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Perspectives of Secondary Metabolites in Reference to Vegetable Crops: A review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Vegetables are known as protective food as they are a rich source of biologically active substances like vitamins, fibers, antioxidants, cholesterol-lowering compounds, etc. The discovery of many health-promoting compounds or nutrients was made possible through various experiments or some by chance in the past. During the time course, some novel technologies like chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance came into known which enabled quantitative, and qualitative measurements of a wide range of plant metabolites. Flavonoids, phenolics, and glucosinolates are the secondary metabolites that are required in plant growth & development, and stress or defence mechanism. Aside from providing strength to a plant's immune system, such metabolites influence the nutritional quality, colour, taste, and smell of the food, as well as medicinal properties. This review intends to gather the scattered information available on the SMs which included their classification, structural differentiation, their potential roles in defence mechanisms, ecological adaptation, and available extraction techniques in reference to vegetable crops.

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1. INTRODUCTION

Plants produce a variety of organic molecules through various metabolic pathways during the synthesis of primary metabolites (carbohydrates, fats, lipids, proteins, & nucleic acids) recognized as 'secondary metabolites' (SMs) [1]. SMs are low molecular weight phytoactive compounds, and their synthesis is specific to tissue/organ/cell. These metabolites composed of distinct carbon skeleton structures as shown in Fig. 1. SMs are not directly involved to perform any cellular functions but their presence does play a significant role in the existence of an organism in the ecosystem [2]. Their level of presence, & types may vary species to species, and even change within a genus. The most affecting factors that can deviate the level of SMs in the individuals are genetic, ecological, and geographical factors. SMs offer plenty of protective functions against various plant stressors including abiotic stress (metal toxicity, high & low temperature, drought & flooding, salt & ionic stress, etc.), and biotic stress (insect attack, bacterial, fungal, & nematode mediated attacks, etc.). SMs have been utilized in the preparation of flavours, dyes, drugs fragrances, and can be used as insecticides too. Many positive effects on human health have been linked to these phytochemicals, including coronary heart disease, diabetes, high blood pressure, degenerative diseases, and obesity [3]. Various vegetables like broccoli, cauliflower, brussels sprouts, turnips, kale, asparagus, spinach, lettuces, and endives contain a large number of phytochemicals with antioxidant, antimutagenic, cytotoxic, antifungal, and antiviral properties [4,5]. Secondary plant metabolite profiles and concentrations have a significant impact on the intrinsic quality of brassicaceous vegetables, including colour, aroma, taste, and beneficial health properties [6]. Significant amounts of data on the identification, biochemical characterization, localization, and health benefits of plant secondary metabolites have been accumulated over the last decade. Furthermore, some studies have highlighted the health benefits of phenolic compounds, and carotenoids, both of which are well-known antioxidants. The profiles, and concentrations of secondary metabolites vary greatly between species, and genetic factors are thought to have the greatest influence on this.

This review attempts to summarize the scattered information based on literature available on secondary metabolites in reference to vegetable crops, which covers the classification of secondary metabolites, their nutritional value, available concentration in different vegetables, potential applications, therapeutic uses, and extraction techniques.

2. CLASSIFICATION OF SECONDARY METABOLITES (SMs)

The concept of secondary metabolites was first of all explained by Albrecht Kossel (1910). Later on, Czapek stated that SMs are the derivatives of nitrogen metabolism (deamination). The phytochemistry field was founded in the 20th century as a result of improvements in chromatography techniques, which led to an increase in the recovery of these chemicals. "The British nutrition foundation's nomenclature divides SMs into four major groups, namely-phenolic & polyphenolic compounds (about 8000 compounds), terpenoids (about 25000 compounds), alkaloids (about 12000 compounds), and sulfur-containing compounds" [5]. Different classes of SMs with their therapeutic uses have been represented in Fig. 2.

2.1 Phenolic & Polyphenolic Compounds

Flavonoids, phenolic acids, and lignans are phenolic & polyphenolic compounds with aromatic or phenolic ring structures. Their high concentration provide resistance against fungal plant pathogens, and protection against feeding by insects, and other animals [7]. Some phenolics influence the colour pattern, fragrance, and attract the pollinators.

2.1.1 Flavonoids

This phenolic group, includes flavonols (quercetin, kaempferol, & isorhamnetin) found in onions, leeks, endives, and broccoli; flavones (apigenin, luteolin, & chrysoeriol) found in parsley, thyme, and celery; anthocyanidins (cyanidin, delphinidin, & malvidin) found in tomatoes; and isoflavones present in soy [8]. Many flavonoids, including anthocyanidins, chalcones, and flavones, are plant pigments that determine vegetable colour. Flavonoids in the diet have antiviral, anti-inflammatory, antihistamine, and antioxidant properties [9].

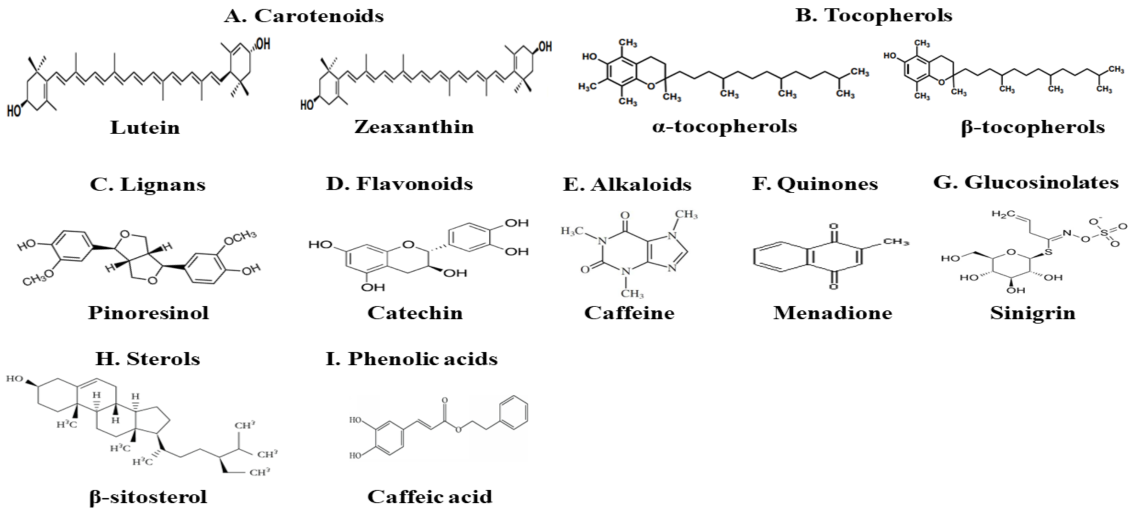

Fig. 1. Secondary metabolites featuring their unique chemical structures

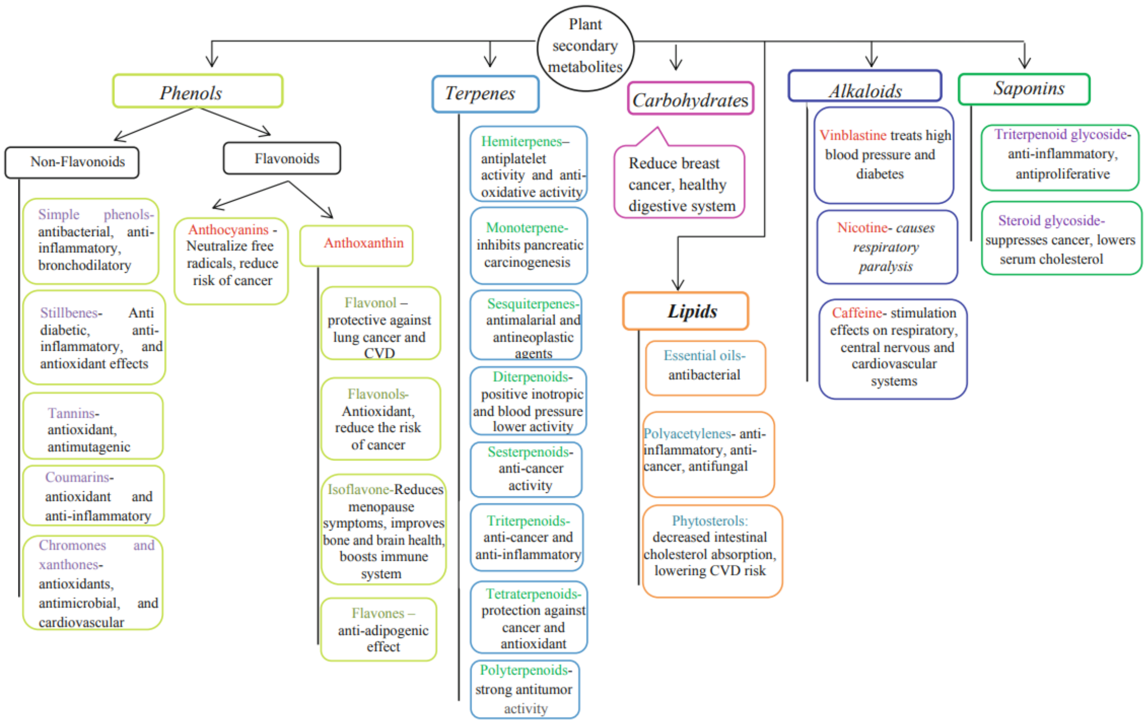

Fig. 2. Classification and therapeutic uses of SMs
[Source: 2]

2.1.2 Phenolic acids

These compounds contain at least one aromatic ring that possess one or more hydroxyl groups. Gallic, and caffeic acid are found in lettuce & pak choi, while vanillic, and cinnamic acids are found in onions, parsley, & spinach, and coumaric acid is found in tomatoes, carrots, and garlic [10]. Caffeic, chlorogenic, sinapic, ferulic, and p-coumaric acid have high antioxidant activity due to inhibition of lipid oxidation, and scavenging reactive oxygen species [11].

2.1.3 Lignans

These are diphenolic compounds that help to produce lignin, a hydrophobic component of plant cell walls. Lignans are abundant in broccoli, kale, brussels sprouts, beans, and garlic. Lignans have several biological activities in humans, such as antioxidant, antiestrogenic properties, and may thus reduce the risk of certain cancers, and cardiovascular diseases [12]. The Table 1 states about the flavonoids, lignans, and carotenoids reported in vegetable crops.

2.2 Terpenoids

Terpenoids are a large chemical compound family formed by the repeated fusion of branched 5-carbon isoprene units. Terpenoids play a variety of roles in plants, including membrane structural components (sterols), photosynthetic pigments (phytol, carotenoids), electron carriers (ubiquinone, plastoquinone), and hormones (gibberellins, abscisic acid) [15]. Carotenoids, tocopherols, tocotrienols, quinones, and sterols are examples of major dietary terpenoids.

2.2.1 Carotenoids

Plant carotenoids (α-carotenes, β-carotenes, xanthophylls, lycopenes) are lipid-soluble pigments found in carrots, tomatoes, pumpkin, and sweet potatoes [10]. Carotenoids in plants protect photosynthetic tissues from photooxidative damage and are precursors of the phytohormone, abscisic acid (ABA), which regulates developmental, and stress processes [16]. Carotenoids with provitamin A activity are required in the human diet. Vitamin A plays a role in hormone synthesis, cell growth, and differentiation regulation, and immune responses. A lack of carotenoids in the human diet can cause xerophthalmia (night blindness), and death [17]. Vegetables like kale, broccoli, brussels sprouts, cabbage, cauliflower, lettuce, asparagus, spinach, sweet potatoes, tomatoes, and turnip contain tocopherols & tocotrienols [18].

Table 1. Flavonoids, lignans, and carotenoids reported in vegetable crops

Compound	Vegetable	Concentration (mg/100g FW)	Reference
Flavonoids			
Cyanidin	Red lettuce	13.7	[13]
Quercetin	Broccoli	3.12	[45]
	Endive	7.71	
	Onion	13.27	
Apigenin	Celery	4.61	[45]
Lignans			
Lariciresinol	Broccoli	972	[14]
	Cauliflower	124	
	Kale	599	
	Sweet pepper	164	
Pinoresinol	Broccoli	315	
	Cabbage	568	
	Kale	1691	
Carotenoids			
α-Carotene	Carrot	4.6	[45]
	Peas	19	
	Sweet pepper	59	
	Tomato	112	
β-Carotene	Broccoli	779	
	Brussels sprout	450	
	Peas	485	
	Tomato	393	
β-Cryptoxanthin	Sweet pepper	2.205	
Zeaxanthin	Carrot	23	
	Kale	173	
	Lettuce	187	
	Spinach	331	
Lycopene	Tomato	3.025	

FW = fresh weight, USDA = U.S. Department of Agriculture National Nutrient Database

2.2.2 Quinones & sterols

Quinones have aromatic rings that have two ketone substitutions. Vitamin K_1 (phylloquinone) can be found in lettuce, spinach, asparagus, cabbage, kale, lettuce, cauliflower, and broccoli [19]. Phylloquinone is involved in electron transport during photosynthesis as well as the generation of active oxygen species observed in response to pathogen attack or stress in plants [20]. On the other side, broccoli, brussels sprouts, cauliflower, and spinach are the rich source of plant sterols (viz., sitosterol, campesterol, brassica sterol, and stigmasterol). Sterols regulate the fluidity, and permeability of phospholipid bilayers in plant membranes. Certain sterols are precursors of plant hormones known as brassinosteroids, which play important roles in embryonic development, cell division, plant growth, and fertility. When exposed to UV light, these sterols produce calciferol (vitamin D_2), which aids in calcium absorption, and bone growth [21].

2.3 Alkaloids

Alkaloids are a class of basic nitrogen-containing compounds derived primarily from amino acids. Because of their distinct physiological, and medicinal properties (for example, caffeine, nicotine, morphine, atropine, and quinine), alkaloids have long been of great interest. Alkaloids are typically classified according to their common molecular precursors, which include pyridine (coniine, nicotine), tropane (atropine, cocaine), isoquinone (morphine, codeine), purine (caffeine), and steroids (solanine) [22]. Saponins for e.g., solanine, tomatine, and chaconine are plant glucoalkaloids that have surfactant properties [23]. These can be found in a variety of crops, including peas, beans, tomatoes, spinach, asparagus, onions, garlic, and potatoes [24]. Saponins have some insecticidal, and molluscicide activity which protect plants from microorganisms, and have allelopathic effects on many weeds as well [25]. Some saponins, such as sapotoxin, are toxic to humans. They can create irritation to the respiratory, & digestive tract membranes, and caused urticaria [26]. Different alkaloids, sterols, tocopherols, quinones, and glucosinolates reported in vegetable crops are presented in the Table 2.

2.4 Sulfur-Containing Compounds

Glucosinolates are a large functional group of sulfur-containing amino acid derivatives with a glucose group. Vegetables like white & red cabbage, brussels sprouts, and cauliflower contain the glucosinolates, progoitrin & sinigrin. Broccoli, red radish, and daikon contain glucoiberin & glucoraphanin. Whereas, mustard, and horseradish contain sinigrin & gluconasturtiin [32]. Substances derived from glucosinolates act as natural pesticides, and herbivore repellents. Some glucosinolates are important flavour compound precursors. Isothiocyanates like allyl isothiocyanate, and benzyl isothiocyanate, also known as "mustard oils," have a pungent or lachrymatory taste, and an acrid odor [33]. Other glucosinolates are undesirable because their breakdown products have unpleasant sensory or physiological properties. Sinigrin, and its degradation product is bitter in flavour. Overconsumption of glucosinolates-rich foods can disrupt thyroid hormone synthesis, and cause inflammation of the stomach mucous membranes.

3. PLANTS SECONDARY METABOLITES EXTRACTION TECHNIQUES

Recent advances in the extraction of bioactive compounds from plant material have been thoroughly reviewed, most likely as a result of increased public awareness of preventive health care, which could be promoted through the consumption of plant material extracts.

3.1 Conventional Extraction Techniques

Various traditional extraction techniques can be used to extract bioactive compounds from plant materials. The majority of these techniques rely on the extraction power of the various solvents in use, as well as the application of heat, and/or mixing. Traditional techniques for extracting bioactive compounds from plants include, soxhlet extraction, maceration, and hydro distillation. These methods, which are generally carried out under atmospheric pressure, use organic solvents, (such as hexane, acetone, methanol, ethanol, and so on) or water. The use of organic solvents and the length of the extraction process are the main disadvantages of traditional methods [34].

3.2 Modern/Non-Conventional Extraction Techniques

Alternative approaches to conventional extraction methods have emerged in an attempt to overcome their limitations. Accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), subcritical water extraction (SWE), pulsed-electric field extraction (PEF), enzyme-assisted extraction (EAE), pressurized liquid extraction (PLE), and rapid solid-liquid dynamic extraction (RSLDE) are the latest extraction methods [35].

In this regard, ASE, SFE, MAE, UAE, and SWE are discussed hereby as they are the most commonly used methods for extracting bioactive molecules from plants.

3.2.1 Accelerated Solvent Extraction (ASE)

ASE, also known as pressurized liquid extraction or pressurized fluid extraction, is a new extraction technique that uses organic solvents like Soxhlet extraction, but the use of elevated temperature, and pressure with ASE allows the extraction process to be completed in a short time, and with a small amount of solvent. Particular attention should be paid to ASE performed at high temperatures, which may result in thermolabile compound degradation [36].

3.2.2 Supercritical Fluid Extraction (SFE)

A critical point in pure matter corresponds to a given pressure & temperature. When subjected to pressures, and temperatures greater than those of its critical point, it enters a phase known as "supercritical" (SC) or supercritical fluid (SF). The SF exhibits intermediate behaviour between the liquid & gaseous states. It has a high density, similar to that of liquids, a coefficient of diffusivity similar to that of liquids, and a low viscosity (similar to that of gases). CO_2 is an example of SF when it exceeds 31.1°C and 7380kPa.

Aside from its favorable physical properties, CO_2 is also cheap, safe, and abundant. However, because CO_2 is polar, and has a low dissolution power, it cannot be used as a solvent, particularly for polar solutes. To overcome this limitation, it is occasionally combined with co-solvents such as ethanol or methanol. However, one significant disadvantage of this method is the high initial cost of the equipment [37].

3.2.3 Microwave Assisted Extraction (MAE)

MAE uses microwave energy with frequencies ranging from 300MHz to 1000GHz to facilitate the partition of analytes from a sample matrix into the solvent [38]. Microwaves have the ability to penetrate biomaterials, and interfere with polar molecules such as water present in biomaterials, resulting in the generation of heat. As a result, microwaves can raise the temperature of an entire material by penetrating deeply into its matrix. MAE is a selective method that prefers polar molecules, and solvents with high dielectric constants. Poor heating occurs in non-polar solvents because energy is only transferred via dielectric absorption [39]. MAE extracts the anthocyanin, phenols, and flavonoids successfully. Other bioactive compounds being studied for MAE include glucosinolates (cabbage), pectin (pomelo peel), and transresveratrol (peony seed) [40].

3.2.4 Ultrasound Assisted Extraction (UAE)

The power of ultrasound can significantly improve the solvent extraction of organic compounds found in plant, and seed bodies. Indeed, the mechanical effects of ultrasound allow for greater solvent penetration into cellular matrices, and improve mass transfer *via* the micro-streaming process. Another advantage of using ultrasound in plant extraction is its ability to disrupt biological cell walls, allowing the cell contents to be released [41]. Overall, UAE with ultrasound frequencies ranging from 20kHz to 2000kHz is an efficient extraction technique that drastically reduces process times, increases yields, and often improves extract quality. In comparison to traditional methods, UAE showed a 143% increase in extraction yield with no loss of carotenoids [42].

3.2.5 Subcritical Water Extraction (SWE)

Extraction of bioactive molecules from plants using subcritical water is an extraction method in which water is used at temperatures ranging from 100°C (boiling point of water) to 374.1°C (critical point of water), and at a pressure that varies with temperature and keeps the water in liquid form [43]. At room temperature, water is a polar solvent with a dielectric constant (') of 75.5. However, in an atmosphere pressurized at high temperatures of the order of 250-300°C, the polarity of the water, and the dielectric constant both decrease.

Plant extracts have been used as medicine, flavouring agents, preservatives, and even poisons for centuries. With recent advancements in extraction, and characterization techniques, it is now possible to separate, and characterize a

Table 2. Alkaloids, sterols, tocopherols, quinones, and glucosinolates reported in vegetable crops

Compound	Vegetable	Concentration	Reference
Alkaloids			
α-Tomatine	Tomato	521 to 795μg/g FW	[27]
α-Solanine	Potato	0.01 to 0.43mg/kg FW	[28]
Sterols			
β-Sitosterol	Broccoli	31mg/100g FW	[21]
	Brussels sprouts	34mg/100g FW	
	Cauliflower	26mg/100g FW	
	Celery	7.3mg/100g FW	
	Kale	7.4mg/100g FW	
	Tomato	2.4mg/100g FW	
Tocopherols			
α-Tocopherol	Broccoli	1.44mg/100g FW	[29]
	Carrot	0.86mg/100g FW	
	Spinach	1.96mg/100g FW	
	Tomato	0.53mg/100g FW	
β-Tocopherol	Carrot, Lettuce, Cucumber	0.01mg/100g FW	
γ -Tocopherol	Broccoli	0.31mg/100g FW	
	Cauliflower & Spinach	0.20mg/100g FW	
	Lettuce	0.11 to 0.74mg/100g FW	
Quinones			
Phylloquinone	Broccoli	102μg/100g FW	[30]
	Celery	29μg/100g FW	
	Cucumber	16.4μg/100g FW	
	Lettuce	24.1 to 127μg/100g FW	
	Sweet pepper	4.9 to 21.4μg/100g FW	
Glucosinolates			
Sinigrin	Brussels sprouts	8.56μmol/100g FW	[31]
	Cauliflower	5.28μmol/100g FW	
	Green cabbage	5.09μmol/100g FW	
Glucoalyssin	Broccoli	3.86μmol/100 g FW	
Glucoraphanin	Broccoli	29.4μmol/100g FW	

FW = fresh weight, USDA = U.S. Department of Agriculture National Nutrient Database

number of bioactive compounds from crude plant extract. Several studies have yielded promising results in terms of the pharmacological significance of the plant secondary metabolites. Furthermore, these secondary metabolite genes are now genetically engineered and can be found in high concentrations in other organisms. More research is needed to investigate and validate the pharmacological potential of such bioactive compounds, as these compounds are a major source of drug candidates for the pharmaceutical industry [44-50].

4. CONCLUSION

Secondary metabolites have a well-established role in the management of general health that has contributed towards the acceptance of them as an alternative to medicines with a certain problem. Future research should be based on identifying novel SMs in vegetable crops to boost biofortification and cure various diseases. There is always a need to give emphasis on their effective distribution, stabilization, extraction, bioavailability, and other linked issues. This review would be helpful to the researchers to understand about the basic concept of secondary metabolites, their classification, structural differences, potential applications, role in plant defence system, extraction methods for the characterization of different secondary metabolites and their role in ecological adaptation of vegetable crops.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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