

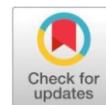
Review Article

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Plants as source of Vitamin-D: A brief review

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**ABSTRACT**

Vitamin D deficiency has become a global problem contributed by the limited sun exposure for a large portion of the world population. Hence dietary intake of vitamin D becomes very essential for every age group. However, dietary recommendations for vitamin D are difficult to meet because of the limited dietary sources of natural vitamin D. Further advanced research has proven that vitamin-D3 is more necessary in human physiological functions and vitamin-D2 has to convert to D3 for it to be utilized by the body. This revelation has made it more difficult for the vegetarian, vegan, and population with compromised health conditions, who are denied to consume meat to a level that can fulfill their daily need for vitamin-D. Hence exploring other alternative sources for catering to the need of vitamin-D in the ever-growing population and food trends (veganism) has gained popularity in recent days. Though microbial production or extract preparations (from animal sources) are performing promisingly in this era, as the world is heading towards a sustainable ecosystem, these methods will not fit with the principles of a sustainable future. Traditionally, animal products have been considered as the only source of vitamin D3, but today we know that vitamin D3 and its metabolites are present in certain plants also. However our knowledge about these plants is still limited in terms of their numbers, effectiveness, and production mechanisms. Hence this review is an attempt to imply that plants can be an alternative source of vitamin-D production, through the results of the studies conducted on the quantification of vitamin-D (D2 & D3) and its metabolites in plants.

Keywords: Vitamin-D2, Vitamin-D3, Metabolites, Plants, Quantification, Solanaceae

Introduction

Vitamin D is a fat-soluble vitamin that is responsible for calcium and phosphorus homeostasis in the human body. It elevates the reabsorption of phosphate from the kidney and stimulates the synthesis of the calcium transport proteins in the small intestine, thereby enhancing the absorption of dietary calcium from gut. Other than macro-mineral homeostasis proper muscle functioning, protection against respiratory disease (especially in children), cardiovascular disease, neurodegenerative diseases, some types of cancer, and both type 1 and type 2 diabetes, etc. are some of the benefits of an adequate vitamin D levels in the body [14], [10], [20], [11]. Though dermal conversion of vitamin -D accounts for 90% of vitamin D replenishment in humans [4], the major influencing factor i.e. the necessary UVB rays (290–315 nm) are only emitted all year round in places that lie below a 35° latitude [19]. Hence 'vitamin D deficiency' has emerged as a major public health problem worldwide in all age groups, even in those residing in countries with low latitudes, where it is generally assumed that UV radiation is adequate enough to prevent this deficiency. It is the most under-diagnosed and undertreated nutritional deficiency in the world [28]. Hence these days, a dietary intake of vitamin D becomes essential to maintain an adequate level of vitamin-D in body which presents a challenge because there are very few foods that naturally contain vitamin D.

Vitamin D3 can be found in oily fish, including sardines, salmon and mackerel, as well as cod liver oil [15]. Vitamin-D is also naturally found in eggs, pork, and some mushrooms but only at a small fraction of the recommended daily dose. As a result, the consumption of supplements of vitamin-D has increased exponentially in the last few decades, which has lead to an exploitation of the existing sources. Therefore, certain investigations that documented the detection of vitamin D or any vitamin D metabolite in plants are covered in this review. These discoveries ushered in a new phase of plant research aimed at identifying components similar to vitamin D for potential multi-factorial applications.

Methodology

A thorough search of the literature was done to find any information on the identification or measurement of vitamin-D or its metabolites in plants and their components. To find relevant scientific studies, PubMed, Mendeley, and Google Scholar searches were conducted. The published literature was unrestricted by dates. For this effort, only reports or articles written in English with English abstracts were taken into consideration. Vitamin D, Vitamin D2, Vitamin D3, Metabolites, Identification, Quantification, Isolation, Intervention, Plant Precursors, and Plant Extracts were among the key phrases chosen in different combinations for literature searches. After downloading the full-text articles of the chosen studies, they were first critically evaluated by their titles, and then their abstracts were analyzed. We also looked through the collected literature's bibliography to find more pertinent works.

Discovery of Vitamin-D

Vitamin D has a long and fascinating history that goes back more than 350 years.

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It started with the discovery of rickets and osteomalacia by two research groups from the Netherlands [42] and England [13] during the early 1600s. By the late 1700s, Percival advocated the effectiveness of cod liver oil compared to gum guaiacum in the treatment of rickets suggesting a nutritional deficiency to be the causative factor [31] of the disease which was later on identified as the vitamin-D [27]. But on the other hand researchers like Sniadecki recorded a difference in incidence rates of rickets in rural and city children of Poland, hinting towards an environmental factor to be the major cause of ricket [39], which again led to the discovery of role of sunlight in rickets and osteomalacia. The dilemma about how both light and a dietary substance cured rickets was eventually resolved by the work of Chick [8] and Steenbock [40] who independently investigated the dual role of nutrition and sunlight exposure in the prevention of rickets. Steenbock was the first person who reported that an inactive non-saponifiable lipid fraction in the diet and skin could be converted by UV light into an active anti-rachitic substance [37]. At this point the exact chemical nature of vitamin D was still unknown to the world. It was in 1932 when for the first time Askew isolated vitamin-D2 from an irradiation mixture of ergosterol and hence it became the first isolated form of vitamin-D. Again the structure of 7-dehydrocholesterol, and vitamin D3 was first time elucidated by Adolf Windaus, who got 1928 Nobel Prize for this discovery [43], [44]. Thereafter further research suggested that vitamin-D3 is only present in animal food and plants contain a precursor of ergocalciferol (Vit-D2/ ergosterol), that converts into vitamin-D3 to be utilized by the human body. However around 2000-2003 cases of calcium intoxication, comparable to vitamin D toxicity reported in grazing animals in some parts of the world due to the consumption of some specific native plants [28]. Thus, scientists came up with a novel theory that: either vitamin D3 or a metabolite of vitamin D3 surely existed in plants and stimulated calcium absorption, leading to hypercalcemia and calcium deposition in soft tissues such as the uterus, kidneys, intestines, and aorta [28]. This theory was later substantiated by many study results where free vitamin-D3 and its metabolites were found in plants belonging to families like- *Solanaceae*, *Fabaceae*, and *Cucurbitaceae*.

Presence of vitamin-D3 and its metabolites in plants

The quest for identifying vitamin D3 or its metabolites in plants was started, when Wasserman, a professor of veterinary medicine and a research scientist with his team tested *Solanum malacoxylon* for it's principle component causing hypervitaminosis-D like activity (calcification) in grazing animals. He published his research findings in 1976 in Science (New York, N.Y.) journal, where he mentioned the principle component of calcification in animals to be “1, 25-dihydroxyvitamin D3,” the active form of vitamin D3. Wasserman used a combined gas chromatography and mass spectrometry method for identifying 1, 25-dihydroxyvitamin D3 [41]. Apart from 1, 25-dihydroxyvitamin D3, *S. malacoxylon* leaves were also reported to contain two other glycoside derivatives of vitamin-D3 (cholecalciferol, and 25-hydroxy-vitamin D3), which served as the first direct evidence of hydroxylation of vitamin D3 in plants [10]. Again in 1996 another group of researchers isolated and quantified cholecalciferol, 25-hydroxycholecalciferol, and 7-dehydrocholesterol from callus cultures extracted from sterile leaves of *S. malacoxylon*. Results of the same study showed that Ca levels of media

Table- I. Summary of literature on the quantification of vitamin-D3 or its metabolites in plants:

Study ID	Specimen Name	Specimen Type	Identification Technique used	Solvents used for extraction	Observed Metabolites	Quantity
Wasserman et al., 1976 [41]	<i>Solanum malacoxylon</i> (<i>Solanaceae</i>)	Leaf (Dried)	GC-MS	Deionized water	1,25(OH)2D3	NQ
Napoli et al., 1977 [30]	<i>Solanum glaucophyllum</i>	Leaf (Fresh)	HPLC	Deionized water	1,25(OH)2D3	NQ
Rambeck et al., 1979 [34]	<i>Trisetum flavescens</i> Beauv. (<i>Solanaceae</i>)	Leaf (Lyophilized)	HPLC & GC-MS	Diethyl ether	D ₃	0.1 µg/g dry wt
Esparza et al.,1982 [10]	<i>Solanum malacoxylon</i> (<i>Solanaceae</i>)	Leaf (Sun-dried)	UV-spectroscopy	Methanol/chloroform (2:1. v/v)	D ₃	NQ
Horst et al.,1984 [16]	<i>Medicago sativa</i> L (Fabaceae)	Whole plant	HPLC	Ethanol	25OHD3	NQ
Prema and Raghuramulu, 1994 [32]	<i>Cestrum diurnum</i> L (<i>Solanaceae</i>)	Leaf (Dried)	Column chromatography &HPLC	Chloroform	1α,25(OH)2D3	0.00063 - 0.001 µg/g dry wt.
Aburjai et al., 1996 [2]	<i>Solanum glaucophyllum</i> Desf. (<i>Solanaceae</i>)	Callus tissue	TLC & HPLC	Chloroform-methanol- NH4OH	D ₃	0.102 µg/g dry wt
Prema and Raghuramulu, 1996 [33]	<i>L. esculentum</i> (<i>Solanaceae</i>)	Leaf (Lyophilized)	HPLC	Chloroform	25OHD3	0.102 µg/g dry wt.
					1α,25(OH)2D3	0.10 µg/g dry wt.
					7-dehydrocholesterol	2.2-42.1µg/g fresh wt
					25OHD3	5-58 µg/g fresh wt
					1α,25(OH)2D3	1.0 µg/g fresh wt.
					D ₃	0.1 µg/g fresh wt
					25OHD3	0.8 µg/g dry wt.
					1α,25(OH)2D3	0.022 µg/g dry wt.
						0.10 µg/g dry wt.

Aburjai et al., 1998 [3]	<i>Solanum glaucophyllum</i> Desf. (Solanaceae)	Leaf (Fresh)	Reverse-phase HPLC	Chloroform	D ₃	0.21 µg/g dry wt.
	<i>Solanum lycopersicum</i> L. (Solanaceae)	Leaf (Fresh)	Reverse-phase HPLC	Chloroform	D ₃	1.1 µg/g fresh wt
	<i>Solanum tuberosum</i> (Solanaceae)	Leaf (Fresh)	Reverse-phase HPLC	Chloroform	25-OHD3	0.15µg/g fresh wt.
	<i>Cucurbita pepo</i>	Leaf (Fresh)	Reverse-phase HPLC	Chloroform	D3	0.15 µg/g fresh wt
Curino et al., 1998 [9]	<i>Solanum glaucophyllum</i> Desf. (Solanaceae)	Callus culture	HPLC	Chloroform/methanol (1:2, v/v)	D ₃	NQ
Skliar et al., 2000 [38]	<i>Nicotiana glauca</i> Graham (Solanaceae)	Leaf and callus cultures	HPLC	Chloroform/methanol (1:2, v/v)	7-dehydrocholesterol 25OHD3	NQ NQ NQ
Björn and Wang, 2001 [7]	<i>Solanum lycopersicum</i> L. (Solanaceae)	Leaf	-	-	1α,25(OH)2D3	0.3-1 µg/g fresh wt.
					D ₃	0.28 µg/g dry wt.
Jäpelt et al., 2013 [20]					7-dehydrocholesterol	0.61 - 0.76 µg/g dry wt.
	<i>Solanum glaucophyllum</i> Desf. (Solanaceae)	Leaf (Lyophilized)	HPLC & LC-ESI-MS/MS	Ethanol	D ₃	3.2-200 (ng/g dry weight)
					25OHD3	0.8-31 (ng/g dry weight)
					1,25(OH)2D3	<0.1-32 (ng/g dry weight)
					Glycosylated 1,25(OH)2D3	<0.1-17 (ng/g dry weight)
	<i>Solanum lycopersicum</i> L (Solanaceae)	Leaf (Lyophilized)	HPLC & LC-ESI-MS/MS	Ethanol	D ₃	1.7-110 (ng/g dry weight)
					25OHD3	<0.02-4.3 (ng/g dry weight)
					1,25(OH)2D3	<0.1 (ng/g dry weight)
					Glycosylated 1,25(OH)2D3	<0.1 (ng/g dry weight)
	<i>Capsicum annuum</i> L.)	Leaf (Lyophilized)	HPLC & LC-ESI-MS/MS	Ethanol	D ₃	<0.2-6.3 (ng/g dry weight)
					25OHD3	<0.02-0.5 (ng/g dry weight)
					1,25(OH)2D3	<0.1 (ng/g dry weight)
					Glycosylated 1,25(OH)2D3	<0.1 (ng/g dry weight)

NQ- Not quantified / mentioned.
D3- Cholecalciferol

25OHD3- 25Hydroxy Cholecalciferol
1,25(OH)2D3- 1,25 DihydroxyCholecalciferol

positively affects the production of cholecalciferol and 25-hydroxycholecalciferol in the callus culture, emphasizing the role of calcium availability and light in the production of vitamin-D₃ or its metabolites in plants [1], [2]. After Wasserman, Napoli was the 2nd researcher to report the presence of vitamin-D₃ metabolite in another crucial member of *Solanaceae* family (i.e. *S. glaucophyllum*) through an *in-vivo* study. He emphasized that aqueous extract of *S. glaucophyllum* resulted accumulation of 1, 25-dihydroxyvitamin D₃ in blood and enhancement of intestinal calcium absorption in rats, suggesting the presence of vitamin-D₃ in it [30]. It was later proven by Japelt, in 2013 that *S. glaucophyllum* had the highest content, of cholecalciferol and 25-hydroxy cholecalciferol. To date this plant is the only plant whose leaves have shown 1, 25-dihydroxy cholecalciferol in both free (32 ng/g dry wt.) and glycosylated form (17 ng/g dry wt.) [20]. Moreover the presence of vitamin-D₃ in *S. glaucophyllum* leaves are light independent [9] rejecting the conventional believes related to the synthesis of vitamin-D₃ in plants. Other member plants like *Cestrum diurnum* L., *Lycopersicon esculentum*, *Solanum tuberosum*, *Nicotiana glauca* and *S. melongena* of solanaceae family were also investigated for possible presence of vitamin-D₃ or its metabolites. *Cestrum diurnum* plant demonstrated the presence of free cholecalciferol, 25-hydroxy cholecalciferol and 1, 25 dihydroxy cholecalciferol at a concentration of 0.102 g/g dry wt., 0.102 g/g dry wt., and 1 g/g dry wt. respectively [32]. Similarly *L. esculentum* leaves showed presence of cholecalciferol, 25-hydroxy cholecalciferol and 1, 25-dihydroxy cholecalciferol, 7-dehydrocholesterol and traces of glycosylated 1, 25-dihydroxy cholecalciferol [3], [7], [20], [33]. Presence of only cholecalciferol was reported in plants likes *Solanum tuberosum*, and *Cucurbita pepo* [3], whereas both cholecalciferol and 25-hydroxy cholecalciferol was detected in *Capsicum annum* L., and *Nicotiana glauca* plant leaves [20], [38]. Plants from families other than *Solanaceae* had also shown considerable amount of vitamin-D₃ or its metabolites suggesting its presence all over plant kingdom. According to literature vitamin-D₃/cholecalciferol was first quantified in the dried leaves of yellow oat grass (*Trisetum flavescens* Beauv.) or golden grass of *Poaceae* family and reported to be 0.1 µg/g of dried leaf sample [34]. *Medicago sativa* commonly known as alfalfa plant a member of *Fabaceae* family showed 0.63ng/g leaf -1.0 ng/g leaf of cholecalciferol in it [16] (Table-I).

Presence of vitamin-D₂ in plants:

Identification of vitamin-D₂ in plants can be traced back to 1924 - 1925 [14]. It was reported to be first isolated from UV irradiated ergosterol solution and was later proven as the plant form of vitamin-D [20]. Provitamin ergosterol gets converted to active vitamin-D₂ when exposed to UV rays due to temperature dependent thermal isomerisation process [22], [21]. Hence ergosterol rich non animal food sources were evaluated by several researchers for their possible use as dietary source of vitamin-D₂. According to the literature before the year 2000 two pivotal studies quantified the presence of vitamin-D₂ in plants and grain. The first study of vitamin -D₂ quantification was conducted by Horst, on *Medicago sativa* (alfalfa) plant that belongs to *Fabaceae* family. They isolated and quantified vitamin-D₂ from two type of alfalfa plants- field grown and laboratory grown. The results of the study showed that sun-

cured, field-grown alfalfa hay contained 48 ng/g plant tissues (1920 IU/kg) of vitamin D₂ and artificially irradiated laboratory grown alfalfa hay contained 80 ng/g plant tissue of vitamin-D₂ in it [16]. Again in 1989 Schwadorf and Muller, studied for the first time the presence of ergosterol in cereals, mixed food components, and mixed feeds (e.g., for swine and poultry) through a sensitive, rapid, reproducible, and reliable liquid chromatographic (LC) method. According to their findings only wheat and broad-bean were detected to contain vitamin-D₂ at a concentration of 7780 µg/kg dry matter, 1030 µg/kg dry matter respectively [35]. However after 2000s only six studies quantified the presence of vitamin-D₂ or its precursor in plants. In 2001, *Solanum lycopersicum* L. leaf was investigated by Björn and Wang, and the presence of D₂ was recorded to be, 8.7 µg/100 g dry wt., and ergosterol at a concentration of 183 µg/100g dry wt. and 223 µg/100g dry wt., respectively when grown under conditions like without UV-B light and with UV-B light [7]. In 2007, Magalhães studied different varieties of hop (*Humulus lupulus* L.) and found both vitamin D₂ and ergosterol in only one variety (Nugget variety). The recovery of ergosterol and ergocalciferol from nugget variety of hop was recorded as 1.84 ± 0.09 µg/g and 1.95 ± 0.05 µg/g respectively [26]. Similarly Jäpelt studied the presence of ergosterol and vitamin D₂ in six varieties of perennial ryegrass (*Lolium perenne* L.) harvested four times during varied seasons. The content of vitamin D₂ and ergosterol was analyzed by LC atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI-MS/MS). They reported that an average content of vitamin D₂ in the plant extract was 2 µg/kg fresh weight. According to the results vitamin-D₂ content in the 6 varieties namely Foxtrot, Tivoli, Turandot, Telstar, Indiana, Kimber were 0.07 - 5.69 µg/kg, 0.07 - 6.18 µg/kg, 0.19 - 0.46 µg/kg, 0.14 - 3.73 µg/kg, 0.11 - 2.91 µg/kg, and 0.4 - 6.39 µg/kg respectively. Similarly the content of ergosterol in all the varieties were 1.5 × 10² - 7.2 × 10³ µg/kg, 1.8 × 10² - 1.1 × 10⁴ µg/kg, 3.4 × 10² - 3.6 × 10³ µg/kg, 5.6 × 10² - 1.3 × 10⁴ µg/kg, 4.2 × 10² - 7.2 × 10³ µg/kg, and 9.5 × 10² - 1.7 × 10⁴ µg/kg fr. wt. respectively [19]. In 2016 Ayyash, used advanced HPLC methods to identify vitamin-D₂ or its metabolites in *Catharanthus roseus* plant. The aim of their study was to investigate the presence of vitamin D₂ in addition to its qualitative and quantitative detection in aqueous, alcoholic extract, and dry powder of plant leaves using HPLC, UV-VIS, IR, and ATR-FTIR. The results revealed that *C. roseus* contained a good concentration of 187.840 IU/gm dry wt. of vit-D₂ in them [6]. Some Australian native edible plants like- *Acacia victoriae*, *Tasmannia lanceolata*, and *Backhousia citriodora* were tested by Hughes et al., in 2018 for presence of vitamin-D metabolites and established the presence of vitamin-D₂ in raw seeds of *A. victoriae* at a concentration of 0.03 µg/100 g. In *Tasmannia lanceolata* dried leaf and dried berries vitamin-D₂ was found at a concentration of 0.67 µg/100 g, and 0.05 µg/100 g respectively. Again in the case of *Backhousia citriodora*, Hughes found 0.03 µg/100 g and 0.24 µg/100 g of vitamin-D₂ in fresh and dried leaves of *B. citriodora* respectively [18]. The same year in 2018 another research group from Germany found vitamin-D₂ in raw cocoa beans at a concentration of 0.20 µg/100 g fresh weight of the sample. They also quantified vitamin-D₂ content in different chocolates and reported that dark chocolate, white chocolate, and chocolate nut spreads contained vitamin-D₂ at a concentration of (1.90 - 5.48) µg/100 g, (0.19 - 1.91) µg/100 g, and 0.15 µg/100 g of chocolate respectively [24] (Table-II).

Table- II. Summary of literature on the quantification of vitamin-D2 or its metabolites in plants:

Study Id	Sample Name	Identification Technique	Metabolites found	Quantity (D2/Ergocalciferol)	Quantity (Ergosterol)
Horst et al., 1984 [16]	<i>Medicago sativa</i> (Alfalfa Plant)	HPLC	D2 & ergosterol (field grown)	48 µg/kg plant tissue	NQ
Schwadorf & Muller, 1989 [35]	Wheat	LC	D2 & ergosterol (Laboratory grown)	80 µg/kg plant tissue	NQ
	Broad-bean		D2	7780 µg /kg dry matter 1030 µg /kg dry matter	NQ NQ
Björn and Wang, 2001 [7]	<i>Solanum lycopersicum</i> L. (Solanaceae)	NQ	D2 & Ergosterol	8.7 µg/100 g dry weight	183 – 223 µg/100 g dry weight
Magalhães et al. 2007 [26]	<i>Humulus lupulus</i> L.	Liquid Chromatography–Diode Array Detection–Electrospray Ionization Tandem Mass Spectrometry	D2 & Ergosterol	1.95±0.05 µg/g	1.84 ± 0.09 µg/g
Jäpelt et al., (2011) [19]	<i>Lolium perenne</i> L (Foxtrot)	Liquid chromatography tandem mass spectrometry	Vitamin-D2 & Ergosterol	0.07 -5.69 µg/kg	1.5×10 ² -7.2× 10 ³ µg/kg
	<i>Lolium perenne</i> L (Tivoli)			0.07 – 6.18 µg/kg	1.8×10 ² – 1.1×10 ⁴ µg/kg
	<i>Lolium perenne</i> L (Turandot)			0.19 – 0.46 µg/kg	3.4× 10 ² – 3.6×10 ³ µg/kg
	<i>Lolium perenne</i> L (Telstar)			0.14 – 3.73 µg/kg	5.6×10 ² – 1.3×10 ⁴ µg/kg
	<i>Lolium perenne</i> L (Indiana)			0.11- 2.91 µg/kg	4.2×10 ² -7.2×10 ³ µg/kg
	<i>Lolium perenne</i> L (Kimber)			0.41 – 6.39 µg/kg	9.5×10 ² -1.7×10 ⁴ µg/kg
Ayyash, et al., 2016 [6]	<i>Catharanthus roseus</i>	HPLC	Vitamin-D2	187.840 IU/gm dry weight	NQ
Hughes et al., 2018 [18]	<i>Acacia victoriae</i> (Raw seed)	Liquid chromatography	Vitamin-D2	0.03 µg/100 g	NQ
	<i>Tasmanian lanceolata</i> (Dried leaf)			0.67 µg/100 g	NQ
	<i>Tasmanian lanceolata</i> (Dried berries)			0.05 µg/100 g	NQ
	<i>Backhousia citriodora</i> , (Fresh leaves)			0.03 µg/100 g	NQ
	<i>Backhousia citriodora</i> , (Dried leaves)			0.24 µg/100 g	NQ
Kühn et al., 2018 [24]	Raw Cocoa beans	HPLC & LC-MS/MS	Vitamin- D2	0.20 µg/100 g fresh weight	NQ

NQ- Not quantified / mentioned

Conclusion and Future Perspectives: In the past, vitamin D₃ was only believed to be found in animal products; however, we now know that vitamin D may also be found in fruits and vegetables. Given the significance of the *Solanaceae* family in human nutrition, it is of particular interest that this family includes high levels of vitamin D₃. This family contains common vegetables like potatoes, tomatoes, and peppers, which are widely used in every kitchen and have been proven to have vitamin D₃—but only in the leaves. Therefore, it has become difficult for researchers to investigate new applications for them. Although we currently only know about the content of the leaves, further research will reveal whether vitamin D₃ is also present in the edible parts of these plants or whether bio-fortification is feasible. Again, our understanding of the basic processes by which photosynthetic species synthesize vitamin D₃ is lacking, and any progress in this area will allow us to produce plants with higher natural concentrations of this vitamin and manipulation of its content will become easier. But before putting a lot of effort into growing plants with a high level of vitamin D, it is crucial to consider the bioavailability of vitamin D₃ from plants. If the vitamin D from plants is not bioavailable, it may limit the potential of plants as a new source of vitamin D. One such attempt has already been initiated by Li in 2022 by developing a vitamin-D₃ biofortified tomato variety whose results showed presence of vitamin-D₃ in both leaves and fruits of the tomato plant with promising bioavailability results [25]. Hence more researches regarding bio-fortification and most importantly identification/quantification of vitamin-D or metabolites in potential medicinal or other plants should be encouraged for future.

Conflict of interest: NA

Acknowledgment: NA

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