy root cultures, the addition of spermidine and putrescine, each at 0.75 mM, greatly increased betalaine production. Moreover, treatment with putrescine at 0.6 mM enhanced the synthesis of polysaccharides in suspension cultures of Dendrobium huoshanense.

8.8. Influence of Plant Growth Regulators on Secondary Metabolites

Several researchers have documented the generation of beneficial secondary metabolites using plant tissue and organ culture. Many initiatives have been taken to increase the productivity of plant tissue cultures, including research on hormone dependence, medium make-up, and light exposure. With the manipulation of phytohormones in cell suspensions of strawberry (Edahiro and Seki 2006), Daucus carota, Ipomoea batatas, and Oxalis reclinata, numerous researchers have attempted to increase anthocyanin synthesis. Plant cell cultures are a great way to produce anthocyanins because of their greater productivity, which can range from 10 to 20% on a dry weight basis. Several growth regulators' effects on biomass accumulation and anthocyanin content in batch cultures of Daucus carota grown in solid-state and liquid states were investigated.

While growth regulators including 2,4-D, IAA, and NAA were added in varying amounts, they supported both growth and anthocyanin production. Kinetin (0.1 and 0.2 mg l-1) supported the highest level of productivity among the cytokinins. IAA at 2.5 mg l-1 and kinetin at 0.2 mg l-1 together performed better than other combinations. Reduced 2,4-D levels in the medium restricted cell growth and improved anthocyanin methylation and synthesis. MeJ treatment led to the greatest increase in the synthesis of anthocyanins. In plants, calcium is a widely distributed chemical that participates in a number of signal

transduction processes. According to research, calcium levels rise in response to stressors like light, salinity, cold, and dryness.

8.9. Influence of Nutrient Stress on Secondary Metabolites

Because growth is frequently hindered more than photosyn-thesis under stress, the carbon fixed is mostly devoted to secondary metabolites, which may lead to an increase in secondary metabolite production. Phosphate stress caused the Daucus carota callus to produce 7.2% dry weight anthocyanin as opposed to 5.4% dry weight (Haslam 1996) in the control. The amount of phenolic compounds in plant tissues is significantly impacted by nutrient stress. Phenyl propanoids build up and lignification are caused by phosphate and nitrogen deficiency. During nutrient stress, anthocyanidin levels in tomatoes increase by three times while quercetin-3-O-glucoside levels simultaneously double. According to (Zeid 2009), the putrescine content of Phaseolus vulgaris cell suspensions was noticeably raised by the increased urea concentration in the feeding solution. It has been discovered that the osmotic stress caused by sucrose and other osmatic substances controls the anthocyanin synthesis in Vitis vinifera cultures.

8.10. Influence of Climate Change on Secondary Metabolites

In the following decades, climate change will have a significant impact on human health and wellbeing as well as biodiversity. Because of anticipated climate change, the productivity of cold-weather crops including rye, oats, wheat, and apples is predicted to reduce by around 15% over the next 50 years, while the productivity of strawberries could fall by as much as 32%. Plants generally do not adjust rapidly to such changes since they are so sensitive to them. Conifer phenolic concentrations have been demonstrated to rise in response to ozone exposure, although monoterpene and resin acid concentrations were unaffected by mild ozone exposure. A small number of crops have had the effects of ozone exposure on their quality examined. For instance, ozone decreased yield in wheat while increasing grain protein concentration. Also, it was shown that ozone improved the quality of potato tubers by lowering sulfates and raising the vitamin C content. The oil, protein, and carbohydrate content of rape seeds has been reported to decrease when O_3 is present. Furthermore, ozone fumi-gation of Ginkgo biloba leaves boosted terpene concentrations while lowering phenolic amounts. High CO_2 levels cause considerable changes in the chemical makeup of plants. The drop in nitrogen (N) concentration in vegetative plant parts, as well as in seeds and grains, which causes a drop in protein levels, is a well-known example of a CO_2 effect. Prior

research has demonstrated that leaves with high CO_2 had more phenolics and condensed tannins. It has been observed that conifers with higher CO_2 levels had lower/higher concentrations of certain monoterpenes and higher levels of total phenolics. In increasing CO_2 composition, higher levels of the monoterpene a-pinene were observed. Williams et al. discovered lower levels of b-pinene in needles when CO_2 levels were high in contrast to this.

8.11. Influence of Environmental Factors on Secondary Metabolites

The geoclimate of the area, seasonal variations, and environmental factors like light, temperature, and humidity have an impact on the makeup of secondary metabolites. Secondary metabolite production, such as the production of saponins, is a response to environmental variables and a mechanism for coping with abiotic challenges. Saponins are found in the roots, leaves, stems, bulbs, flowers, and fruit of Panax ginseng, and environmental and abiotic conditions have an impact on their content. The buildup of saponins in plant reproductive organs contributes to chemical defense and the response of the plant to environmental conditions. Recently, it has been discovered that the plant kingdom produces melatonin, a neurohormone produced by the pineal gland. An environmentally friendly chemical with strong antioxidant properties is melatonin. The water hyacinth, an aquatic plant that is extremely resilient to environmental contaminants, contains significant quantities of melatonin. Melatonin levels that are higher may aid plants in fending off environmental stress brought on by soil and water contaminants. The potential connections between melatonin supplementation and plant environmental tolerance have recently come to light.

Table 7. Metabolite Responses to Environmental Factors in Various Plant Species

Secondary Metabolites	Plants	Categories	Resistance against
Terpenoids	Citrus	Terpenoid Limonene	Atta cephalotes
Pine and fir	Monoterpenes	bark beetle	
Steroids	Common fern	Phytoecdysones	Insect
Terpenoids	Tobbaco	Trans-anethole and thymol, citronellal,	Spodoptera litura
Phenolics	Wheat	Phenolics	Rhopalosiphum padi
Phenolics	Willow plant	Phenolics	Galerucella lineola
Benzoic acid	Salix	Benzoic acid	Operophtera brumata

Phenolics	Strawberry	Phenolics	Tetranychus urticae
Phenolics	Cotton	Gossypol	Heliothis virescens, Heliothis zea
Alkaloids	Nightshade potato	Alkaloid demissine	Leptinotarsa decemlineata
Benzoxazinoides	Gramineae	DIMBOA	Ostrinia nubilalis
Cyanogenic Glucosides	Cassava	CNglcs	Cyrtomenus bergi
Cyanogenic Glucosides	Bitter almond plants	Amygdalin and prunasin	Capnodis tenebronis
Cyanogenic Glucosides	Trifolium repens	Amygdalin and prunasin	Hypera postica
Cyanogenic Glucosides	Lotus	Cyanogenic glucosides	Zygaena filipendulae
Cyanogenic Glucosides	P.lunatus	CNglcs	Spodoptera eridania

9. TOXICITY AND SAFETY IMPLICATIONS OF HERBAL MEDICINES

Herbal medicines have been used for centuries in various traditional healing practices around the world. While they are generally considered natural and safe, it is important to recognize that herbal medicines, like any other therapeutic agents, can have potential toxicity and safety implications.

1. **Quality Control:** One of the primary concerns with herbal medicines is the lack of standardized quality control measures. The composition and potency of herbal products can vary significantly due to factors such as plant species, growing conditions, harvesting methods, and processing techniques. Contamination with heavy metals, pesticides, or adulterants can also occur. These variations and contaminants can influence the safety and efficacy of herbal medicines.
2. **Toxicity and Side Effects:** Herbal medicines can contain active compounds that may have toxic effects if used improperly or in excessive amounts. Some herbs may have intrinsic toxic properties, and certain individuals or animal species may be more susceptible to adverse effects. Herbal medicines can also interact with conventional medications, leading to potentially harmful interactions or reduced effectiveness of prescribed treatments. Side effects such as allergic reactions, gastrointestinal disturbances, or liver toxicity may occur in some cases.
3. **Lack of Standardized Dosage Guidelines:** Unlike conventional pharmaceuticals, herbal medicines often lack standardized dosage guidelines. Determining the appropriate dosage for specific conditions and individual animals can be challenging. Improper dosage can lead to ineffective treatment or adverse reactions.
4. **Herb-Drug Interactions:** Herbal medicines can interact with prescription medications, leading to potential complications. Some herbs may affect drug metabolism, altering drug levels in the body. These interactions can result in reduced drug efficacy, increased drug toxicity, or unpredictable effects. It is crucial for veterinarians and pet owners to be aware of potential herb-drug interactions when combining herbal medicines with conventional treatments.
5. **Contamination and Adulteration:** Herbal medicines sourced from unreliable or unregulated suppliers may be contaminated or adulterated. Contamination can occur due to the presence of microbial pathogens, heavy metals, or pesticide residues. Adulteration involves the intentional or unintentional addition of substances that may mimic the effects of the

original herb or be harmful to health. Contaminated or adulterated herbal medicines can pose significant safety risks.

6. **Lack of Scientific Evidence:** Many herbal medicines have limited scientific evidence regarding their safety and efficacy. Traditional use and anecdotal evidence may support their use, but comprehensive clinical trials and toxicological studies are often lacking. The lack of robust scientific data makes it challenging to accurately assess the safety profile of herbal medicines.

To ensure the safety of herbal medicines, it is crucial to consider the following measures:

a) **Consultation with Veterinary Professionals:** Always seek advice from qualified veterinarians who have knowledge and experience in herbal medicine. They can provide guidance on appropriate herbal remedies, dosage, and potential interactions with other medications.
b) **Quality Assurance:** Choose herbal medicines from reputable manufacturers or suppliers who adhere to stringent quality control practices. Look for products that have been independently tested for purity, potency, and absence of contaminants.
c) **Individual Variations:** Recognize that individual animals may react differently to herbal medicines. Monitor the animal closely for any adverse reactions, and discontinue use if any concerning symptoms arise.
d) **Full Disclosure:** Inform your veterinarian about any herbal medicines or supplements being used. This will help identify potential interactions with conventional treatments and ensure comprehensive healthcare management.
e) **Evidence-Based Information:** Seek herbal medicines that are supported by scientific research, clinical trials, or established traditional use.
f) **Adherence to Dosage Instructions:** Follow recommended dosage instructions carefully. Avoid exceeding the recommended dosage without professional guidance.

In summary, while herbal medicines can offer potential benefits, it is important to be aware of the potential toxicity and safety implications. By practicing informed and responsible use, involving veterinary professionals, and ensuring product quality and integrity, the safety of herbal medicines can be maximized.

10. HERBAL MEDICINE USE DURING PREGNANCY: BENEFITS AND UNTOWARD EFFECTS

The use of herbal medicine during pregnancy is a topic that requires careful consideration. While herbal medicines are often perceived as natural and safe, it is important to recognize that they can have potential benefits as well as untoward effects.

1. **Benefits of Herbal Medicine Use during Pregnancy:**
   a) **Symptom Relief:** Herbal medicines can provide relief from common pregnancy-related symptoms such as nausea, heartburn, constipation, and insomnia. For example, ginger has been traditionally used to alleviate morning sickness, and peppermint tea may help with digestive discomfort.
   b) **Nutritional Support:** Certain herbal supplements can offer nutritional support during pregnancy. For instance, red raspberry leaf is often used to promote uterine tone and prepare the body for labor.
   c) **Stress and Anxiety Management:** Some herbal remedies, such as chamomile or lavender, may help reduce stress and anxiety during pregnancy.

2. **Untoward Effects and Considerations:**
   a) **Safety Concerns:** Many herbal medicines have not been extensively studied in pregnant women, and safety data may be limited. The potential risks associated with herbal medicine use during pregnancy depend on several factors, including the specific herb, dosage, duration of use, and individual circumstances.
   b) **Lack of Standardization:** Herbal medicines can vary in composition and potency due to factors such as plant species, growing conditions, and processing methods. This lack of standardization makes it difficult to determine the exact dose and potential risks associated with their use.
   c) **Potential Harmful Effects:** Some herbs have known potential risks during pregnancy. For example, certain herbs, including black cohosh, pennyroyal, and blue cohosh, have been associated with uterine contractions and may increase the risk of miscarriage or premature birth. Additionally, some herbs may have hormonal effects that could interfere with fetal development or hormonal balance.
   d) **Herb-Drug Interactions:** Herbal medicines may interact with conventional medications prescribed during pregnancy. These interactions can affect the efficacy or safety of both the herbal medicine

and the prescribed medication. It is important to consult with a healthcare professional to assess potential interactions before using herbal remedies during pregnancy.

e) **Contamination and Adulteration:** Herbal products can be subject to contamination or adulteration, which can pose additional risks during pregnancy. Contamination with heavy metals, microbial pathogens, or pesticide residues may occur, and adulteration with other substances can lead to unpredictable effects.

f)**Individual Variations:** Each pregnancy is unique, and individual variations in response to herbal medicines should be considered. Some pregnant individuals may be more sensitive or have specific health conditions that require extra caution when using herbal remedies.

It is essential to consult with a qualified healthcare provider, such as an obstetrician, midwife, or herbalist experienced in pregnancy care, before using herbal medicines during pregnancy. They can provide personalized guidance based on your specific health needs and circumstances. Additionally, consider the following recommendations:

Disclosure: Inform your healthcare provider about any herbal medicines or supplements you are considering or currently using during pregnancy. This includes both over-the-counter and herbal products.

Professional Guidance: Seek guidance from qualified healthcare professionals who are knowledgeable about herbal medicine use during pregnancy. They can help assess the safety, potential benefits, and possible risks associated with specific herbs.

Quality Assurance: Choose herbal products from reputable manufacturers or suppliers that adhere to stringent quality control practices. Look for products that have been independently tested for purity, potency, and absence of contaminants.

Individualized Approach: Recognize that each pregnancy is unique, and individual responses to herbal remedies may vary. What works for one person may not work the same way for another. Personalized guidance can help ensure safe and appropriate herbal medicine use during pregnancy.

In summary, the use of herbal medicine during pregnancy requires caution and informed decision-making. While some herbal remedies may offer potential benefits, it is crucial to consider safety, potential risks, and individual variations. Consulting with a qualified healthcare professional experienced in pregnancy care is essential for personalized guidance and to make informed choices regarding herbal medicine use during pregnancy.

11. BIOLOGICAL PROPERTIES OF SECONDARY METABOLITES

11.1. Secondary metabolites against plant insect

Secondary metabolites have an impact on the feed value of plant tissues rather than reducing plant growth and development. They are either kept inactively (phytoanticipins) or produced to protect against the attack of bacteria and insects (phytoalexins) (Fig. 2). (Table 1). Biocidal aglycones are released during herbivory as a result of -glucosidase activated phytoanticipins. The traditional example of phytoanticipins is the hydrolysis of glucosinolates by myrosinases after tissue rupture. Another type of phytoanticipin found in Poaceae are benzoxazinoids (BXs). Their breakdown by plastid-targeted -glucosidase after tissue injury results in biocidal aglycone BXs, which serves as an insect deterrent. The performance of herbivores is impacted by phytoalexins, which also include alkaloids, terpenoids, isoflavonoids, etc. Secondary metabolites help plants become more fit in addition to enhancing their defense. Nuessly reported in 2007 that the C-glycosyl flavone maysine and chlorogenic acid are responsible for maize HPR to Helecoverpa zea (corn eatworm). Sorghum's resistance against shoot flies is provided by 4,4-dimethyl cyclooctene.

11.2. Secondary metabolites against plant viruses

Alkaloids, phenolics, and flavonoids are examples of secondary plant metabolic chemicals that have antiviral properties. Alkaloids have a wide variety of physiologically active substances that have an impact on living things. Around 18000 alkaloids have been found in old Chinese plants that have antiviral capabilities, according to studies. The anti-TMV alkaloid 7-deoxy-trans-dihydronarciclasine, which was isolated from plantain lilies (Hosta plantaginea), has an IC50 value of 1.80 M at the lowest concentration. Similar to Bruceine-D, which is contained in Brassica javanica extract, it has an inhibitory impact on PVY, CMV, and TMV. According to research, using a white goosefoot (Chenopodium amaranticolor) extract embedded with 100 g/L Bruceine-D prevented more than 90% of PVY and CMV infection after 15 minutes. With an IC50 value of 3.42 to 5.66, seventeen quassinoids have been found to be anti-TMV infection. A quassinoid with promising results was found by (Chen, Ludwiczuk et al. 2018) when they examined the anti TMV capabilities of Picarma quassioides wood extract, along with a number of other -carboline alkaloids. His subsequent research indicated that the combination application of -carboline and quassinoids results in an infection inhibition of

36.4%–68.4% as opposed to a solo treatment of -carboline @ 50 gm/l that only results in an infection inhibition of 25%–47.4%. Similar to this, An et al. reported that application of Cynanchum komarovii extracts containing (7-demethoxytylophorine and 7-demethoxytylophorine N-oxide) two alkaloids at a concentration of 500 gm/L resulted in anti-TMV activity of 60 and 65%.

11.3. Plant secondary metabolites as antifungal compounds

According to studies, the majority of secondary metabolites have antifungal properties. One significant group of phytochemicals is made up of related phenolics and flavonoids. These substances contribute to a plant's defense against pigmentation, UV resistance, and disease resistance and are concentrated in fruit skins and leaves. Phenolics are known to disrupt the pH gradient, the ATP production and conservation system, membrane-bound enzymes, and substrate usage for ATP production by altering the permeability of microorganisecondary metabolites' cells and disrupting the structural and functional integrity of membrane proteins.

Table 8. List of Phenolic compound role against Fungus.

Chemical	Fungus
benzaldehyde	*Botrytis cinerea*
protocatechuic acid	*Colletotrichum circinans*
Salicylic acid	*eutypa lata*
Vanillic acid	*Phytophthora infestans*
Chlorogenic acid	*Fusarium oxysporum*
Naringin	*Penicillium digitatum*
Flavones	*Aspergillus*
Oleuropein	*Phytophthora*
Nobiletin	*Phoma tracheiphila*
Genistein	*Monilinia fructicola*
Hordatin A	*Helminthosporium sativum*

11.4. Plant secondary metabolites as antibacterial compounds

The protective role of antimicrobial chemicals found in root exudates against Pseudomonas syringae was discovered through analysis of Pseudomonas syringae-infected Arabidopsis root exudates. Seven of the eight variants cannot

infect humans, and when plants were exposed to non-pathogenic strains, they produced more secondary metabolites. Non-pathogenic bacteria acquired modest levels of antibacterial activity against non-infectious strains. Root exudation caused by infected strains had negligible antibacterial action. Research have been conducted to unequivocally show the role of phytoalexins and phytoanticipins in resisting the growth of infections. The quality of the key global vegetable crop Brassica rapa is impacted by the Xanthomonas campestris pv. campestris disease (Xcc). Glucosinolates and phenolic substances can give resistance to Brassica, however little research has been done in this area.

12.THERAPEUTIC EFFECTS OF SECONDARY METABOLITES

12.1. Effects of Secondary metaboites on human colorectal carcinoma

Colorectal carcinoma, commonly known as colorectal cancer (CRC), is a significant global health concern with increasing incidence rates worldwide. The development and progression of CRC involve complex interactions between genetic, environmental, and lifestyle factors. In recent years, there has been growing interest in exploring the potential role of secondary metabolites derived from natural sources in preventing and treating CRC. Secondary metabolites are chemical compounds produced by plants, fungi, and microorganisecondary metabolites, which often possess diverse biological activities.

Secondary metabolites with anticancer properties:

Polyphenols: Polyphenols are a diverse group of compounds found abundantly in fruits, vegetables, and plant-based foods. They exhibit various anticancer properties, including antioxidant, anti-inflammatory, and anticarcinogenic effects. Studies have shown that polyphenols such as curcumin, resveratrol, and epigallocatechin gallate (EGCG) can inhibit colorectal cancer cell proliferation, induce apoptosis, and suppress tumor growth through multiple signaling pathways.

Flavonoids: Flavonoids are another class of secondary metabolites widely distributed in plants. They possess potent anticancer activities, acting as antioxidants, anti-inflammatory agents, and modulators of cell signaling pathways. Quercetin, a flavonoid found in many fruits and vegetables, has been shown to inhibit CRC cell proliferation and induce cell cycle arrest and apoptosis. Additionally, flavonoids like apigenin and luteolin exhibit antiangiogenic effects, suppressing the formation of new blood vessels that support tumor growth.

Alkaloids: Alkaloids are nitrogen-containing compounds derived from plants and microorganisecondary metabolites. Some alkaloids, such as camptothecin and vinblastine, have shown promising anticancer effects against CRC. Camptothecin derivatives, like irinotecan and topotecan, are used in clinical practice as chemotherapeutic agents. These alkaloids act by inhibiting topoisomerase I, an enzyme involved in DNA replication, thereby inducing DNA damage and cell death in CRC cells.

Terpenoids: Terpenoids are a diverse group of secondary metabolites found in plants, fungi, and marine organisecondary metabolites. They exhibit numerous biological activities, including anticancer effects. Certain terpenoids, such as taxol (paclitaxel) and its derivatives, are used in chemotherapy for CRC.

Taxol inhibits microtubule depolymerization, disrupting cell division and leading to cell death. Other terpenoids, such as curcuminoids, exhibit anticancer effects through modulation of signaling pathways involved in cell proliferation, apoptosis, and inflammation.

Mechanisecondary metabolites of action:

Secondary metabolites exert their anticancer effects through various mechanisecondary metabolites, including:

Antioxidant activity: Many secondary metabolites possess antioxidant properties, neutralizing reactive oxygen species (Figueiredo, Barroso et al. 2008) and reducing oxidative stress, which contributes to carcinogenesis.

Anti-inflammatory activity: Chronic inflammation is closely linked to CRC development. Secondary metabolites can inhibit pro-inflammatory signaling pathways and reduce the production of inflammatory mediators, thereby attenuating inflammation-associated carcinogenesis.

Modulation of cell signaling pathways: Secondary metabolites can interact with key signaling pathways involved in cell proliferation, apoptosis, and angiogenesis. For instance, polyphenols and flavonoids can target the Wnt/β-catenin pathway, which plays a crucial role in CRC progression.

Epigenetic modifications: Some secondary metabolites can modify gene expression patterns by affecting DNA methylation, histone modifications, and microRNA regulation, leading to the suppression of oncogenes and the activation of tumor suppressor genes.

Secondary metabolites derived from natural sources have shown great potential as therapeutic agents in the prevention and treatment of human colorectal carcinoma. Their diverse biological activities, including antioxidant, anti-inflammatory, and anticancer effects, make them promising candidates for further investigation. However, more research is needed to fully understand the mechanisecondary metabolites of action, optimize delivery methods, and assess their efficacy and safety in clinical settings. The exploration of secondary metabolites opens up new avenues for the development of novel and more targeted approaches to combat CRC, ultimately improving patient outcomes in the battle against colorectal carcinoma.

12.2. Effects of secondary metaboites on human breast cancer

Breast cancer is one of the most prevalent malignancies affecting women worldwide. Despite significant advancements in diagnosis and treatment, the search for effective therapeutic strategies continues. In recent years, there has been increasing interest in exploring the potential of secondary metabolites derived from natural sources as therapeutic agents against breast cancer. Secondary metabolites are chemical compounds produced by plants, fungi, and microorganisecondary metabolites, known for their diverse biological activities. This essay aims to investigate the effects of secondary metabolites on human breast cancer and highlight their potential as valuable tools in breast cancer management.

Polyphenols: Polyphenols are a group of secondary metabolites found abundantly in fruits, vegetables, and plant-based foods. They possess potent anticancer properties, including antioxidant, anti-inflammatory, and anti-proliferative effects. Polyphenols such as curcumin, resveratrol, and quercetin have demonstrated promising results in inhibiting breast cancer cell growth, inducing apoptosis, and suppressing tumor formation through multiple molecular pathways. These compounds can modulate various cellular signaling pathways involved in cell survival, proliferation, and angiogenesis.

Flavonoids: Flavonoids are secondary metabolites widely distributed in plants and exhibit diverse pharmacological activities. Some flavonoids, including genistein, apigenin, and kaempferol, have been extensively studied for their potential anticancer effects against breast cancer. Flavonoids can inhibit cell proliferation, induce cell cycle arrest, and promote apoptosis in breast cancer cells. Additionally, they possess antiangiogenic properties, suppressing the formation of new blood vessels crucial for tumor growth and metastasis.

Terpenoids: Terpenoids are a large group of secondary metabolites found in plants, fungi, and marine organisecondary metabolites. Several terpenoids, such as paclitaxel (Taxol) and its derivatives, have been used in breast cancer chemotherapy due to their ability to inhibit microtubule polymerization, leading to cell cycle arrest and apoptosis. Other terpenoids, including betulinic acid and ginsenosides, exhibit anticancer effects through various mechanisecondary metabolites such as modulating cell signaling pathways, inhibiting metastasis, and enhancing the immune response against cancer cells.

Alkaloids: Alkaloids are a diverse class of secondary metabolites commonly found in plants, particularly in medicinal herbs. Several alkaloids have shown potential in breast cancer treatment. For example, vincristine and vinblastine, derived from the Madagascar periwinkle plant, are used in combination

chemotherapy regimens for breast cancer. These alkaloids interfere with microtubule assembly, disrupt cell division, and induce apoptosis in cancer cells. Moreover, other alkaloids, such as berberine and noscapine, have demonstrated anti-proliferative and anti-invasive effects in breast cancer models.

Mechanisecondary metabolites of action:

Secondary metabolites exert their effects against breast cancer through various mechanisecondary metabolites, including:

a) **Induction of apoptosis:** Many secondary metabolites can trigger programmed cell death by activating apoptotic pathways in breast cancer cells. This includes modulation of Bcl-2 family proteins, caspases, and the intrinsic and extrinsic apoptotic pathways.
b) **Cell cycle arrest:** Secondary metabolites can disrupt the cell cycle progression of breast cancer cells, leading to cell cycle arrest at specific phases, such as G1 or G2/M. This interference prevents uncontrolled cell proliferation and promotes cancer cell death.
c) **Modulation of signaling pathways:** Secondary metabolites can interact with critical signaling pathways involved in breast cancer progression, such as the PI3K/AKT/mTOR pathway and the estrogen receptor signaling pathway. By modulating these pathways, secondary metabolites can inhibit cell survival, proliferation, and metastasis.
d) **Anti-angiogenic effects:** Some secondary metabolites can inhibit angiogenesis, the process of new blood vessel formation. By suppressing the formation of new blood vessels, secondary metabolites restrict the nutrient and oxygen supply to tumors, leading to their regression and reduced metastatic potential.

Secondary metabolites derived from natural sources offer promising potential in the prevention and treatment of human breast cancer. Their diverse biological activities, including antioxidant, anti-inflammatory, and anticancer effects, make them attractive candidates for further exploration. While significant progress has been made in understanding the effects of secondary metabolites on breast cancer, further research is needed to uncover their precise mechanisecondary metabolites of action, optimize dosage and delivery methods, and evaluate their safety and efficacy in clinical settings. The study of secondary metabolites provides a promising avenue for the development of novel and more effective strategies in the fight against breast cancer, ultimately leading to improved outcomes for patients.

12.3. Effects of secondary metaboites on human prostate cancer

Prostate cancer is a significant health concern and one of the most commonly diagnosed malignancies among men worldwide. Despite advances in detection and treatment, the search for effective therapeutic strategies continues. In recent years, there has been growing interest in exploring the potential of secondary metabolites derived from natural sources as therapeutic agents against prostate cancer. Secondary metabolites are chemical compounds produced by plants, fungi, and microorganisecondary metabolites, known for their diverse biological activities. This essay aims to investigate the effects of secondary metabolites on human prostate cancer and highlight their potential as valuable tools in prostate cancer management.

Polyphenols: Polyphenols are a group of secondary metabolites found abundantly in fruits, vegetables, and plant-based foods. They possess potent anticancer properties, including antioxidant, anti-inflammatory, and antiproliferative effects. Polyphenols such as curcumin, resveratrol, and epigallocatechin gallate (EGCG) have demonstrated promising results in inhibiting prostate cancer cell growth, inducing apoptosis, and suppressing tumor formation through multiple molecular pathways. These compounds can modulate various cellular signaling pathways involved in cell survival, proliferation, and angiogenesis.

Flavonoids: Flavonoids are secondary metabolites widely distributed in plants and exhibit diverse pharmacological activities. Some flavonoids, including quercetin, apigenin, and genistein, have been extensively studied for their potential anticancer effects against prostate cancer. Flavonoids can inhibit cell proliferation, induce cell cycle arrest, and promote apoptosis in prostate cancer cells. Additionally, they possess antiangiogenic properties, suppressing the formation of new blood vessels crucial for tumor growth and metastasis.

Terpenoids: Terpenoids are a large group of secondary metabolites found in plants, fungi, and marine organisecondary metabolites. Several terpenoids have shown potential in prostate cancer treatment. For example, betulinic acid, derived from birch bark, exhibits antiproliferative effects on prostate cancer cells by inducing apoptosis and inhibiting cell migration and invasion. Other terpenoids, such as reserpine and triptolide, have demonstrated promising results in inhibiting prostate cancer growth and metastasis through various mechanisecondary metabolites, including modulation of signaling pathways and inhibition of angiogenesis.

Alkaloids: Alkaloids are a diverse class of secondary metabolites commonly found in plants. Several alkaloids have shown potential in prostate cancer treatment. For instance, berberine, derived from various plants, has

demonstrated antiproliferative effects on prostate cancer cells by inducing cell cycle arrest and apoptosis. Additionally, vincristine, derived from the Madagascar periwinkle plant, has been used in combination chemotherapy for prostate cancer, inhibiting microtubule assembly and disrupting cell division.

Mechanisecondary metabolites of action: Secondary metabolites exert their effects against prostate cancer through various mechanisecondary metabolites, including:

a) **Induction of apoptosis:** Many secondary metabolites can trigger programmed cell death by activating apoptotic pathways in prostate cancer cells. This includes modulation of Bcl-2 family proteins, caspases, and the intrinsic and extrinsic apoptotic pathways.
b) **Cell cycle arrest:** Secondary metabolites can disrupt the cell cycle progression of prostate cancer cells, leading to cell cycle arrest at specific phases, such as G1 or G2/M. This interference prevents uncontrolled cell proliferation and promotes cancer cell death.
c) **Modulation of signaling pathways:** Secondary metabolites can interact with critical signaling pathways involved in prostate cancer progression, such as the PI3K/AKT/mTOR pathway and the androgen receptor signaling pathway. By modulating these pathways, secondary metabolites can inhibit cell survival, proliferation, and metastasis.
d) **Anti-angiogenic effects: Some** secondary metabolites can inhibit angiogenesis, the process of new blood vessel formation. By suppressing the formation of new blood vessels, secondary metabolites restrict the nutrient and oxygen supply to tumors, leading to their regression and reduced metastatic potential.

Secondary metabolites derived from natural sources offer promising potential in the prevention and treatment of human prostate cancer. Their diverse biological activities, including antioxidant, anti-inflammatory, and anticancer effects, make them attractive candidates for further exploration. While significant progress has been made in understanding the effects of secondary metabolites on prostate cancer, further research is needed to uncover their precise mechanisecondary metabolites of action, optimize dosage and delivery methods, and evaluate their safety and efficacy in clinical settings. The study of secondary metabolites provides a promising avenue for the development of novel and more effective strategies in the fight against prostate cancer, ultimately leading to improved outcomes for patients.

12.4. Effects of secondary metaboites on human lung cancer

Lung cancer is a devastating disease that poses a significant global health burden. Despite advancements in diagnosis and treatment, the need for effective therapeutic strategies remains crucial. In recent years, there has been growing interest in exploring the potential of secondary metabolites derived from natural sources as valuable tools for lung cancer management. Secondary metabolites, produced by plants, fungi, and microorganisecondary metabolites, possess diverse biological activities and exhibit promising anticancer properties. This essay aims to explore the effects of secondary metabolites on human lung cancer and shed light on their potential as therapeutic agents.

Polyphenols: Polyphenols are a group of secondary metabolites widely distributed in fruits, vegetables, and plant-based foods. They exhibit remarkable anticancer properties, including antioxidant, anti-inflammatory, and antiproliferative effects. Polyphenols such as resveratrol, curcumin, and epigallocatechin gallate (EGCG) have demonstrated promising results in inhibiting lung cancer cell growth, inducing apoptosis, and suppressing tumor formation through various molecular pathways. These compounds can modulate signaling pathways involved in cell survival, proliferation, and angiogenesis.

Flavonoids: Flavonoids, another group of secondary metabolites abundantly present in plants, possess diverse pharmacological activities. Several flavonoids, including quercetin, apigenin, and luteolin, have been extensively studied for their potential anticancer effects against lung cancer. Flavonoids can inhibit cell proliferation, induce cell cycle arrest, and promote apoptosis in lung cancer cells. Moreover, they exhibit anti-angiogenic properties, which impede the formation of new blood vessels crucial for tumor growth and metastasis.

Terpenoids: Terpenoids constitute a large group of secondary metabolites found in plants, fungi, and marine organisecondary metabolites. Various terpenoids have shown promise in lung cancer treatment. For example, paclitaxel (Taxol) and its derivatives, derived from the Pacific yew tree, have been successfully used in chemotherapy for lung cancer. These terpenoids act by inhibiting microtubule depolymerization, disrupting cell division, and inducing cell death. Additionally, other terpenoids, such as curcuminoids and betulinic acid, exhibit anticancer effects through modulation of signaling pathways involved in cell proliferation, apoptosis, and inflammation.

Alkaloids: Alkaloids, diverse secondary metabolites found in plants, have demonstrated potential in lung cancer treatment. For instance, vinblastine and vincristine, derived from the Madagascar periwinkle plant, are used in combination chemotherapy regimens for lung cancer. These alkaloids interfere with microtubule assembly, disrupt cell division, and induce apoptosis in cancer

cells. Additionally, other alkaloids, such as camptothecin and noscapine, exhibit antiproliferative and anti-invasive effects in lung cancer models.

Mechanisecondary metabolites of Action: Secondary metabolites exert their effects against lung cancer through various mechanisecondary metabolites, including:

a) **Induction of apoptosis:** Many secondary metabolites trigger programmed cell death by activating apoptotic pathways in lung cancer cells. They modulate key proteins involved in apoptosis, such as Bcl-2 family proteins, caspases, and the intrinsic and extrinsic apoptotic pathways.
b) **Cell cycle arrest:** Secondary metabolites can disrupt the cell cycle progression of lung cancer cells, leading to cell cycle arrest at specific phases, such as G1 or G2/M. This interference prevents uncontrolled cell proliferation and promotes cancer cell death.
c) **Modulation of signaling pathways:** Secondary metabolites interact with critical signaling pathways involved in lung cancer progression, such as the PI3K/AKT/mTOR pathway and the EGFR signaling pathway. By modulating these pathways, secondary metabolites can inhibit cell survival, proliferation, and metastasis.
d) **Anti-angiogenic effects:** Some secondary metabolites exhibit anti-angiogenic properties by inhibiting the formation of new blood vessels that support tumor growth and metastasis. This deprivation of oxygen and nutrients can suppress lung cancer progression.

Secondary metabolites derived from natural sources offer promising potential as therapeutic agents for human lung cancer. Polyphenols, flavonoids, terpenoids, and alkaloids possess diverse biological activities and have demonstrated anticancer effects through various mechanisecondary metabolites. Their ability to induce apoptosis, arrest cell cycle progression, and modulate key signaling pathways involved in lung cancer progression highlights their potential as valuable tools for therapeutic intervention. Further research is necessary to uncover the precise mechanisecondary metabolites of action, optimize dosage and delivery methods, and evaluate the efficacy and safety of secondary metabolites in clinical settings. Exploring secondary metabolites provides a novel and exciting avenue for developing effective strategies against lung cancer, offering hope for improved patient outcomes in the fight against this devastating disease.

12.5. Effects of secondary metaboites on human brain cancer

Brain cancer, including primary and metastatic tumors, is a challenging and often fatal disease. Despite significant advancements in diagnosis and treatment, effective therapeutic options are limited. In recent years, there has been growing interest in exploring the potential of secondary metabolites derived from natural sources as valuable tools for brain cancer management. Secondary metabolites, produced by plants, fungi, and microorganisecondary metabolites, possess diverse biological activities and exhibit promising anticancer properties. This essay aims to investigate the effects of secondary metabolites on human brain cancer and shed light on their potential as therapeutic agents.

Polyphenols are a group of secondary metabolites widely present in fruits, vegetables, and plant-based foods. They exhibit potent antioxidant, anti-inflammatory, and antiproliferative effects. Polyphenols such as curcumin, resveratrol, and epigallocatechin gallate (EGCG) have demonstrated promising results in inhibiting brain cancer cell growth, inducing apoptosis, and suppressing tumor formation through various molecular pathways. These compounds can modulate signaling pathways involved in cell survival, proliferation, and angiogenesis. Several flavonoids, including quercetin, apigenin, and kaempferol, have been extensively studied for their potential anticancer effects against brain cancer. Flavonoids can inhibit cell proliferation, induce cell cycle arrest, and promote apoptosis in brain cancer cells. Moreover, they exhibit anti-angiogenic properties, suppressing the formation of new blood vessels crucial for tumor growth and metastasis. Various terpenoids have shown promise in brain cancer treatment. For example, paclitaxel (Taxol), derived from the Pacific yew tree, has been used in chemotherapy for brain cancer. Terpenoids act by inhibiting microtubule depolymerization, disrupting cell division, and inducing cell death. Additionally, other terpenoids, such as betulinic acid and ginsenosides, exhibit anticancer effects through modulation of signaling pathways involved in cell proliferation, apoptosis, and angiogenesis. Several alkaloids have shown potential in brain cancer treatment. For instance, vincristine and vinblastine, derived from the Madagascar periwinkle plant, are used in combination chemotherapy regimens for brain cancer. These alkaloids interfere with microtubule assembly, disrupt cell division, and induce apoptosis in cancer cells. Additionally, other alkaloids, such as berberine and harmine, exhibit antiproliferative and anti-invasive effects in brain cancer models. Secondary metabolites exert their effects against brain cancer through various mechanisecondary metabolites, including: Many secondary metabolites trigger programmed cell death by activating apoptotic pathways in brain cancer cells. They modulate key proteins involved in

apoptosis, such as Bcl-2 family proteins, caspases, and the intrinsic and extrinsic apoptotic pathways.

Secondary metabolites can disrupt the cell cycle progression of brain cancer cells, leading to cell cycle arrest at specific phases, such as G1 or G2/M. This interference prevents uncontrolled cell proliferation and promotes cancer cell death. Secondary metabolites interact with critical signaling pathways involved in brain cancer progression, such as the PI3K/AKT/mTOR pathway and the Wnt/β-catenin pathway. By modulating these pathways, secondary metabolites can inhibit cell survival, proliferation, and metastasis. Some secondary metabolites exhibit anti-angiogenic properties by inhibiting the formation of new blood vessels that support tumor growth and metastasis. This deprivation of oxygen and nutrients can suppress brain cancer progression.

Secondary metabolites derived from natural sources offer promising potential as therapeutic agents for human brain cancer. Polyphenols, flavonoids, terpenoids, and alkaloids possess diverse biological activities and have demonstrated anticancer effects through various mechanisecondary metabolites. Their ability to induce apoptosis, arrest cell cycle progression, and modulate key signaling pathways involved in brain cancer progression highlights their potential as valuable tools for therapeutic intervention. Further research is necessary to uncover the precise mechanisecondary metabolites of action, optimize dosage and delivery methods, and evaluate the efficacy and safety of secondary metabolites in clinical settings. Exploring secondary metabolites provides a novel and exciting avenue for developing effective strategies against brain cancer, offering hope for improved patient outcomes in the battle against this devastating disease.

13.USEAGE OF SECONDARY METABOLITES IN THE FIELAD OF VETERINARY

Secondary metabolites play a significant role in the field of veterinary medicine. They have various applications in veterinary healthcare, including animal nutrition, disease management, and as potential therapeutic agents. Here are some key uses of secondary metabolites in veterinary:

a) **Animal Feed Additives:** Secondary metabolites derived from plants, such as polyphenols and flavonoids, are often used as feed additives in animal nutrition. These compounds can improve animal health and performance by enhancing nutrient utilization, modulating gut microbiota, and exerting antioxidant and anti-inflammatory effects. For example, tannins derived from tree barks are used as natural feed additives to reduce methane emissions from ruminant animals and improve their feed efficiency.

b) **Herbal Medicines:** Many traditional herbal medicines contain secondary metabolites that have been used for centuries in veterinary healthcare. These natural compounds can have antimicrobial, antiparasitic, and immunomodulatory properties. For instance, extracts from plants like neem, garlic, and turmeric are used as natural alternatives to conventional treatments for various animal infections and infestations.

c) **Wound Healing and Tissue Repair:** Certain secondary metabolites, such as tannins and terpenoids, possess wound healing properties. They can promote tissue regeneration, enhance wound contraction, and exhibit antimicrobial effects. These compounds are used in veterinary medicine for managing wounds, burns, and skin infections in animals.

d) **Antimicrobial Agents:** Some secondary metabolites have antimicrobial properties and can serve as alternatives to conventional antibiotics in veterinary medicine. For example, essential oils derived from plants, rich in terpenoids, have shown antimicrobial activity against various pathogens. These natural compounds can be used to control bacterial, fungal, and parasitic infections in animals, reducing the reliance on synthetic antibiotics.

e) **Reproductive Health:** Secondary metabolites have also been explored for their effects on animal reproductive health. Compounds such as phytoestrogens, found in certain plants, have shown potential in managing reproductive disorders in livestock animals. These

compounds can modulate hormone levels, improve fertility, and regulate reproductive functions in animals.

f) **Immunomodulation:** Some secondary metabolites possess immunomodulatory properties and can help strengthen the immune system in animals. For instance, polysaccharides derived from medicinal mushrooms have been studied for their immune-enhancing effects in animals, improving resistance against infections and diseases.

g) **Anti-inflammatory and Analgesic Effects:** Certain secondary metabolites have anti-inflammatory and analgesic properties, making them useful in managing pain and inflammation in animals. For example, curcumin, a polyphenol found in turmeric, has shown anti-inflammatory effects and is used in veterinary medicine for managing pain and inflammation associated with conditions like arthritis in animals.

It is important to note that while secondary metabolites offer potential benefits in veterinary medicine, their use should be approached with caution. Proper dosage, formulation, and safety considerations should be taken into account to ensure the well-being and health of the animals being treated. Additionally, further research and clinical studies are needed to validate the efficacy and safety of secondary metabolites in veterinary applications.

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