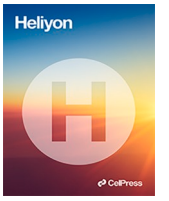
Heliyon 10 (2024) e34890

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

Plant proteins, peptides, and non-protein amino acids: Toxicity, sources, and analysis

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ARTICLE INFO

Keywords:
Plant toxin
Plant protein
Plant peptide
Plant non-protein amino acid
Food toxin

ABSTRACT

Plants have evolved various mechanisms to synthesize diverse range of substances that contribute to their survival against pests, pathogens, predators, and adverse environmental conditions. Although several plant metabolites possess therapeutic potential, some can be potentially harmful to human and animal health when consumed in large proportion. Proteins, peptides, and non-protein amino acids are products of plant biochemical pathways with proven beneficial and nutritional effects. Despite these benefits, the *in vivo* toxicities associated with certain plant-derived proteins, peptides, and non-protein amino acids pose a significant risk to humans and animals. Symptoms of poisoning include nausea, vomiting, diarrhea, hair and weight loss, goiter, cataracts, and infertility. Even though plant processing methods such as soaking and drying can reduce the amount of toxin contained in plants, complete riddance is often impossible. As such, food regulatory bodies need to prevent uncontrolled consumption of the listed and many other toxin-containing plant species to keep the public safe. For this purpose, this review collates crucial insights into the sources, and *in vivo* toxicity associated with certain plant-derived proteins, peptides, and non-protein amino acids that have the clear potential to adversely affect human health. Additionally, this review provides information on analytical methods suitable for the detection of these substances in plants.

1. Introduction

Plants synthesize a broad range of metabolites that are often not essential to their growth and metabolism [1] but combat environmental stress and ward off predators and pathogens in their ecological habitat [2]. These metabolites display therapeutic properties in the treatment of disorders making them suitable candidates for drug development. Despite the remarkable biological activities associated with plant metabolites, reports regarding their *in vivo* toxicity in humans and other mammals cannot be ignored. Toxic metabolites in plants include but are not limited to certain proteins, peptides, and non-protein amino acids which have been identified in a wide variety of wide and edible plants. For example, edible plants such as soybeans and tomato contain toxic lectins which can be poisonous if not well degraded by digestive enzymes [3]. Depending on the type and amount of toxin consumed, common symptoms of poisoning include loss of weight and failure of organs, altered metabolic rates, reduced digestibility of nutrients, compromised expenditure of energy and even death [4].

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https://doi.org/10.1016/j.heliyon.2024.e34890
Received 4 February 2024; Received in revised form 17 July 2024; Accepted 18 July 2024
Available online 21 July 2024

Existing studies on plant protein have emphasized the classes, toxicity, mode of action, potential applications, and proteomic methods of detection and quantification [5–7]. Plant peptide studies have explored its use to enhance biosecurity, biological activities, mechanisms of action, utilization in food development, and application in human health [8–10]. Studies on non-protein amino acids focused primarily on toxicity and mode of action [11,12]. For this review, scientific databases including PubMed, Google Scholar, Science Direct, Web of Science and Scopus databases were utilized to gather articles used for this study. Search words used include "plant toxins," "plant protein," "plant peptides," "plant non-protein amino acids," "*in vivo* toxicity of plant proteins," "*in vivo* toxicity of plant peptides," "*in vivo* toxicity of plant non-protein amino acids," "extraction and analysis of plant protein," extraction and analysis of plant peptides," extraction and analysis of plant non-protein amino acids", and "reduction of toxin content of edible plants." The 3D crystal structures of some proteins and peptides [13–22] which were retrieved from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb) are shown in Fig. 1.

While the extrapolation of animal findings to humans should be done cautiously, the existence of toxic effects in animals warrants caution regarding human consumption. This study focused on *in vivo* toxicities in humans, where unavailable, data on *in vivo* toxicity in animal models were used to support the potential toxicity of certain plants and their metabolites to humans. Despite the information currently available on plant toxins, a review that discusses the sources, *in vivo* toxicity to humans and some mammals, and analytical methods is particularly lacking. Therefore, this review focuses on the toxicity of naturally occurring proteins, peptides, and non-protein amino acids from plants, with particular emphasis on their *in vivo* toxicity and analytical techniques. Furthermore, how processing techniques can help to minimize toxin levels in plants was discussed.

2. Toxicity

A plant is considered toxic when it causes deleterious effects in humans and animals irrespective of the route of administration. For this review, *in vivo* toxicity of plants to humans and laboratory animals will be discussed. Even though plants form a basic part of human and animal nutrition and therapy, when consumed or administered in excess they can become toxic. Following the ingestion of toxin-containing plants, the symptoms of toxicity or poisoning in humans and animals have been associated with the presence of certain proteins, peptides, and non-protein amino acids. The toxicity of these can however be dependent upon the dose and route of administration. Here, the toxicities of different kinds of protein, peptides, and non-protein amino acids will be discussed. The *in vivo* toxicities of these substances in humans and animals are listed in Table 1.

2.1. Lectins

Lectins are carbohydrate-binding proteins that have been reported as toxic to humans and other mammals. The proportion of lectin varies from plant to plant. For example, non-leguminous plants have lower lectin content compared to legumes [40]. When lectins are

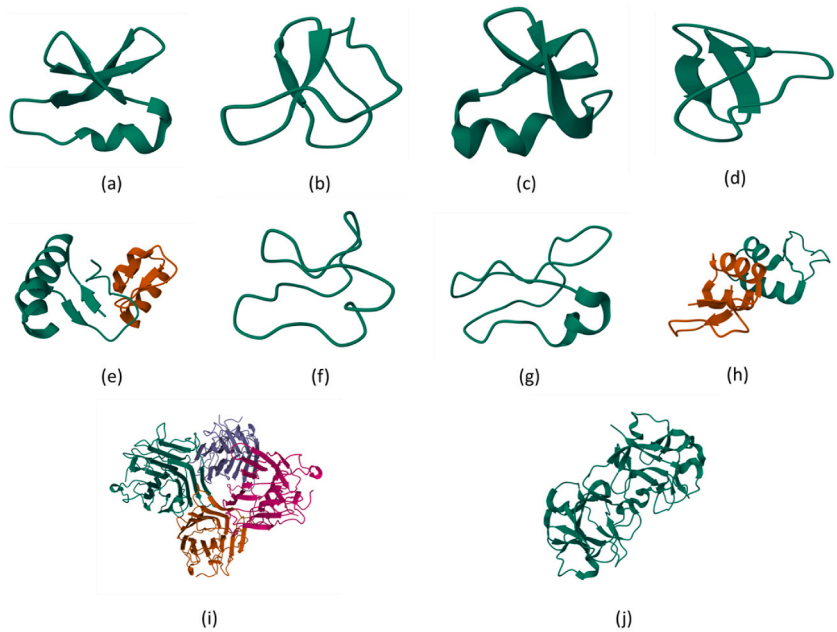

Fig. 1. The 3D crystal structures of peptides and proteins retrieved from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb) with (a) cyO2 (PDB: 2KNM) [13] (b) varv F (PDB: 3E4H) [14] (c) hyen D (PDB: 7RII) [15] (d) kalata B1 (PDB: 1N1U) [17] (e) viscotoxin A1 (PDB: 3C8P) [18] (f) circulin A (PDB: 1BH4) [16] (g) palicourein (PDB: 1R1F) [19] (h) β-hordothionin (PDB: 1WUW) [20] (i) ConBr (PDB ID: 1AZD) [21] and (j) *Sambucus nigra* agglutinin II (PDB ID: 3CA0) [22].

Table 1
Toxic effects of plant-derived proteins, peptides, and non-protein amino acids.

Toxin	Toxicity	Reference
Cyclotides	*In vivo* toxicity - Repeated injection of 1 mg/kg body weight dose of cyO2 caused local inflammation at the site of injection and acute toxicity at 2 mg/kg body weight in mouse xenograft models	[23]
	In vivo toxicity – hemolytic activity in human and rat with HD_{50} values ranging from 10.2 to 13.4 μM	[24]
	In vivo toxicity –Intravenous injection of kalata B1 showed strong hemolytic activity with LD_{50} of 1.0 mg/kg and 1.2 mg/kg, in rats and rabbits	[24]
	Increased arterial blood pressure and tachycardia	[25]
Thionins	*In vivo* toxicity - Intravenous injection of viscotoxin (35 μg/kg body weight) or phoratoxin (1 mg/kg body weight) causes a gradual decrease in blood pressure, contractile force, and heart rate and eventual death	[26]
Lectins	Acute toxicity symptoms include nausea, vomiting, and diarrhea	[3]
	In vivo toxicity - Death of mice injected with ebulin f via i.p. administration (LD_{50} of 2.8 mg/kg body weight)	[27]
	In vivo toxicity - Damage to lungs, intestine, and organs of mice at ≥2.1 mg/kg body weight i.p.	[28]
	In vivo toxicity - Mucosal damage to the digestive tract	[28]
Canatoxin	*In vivo* toxicity - Induction of hypothermia, ataxia, dyspnea, tonic convulsions, coma, and eventually death of mice administered about 100–200 mg/kg body weight i.p.	[29]
	In vivo toxicity - Lethal seizure at LD_{50} of 2 mg/kg bodyweight in rodents	[30]
	In vivo toxicity - Biphasic alteration in blood glucose levels of mice and rats and an increased plasma level of insulin by i.v. route	[31,32]
	Oedema in rats	[33]
	Disruption of calcium transport	[33]
Soyatoxin	*In vivo* toxicity - Toxic to mice via i.p. route, causing chronic tonic convulsions and flaccid paralysis followed by eventual death	[34]
L-Mimosine	*In vivo* toxicity - Decrease in weight gain, cataracts, goitre, infertility, and hair loss in cattle and sheep	[35]
	Hyperthyroidism	[36]
	In vivo toxicity - Impairment of male and female reproductive organs	[35,37]
L-DOPA	Nausea, vomiting, cramping	[38]
	Neurological disorders	[39]

not properly degraded during digestion, they can become poisonous. Typical symptoms of acute lectin toxicity include nausea, diarrhea, vomiting and clumping of the red blood cell [3]. In humans, consumption of large proportion of lectin-containing plants has been implicated in common human health problems including inflammation, leaky gut, autoimmune diseases, obesity, and cancer. In rodents, long period of lectin intake resulted in weight loss epithelial cell necrosis and hyperplasia of intestinal cells [41].

Due to the diverse nature of lectins, their toxicity upon injection can exhibit considerable variation. For instance, in experiments conducted with mice, intraperitoneal (i.p.) administration of approximately 5 mg ebulin f/kg body weight was adequate to induce mortality within a span of 3 days. Conversely, equivalent doses of another lectin derived from dwarf elder (*Sambucus ebulus* L.) fruits, administered intraperitoneally, resulted in neither mortality nor observable toxicity over a period of 14 days [27]. Interestingly, the route of administration significantly influenced the toxic action, as intraperitoneal administration produced a greater toxicity effect compared to oral administration at the same dose in mice. This highlights the importance of considering not only the type of lectin but also the route of exposure when assessing toxicity levels.

The response to poisoning can indeed be age- or organ-dependent, as demonstrated by research conducted by Garrosa et al. In their study, it was found that six-month-old mice exhibited greater sensitivity to ebulin administration compared to their six-week-old counterparts. Despite a dosage of approximately 2.1 ebulin f mg/kg body weight being toxic to all organs studied, elderly mice showed varying degrees of sensitivity to lower doses in various organs. Specifically, relatively low doses of 1.4 mg ebulin f/kg body weight caused no observable damage to the intestines of elderly mice, while significant damage was observed in their lungs at the same dose [28]. This age-or organ-dependent response highlights the importance of considering factors such as age and organ when evaluating the toxic effects of substances like lectins. Signs of toxicity include lung damage leading to pneumonia and mucosal damage to the digestive tract. Mucosal damage was also detected in mice administered intravenous (i.v.) injections of ribosome-inactivating lectins, such as nigrin b and ricin [28].

Processing techniques which involve peeling of skin, removal of seeds of fruits and vegetables, and soaking and rinsing of lectin-containing plants can help reduce lectin levels. Other methods to inactivate lectin in plants include boiling or steaming, especially at high heat. Since lectins are water soluble, exposure to water removes the lectins thus reducing their lectin content [42]. In addition, fermentation breaks down lectins and can decrease their toxicity [43].

2.2. Canatoxin and soyatoxin

Canatoxin is a plant toxin that acts on the central nervous system. When injected intraperitoneally in rodents, the toxin induced convulsions at LD_{50} of 2 mg/kg meanwhile no response was observed when given orally [44]. This shows the significance of route of administration to the toxic action of canatoxin. The pattern of toxicity through intraperitoneal, intravenous, intramuscular and subcutaneous was the same however the time of expression was shortest for intravenous route, producing lethal convulsion within 15 min after 2–3 mg canatoxin/kg injection [30]. Signs of canatoxin toxicity include induced hypothermia, ataxia, dyspnea, tonic convulsions, coma, and eventually death [31]. Other reported toxic action of canatoxin include biphasic alteration of blood glucose levels in mice and rats [30], increased plasma insulin levels [29], induction of hind-paw oedema and Ca^{2+}-transport disrupting ability in rats [32].

Soyatoxin is a canatoxin-like plant toxin which is immunologically related to canatoxin and shares several pharmacological properties with it [33]. Soyatoxin is highly toxic to mice with LD_{50} 7–8 mg/kg body weight. The toxic effect of this metalloprotein is dependent on both the dose and route of administration. Intraperitoneal injection of soyatoxin was found to be extremely toxic to mice, resulting in tonic–clonic convulsions, flaccid paralysis, and eventual death [45].

2.3. Cyclotides

Cyclotides, a class of cyclic peptides, have been noted for their various toxic effects. One notable effect is their ability to induce hemolysis, the rupture of red blood cells. For example, the cyclotide kalata B1 has been found to induce hemolysis in both human and rat erythrocytes, with half-maximal hemolytic dose (HD_{50}) ranging from 10.2 to 13.4 μM [34]. *In vivo* studies using mouse xenograft models have revealed additional toxic effects. Repeated doses of 1 mg/kg body weight of cyO2, another cyclotide, led to local inflammation at the injection site after two to three administrations. Acute toxicity was observed with the administration of 2 mg/kg body weight of cyO2 [24].

Furthermore, intravenous injection of kalata B1 demonstrated potent hemolytic activity, with LD_{50} values of 1.0 mg/kg and 1.2 mg/kg in rats and rabbits, respectively. Additionally, kalata B1 administration was associated with an increase in arterial blood pressure and induced tachycardia [23]. These findings highlight the diverse and potentially hazardous effects of cyclotides, particularly in relation to cardiovascular function and erythrocyte integrity.

2.4. Thionins

Thionins are considered important for plant defense against pathogens and are toxic to a wide range of other life forms [25]. Ingestion of phoratoxin, a typical example of thionin may cause nausea, vomiting, abdominal cramps, diarrhea, and in extreme cases multiple organ system failure [46]. Other toxic thionin such as viscotoxin pose significant risks on cardiovascular function. Studies investigating the toxic effects of viscotoxin on cat models have revealed gradual decline in blood pressure, contractile force, and heart rate from intravenous injection of 35 μg viscotoxin/kg body weight. Moreover, repeated administration of viscotoxin totaling 100 μg/kg body weight over 4–6 injections led to more severe consequences, including asystole and eventual death in the cat models.

Similar effects were observed phoratoxin, albeit at a higher dose of 1 mg/kg body weight [47]. These physiological changes indicate a direct impact of thionins on cardiovascular function.

The fact that both viscotoxin and phoratoxin induce vasoconstriction and cardiac dysfunction underscores the general risk associated with thionins on heart function. This suggests that thionins can exert a cumulative and dose-dependent effect on cardiovascular function, leading to fatal outcomes, highlighting the importance of regulating the use of thionin-producing plants to mitigate the risks posed to both human and animal health.

2.5. l-mimosine

L-mimosine is found in *Leucaena* species and has been associated with potential toxic effects. Although specific data on human toxicity are scarce, animal studies have provided valuable insights into the potential risks, with several investigations cautioning against excessive consumption of *Leucaena* due to adverse effects observed in other animals [26].

Unlike some other animals, sheep and cattle lack the necessary enzymes to detoxify mimosine after absorption. Instead, mimosine is decomposed to 2,3-DHP and 3,4-DHP in the rumen [35]. Therefore, mimosine poses a risk to animals either because they lack the specific metabolic pathways to break it down effectively or complete detoxification may not occur. As a result, mimosine can accumulate in their bodies, potentially leading to toxicity. Symptoms of mimosine toxicity in *Leucaena*-fed cattle include decreased weight gain, cataracts, goiter, infertility, and hair loss [26]. Cattles and goats can tolerate mimosine levels of up to 0.18 g/kg, while sheep can tolerate *Leucaena leucocephala* intakes of approximately 0.14 g mimosine/kg bodyweight without any critical toxic symptoms [48]. The increment of daily intake in sheep to approximately 0.2–0.3 g/kg body weight caused serious fleece shedding within 7–10 days.

Meanwhile, mimosine also impacts thyroid function, with hyperthyroidism being a potential result of mimosine toxicity in cattle fed diets containing over 1 % mimosine. However, below this threshold, typically no considerable adverse effects on thyroid function are observed [49]. Additionally, mimosine induces anti-cell proliferation of male germ cells [36,37]. Kanla et al. reported that mimosine damages testicular tissue, often resulting in decreased epididymal sperm concentration. As such, the consumption of high mimosine levels via *L. leucocephala* intake should be avoided to prevent male reproductive impairment [26].

Processing techniques such as drying at high temperatures, soaking in water for a long time, ensiling, and fermentation can help reduce the mimosine content in plants [50,51]. Despite other known benefits of L-mimosine, a high intake of L-mimosine-containing plants needs to be avoided to evade these associated consequences.

2.6. Levodopa (L-DOPA)

Either synthetic or natural, L-DOPA is readily converted into dopamine in the GI tract [52]. Indeed, the amount of L-DOPA present in plants can vary, with some sources containing higher concentrations than others. For instance, approximately 1.5 g of L-DOPA has been reported to be obtained from 100 g of *Mucuna* seed flour. While lower quantities of L-DOPA can be found in other plant sources, *Mucuna* stands out due to its relatively high content [38,53]. *Mucuna* has a long history of use in traditional medicine systems such as Ayurveda, where it has been described as a treatment for neurological diseases [54]. However, ingestion of *Mucuna* or other L-DOPA-containing plants can lead to adverse effects like those associated with the pharmaceutical L-DOPA used in Parkinson's Disease (PD) treatment [52,55]. Basically, the conversion of L-DOPA by aromatic amino acid decarboxylase into dopamine in the GI tract leads to nausea, vomiting, cramping, with other neurological side effects [52]. Other neurological complications associated with L-DOPA include motor development issues (such as wearing off, on-off phenomena, and dyskinesias) and cognitive dysfunction [56].

Although, processing techniques such as cracking and rinsing of seeds under running water for 72 h helps to reduce L-DOPA levels in seeds [39], varying opinions within the scientific community regarding the toxicity of L-DOPA, highlights the need for further research to better understand its potential risks.

3. Sources

Toxic protein, peptides, and non-protein amino acids can be found in many plant species but are more predominant among leguminous plants. They exist in edible plants or wild species [11]. These metabolites can be localized to the seeds [57], stem [58], bark [59], root [60], aerial parts [61], or the entire plant [62]. Some sources of plant protein, peptides, and non-protein amino acids are summarized in Table 2a–c.

3.1. Proteins

Proteins are long sequences of amino acids monomers that have a great variety of functional, structural, and regulatory roles in organisms. Many monocot and dicot plants are rich in plant proteins [77]. Some toxic protein sources are discussed below.

3.1.1. Lectins

Lectins are carbohydrate-binding proteins that are present in diverse living organisms, mostly from plants. Lectins are widely distributed in the plant kingdom, particularly in the Family Fabaceae [114,63]. Examples of lectins isolated from the Fabaceae family include ConA, ConBr, ConBol, ConGF, and ConM, obtained from seeds of different *Canavalia* species such as *Canavalia ensiformis* (Jack bean), *C. brasiliensis* (Barbicou bean), *C. boliviana*, *C. grandiflora*, and *C. maritima* (Bay bean), respectively [64]. Similarly, Abrin, isolated from the seeds of *Abrus precatorius* (Rosary pea), represents another lectin from the genus *Abrus* [65]. Leguminous sources of

Table 2a
Sources of plant protein toxins and analytical methods.

Toxin	Analytical methods (Sample preparation)	Sources	Reference
Lectins	Affinity chromatography, ion exchange chromatography, MALDI-TOF MS (Homogenization, centrifugation, filtration, protein precipitation with ammonium sulfate, dialysis)	Widely distributed in Fabaceae (Legumes); *Canavalia ensiformis* (Jack bean), *C. brasiliensis* (Barbicou bean), *C. boliviana, C. grandiflora, C. maritima* (Bay bean), *Glycine* max (Soybean), *Cicer arietinum* (Chickpea), *Phaseolus lunatus* (Lima bean), *P. vulgaris* (Black bean), *Vicia faba* (Broad bean), *Lens culinaris* (Lentil), *Pisum sativum* (Pea), *Vigna angularis* (Adzukki bean), *V. mungo* (Urid bean), *V. radiata* (Mung bean), *V. unguiculata* (Cowpea)	[29,63–65, 66]
		Family Euphorbiaceae; *Ricinus communis* (Castor bean), *Jatropha curcas* (Physic nut)	[58,67]
		Family Santalaceae; *Viscum album* (Mistletoe)	[68,69]
		Family Adoxaceae; *S. ebulus* (Dwarf elder)	[27]
		Family Amaryllidaceae; *Morus indica* (Mulberry)	[70]
		Family Amaranthaceae; *Chenopodium quinoa* (Quinoa)	[71]
		Family Poaceae; *Triticum aestivum* (Wheat), *Oryza sativa* (Rice)	[72,73]
		Family Solanaceae; *Solanum lycopersicum* (Tomato), *Solanum tuberosum* (Potato)	[74]
Canatoxin	Ammonium sulfate fractionation, ion exchange chromatography; DEAE–cellulose, DPA–Sepharose 6B column, gel filtration, SDS–PAGE (Homogenization in Tris-HCl, ethanol treatment, dialysis)	Fabaceae – *Canavalia ensiformis* (Jack bean)	[29,75,76]
Soyatoxin	Ammonium sulfate fractionation SDS–PAGE, gel filtration, FPLC (Pulverization, defatting, vortexing, centrifugation)	Fabaceae – Glycine max (Soybean)	[34]

lectins extend beyond the genus *Canavalia* to include plants like *Lens culinaris* (lentil), various *Vigna* species such as *Vigna angularis* (adzuki bean), *V. mungo* (urid bean), *V. radiata* (mung bean), and *V. unguiculata* (cowpea) [115]. A typical lectin structure from Lens culinaris (lentil) is depicted in Fig. 2a.

Table 2b
Sources of plant peptide toxins and analytical methods.

Toxin	Analytical methods (Sample preparation)	Sources	Reference
Cyclotides	RP-HPLC, MALDI-TOF MS, MALDI-TOF/TOF MS, Triple TOF LC-MS/MS, ESI-QTOF MS, FT-ICR MS, LCQ Deca ion trap MS, LTQ-Orbitrap Fusion Tribrid MS, nano-LC-FTMS (Maceration, hot extraction, refluxing, filtration, partitioning, centrifugation, lyophilization, LLE, SPE)	Violaceae (violet); *Viola hederacea* (Ivy-leaved violet), *V. odorata* (Sweet violet), *V. tricolor* (Wild pansy), *V. arvensis* (Field pansy), *V. ignobilis, V. cotyledon, V. biflora* (Arctic yellow violet), *Leonia cymosa, Hybanthus parviflorus, H. floribundus* (Shrub violet), *H. enneaspermus* (Spade flower), *Pombalia calceolaria, Melicytus ramiflorus* (Whiteywood)	[59–62,77,78, 79–82,83]
		Rubiaceae (coffee); *Oldenlandia affinis* (Kalata-kalata), *Psychotria suterella, P. leptothyrsa, P. solitudinum, P. suerensis, P. poeppigiana* (Sore-mouth-bush), *P. deflexa* (Nodding wild coffee), *P. brachiate, Palicourea condensata, P. tetragona, Chassalia discolor, C. chartacea, C. curvifolia* (curved flower woody chassalia), *Hedyotis biflora* (Palarapdap), *H. diffusa* (Snake-needle grass), *Notopleura capacifolia, Carapichea ipecacuanha* (Ipecac)	[78,84–90]
		Fabaceae (pea); *Clitoria ternatea* (Butterfly pea)	[91,92,93]
		Solanaceae (potato); *Petunia x hybrida* (Garden petunia)	[94]
		Poaceae (grass); *Panicum laxum* (Lax gaping grass)	[95]
		Cucurbitaceae (cucumber); *Momordica dioica* (Spine gourd), *M. cochinchinensis* (Spiny bitter-cucumber)	[96,97]
Thionins	RP-HPLC (Centrifugation, dialysis, precipitation with ammonium sulfate)	Widely distributed in Poaceae (cereals); *Triticum vulga* (Wheat), *Hordeum vulgare* (Barley), *Avena sativa* (Oat), Zea mays (Maize), *Secale cereale* (Rye)	[98–100]
		Family Solanaecae; *Lycopersicum esculenta* (Tomato), *Capsicum annuum* (Bell pepper)	[101,102]
		Family Anacardiaceae *Mangifera indica* (Mango)	[101]
		Family Caricaceae; *Carica papaya* (Pawpaw)	[101]
		Family Juglandaceae; *Juglans regia* (Walnut)	[101]
		Family Santalaceae; *Viscum album* (European mistletoe), *Phoradendron tomentosum* (Christmas mistletoe), *Pyrularia pubera* (Buffalo-nut)	[99,103,104]
		Family Brassicaceae; *Arabidopsis thaliana* (Mouse-ear cress), *Crambe abyssinica* (Crambe)	[46,105]
		Family Liliaceae; *Tulipa gesneriana* (Didier's tulip)	[99]

Furthermore, lectin-containing plants are found in other non–leguminous families such as Euphorbiaceae, Santalaceae, Adoxaceae, Amaryllidaceae, Amaranthaceae, Poaceae, and Solanaceae. For instance, Ricin from *Ricinus communis* (Castor bean) and Curcin from *Jatropha curcas* (Physic nut) are examples from family Euphorbiaceae [57,66], CqLec from *Chenopodium quinoa* (Quinoa) belongs to family Amaranthaceae [67], while wheat seed lectins from *Triticum aestivum* (Wheat) [71] and rice bran lectins (RBA1 and RBA2) from *Oryza sativa* (Rice) [72] are examples from the Poaceae family. Other examples include Agglutinin II from the bark of *Sambucus nigra* (European elder) [22], Ebulin f from the fruits of *S. ebulus* (Dwarf elder) [27], lectins from the latex of *Morus indica* (Mulberry) [73], *Solanum lycopersicum* (tomato), and *S. tuberosum* (potato) [70].

3.1.2. Canatoxin and soyatoxin

Canatoxin is an isoform of urease and a covalently linked dimer of 95-kDa subunits [74] found in *Canavalia ensiformis* (Fabaceae), commonly known as Jack bean. Jack bean is a widely distributed legume in Africa, Asia, and America, serving as a food source for both livestock and, occasionally, humans [75].

On the other hand, Soyatoxin is a single-chain acidic protein with a molecular weight of 21 kDa [45] found in soybeans (*Glycine max*), another leguminous plant belonging to the Fabaceae family. Soybean is extensively cultivated in countries including the United States, Brazil, Argentina, China, India, Canada, and Paraguay where it is recognized as a valuable source of protein and edible oil for humans and animals and humans [116].

3.2. Peptides

Peptides are composed of 2–50 covalently bonded amino acid constituents. They are known to regulate physiological and developmental, and defense processes in plants [117]. Sources of some peptides such as cyclotides and thionins are discussed below.

3.2.1. Cyclotides

These are macrocyclic peptides containing approximately 30 amino acids and comprising a characteristic head-to-tail cyclic backbone and cystine knot motif [118]. Over 260 cyclotides have been characterized from plants predominantly belonging to the Violaceae (violet), Rubiaceae (coffee), Fabaceae (pea), Solanaceae (potato), and Cucurbitaceae (cucumber) families [95,96,119,120]. Among these families, Violaceae and Rubiaceae are the major cyclotide sources [94]. All examined Violaceae family plants contained cyclotides, while only less than 5 % of Rubiaceae family plants possessed cyclotides [94]. Other sources of cyclotides include *Petunia x hybrida* (Garden petunia) which belong to Family Solanaceae [95]; two Cucurbitaceae species, *Momordica dioica* (Spine gourd) and *M. cochinchinensis* (Spiny bitter-cucumber) [120,78]; and *Clitoria ternatea* (Butterfly pea) from the Fabaceae family [97]. Although most cyclotides have been detected in dicots, panitides L1–9 from *Panicum laxum* (Lax gaping grass) represent a typical linear cyclotide from a monocot [96].

Cyclotide distribution in a plant potentially varies across different parts, such as the roots, stems, flowers, leaves, and seeds [58, 120,91]. While some cyclotides may be found in various tissues of an individual plant, some can be specific to a given tissue [58]. Additionally, some cyclotides occur in multiple plants, for example, Varv A can be found in *Oldenlandia affinis* (Kalata-kalata), *Viola odorata* (Sweet violet), *V. tricolor* (Wild pansy), and *V. arvensis* (Field pansy) [94].

The violet family is an extensively distributed plant family, comprising approximately 1100 species [121]. All plants belonging to the Violaceae family contain cyclotides either in the leaves, roots, bark, seeds, flowers, aerial parts, or entire plant. Mra 1–5 was isolated from the leaves of *Melicytus ramiflorus* (Whiteywood) [122], Cycloviolin A–D were obtained from the bark of *Leonia cymose* [59], Tricyclon A was obtained from the flowers of wild pansy [79], while Poca A and B were obtained from the roots of *Pombalia calceolaria* [60]. Vigno 1–10 and Hyfl A–C were also found in the aerial parts of *V. ignobilis* and *Hybanthus floribundus* (Shrub violet), respectively [61,80]. Other examples are the Vibi D, E, G, and H from *V. biflor* (Arctic yellow violet) [81]. Varv peptides A–H and Vitri A–F were also reported in wild pansy [62].

The Rubiaceae family ranks among the largest angiosperm families [82]. However, cyclotide-containing species within this family represent less than 5 % of the total Rubiaceae species diversity [94]. Kalata B cyclotides, for instance, were identified in the aerial parts of *Oldenlandia affinis* (Kalata-kalata) [123,84]. Studies on the genus *Psychotria* have unveiled numerous cyclotides, including Psyles A–F from *Psychotria leptothyrsa* [85], PS-1 from *P. suterella* [94], and Pysol 1 and 2 from *Psychotria solitudinum* [86,87]. *Chassalia*, another cyclotide-producing genus within the Rubiaceae family, has yielded cyclotides like Circulin A–F, and Chassatides C1–C6, and Chacur 1 [94,87,88]. Palicourein was discovered in the bark of *Palicourea condensata*. Further examples of cyclotides found in Rubiaceae include Diffusa cyclotides 1–3 (DC1–3) from the leaves and roots of *Hedyotis diffusa* (Snake-needle grass) [89].

The Fabaceae family comprises herbs, shrubs, trees, or vines, boasting a worldwide distribution. Notable members of this family include peanuts, soybeans, peas, and lentils [90]. *Clitoria ternatea*, commonly called Butterfly pea, is the only known cyclotide-containing species in the Fabaceae family [124]. Cyclotides, specifically Cter A–L, are found in the seeds of Butterfly pea [125,126], while the leaf and flower tissues contain Cter M–R [97]. Additionally, cliotides T1–T12 are present throughout the entire plant [92]. Interestingly, Cter R, also known as cliotide T7, is not only found in the leaf and flower tissues but also occurs in the seeds of Butterfly pea [126].

3.2.2. Thionins

Thionins are small, cysteine-containing basic proteins with a molecular weight of approximately 5 kDa that are found in various monocot and dicot plants and comprise 45–48 amino acids linked by three or four disulfide bonds [93]. Thionins were initially identified in wheat (*Triticum vulga*) and barley (*Hordeum vulgare*) extracts. These peptides are not only found in cereals but also other

Table 2c
Sources of plant non-protein amino acid toxins and analytical methods.

Toxin	Analytical methods (Sample preparation)	Sources	Reference
L-Mimosine	Ion exchange, PC, TLC, RP-HPLC (Pulverization, maceration, ethanol extraction)	Predominant in the Fabaceae family; *Mimosa pudica* (Sensitive plant), *Leucaena leucocephala* (Lead tree), *L. diversifolia* (Wild tamarind), *L. collinsii, L. greggii, L. esculenta, L. pallida*	[106–108,109,110,111–113]
L-DOPA	UV spectrophotometry, RP-HPLC, HPTLC, LC-QTOF-MS, LC-MS/MS (Solid-liquid extraction using ethanol, centrifugation, filtration)	Predominant in the Fabaceae family; *Mucuna pruriens* (Velvet bean), *M. monosperma* (Negro bean), *M. gigantean* (Sea bean), *Vicia faba* (Broad bean), *V. narbonensis* (French vetch), *Alysicarpus rugosus* (Red moneywort), *Bauhinia* sp., *Dalbergia retusa* (Cocobolo), *Canavalia* sp., *Cassia* sp., *Baptisia* sp., *Lupinus* sp., *Parkinsonia aculeate* (Palo verde), *Vigna* sp., *Phanera vahlii* (Maloo Creeper), *Pileostigma malabaricum* (Purple orchid tree), *Prosopis chilensis* (Chilean mesquite), *Robinia pseudoacacia* (Black locust), *Sarothamnus scoparius* (Scotch broom), *Phaseolus vulgaris* (Common bean), *Teramnus labialis*	[51,55,126,129,142,148,152]

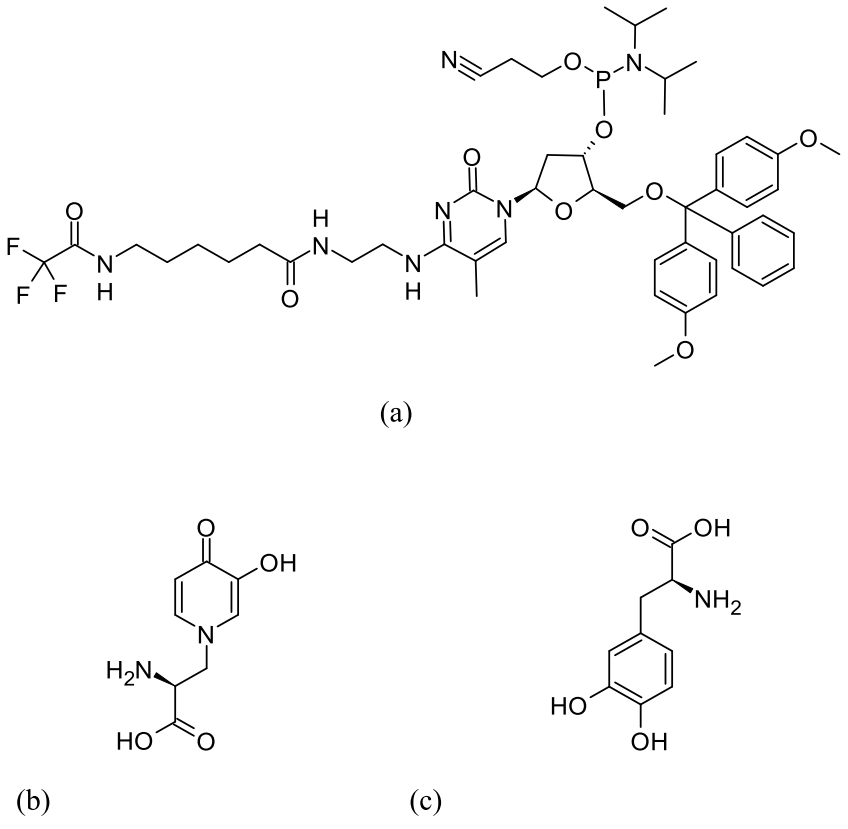

Fig. 2. Chemical structures of some non-protein amino acids and protein (a) *Lens culinaris* agglutinin (b) L-Mimosine (c) L-dopa.

dicotyledons [127]. Cereal examples include barley, oat, maize and rye [98,99]. Other dicots known to contain thionins include tomato leaves (*Lycopersicum esculenta*), bell pepper (*Capsicum annuum*), mango (*Mangifera indica*), papaya (*Carica papaya*), and walnut (*Juglans regia*) seeds [100,101]. The thionins obtained from the seeds of wheat, barley, and crambe (*Crambe abyssinica*) are Purothionin, Hordothionin, and Crambin, respectively [102,105]. Viscotoxin is present in European mistletoe (*Viscum album*) [128]. Similarly, thionins have been detected in the leaves of *Phoradendron tomentosum* (Christmas mistletoe) [129]. Another species of the Brassicaceae family, *Arabidopsis thaliana*, commonly known as Mouse-ear cress also contains thionins [25]. Other sources include *Pyrularia pubera* (Buffalo nut) and *Tulipa gesneriana* (Didier's tulip), from which Pp-TH and Thionin4.1 were obtained, respectively [98, 103].

3.3. Non-protein amino acids

Non-protein amino acids are amino acids other than the 20 amino acids incorporated in protein. These forms of amino acids have been reported from a wide variety of plants including Fabaceae, Liliaceae, Sapindaceae, Cycadaceae, Compositae, Rubiaceae, and Lecythidaceae [104]. Sources of some non-protein amino acids such as L-mimosine and L-DOPA are discussed below.

3.3.1. L-mimosine

Mimosine, β-(3-hydroxy-4-pyridon-1-yl)-L-alanine (Fig. 2b), is a toxic, non-protein amino acid with a molecular weight of 198 Da [130]. It is exclusively found in *Mimosa pudica* and certain *Leucaena species* within the Fabaceae family [131]. According to Jones, the mimosine content of *Leucaena* species tends to be significantly higher than that of *Mimosa* [132].

Mimosa pudica, commonly called sensitive plant, is characterized as a creeping annual or perennial herb, and the first mimosine was obtained from the sap of this herb [133]. Among *Leucaena* species, mimosine have been found in the leaves, pods, and seeds of various parts of *L. leucocephala* (Lead tree), *L. diversifolia* (Wild tamarind), *L. collinsii*, *L. greggii*, *L. esculenta*, and *L. pallida* [134]. *Leucaena leucocephala*, an evergreen, fast-growing, multipurpose leguminous tree species within the Fabaceae family, also contains this toxic amino acid [106]. In regions like Central America, Indonesia, and Thailand, *Leucaena* is consumed by humans for food and medicinal values [107].

3.3.2. L-DOPA

L-DOPA (Fig. 2c) is a non-protein amino acid with a molecular weight of 197 Da (Da), and it is found naturally in animals and

certain plants [108]. The occurrence of L-DOPA in legumes, particularly those of the Fabaceae family, has drawn interest owing to its high content in these species [135,136]. L-DOPA is also present in the seeds of *Mucuna* species, such as *M. pruriens* (Velvet bean), and *M. gigantean* (Sea bean) [52,55]. Other known plants from the Fabaceae family that contain L-DOPA include *Vicia faba* (Broad bean), *V. narbonensis* (French vetch), *Alysicarpus rugosus* (Red moneywort), *Parkinsonia aculeate* (Palo verde), *Vigna* sp., *Pileostigma malabaricum* (Purple orchid tree), *Phaseolus vulgaris* (Common bean), among many others [137,138].

4. Analytical methods

Currently available analytical techniques for plant-derived proteins, peptides, and non-protein amino acids are summarized in Table 2a–c under extraction and pretreatment, chromatographic, and spectroscopic techniques.

4.1. Extraction and pretreatment techniques

Each extraction method is tailored to the metabolite of interest and may involve various steps to obtain lectins, canatoxin, soyatoxin, cyclotides, thionins, L-mimosine, and L-DOPA. effectively. Extraction methods such as maceration [139], hot extraction [140], and refluxing [109] using a single solvent [140,141], miscible solvents [126,110], or an extraction buffer [27] are the predominantly employed techniques. For cyclotide extraction, a combination of polar solvents, such as water, methanol, and acetonitrile [126,110] has been widely utilized in various studies, demonstrating its effectiveness in isolating cyclotides from different plant sources. While some researchers employed the combination of moderately polar dichloromethane with methanol [139,83], others added formic acid (FA) buffer to the extraction solvent [97,125]. These variations in extraction methods highlight the flexibility in analytical procedures to optimize the extraction of plant toxins from different plant sources.

For cyclotide analysis, extraction is often followed by successive liquid–liquid extraction (LLE) and solid-phase extraction (SPE). This successive method of extraction ensures the total removal of all polar compounds and the strong retention of cyclotides on RP-material as a result of the exposed hydrophobic faces of the protein [81,142]. While LLE involves partitioning of the extract between two immiscible solvents, an aqueous and an organic solvent [60,126], SPE on the other hand allows for the pre-purification of extracts on C18 columns. Elution on SPE columns can be performed using methanol [60,126], ethanol [143], or acid-buffered aqueous acetonitrile [139], such that only polar compounds are eluted from the column before chromatographic analysis. Hashempour et al. dissolved the sample in ammonium bicarbonate buffer (pH 8) before loading it on the SPE column [143]. While LLE and SLE are important for efficient cyclotide analysis, they were excluded for certain cyclotide analyses [83].

In the case of thionin peptides, extraction of ground plant samples with done with extraction buffer. It is then subjected to ammonium sulfate precipitation between 0 % and 70 % saturation. The precipitate is re-dissolved in distilled water and heated. Following this, the suspension is clarified by centrifugation, and the resulting supernatant is extensively dialyzed against distilled water. After dialysis, the solution is freeze-dried to obtain a crude extract for further purification [101].

For proteins, the extraction of soyatoxin from soybean seed meals, a crucial initial step involves defatting the samples to remove lipid components that may interfere with the extraction process. This can be achieved using a denaturing sample buffer containing 0.1 % sodium dodecyl sulfate for about 2–3 h at room temperature, followed by centrifugation to separate the supernatant from the lipid layer [45]. For canatoxin analysis, homogenization and subsequent dialysis are essential steps [31]. Plant material is typically homogenized in an ice-cold buffer (25 mM Tris-HCl, pH 7.5), followed by treatment with 30 % ethanol to induce precipitation. The supernatant obtained after centrifugation is then dialyzed against the same buffer to remove unwanted salts and impurities [30,144]. Salting out with ammonium sulfate and repeated dialysis with the same buffer further aid in complete salt removal before subsequent purification steps [74,144].

Similarly, for lectin extraction, salting out with ammonium sulfate and dialysis are critical steps. However, dialysis is typically performed against phosphate-buffered saline to maintain the appropriate buffer conditions for lectin stability [76,68]. In some cases, a double filtration process is employed to remove mucilaginous substances from the extract, enhancing the efficiency of the isolation procedure [27].

For non-protiein amino acids, L-DOPA extraction from plant samples, various extraction buffers have been utilized, including a mixture of acetate buffer, octyl sulfate, and NaEDTA, FA and ethanol (1:1 v/v) [108] and 0.1 N HCl [109,69]. Boiling water or 0.1 N HCl is commonly used to extract L-mimosine [140,141]. After extraction, the samples are subjected to centrifugation and/or filtration to obtain the filtrate or supernatant required for further analysis. These extraction methods ensure the efficient recovery of toxic substances for further analysis and characterization. It is evident that specific extraction buffers and processes are employed for canatoxin, lectins, thionins, and L-DOPA extraction.

4.2. Chromatographic methods

Reversed-phase high-performance liquid chromatography (RP-HPLC) is the preferred method of choice for fractionating cyclotide extracts employing various eluents such as methanol [81,126], ethanol gradient [143], 20–80 % acetonitrile with or without 1 % FA [97,125], acetonitrile/ddH_2O [83] and other buffer mixtures. Similarly, thionins are commonly separated from crude extracts using RP-HPLC [101]. Affinity chromatography on a hydrolyzed Sepharose column is utilized for lectin fractionation, while ammonium sulfate fractionation is employed for canatoxin extraction [27,76]. Ammonium sulfate fractionation (35–55 % saturation) has mostly been employed to extract canatoxin. Other known chromatographic techniques include diethylaminoethyl (DEAE)–cellulose, the Zn^{2+}-chelated form of dipicolylamine (DPA)–Sepharose 6B column, sodium dodecyl sulfate–polyacrylamide gel electrophoresis

(SDS–PAGE), and gel filtration [74,144]. Fast protein liquid chromatography (FPLC), SDS–PAGE, and gel filtration using 12 % polyacrylamide/0.1 % SDS gels are applicable techniques for soyatoxin analysis [45].

Ion exchange resin chromatography is an ideal method for obtaining mimosine in substantial quantities [140,145]. Other mimosine analysis methods include paper chromatography [111], thin layer chromatography [145], and gas chromatography [112]. While paper chromatography is considered to be relatively less selective and sensitive for mimosine analysis, gas chromatography is reportedly more tedious and time-consuming [112]. On the other hand, RP-HPLC is a simple, sensitive, rapid, and accurate method [113]. In addition, a combination of techniques, such as TLC + RP-HPLC, is useful to verify and quantify the degree of purity of mimosine [145].

For L-DOPA analysis, RP-HPLC [146] and high-performance thin-layer chromatography (HPTLC) [147,148] are commonly used methods. While RP-HPLC may be time-consuming and costly, it remains a widely used method. According to Topal and Bozoğlu, RP-HPLC is time-consuming, costly and requires considerable selectivity and complex sample preparation procedures [146]. The relatively low sensitivity and selectivity of RP-HPLC when compared to other analytical methods like LC-MS might make it a less popular method for L-DOPA analysis in the future. On the other hand, HPTLC offers simplicity, rapidity, precision, cost-effectiveness, and reproducibility for L-DOPA determination [109].

4.3. Spectrometry-based methods

Methods employed for cyclotide analysis include matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) [60,139], matrix-assisted laser desorption ionization–tandem time-of-flight mass spectrometry (MALDI-TOF/TOF MS), triple time-of-flight liquid chromatography–tandem mass spectrometry (triple TOF LC-MS/MS) [110], electrospray ionization–quadrupole time-of-flight mass spectrometry (ESI-QTOF MS) [124], Fourier transform–ion cyclotron resonance mass spectrometry (FT-ICR MS) [143], liquid chromatography quadrupole (LCQ) Deca ion trap MS [126], nano-liquid chromatography–Fourier transform mass spectrometry (nano-LC-FTMS) [143] and linear trap quadrupole (LTQ)-Orbitrap™ Fusion Tribrid™ MS [83]. LTQ-Orbitrap Fusion Tribrid MS improves the quality and sensitivity of data [83]. The use of MALDI-TOF MS has also been reported for the analysis of lectin [27]. No report on the use of spectrometry for canatoxin and soyatoxin detection were found, except atomic absorption spectrophotometry for the detection of the metal content of canatoxin [74]. Spectroscopic methods such as near-infrared reflectance spectroscopy [149] and spectrophotometry [141] have been reported for mimosine detection; nonetheless, they have proven less selective and sensitive. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is reportedly tedious and time-consuming [150].

Methods available for L-DOPA detection include the ultraviolet (UV) spectrophotometric method, which is based on the nitrosation of L-DOPA [138], liquid chromatography–mass spectrometry (LC-MS) [151], and LC-MS/MS [108]. UV spectrophotometry has been described as a user-friendly, rapid, and economical method for L-DOPA detection [138]. LC-MS was used by Hu et al. for the chromatographic separation and quantification of L-DOPA in *Vicia faba*. The LC-MS system comprises a Waters UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm) accompanied by a Synapt G2 high-definition hybrid quadrupole/time-of-flight mass spectrometer [151].

In another study where LC-MS/MS used for L-DOPA determination in *V. faba*, it was described as rapid, sensitive, precise, and accurate, facilitating a well-resolved detection of L-DOPA [108]. While LC-MS/MS might be rapid, accurate, and sensitive for detecting L-DOPA, it is not the best choice for analyzing mimosine where it is reportedly tedious and time-consuming. This highlights the need to consider the unique characteristics of each toxin and choose the method that fits best.

5. Conclusion

Proteins, peptides, and non-protein amino acids are examples of biochemical pathways products often produced for the growth and survival of plants in their habitat. From this review, edible plant species contain certain plant proteins, peptides, and amino acids that can pose health risks when consumed excessively. The toxic effects associated with excessive consumption include nausea, vomiting to more severe symptoms such as goiter and infertility. Where *in vivo* toxicity in animals was used to infer *in vivo* toxicity in humans, it is important to state that the use of animal models to assess the likelihood of a metabolite to prove toxic to humans cannot be relied upon. Therefore, it is important to conduct appropriate *in vivo* toxicity test of such metabolite in humans. In the case of soyatoxin in Soyabean (*Glycine max*), where there is paucity of data for *in vivo* toxicity in humans, this study recommends quantitative *in vitro* to *in vivo* extrapolations using computational tools to complement the available data and predict *in vivo* toxicity.

In cases where the consumption of a toxic plant cannot be entirely avoided, appropriate processing methods such as soaking for long hours, drying at high temperatures, and fermentation should be applied to considerably reduce the toxin content.

Furthermore, the analytical techniques mentioned in this manuscript have enabled the accurate identification, quantification, and characterization of these plant-based compounds. This study has shown that the older identification and quantification methods for protein such as gel electrophoresis have now been replaced by methods such as HPLC or hyphenated techniques such as LC-ESIMS or MALDI-TOF-MS which allows for the analysis of complex matrices with less rigorous sample pretreatment procedure. As shown in this review, appropriate detection methods can vary from toxin to toxin. For instance, LC-MS/MS is effective for L-DOPA analysis due to its capacity to identify complex mixtures with high sensitivity, but not for L-Mimosine detection. Methods like ion exchange resin chromatography and HPLC are typically preferred due to their higher sensitivity and specificity in detecting low concentrations in biological samples.

In conclusion, these findings underscore the dose- and route-dependent toxic effects of plant proteins, peptides, and non-protein amino acids and highlight the risks associated with their unregulated utilization. Proper caution and regulation are essential in

handling and employing such plants to mitigate potential harm to both humans and animals. Efforts should be made or maintained to issue regulations to farmers and food producers on maximum toxin levels and to ensure that hazard controls are complied with. Furthermore, this study recommends frequent monitoring and analysis of plant species for possible toxic components to help prevent and reduce the chances of exposure.

Consent for publication

All the authors read and agreed to publish this article.

Funding/Acknowledgements

This study was supported by the Research Program (2023-1235) funded by SeoulTech (Seoul National University of Science and Technology).

Data availability

No data was used for the research described in the article.

CRediT authorship contribution statement

Ganiyu Akinniyi: Writing – original draft, Methodology, Visualization, Writing – review & editing. **Adebayo J. Akinboye:** Writing – review & editing, Data curation, Investigation. **Inho Yang:** Project administration, Supervision, Validation. **Joon-Goo Lee:** Supervision, Investigation, Project administration, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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