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Secondary metabolites-God gifted arsenal for plants

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Abstract

Plant secondary metabolites refer to various chemical compounds that plants produce. Still, they are not directly involved in essential growth processes like primary metabolites (such as sugars, amino acids, and lipids). The term "secondary metabolite" has generated controversy, as it implies these compounds are of minor importance to plants. "Special metabolites" may be a more suitable term. The plants are protected from both abiotic and biotic stress by these metabolites. The vast diversity of plant secondary metabolites showcases plants' incredible adaptability and versatility, providing an arsenal of chemical tools to defend against herbivores, protect from pathogens, attract pollinators and seed dispersers, adaptability and thrive and environmental interactions. Plants can therefore survive under less-than-ideal conditions. The profound impact of plant secondary metabolites highlights the intricate relationship between plants and humanity, opening doors to innovative applications with far-reaching implications.

Keywords: Bioactive compounds, phytochemicals, secondary metabolites, plant defense

1. Introduction

Our daily diet must include plants, and their nutritional worth and contents have been the subject of extensive research for many years. In addition to essential primary metabolites, including lipids, amino acids, and carbohydrates, higher plants are capable of producing a variety of chemical molecules referred to as secondary metabolites ^[1].

The chemical compounds formed from a series of enzyme-dependent chemical reactions are metabolites. This sum of the chemical reactions in an organism for the proper functioning of cells and organisms is called metabolism. The sequence of chemical reactions is known as metabolic pathways. The citric acid cycle and glycolysis produce energy used in these metabolic processes. The metabolism provides chemical compounds essential to maintain life, such as growth, development or reproduction in living organisms known as primary metabolism. The resulting end product(s) is termed primary metabolites. The term "secondary metabolite" refers to an organic substance that serves a purpose in a plant's ecology and is crucial to the defense of the plant but is not necessary for its growth and reproduction. The production of secondary metabolites may be limited to particular families, genera, and maybe even species, but levels are typically less, though not always. Secondary metabolites in annual plants are mostly contained in trichomes or glandular hairs (terpenoids in Asteraceae and Labiatae), stinging hairs (Amines in Urticaceae), or the epidermis itself (alkaloids, flavonoids of various classes, anthocyanins, cyanogenic glycosides, coumarins, etc.). Usual sources of secondary metabolites include flowers, fruits, and seeds. Secondary metabolites are mostly found in perennial species' roots, rhizomes, bulbs, bark and stems. Primary metabolites provide building blocks for secondary metabolites. The type of primary metabolites provides building blocks for secondary metabolites such as S-methyl of L-methionine (C1 methyl group); Acetyl-Co A (C2 ethyl group); Mevalonic acid (C5 branched-chain five carbon unit "isoprene"); L-ornithine (non-protein amino acid); C4N unit (heterocyclic pyrrolidine system); L-phenylalanine or L-tyrosine (The phenyl propyl unit C_6C_3 and C_6C_2N); L-lysine (C_5N as a piperidine ring system) and L-tryptophan (Indole C₂N). Secondary metabolites are synthesized by building blocks provided by primary metabolism. Carbohydrates, proteins, fats and nucleic acids are examples of primary metabolites. Secondary metabolites include alkaloids, essential oil, glycosides, tannins etc. Different metabolic pathways for secondary metabolites and their end products are:

- a. Acetate pathway: Aliphatic amino acids, alkaloids and proteins.
- b. Amino acids pathway: Alkaloids and protein.
- c. Malonic acid pathway: Flavonoids, tannins, fat and waxes.
 - Mevalonic acid: Steroids and terpenes.

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e. Shikimic acid: Aromatic alkaloids, aromatic amino acids (phenylalanine, tyrosine and tryptophan), cyanogenic glycosides, glucosinolates, lignin, phenols and tannins ^[2, 3].

2. How Are Secondary Metabolites Defined?

A. Kossel first identified and described secondary metabolites in 1891. According to A. Kossel, "Organic compounds as incidentally occurring and not of paramount significance to plant life. Most of these compounds do not directly participate in plants' growth, development and reproduction, hence named secondary metabolites"^[1].

It is now clear that these notions were false and misleading. The name "secondary metabolite," which implies that they are of only marginal significance to the plant, is controversial. The phrase "special metabolites" is perhaps more suitable for secondary metabolites. The adage "necessity is the mother of innovation" appears to be true when we consider plants, which, although non-motile and without an immune system, are not powerless against a wide range of biotic and abiotic challenges; instead, they wield an arsenal of chemicals in the form of secondary metabolites to discourage adversaries, ward off diseases, and overcome environmental limits. Secondary metabolites play a vital and active part in possible defense systems, particularly in the chemical conflict between plants and pathogens. Additionally, it has been demonstrated that some of these substances can deter herbivores, draw pollinators, offer toxicity protection, and serve as UV radiation shields. To lure, catch, digest, and assimilate their prey, carnivorous plants also require secondary metabolites. Because it implies that they are inconsequential while playing a variety of roles in plant life, the name "secondary metabolite" seems unsuitable from their current vantage point [4, 5]

3. Factors Affecting Secondary Metabolites Production

Secondary metabolites need a different cellular mechanism for their transit, storage, and turnover due to their hydrophilic and lipophilic natures. Following production in the cytoplasm, hydrophilic secondary metabolites are frequently stored in vacuoles (alkaloids, saponins, glycosides, flavonoids, anthocyanins, cyanogens, glucosinolates, amines), laticifers (alkaloids of Lobelia, Papaver and Chelidonium; cyanogens, cardiac glycosides of Nerium), apoplast or cell wall (tannins). Whereas lipophilic substances are sequestered in the cuticle lipophilic flavonoids, terpenoids), trichome (waxes. (monoterpenes, sesquiterpenes, quinones), resin ducts (terpenoids, lipophilic flavonoids), laticifers (polyterpenes, diterpenes, lipophilic flavonoids, quinones) oil cells (anthraquinones, naphthodianthrones, terpenoids) and plastid membrane (Ubiquinones, tetraterpenes)^[2, 6].

The following elements have an impact on secondary metabolite synthesis in plants.

3.1 Genes: Genes play a significant role in determining the type and quantity of a plant's secondary metabolites. Plant secondary metabolite concentration varies between species and within the same species due to the presence of specific genes. Understanding how genes impact secondary metabolite production is essential for various applications, including developing crops with improved nutritional value, enhanced medicinal properties, and increased resistance to environmental stresses. Additionally, this knowledge can aid in the sustainable utilization of plant secondary metabolites for pharmaceutical, agricultural, and industrial purposes.

3.2 Specific plant tissues: Due to the presence of laticiferous cells, the alkaloids morphine, thebaine, and codeine are mostly deposited in fully-grown capsules of *Papaver somniferum*. At the same time, a significantly less quantity is present in roots.

3.3 Different development stages

3.3.1 Plant maturation

Plant secondary metabolites show mixed responses in plant maturation. The young plant of *Cannabis sativa* contains (±)-Cannabichromene, which converts into Δ9tetrahydrocannabinol in the matured plant. Young leaves of Mentha piperita (peppermint) contain pulegone, but mature leaves contain menthol and menthone. Lysergic acid and chanoclavine are high in maturing Ipomoea violacea (morning glory), whereas young seeds contain a low quantity. Camphor accumulates in Cinnamomum camphora (camphor tree) and is ready for collection from the heartwood of 40 years trees. In Datura stramonium (thornapple), the young plant contains hyoscine and hyoscyamine (80%), decreasing to 30% in matured fruiting plant.

3.3.2 Fructification stage (fruit bearing stage)

Astragalus compactus in the fructification stage contains high phenolic contents.

3.3.3 Flower pollination

The biosynthesis of vanillin in *Vanilla planifolia* (vanilla) increases eight months after flower pollination.

3.4 Seasonal variation: In, *Taxus baccata* (European yew), the ω -deacetyl baccatin present in summer which convert into pharmacologically active 2,4-di methoxy phenol in winter.

3.5 Biological agents: Attacking bacteria, fungi, viruses, nematodes, and parasites to plants triggers some secondary metabolites, especially phenolic compounds, to show resistance against these organisms.

3.6 Insufficient availability of water (Drought): A lack of water constraints plants' ability to biosynthesize secondary metabolites. In places with limited water resources, Trachyspermum ammi (ajowan) plantings raise the total phenol content. The essential oil content in Matricaria chamomilla (chamomile), reduces under the same condition. Artemisinin. betulinic acid, quercetin, and rutinin concentrations in Artemisia and Hypericum brasiliense are all increased by a lack of water. This condition increases the essential oil percentage and decreases nitrogen, phosphorus, potassium and protein content in Ocimum basilicum (sweet basil). Chenopodium quinoa produces more saponins when it is under stress from drought. The production of flavonoids was aided by the drought's oxidative stress, which was also thought to be defending plants growing in soils containing harmful metals like aluminum. To participate in the production of different phenolics and flavonoids, the Lactuca sativa (lettuce) gene PAL (phenylalanine ammonia-lyase) can be activated under conditions of water deprivation. The Antirrhinum majus (snapdragon) AmDEL gene is overexpressed in response to a water shortage, giving the fruit's peel and flesh an intense purple coloration and much more flavonoid accumulation.

3.7 The high soil salinity: In essential oil production, there is a mixed salt stress response. Some plants, like *Matricaria chamomilla*, *Mentha piperita*, *Origanum vulgare* (oregano), *Salvia officinalis* (sage) and *Trachyspermum ammi*, show a decrease in essential oil content. Although others, including *Matricaria recutita* (German chamomile) and *Satureja hortensis* (Summer savory), exhibit an increase in essential oil content when subjected to salt stress. Salinity stress also decreased phenolic compounds (chlorogenic and sinapic acid derivatives and flavonoids) in leaves of *Brassica oleracea* (broccoli).

3.8 Temperature of environment: In Panax guinguefolius (American ginseng), the amount of root ginsenosides increases as the temperature rises. Low temperatures (20 °C) can slow plant growth, lowering photosynthesis and pepper) reducing Capsicum annuum (bell output. Temperature rise causes Stevia rebaudiana roots to produce more stevioside. The biosynthesis of alkaloids-the overall accumulation of alkaloids (morphine, naphthyl isoquinoline, and benzyl isoquinoline) in Papaver somniferum-prefers high temperatures. Anthocyanin accumulation is triggered by low temperatures and prevented by high temperatures in Melastoma malabathricum (Singapore rhododendron) and Citrus sinensis (sweet orange) fruits. The total anthocyanin content of Vitis vinifera (common grapes) berries was lowered by high temperature (35 °C) to less than half of the control berries (25 °C). The high terpene content in Daucus carota (wild carrot) roots can cause a strong bitterness that is unpleasant to consumers. Therefore, to cultivate good carrots, high temperatures must be avoided.

3.9 Sun light period: Growing plants require light to carry out their biosynthetic activities. Light is indispensable when it comes to encouraging plant development and triggering or controlling plant metabolism. In *Muhlenbergia glomerata* (marsh muhly) stems and leaves, shorter photoperiods resulted in lower coumarin concentrations, whereas longer photoperiods resulted in significantly higher coumarin concentrations. *Panax quinquefolius* plants produce more ginsenosides in their roots when they are exposed to sunlight for an extended period. The presence of phenolic acids (hydroxycinnamic and hydroxybenzoic acids) and flavonoids (anthocyanins, catechins, and flavonols) was dramatically enhanced in *Ipomoea batatas* (sweet potato) leaves after prolonged (16 h) light exposure, confirming the idea that compounds like acid can shield plants from the sun.

3.10 Impact of location: Different regions result in different yields of secondary plant metabolites. *Mentha piperita* exhibits more antimicrobial activity at higher altitudes due to the considerable synthesis of secondary metabolites with antimicrobial activities.

3.11 Nutrients: The availability of micronutrients can affect biosynthetic processes, such as enzyme activators, which can influence the synthesis of bioactive compounds. As opposed to that, macronutrients, including carbon and nitrogen, are essential for synthesising biomass and secondary metabolites. A phosphorus deficiency causes stunting because phosphorus is essential for plants' primary metabolism and is a part of energy-rich molecules like ATP and ADP. In plants, magnesium is involved in many processes, such as ATP synthesis, CO_2 fixation, and the creation of chlorophyll, which serves as the building block of secondary metabolism.

The production of putrescine and cadaverine, which reduce the diamine oxidase activity, relies on copper-dependent oxygenases and oxidases. The flavonoid content of *Hypericum perforatum* (St. John's wort) and leaf biomass in *Salvia miltiorrhiza* (red sage) was increased by nitrogen and phosphorus ^[7-10].

4. Secondary Metabolites' Role in Plants

The productivity of crops is impacted by a wide range of environmental challenges that plants are subjected to, including biotic and abiotic factors. Variations in physical or chemical elements like droughts, salinity, floods, heavy metals, severe temperatures, etc., produce abiotic stress. In contrast, biotic stress is caused by living things like weeds, nematodes, bacteria, fungi, insects, herbivores, nematodes, and oomycetes. The lack of nutrients, illnesses, and infections brought on by biotic agents cause plants to die, which causes significant pre- and postharvest losses. The principal causes of soil-borne disorders that result in restricted plant development, nutrient shortages, and wilting are nematodes, which feed on plants. Viruses also produce systemic and localized harm, such as chlorosis and stunting. In contrast, insects and mites pierce and suck on plants to feed, lay their eggs, and act as carriers of bacteria and viruses that harm humans and other animals. Plants, unlike animals, are sessile and lack an adaptive immune system. Thus they are unable to flee from stress. As a result, they have evolved sophisticated strategies to deal with both biotic and abiotic challenges. Phytoanticipins, which are consistently present in plant cells, and phytoalexins, created in response to infections but absent from healthy plant tissues, are two types of plant-derived chemicals involved in chemical defence [11]. Different secondary metabolites perform different functions in plants are as follows.

4.1 Gums and Mucins

The plant is shielded against microbial attack by the cell wall's matrix, which is made up of gum and mucus. Additionally, plant wounds on leaves and stems are sealed with gum to stop plant infection. Lignin acts as a wall stabilizer. Waxes, cutin and suberin improve the physical resistance of plants and the resistance to microbial attack.

4.2 Saccharides and Glycosides

Different monosaccharides, sugar alcohols, or oligosaccharides are the sources of sweetness present in the fleshy fruit and dispersed by animal after eating. Plants of the cabbage or mustard family (Brassicaceae or Cruciferae) produce isothiocyanate glycosides which are highly toxic to most insects. At the same time, these compounds seem to stimulate the adult female cabbage butterfly, *Pieris brassicae*, to lay her egg on the plant ^[12].

4.3 Waxes

Waxes improve the physical resistance of plants and the resistance to microbial attack. The wax layer of *Vitis vinifera* (grapes), *Juniperus communis* (juniper) and *Ficus carica* (figs), increases UV reflection and hence their visibility for birds and accordingly provides aid in pollination. In carnivorous or insectivorous plants such as *Catopsis berteroniana*, *Brocchinia reducta*, *Cephalotus follicularis*, *Sarracenia*, *Darlingtonia* and *Heliamphora* wax provides slippery surfaces in their traps due to the presence of anti-adhesive wax for capture and reducing the prey's ability to escape ^[4].

4.4 Phenolic compounds (Floral scent volatiles and pigments)

The colors, smell and taste of ripe fruits attract animals suitable for food. Animals consume the fruit (together with the seeds it contains), break down the pulp, and then excrete it in feces. The phenolic compounds act as signals for animals that eat the fruits and disperse their seeds. The yellow, orange, red-colored carotenoids; red, pink, purple, violet, and bluecolored anthocyanins are present in flowers and fruits to attract pollinators. Xanthophyll (carotenoid) turns extra light in plant cells into safe heat energy. In carnivorous plants, anthocyanins give coloration to trap leaves, and other phenolics display UV reflectance patterns for prey attraction function. Specific tastes and odors are produced by plant phenolic chemicals that act as anti-grazing for animals, insects and humans. Flavonoids (anthocyanins, flavones, flavonols and isoflavones) play an essential role in plant growth and defense against pathogens and insects. This class of compounds can scavenge ROS, which can harm plant cells. Young willow plants Salix dasyclados contained high concentrations of phenolics, which repel leaf beetle Galerucella lineola. The phenolic cotton pigment known as gossypol has unintentionally developed into an effective pest repellent such as Heliothis virescem (Tobacco budworm) and Heliothis zea (Corn earworm). Resistance in Pisum sativum (pea) to powdery mildew Erysiphe polygoni strongly correlates with the leaves' ortho-dihydroxyphenols. Malus domestica (apple) fruits contain epicatechin and 4'caffeoylquinic acid, which provide resistance against scab, a fungal infection caused by the Cladosporium cucumerinum. Phytoalexins in *Glycine max* (soybean) and *Medicago sativa* (alfalfa) are produced in response to the fungus *Phytophthora megasperma* invasion to serve a defensive response function against the same fungus ^[10, 12-14]. Polyphenols are predominant in the outer parts of fruits and vegetables, serve as UV protectors and prevent spoilage. Since phenols rise in response to hazardous chemical exposure and plant stress, they can also be employed as stress indicators. Lignans (phenylpropanoid derivatives) also absorb UV radiation and reduce water loss through cellulose

membranes. The leaves of the creosote bush *Larrea tridentata* contain high concentrations of lignans in their resin which can be complex with proteins and reduce the degradability of plant material by herbivores ^[9, 15, 16]. Onion scales with *Colletotrichum circinans* infection build up catechol and protocatechuic acid to protect themselves from onion smudge disease. *Solanum lycopersicum* (tomato) plants react to *Fusarium oxysporum* infection, which causes fusarium wilt, by accumulating phenolic compounds including ferulic, caffeic, and vanillic acid in recovered leaves and roots ^[11].

A group of chemical compounds produced from naphthalene is known as naphthoquinones. Naphthoquinone from zevlanica (Ceylon leadwort), Plumbago which has fungicidal bactericidal and properties, discourages herbivorous insects from feeding on non-carnivorous plants. Plumbagin is found on the rim of species of carnivorous plants such as Aldrovanda vesiculosa, Dionaea muscipula, Drosera species, Drosophyllum lusitanicum, Nepenthes species (N. gracilis, N. khasiana, N. rafflesiana) and Triphyophyllum peltatum inflicting an anaesthetic or toxic influence on prey, inhibiting their escape responses after capture with the sticky surface ^[4].

The coumarins scopoletin and umbelliferone protect *Platanus occidentalis* (American sycamore) leaves from the fungus *Ceratocystis fimbriata*. Abiotic stress, such as wind or heavy

rain, can cause stems to bend or break, while lignin deposition in plant cell walls helps to strengthen mechanical strength and rigidity^[11].

4.5 Terpenes

The smell of fruits is caused by mono and sesquiterpenes, i.e. components of essential oils that attract birds for pollination. Terpenes are necessary to plant defense. In response to mechanical damage, some plants emit volatiles that attract natural enemies - predators or parasites - toward the attacking herbivore to find their prey. In Nicotiana tabacum (Tobacco plant) terpenoids, trans-anethole, thymol and citronellal protect these plants against insects Spodoptera litura. Tobacco increases phenylalanine ammonia lyase (PAL) activity and phytoalexins (sesquiterpene), conferring resistance to fungal infections after fungal attack. In Citrus plants limonene (terpenoid) protects these plants against insects Atta cephalotes. Volatile terpenes (essential oil) such as limonene in lemon oil and menthol in peppermint oil have insect repellent properties. Pyrethroids (monoterpene esters), founds in the leaves and flowers of Chrysanthemum cinerariifolium having insect repellent and insecticidal activity. Gossypium herbaceum and Gossypium hirsutum (cotton plants) have four sesquiterpene phytoalexins named as deoxyhemigosypol, hemigosypol, deoxy-6-methoxy gossypol, and 6-methoxy gossypol that confer resistance to Verticillium dahliae (fungus) infection. Melampodium divaricatum (Butter Daisy) produces a volatile insect repellant called caryophyllene epoxide (a terpenoid derivative) to protect their leaves by *Atta cephalotes* ants. Terpene glycosides (saponins) also behave as a toxic compound to mammals. Due to the high concentration of saponins in their leaves, *Ilex aquifolium* (European holly) leaves defend themselves against insects. Three sesquiterpene lactones—8-deoxylactucin, lactucin, and lactupicrin-protect Cichorium intybus (chicory) from insect attack [17-19].

Terpenes attract prey in carnivorous plants as floral or fruit scent which is downregulated after prey capture such as 3carene, sabinene, tricyclene, α -phellandrene, γ -terpinene, (monoterpene) and trans-geranylacetone (sesquiterpene) in *Dionaea muscipula*; eudesmol (sesquiterpenoid), isochiapin B (sesquiterpene), pulegone (monoterpene) in *Sarracenia* spp.; (E)-ocimene, (Z)-ocimene, perillene, β -pinene (monoterpenoid) and (E)-nerolidol, (Z)-dendrolasine, germacrene A, (sesquiterpenoid) in *Nepenthes rafflesianas*^[4].

4.6 Nitrogen-containing compounds

Compounds containing nitrogen include alkaloids, glucosinolates, and cyanogenic glycosides. Since most alkaloids are poisonous to humans and animals, herbivores cannot consume them; instead, they serve as protective elements in plants. Nicotine is the most prevalent alkaloid in tobacco leaves and is always present at baseline levels in (tobacco) Nicotiana tabacum plants. The findings demonstrate that biological stress increases the synthesis and, thus, increases plant defense. The rise in nicotine alkaloid production in Nicotiana sylvestris (a wild tobacco plant) peaked ten days after feeding stimulation in Manduca sexta moth larvae. It might become more harmful and unpleasant for herbivores. Pyrrolizidine alkaloids are poisonous by nature and protect against microbiological diseases and herbivore attacks. The nectar of Catalpa speciosa (Northern Catalpa) is poisonous to nectar robbers like ants because it contains catalpa (iridoid glycosides). The bandits who ingested nectar had loss of mobility. Legitimate pollinators, such as bumblebees and moths, can still consume it ^[19, 20]. Gramineous alkaloids from *Hordeum vulgare* (barley) severely impair the aphid *Schizapis graminum* ability to feed and develop ^[14]. It has been discovered that the carnivorous plant *Sarracenia* spp., creates the alkaloid coniine in their pitchers and lids and may play a role in retaining prey as an insect-stunning agent ^[4]. In addition to being dangerous, cyanogenic glycosides have a harsh taste; hence, these plants are typically avoided. It has been discovered that *Sorghum bicolor* (sorghum) stores the cyanogenic glycoside dhurrin, rendering it immune to pests like *Diabrotica* spp. (rootworms).

Prunus amygdalus var. *amara* (Bitter almond) plants contain cyanogenic glycosides amygdalin and prunasin protect them against *Capnodis tenebronis* insect. *Manihot esculenta* (cassava) tubers have a high concentration of cyanogenic glycosides, which can paralyze insect limbs. *Phaseolus lunatus* contain cyanogenic glycosides that protect them against Spodoptera eridania insects ^[9, 14, 17, 20]. The most prevalent cyanogenic glycosides are lotaustralin and linamarin, which can be found in plants including Trifolium repens (clover), Linum usitatissimum (flax), and Lotus corniculatus (birdsfoot trefoil). The presence of amygdalin may be seen in the seeds of Prunus avium (sweet cherry) and Prunus armeniaca (apricot), and Prunus persica (peach), while dhurrin is found in Sorghum bicolor (millet). In plant defence systems, cyanogenic glycosides exhibit anti-herbivore characteristics. Cyanogenic glycosides in Trifolium repens protect young seedlings from snails and slugs ^[11]. Vegetables' flavor and aroma are brought about by glucosinolates such as oleracea var. capitata (cabbage), Brassica Brassica oleracea var. italica (broccoli), Raphanus sativus (radish) etc. The glucosinolate content of the developing cotyledons of the Brassica napus (oilseed rape) discouraged the slug species Deroceras reticulatum from eating on them [9, 14, 17, 20].

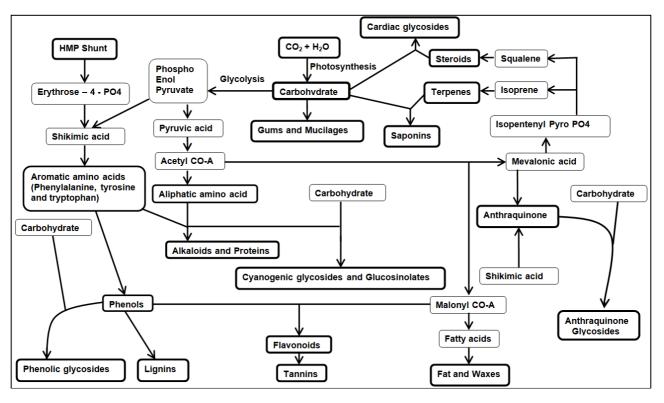


Fig 1: Principle biosynthetic pathway leading to secondary metabolite synthesis ^[21-23].

5. Conclusion

Plants developed defense systems against several biotic and abiotic stresses with time. This study assesses a select group of secondary metabolites from plants and their potential applications as defensive compounds. Secondary metabolites serve diverse and multifaceted purposes, making them vital for plant development, survival, and growth. Mainly when pathogens or herbivores are attacking them, plants release these substances. To protect themselves from being overgrazed by herbivores, plants have developed defensive measures that primarily rely on secondary metabolism. Additionally, these substances are created when plants are subjected to abiotic stressors such as salinity, UV radiation, drought, heavy metals, and extreme weather. The luring, catching, digestion, and assimilation of prey are all accomplished by carnivorous plants using secondary metabolites. Much more research is needed in every aspect of secondary metabolite biology (physiology, biochemistry, and ecology) to comprehend secondary metabolites' role in plants completely.

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