International Journal of Food Science and Technology, 2025, 60(1), vvae013
https://doi.org/10.1093/ijfood/vvae013
Advance access publication: 8 January 2025
Original Article

Methodology for accessing cyanogen glycoside and alkaloid content in Traditional foods

Thomas Owen Hay[1,*], Joseph Robert Nastasi[1], Gerry Turpin[2], Dale Chapman[1], Bronwyn Fredricks[1], Suzanne Thompson[3], and Melissa Fitzgerald[1]

[1]School of Agriculture and Food Sustainability, University of Queensland, Brisbane, Queensland, Australia
[2]Australian Tropical Herbarium, James Cook University, Townsville, Australia
[3]The Yumbangku Aboriginal Cultural Heritage and Tourism Development Aboriginal Corporation (YACHATDAC), Barcaldine, Australia
*Corresponding author: School of Agriculture and Food Sustainability, University of Queensland, Hartley Teakle Building (Building 83), St Lucia Campus, Brisbane, Queensland 4067, Australia. Email: t.hay@uq.edu.au

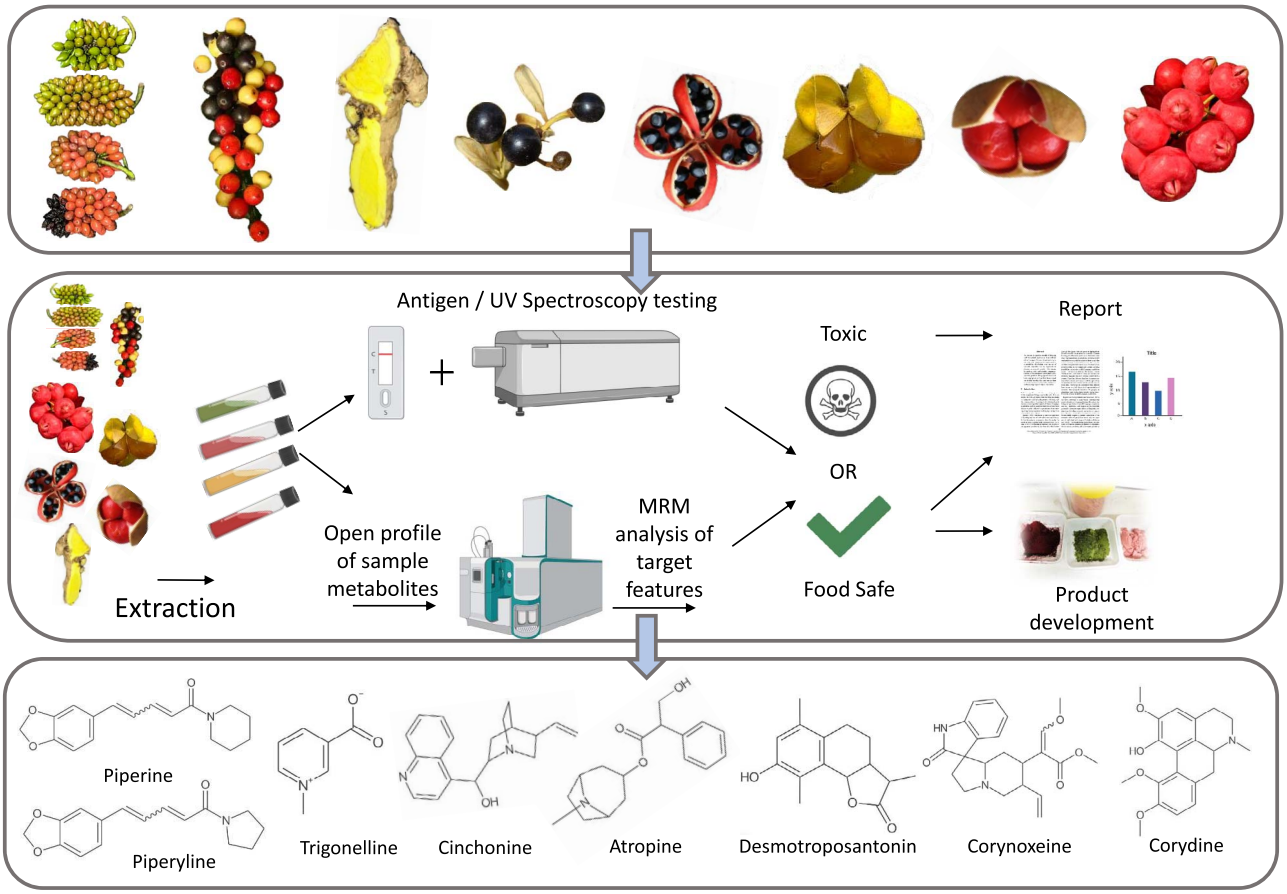
Abstract

This study aimed to assess the food safety of 8 Traditional Australian foods by analysing their cyanogen glycoside and alkaloid content. The research utilized a combination of ultra performance liquid chromatography-quadrupole time of flight tandem mass spectrometry, UV–Vis spectroscopy, and indicator strip assays to identify and quantify toxic compounds. The study found no cyanogen glycosides across all tested species above a limit of detection of 1.59 μg g^{-1} cyanide equivalent. Eight alkaloids were identified, including trigonelline, piperine, atropine, piperyline, corydine, cinchonine, corynoxiene, and desmotroposantonin. Notably, atropine, which was detected in trace amounts in native pepper, was found to be below the detection limit of 1.30 ± 0.60 mg per 100 g. The presence of trigonelline and piperine was significant in several species, particularly the native pepper. These findings suggest that the tested Traditional foods are safe for consumption concerning cyanogen glycosides and alkaloids. The systematic approach to toxin detection in these foods supports the use of Traditional knowledge, aids in validating their food safety, and provides a framework for future toxicological assessments.

Keywords: Traditional foods, food safety, metabolomics, bushfoods, alkaloids, cyanogen glycosides

Received: 26 August 2024. **Accepted:** 7 November 2024

Graphical abstract



Introduction

Traditional foods refer to culinary preparations deeply rooted in a particular culture or region, with relevant knowledge often passed down through generations (Lopes et al., 2023). Modern food systems benefit from Traditional foods because the diversification of agricultural biodiversity is a primary resource for food system resilience, security, and nutrition (Bharucha and Pretty, 2010; Frison et al., 2006; Tong et al., 2022). Moreover, investigating Traditional foods allows the discovery of novel functional foods and food additives (Hay et al., 2024). Traditional foods were the subject of thousands of years of human trials and knowledge gained and is evidence of their nutritional suitability (Hidalgo-Mora et al., 2020). However, while Traditional food safety practices were effective within their cultural and environmental contexts, they may only sometimes align with global and nation-state food safety standards and regulations. Changes in food production, processing techniques, and storage methods have influenced the evolution of food safety practices globally. Moreover, the disruption of Traditional Knowledge in many global regions by the practices of colonization suggests that a degree of caution may be necessary when considering the food safety of underutilized species (Williams et al., 2023; Hay et al., 2022). Comprehensive toxicology workflows are highly effective for evaluating the food safety of Traditional foods, but they are costly and time-intensive (Bessaire et al., 2021; Madariaga-Mazon et al., 2019). Therefore, workflows and analytical techniques that streamline the assessment of Traditional foods require further research and development.

There are seven classes of toxins found in plants (World Health Organisation, 2018). Among these, cyanogen glycosides and several classes of alkaloids are of primary concern, as their ingestion can be anti-nutritive or fatal (Aniszewski, 2007; Bolarinwa et al., 2016). Cyanogen glycosides are phytotoxins found in over 2000 plant species, such as cassava, taro, and many other fruit seeds (Burns et al., 2012; Bolarinwa et al., 2016). Thermal treatment or fermentation can reduce cyanogen glycoside content, but its presence at concentrations as low as 0.5 mg L^{-1} is hazardous in raw foods (Bolarinwa et al., 2016). Many types of alkaloids, including pyrrolizidines, indole, and tropane alkaloids are toxic (Bessaire et al., 2021). However, within the xanthine and purine alkaloid classes, compounds like caffeine and theophylline are considered safe when consumed in moderate amounts. Therefore, the ability to rapidly discern between high and low-risk alkaloid classes is important for assessing the safety of Traditional foods. Ultra performance liquid chromatography-quadrupole-time of flight tandem mass spectrometry (UPLC-Q-ToF-MS/MS) is a reliable analytical technique for elucidating compound structure, which makes it highly applicable for ontological analysis (Nastasi et al., 2023). In addition, UV–Vis spectroscopy-based assays and indicator strip testing can be used for the rapid quantitative and qualitative determination of target compounds and their classes (Appenteng et al., 2021; John et al., 2014).

Eight underutilized Traditional foods native to Australia were selected for analysis (Figure 1). Firstly, *Piper hederaceum* (native pepper) is akin to black pepper and grows natively along coastal New South Wales and Queensland (The Australasian Virtual Herbarium, 2024b). This spice is likened to *Piper nigrum*

and is a potential new crop with commercial prospects for indigenous Traditional Owners (Drew and Bailey, 2018). The pepper is flavourful when the fruit turns bright red or is dried to black. *Antidesma erostre* (Australian currants) includes varieties producing small, mildly sweet berries, with dark red to purple juice, historically, which grow throughout Northern Queensland and Northern Territory (The Australasian Virtual Herbarium, 2024a). *Syzygium fibrosum* (Satinash) produces fruits like small apples, with smooth, thin skin and crisp, juicy flesh. It grows wild in Northern Queensland and pockets along southeast Queensland (The Australasian Virtual Herbarium, 2024c). *Sterculia quadrifida* (Native peanut), also found in Southeast Asia, yields fruits resembling peanuts and has shown high antioxidant levels in various plant parts (Saragih and Siswadi, 2019). Its potential in combating breast cancer due to 8-hydroxydehydrodunnione has been reported in the literature (Rollando, 2018). Native peanut, traditionally consumed by Indigenous community groups such as the Mbabaram people from the Mareeba/Atherton Tablelands region, grows in large red seed pods, with an unpalatable black husk but a peanut-like edible portion. *Curcuma australasica* (Australian turmeric) has been reported to have high bioactivity and potential health benefits (Rajkumari & Sanatombi, 2017). It is similar to Indian turmeric, though paler when dried, and has been part of the diet of the Wurundjeri community in Victoria and community in New South Wales. *Diploglottis campbellii* (Australian tamarind), native to the Gumbaynggirr and Bundjalung regions in New South Wales, yields tart, acidic fruits with a sweet undertone, similar to unripe nectarines. *Diploglottis diphyllostegia* (Northern tamarind) bears a small, orange fruit known for its tart sourness and also was consumed by the Mbabaram people. Lastly, *Carissa lanceolata* (conker berry or colloquially known as 'Burra' berry) yields fleshy red berries with a sweet, tangy taste (Patel, 2013), traditionally eaten by the Iningai peoples of central Queensland.

This study proposed a systematic workflow to qualify cyanogen glycosides and quantify alkaloid content within the fruit, seeds, and tubers of these eight Traditional foods. Firstly, untargeted metabolomics via UPLC-Q-ToF-MS/MS was employed to qualify the presence of cyanogen glycoside and alkaloid compounds. Secondly, a combination of indicator and UV–Vis-based assays was used to further screen the Traditional food species for the presence of cyanogen glycosides and quantify the total content of alkaloids. Next, targeted metabolomics using a multiple reaction monitoring (MRM) method analysis was employed to quantify the different alkaloid compounds using analytical standards and database matching of the compound fragmentation patterns. This study further investigates the notion that Traditional Knowledge can be used as a reliable basis for food safety investigations of native foods. The methods reported in this study can be used to rapidly evaluate the expanding list of new, emerging, Australian Traditional food species.

Materials and methods

Materials

Traditional food samples, *Sisyrinchium fibrosum* and *S. quadrifida* were collected from Sevgen farm (GPS coordinates −26.44710, 152.88974) and samples of *Philodendron hederaceum* and *Antidesma erostre* from Widjutgrub Bush Food Nursery (GPS Coordinates −26.64148, 152.81429). *D. Australis* and *C. australis* were harvested from the bushfood garden at the School of Agriculture and Food Sustainability, University of Queensland, St. Lucia at (GPS coordinates −27.49553, 153.01071). *C. lanceolatta* was supplied by YACHATDAC (Iningai traditional land) located in Central Queensland, while *Syzygium aqueum* was supplied by our indigenous collaborator Gerry Turpin located in Watsonville, Queensland. Specific locations are undisclosed at the request of the traditional landowners. All samples were collected directly from plants and kept on ice in transit. All chemicals, organic solvents, and materials were supplied by Sigma Aldrich (St. Louis, MO, USA) and reagents were prepared freshly on the day of analysis.

Cyanogenic glycosides indicator test

Cyanogen glycoside test samples were prepared using a 500 mg fresh sample that was crushed and wetted with Milli-Q water. The method for qualifying the presence of cyanogenic glycoside was adapted from Appenteng et al. (2021). A 1 cm x 3 cm grade 1 Wattman filter paper strip (item number: 1001 110) was dipped in a 1.4% w/v picric acid solution that was diluted in a 2.5% w/v Na_2CO_3 solution. Strips were attached to the lid of a plastic screw-top specimen jar while still moist. A Bromocresol green (BCG) indicator solution (10^{-4} M) was prepared by heating 68 mg of BCG in 3 ml of 2 M Na_3PO_4 buffer (made to 4.77 pH). Lids containing the strip were fastened and hermetically sealed to specimen jars containing the fresh food samples. Positive and negative controls were made using *Prunus* seed and deionized water respectively. Specimens were inspected every 10 min until no further colour change was observed in the positive control and a total time of 40 min had elapsed. Samples were left 24 h to confirm no further colour change. Testing was conducted in triplicate both inter/intraday.

Sample preparation for alkaloid analysis

Samples were washed with milliQ water to remove dirt and stored at −80 °C before being analysed. Analytical samples were prepared by freeze-drying for 72 h in a Christ alpha 1–2 LD freeze drier (Martin Christ, Osterode, Germany) at 1.0 mPa. Dried sample matter was immersed in liquid nitrogen, ground in a Tissuelyser II ball and socket mill (Qiagen, Tokyo, Japan) at 30x1/s Hz for 30 s and seized through a 50 μm mesh. Sample (5 g) was twice extracted in 25 ml of 80% methanol/0.01% formic acid solution, sonicated (Soniclean 160TD, Mektronics, Australia) for 15 min at 100 Hz, vortexed for 30 min in the dark, and incubated at −20 °C for 2 h. Next, the solution was centrifuged (Thermo Fischer Scientific, Waltham, MA, USA) at 4500 *g* for 15 min to separate the insoluble plant matter. The supernatant for each sample was filtered through 0.45 μm syringe filters (AF3–3107-52: Phenomenex, Torrance, USA), transferred to 2 ml Eppendorf tubes, and vacuum evaporated at 40 °C in a JMS Christ AVC 2–18 CD vacuum evaporator (Martin Christ, Osterode, Germany) until complete dryness.

Total alkaloid content

The total alkaloid assay was adapted from John et al. (2014). Plant samples were prepared as described above. The sample was weighed to 5 g and then 25 ml of solvent (80% Methanol, 0.01% formic acid) was added. The samples were sonicated (Soniclean 160TD, Mektronics, Australia) for 15 min at 100 Hz, then vortexed for 30 min in the dark before being incubated at −20 °C for 2 h. Samples were centrifuged at 4500 *g* (Thermo Fischer Scientific, Waltham, MA, USA) for 15 min, and the supernatant was collected. The pellet was further extracted with another 25 ml of solvent. Sonication and vortex were repeated as above and the supernatants of the extraction were combined and made up to 50 mL. The extract was filtered through 0.45 μm syringe

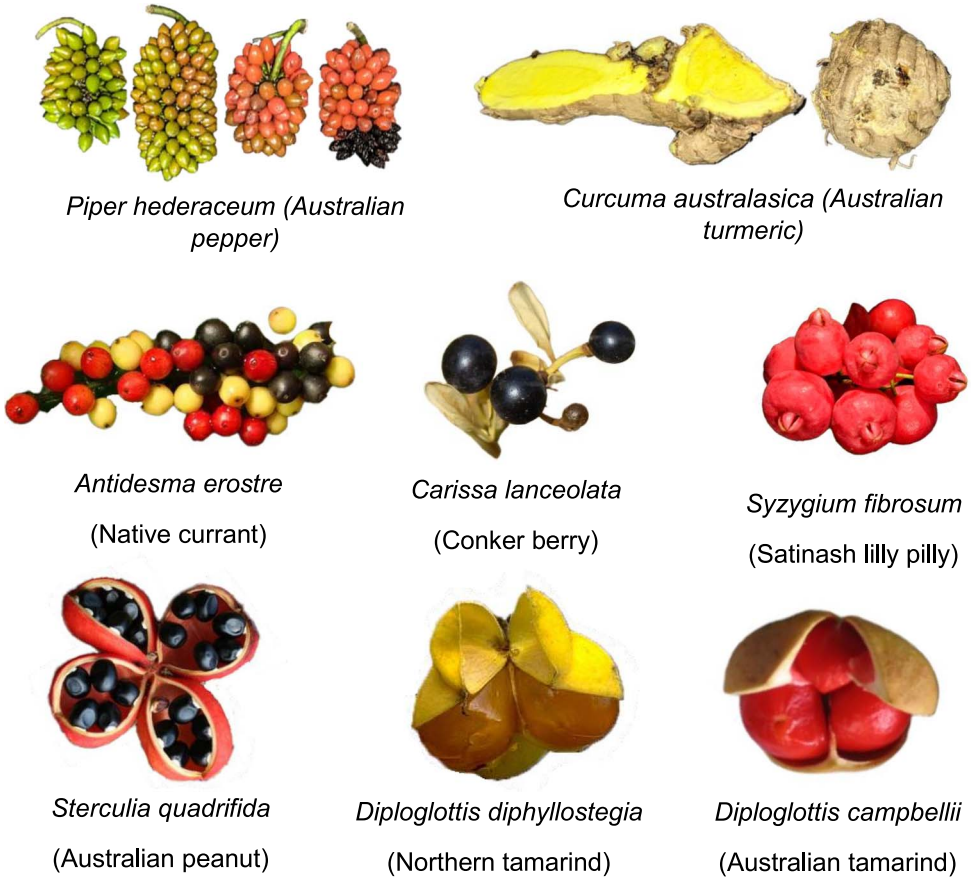


Figure 1. Images of eight underutilized Australian traditional foods selected for food safety analysis.

filters (AF3–3107-52: Phenomenex, Torrance, USA), and vacuum evaporated until dryness. The residue was suspended in 2 N HCl and filtered through 0.45 μm syringe filters (AF3–3107-52: Phenomenex, Torrance, USA). The filtered extract suspended in 2 M HCl was transferred into a 50 ml separatory funnel and 5 ml of BCG solution (prepared as above) was added. Funnels were shaken vigorously for 30 s and then 8 ml of chloroform was added. Funnels were shaken again for 30 s, and the chloroform extract was separated into 10 ml volumetric flasks and made to volume with chloroform. This process was repeated for all samples, atropine aliquots and positive (poppy seed) and negative (lavender) controls. Absorbance was measured spectrophotometrically in all extracts at 410 nm (Shimadzu UV1800, Shimadzu, Kyoto, Japan).

UPLC-Q-ToF-MS/MS analysis and instrument conditions

Samples were prepared according to section "UPLC-Q-ToF-MS/MS analysis and instrument conditions" and analysed *via* UPLC-Q-ToF-MS/MS. Samples (1 μl) were injected into an ExionLC AC system coupled to a Sciex X500B QToF mass spectrometer (AB SCIEX, Toronto, Canada) with an ESI operating in Data Independent Acquisition (DIA) mode (Nastasi et al., 2023). Analysis was conducted in both positive and negative ionisation modes. Metabolites were separated on a Waters ACQUITY UPLC BEH C18 column (130 Å, 1.7 μm, 2.1 mm × 50 mm, SKU: 186002350) (Milford, MA, USA) and the column oven temperature was held at 45 °C. Chromatography conditions were as followed: Solvent A: dH_2O + 1% Acetonitrile +0.1% Formic acid, Solvent B: 90% Acetonitrile +10% dH_2O + 0.1% Formic acid; flowrate: 0.40 ml min^{-1}; 0–0.20 min 5% B, 0.20–6.0 min 100% B, 6.00–6.10100% B, 6.10–7.90 min 100% B, 7.90–8.00 5% B, 8.00–10.00 min 5% B. Source settings were as followed: cur gas = 25 psi, GS1 = 50 psi, GS2 = 50 psi, CAD = 7, 500 °C. Ion spray voltage = 5500 V positive and − 4500 V negative mode. A ToF MS scan was performed across 100–1000 *m/z* (0.125 s) followed by DIA using 20 *m/z* isolation windows across 100–900 *m/z* (0.05 s) with CE = 35 ± 15 V. Positive and negative mode data was analysed using MS-Dial version 4.80 (Tsugawa et al., 2015). Selected alkaloid species were quantified with MRM in positive mode over 0–50 μM following the precursor fragment ion transitions as follows: atropine 290.181/124.109 and 290.181/93.067, trigonelline 138.055/65.043, 138.055/76.034, and 138.055/93.053, piperine 286.142/201.053 and 286.142/143.053.

Results

UPLC-Q-ToF-MS/MS based identification of alkaloid species

Untargeted metabolomic profiling was performed to identify species of alkaloids present in the traditional foods that may pose a risk to human health. Metabolites between 100 and 900 *m/z* were scanned for in positive and negative mode and 4002 potential features were identified using the MS-Dial public libraries for both positive and negative mode. After manual curation of the data set, removal of false positives and blank contamination, eight alkaloids were identified across the eight traditional food species, and three curcuminoids were present in the native turmeric sample. The annotated alkaloid species are reported in Table 2 according to the best-reported practices

suggested by Alseekh et al. (2021). Of these eight alkaloids, three were resolved with analytical standards (trigonelline, piperine, and atropine), two were putatively identified using their MS2 spectra (piperyline and corydine) and three were tentatively identified (desmotroposantin, cinchonine, and corynoxeine). The curcuminoids were identified using their MS2 spectra.

Annotation of the alkaloid species *via* their fragmentation pattern in positive ionization mode is described as follows. Trigonelline had a precursor ion of 138.055 *m/z* and produced product ions of 92.053 [M + H—COOH], 65.043 [M + H—$C_2H_4O_2$]. Piperine had a precursor ion of 286.142 *m/z* with product ions of 201.053 *m/z* [M + H—$C_5H_{10}N$] and 143.053 *m/z* [M + H—$C_7H_{12}NO_2^-$]. The Piperyline has a precursor ion of 272.125 *m/z*, product ions of 201.055, [M + H—C_4H_8N], and 171.044 *m/z* [M + H—$C_8H_{11}NO$]. Atropine precursor was determined at 290.181 *m/z*, and fragments were at 124.109 *m/z* [M + H—$C_9H_{10}O_3$], and 93.067 *m/z* [M + H—$C_{11}H_{19}NO_2$]. Corydine had a precursor ion of 342.173 *m/z*, and product ions of 311.121 *m/z* [M + H—CH_4O], and 296.101 *m/z* [M + H—C_2H_8N].

Analysis *via* UPLC-Q-ToF-MS/MS identified no other features in any sample that could present a toxicology concern, i.e., cyanogen glycosides. Trigonelline was present in all eight traditional food samples: Australian turmeric, native pepper, Australian tamarind, native currant, native peanut, satinash, northern tamarind, and conker berry. Piperine was identified in native pepper, native currant, satinash, and native peanut as well as the black pepper control sample, while Piperyline was present in the native pepper and black pepper. Corydine was present in native pepper. Cinchonine and Corynoxeine were present in the native turmeric. Desmotropo-santonin was present in native peanut. Atropine was identified in the native pepper. Three curcuminoids: curcumin, bisdemethoxycurcumin, and curcumenol were identified in the native turmeric and included for their contribution to the total alkaloid UV–Vis spectral result.

Cyanogen glycoside and UV–vis spectral total alkaloid content

The results from the cyanogenic glycosides indicator test and total alkaloids assay are displayed in Table 1. All samples returned a negative response for the cyanogen glycosides indicator assay except for the positive control. The lower limit of detection for the cyanogen indicator test is 1.59 μg cyanide equivalent (Appenteng et al., 2021). The total alkaloid content in the samples (without the negative and positive control) ranged from 210.11 ± 22.12 to 4504.57 ± 398.58 mg AE 100 g^{-1}. Notably, Australian turmeric and native pepper contained a higher alkaloid content than both the control samples which were black pepper (4382.62 ± 225.93 mg AE g^{-1}) and poppy seed (3993.90 ± 205.27 mg AE g^{-1}). Northern tamarind contained sixfold lower total alkaloids compared to Australian tamarind. Overall, majority of the traditional food samples had a lower alkaloid content compared to the positive controls.

Quantification of alkaloids in traditional food samples

A high-resolution Q-ToF-MRM method demonstrated effective quantitation of atropine, piperine, and trigonelline reference standards (Figure 2 and Table 3) in the eight traditional food samples, with the black pepper used as a control comparison. Native pepper was the sole atropine-containing sample which reflects the untargeted study result. The atropine content was detected below the limit of detection at 1.30 ± 0.60 mg 100 g^{-1}. Trigonelline was putatively matched in Australian turmeric, native pepper, Australian tamarind, native currant, native peanut, satinash, northern tamarind, conker berry and black pepper (control), with significantly high levels in the native currant and Australian tamarind (1107.34 ± 14.55 and 1039.84 ± 8.07 mg 100 g^{-1}, respectively). Piperine content was 6921.72 ± 391.75 and 4101.23 ± 412.84 mg 100 g^{-1} in native pepper and black pepper respectively, and piperyline was 2344.59 ± 429.02 and 1003.45 ± 3.45 mg 100 g^{-1} in native pepper and black pepper, respectively.

Discussion

This study aimed to evaluate the food safety of eight traditional foods that have been eaten for thousands of years by Indigenous Australians (Hay et al., 2022) (Figure 1). These foods included a range of nuts, fruits, tubers, and spices from arid and tropical biomes. The food safety of these species was investigated by qualifying and quantifying any alkaloid or cyanogenic glycoside compounds. Cyanogen glycosides were not found in any of the traditional food samples (Table 1); however, alkaloids were detected across all of the Traditional foods. The study by Burns et al. (2012) on the cyanide content of cassava food products in Australia and the current study both focus on the safety of food products containing cyanogenic compounds. However, Burns et al. highlight the dangers of high cyanide content in commercially available cassava products, with some samples exceeding safe consumption limits. Burns et al. (2012) used the picrate method to analyse the total cyanide content, finding concerning levels in cassava chips, where values as high as 262 ppm were recorded. In contrast, the current study did not detect cyanogen glycosides in the tested traditional Australian foods, suggesting they are safe in terms of cyanide presence. The methods used in the current study, such as UPLC-Q-ToF-MS/MS, offer a higher-resolution assessment of compounds, providing a more detailed chemical profile than the picrate method used in Burns' work. Despite the shared focus on food safety, the current study expands the toxin analysis to include alkaloids, providing a broader toxicological evaluation.

Untargeted metabolomic profiling of methanolic extracts identified a variety of alkaloids in high abundance across several species. Among the eight traditional foods analysed, eight alkaloids and three curcuminoid compounds were detected (Table 2). Therefore, the present work identified the need to quantify the total alkaloid content in these underutilised traditional foods, as scientists can use the presence of these compounds to rapidly assess the risk within traditional food species (Adamski et al., 2020; Schrenk et al., 2020). As such, the quantification *via* a UV–Vis Spectrophotometric assay adapted from John et al. (2014), and MRM quantification was performed. Previously, caffeine has been used as a reference standard for alkaloid quantification in benchtop spectrophotometric methods (Aniszewski, 2007). Given the varied nature of alkaloids (Adamski et al., 2020), quantitative methods should employ reference standards that represent toxic ontologies that are damaging to human health, such as tropane or pyrrolizidine alkaloids. Therefore, in the present investigation, atropine was used as a reference standard for UV–Vis spectroscopy after being identified in the untargeted metabolomic analysis.

The quantification of the total alkaloids *via* the UV–Vis Spectroscopy assay included positive control samples of poppy seed and black pepper for comparison (Table 1). The total alkaloids for poppy seed (3993.90 ± 205.27 mg AE 100 g−1) and black pepper (4812.3 ± 131.91 mg AE 100 g^{-1}) were within the range of previously reported content (Mani & Dhawan, 2011; Vieira

Table 1. Alkaloid content and cyanogen glycoside presence in Australian traditional food (bushfoods) species.

Pos/neg control	Common name	Scientific name	Spectrometric total alkaloids (mg AE 100 g^{-1})	UPLC-Q-ToF-MS/MS total alkaloids (mg AE 100 g^{-1})	Cyanogen glycosides
TA positive control	Black pepper	*Piper nigrum*	4812.30 ± 131.91[a]	5104.68 ± 416.29[b]	Negative
-	Australian turmeric	*Curcuma australasica*	4504.57 ± 398.58[a]	1065.63 ± 1.82[e]	Negative
-	Native pepper	*Piper hederaceum*	4382.62 ± 225.93[b]	9419.823 ± 8.07[a]	Negative
TA positive control	Poppy seed	*Papaver somniferum*	3993.90 ± 205.27[c]	*	Negative
-	Australian tamarind	*Diploglottis campbellii*	2751.52 ± 29.18[d]	1239.86 ± 8.07[c]	Negative
-	Native currant	*Antidesma erostre*	1257.62 ± 188.56[e]	1109.22 ± 14.55[d]	Negative
-	Native peanut	*Sterculia quadrifida*	1234.756 ± 201.47[e]	961.35 ± 16.73[f]	Negative
-	Satinash	*Syzygium fibrosum*	754.57 ± 45.73[f]	182.61 ± 17.25[h]	Negative
-	Northern tamarind	*Diploglottis diphyllostegia*	420.14 ± 15.87[g]	379.49 ± 1.34[g]	Negative
-	Conker berry	*Carissa lanceolata*	210.11 ± 22.12[h]	110.3 ± 11.67[i]	Negative
TA negative control	Lavender	*Lavandula angustifolia*	152.44 ± 35.24[i]	*	Negative
CG positive control	Apricot seed	*Prunus* seed	*	*	Positive

Note. TA = total alkaloids; CG = cyanogen glycosides; AE = atropine equivalents. Total alkaloid values are averaged (n = 3) with standard deviation. Superscript indicates significance within column *via* Tukey analysis ($p \leq .05$). *Lavandula angustifolia* was the negative control for total alkaloids, while *Prunus* seed gave a positive result in the indicator assay.
*Sample not tested. Lower limit of detection for the cyanogen glycosides indicator test is 1.59 μg cyanide equivalents.

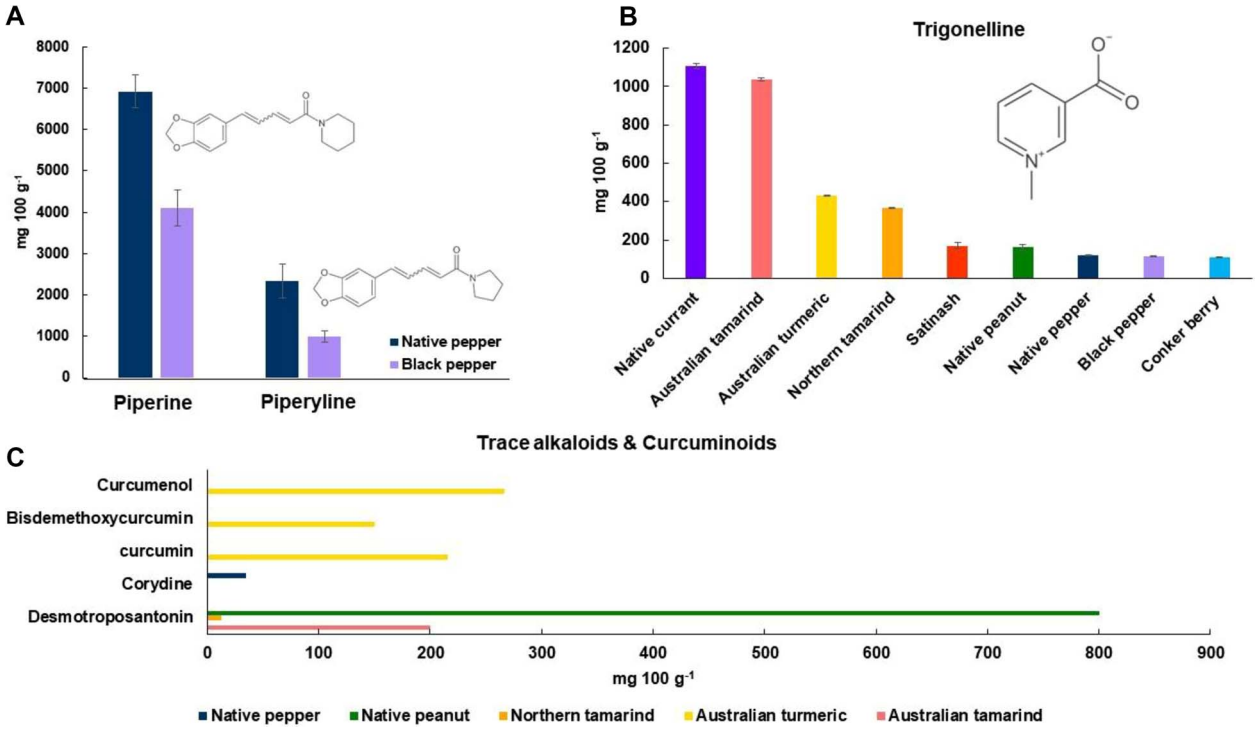


Figure 2. UPLC-Q-ToF-MS/MS MRM quantification results for eight traditional food species. (A) Piperine content of native pepper and black pepper. (B) Trigonelline content within eight traditional food species. (C) Corydine, desmotroposantonin content present in native pepper, native peanut, northern tamarind, Australian tamarind, and curcuminoids content in Australian turmeric. Trigonelline and piperine were quantified by use of their respective reference standards. All other alkaloids were presented and reported as trigonelline equivalents. Curcuminoids are reported as trigonelline equivalents.

et al., 2022). Australian turmeric and native pepper contained similar alkaloid content to black pepper and poppy seed per dry weight. Therefore, native pepper was identified as the most significant toxicity concern, because of its high total alkaloid content and presence of atropine. Moreover, Australian turmeric had 57% greater total alkaloid content than Indian turmeric (Cyril, 2023), and native pepper had 9% lower content compared to the black pepper control. Of the remaining six samples tested by the UV–Vis methodology, Australian tamarind, native currant and native peanut contained moderate alkaloid contents relative to the control species, while satinash, northern tamarind and conker berry were considered to be very low. The alkaloid analysis of lesser-known traditional foods is necessary to progress their commercial pathways because food safety is a primary concern. However, bench top assays are not a high-resolution technique and a better assessment of food safety can be achieved *via* UPLC-Q-ToF-MS/MS, where specific compounds can be individually quantified.

Quantification of the alkaloids was conducted with the use of an MRM approach *via* UPLC-Q-ToF-MS/MS. All spectral fragmentation reported in Table 2 is consistent with previous investigations for trigonelline (Chen et al., 2013), piperine (Kotte, Dubey, & Murali, 2014), piperyline (Wang et al., 2016), atropine (Marques et al., 2018), and corydine (Macedo et al., 2021). Atropine was used as a reference standard to assess the food safety of native pepper, alongside trigonelline and piperine, which were present in high

Table 2. Alkaloids and curcuminoids identified in traditional foods undergoing food safety analysis.

RT (min)	Putative ID metabolite	Metabolite class	Molecular formula	Adduct	ES (+) found m/z	ES (+) theoretical m/z	m/z error (Da)	MS/MS ES (+) Fragments	Ref. (ID)	ID
0.56	Trigonelline	Alkaloids and derivatives	$C_7H_7NO_2$	[M + H] +	138.055	138.14	0.085	138.055 $[M + H]^+$, 92.053 [M + H—COOH], 65.043 $[M + H—C_2H_4O_2]$	Standard	1
4.97	Piperine	Benzodioxoles	$C_{17}H_{19}NO_3$	[M + H] +	286.142	286.341	0.019	286.142 $[M + H]^+$, 201.053 $[M + H—C_5H_{10}N]$, 143.053 $[M + H—C_7H_{12}NO_2^-]$	Standard	1
4.57	Piperyline	Benzodioxoles	$C_{16}H_{17}NO_3$	[M + H] +	272.125	272.121	0.051	272.125 $[M + H]^+$, 201.055 $[M + H—C_4H_8N]$, 171.044 $[M + H—C_8H_{11}NO]$	636,537	2
4.24	Atropine	Tropane alkaloids	$C_{17}H_{23}NO_3$	[M + H] +	290.174	290.181	0.006	290.181 $[M + H]^+$, 124.109 $[M + H—C_9H_{10}O_3]$, 93.067 $[M + H—C_{11}H_{19}NO_2]$	Standard	1
2.67	Corydine	Aporphines	$C_{20}H_{23}NO_4$	[M + H] +	342.171	341.163	0.007	342.173 $[M + H]^+$, 311.121 $[M + H—CH_4O]$, 296.101 $[M + H—C_2H_8N]$	10,153	2
4.67	Desmotropo-santonin	Naphthofurans	$C_{15}H_{18}O_3$	[M + H] +	247.131	246.126	0.005	247.132 $[M + H]^+$	6,543,723	3
2.35	Cinchonine	Cinchona alkaloids	$C_{19}H_{22}N_2O$	[M + Na] +	317.156	294.173	0.002	294.391 $[M + Na]^+$	90,454	3
2.91	Curcumenol	Guaianes	$C_{15}H_{22}O_2$	[M + H] +	235.166	235.169	0.003	235.166 [M + H] +, 217.159 $[M + H - C_{15}H_{22}O]$, 133.101 $[M + H—C_{10}H_{18}]$	167,812	2
4.06	Curcumin	Curcuminoids	$C_{21}H_{20}O_6$	[M + Na] +	391.115	391.115	0.006	391.115 $[M + Na]^+$	969,516	3
4.76	Bisdemethoxy-curcumin	Curcuminoids	$C_{19}H_{16}O_4$	[M + H] +	309.108	309.109	0.013	309.108 [M + H] +, 225.089 $[M + H - C_{11}H_{10}O_3]$	5,315,472	2
6.26	Corynoxeine	Indolizidines	$C_{22}H_{26}N_2O_4$	[M + H] +	383.204	382.189	0.014	383.201 $[M + H]^+$	44,568,160	3

Note. RT = Retention time. Compound reporting is presented as best practice according to Alseekh (2021). Identification levels represented as 1: analytical standard, 2: putative match on high MS2 spectral library match (> 85%), 3: Tentative matches where spectra < 85% library match, where *in silico* model support annotation. All matches represent MS1 and MS2 spectra matches from two databases: MS Dial Positive database and Sciex Pos QTOF ESI database. ID is pub chem CID number.

concentrations in native pepper, native currant, and Australian turmeric.

Trigonelline was detected in all eight samples, with native currant (1107.34 ± 14.0 mg 100 g^{-1}) and Australian tamarind (1039.84 ± 8.1 mg 100 g^{-1}) showing the highest concentrations (Figure 2). These levels are substantially higher than other species, indicating that these fruits may be particularly rich sources of this bioactive compound. Trigonelline is synthesized from nicotinic acid, a breakdown product of pyridine nucleotides (Ashihara et al., 2015). Trigonelline is commonly found in high concentrations in foods consumed by humans, namely in coffee, which contains 1–3% dry weight for green coffee beans (seeds) (Ashihara et al., 2015), or 7.2 g kg^{-1} (Konstantinidis et al., 2023). Comparatively, both the native currant and Australian tamarind trigonelline contents were higher than the reported trigonelline content in coffee. Konstantinidis et al. (2023) highlighted that while no chronic exposure to trigonelline has been investigated, the risk posed by dietary coffee consumption is minimal. Furthermore, trigonelline is protective of hepatic induced-oxidative stress and to lower the risk of colon cancer, type II Diabetes and Alzheimer's disease (El Bairi et al., 2017). As such, the high trigonelline content in the native currant and Australian tamarind promotes these species for dietary inclusion.

Piperine is the main compound contributing to the 'pepper' flavour, which was detected in the black pepper control and the native pepper (Lee et al., 2021). Piperine is synthesized in plants from L-lysine, which undergoes several transformations to yield the piperidine ring. Several derivatives of piperine, often termed piperidines or piperidine alkaloids, share the basic piperidine ring structure but differ in their side chains or functional groups (Haq et al. 2021). The native pepper has a significantly higher concentration (6921.72 mg 100 g^{-1}) compared to black pepper (4101.23 mg 100 g^{-1}) ($p < .05$). Native pepper also contains more piperyline (2344.60 mg 100 g^{-1}) compared to black pepper (1003.45 mg 100 g^{-1}). The piperine content of *Piper* species typically ranges from 1% to 7%, where yield is highly subjective to extraction methods (Gigliarelli et al., 2017). The piperine content of the native pepper was greater than that of the black pepper, which is consistent with Vieira et al. (2022) who reported 48.8 ± 0.4 mg g^{-1}. As such, native pepper is shown here to be a good source of piperine, having upwards of 9% combined content of piperine and its derivatives. The toxicology of piperine has been reviewed by Haq et al. (2021), who summarized that studies assessing genotoxicity, immunotoxicity, and reproductive health found no clear evidence of toxic effects from piperine.. Moreover, the bioactivity of piperine is used in some medicines to increase drug bioavailability and modulate several transporter and enzyme activities (Tiwari et al., 2020; Haq et al., 2021).

The principal concern among the eight Traditional food species was the native pepper due to the putative annotation for atropine. As a tropane alkaloid, atropine is an antagonist of muscarinic acetylcholine receptors, which are integral to the functionality of the central nervous system (Lakstygal et al., 2018). In practice, atropine is used to treat motion sickness, and for ophthalmic effects (Lakstygal et al., 2018). However, atropine has also been identified to cause toxicity in mice models, where

Table 3. Calibration curve and validation parameters of alkaloid standards.

Compound	LoD (μM)	LoQ (μM)	Range (μM)	R^2	Equation
Trigonelline	3.407	10.324	1.56–50	0.997	y = 1479.6x + 690.48
Piperine	2.613	7.918	1.56–50	0.998	y = 5342.5x—5036.3
Atropine	3.985	12.075	1.56–50	0.995	y = 21,938x + 19,736

Note. LoD = Limit of detection; LoQ = Limit of quantitation. LoD = σ/S x 3.3, LoQ = σ/S x 10 where σ is the standard error and S is the gradient of the standard curve.

LD_{50} = 75 mg kg^{-1} induced paralysis of parasympathetically innervated organs (Wishart et al., 2018; Wu et al., 2019). Atropine was detected in native currant at 1.3 ± 0.60 mg 100 g^{-1}; however, the quantitation was below the limit of detection. The limit of detection for atropine (Table 3) is below the previously reported toxicity level of atropine (Wishart et al., 2018). As such, this study determines native pepper has no atropine toxicity concern for human consumption within the detectable range using the methodology in this study.

Desmotroposantonin was identified in the native peanut (800 mg 100 g^{-1}), though it is not as widely studied as other alkaloids like piperine or atropine. However, it is a sesquiterpene lactone, which has been suggested as being underutilized for its bioactive and pharmaceutical potential (Moujir et al. 2020; Matos et al., 2021). While the specific structure of desmotroposantonin is less documented in the literature, it is likely a positional isomer of α-Santonin (Yang et al., 2024). Being a less-studied derivative, there is less information available about its stability, metabolism, or toxicity. However, as a sesquiterpene lactone derivative, it may share some common pathways of degradation or transformation in biological systems, though these would depend on the exact structural differences.

All alkaloid compounds identified are considered safe in naturally occurring quantities, aside from atropine, which was identified in the native pepper. The alkaloids present mainly consisted of trigonelline and piperine. The results of this study have indicated that the traditional knowledge surrounding the safe consumption of the eight traditional foods is a reliable basis for modern food safety assertion. However, follow-up analysis *via* high-resolution mass spectrometry-based methods is highly recommended to validate assertions made from traditional knowledge. Such traditional knowledge provides a valuable asset in facilitating the exploration of new food crops. There was a precedent for overconfidence in the food safety of a traditional food source based on traditional knowledge (Burns et al. 2012). A 2008 survey conducted by the New South Wales government revealed that cassava chips contained higher-than-expected levels of cyanogenic glycosides. This led to a product recall and the introduction of a new safety standard (NSW Food Authority, n.d.). Currently, traditional food status can be achieved by way of confirming traditional knowledge for food use by an indigenous elder or community leader. The current system is flawed in such cases as the cassava chips case, and rapid toxicology workflows are key to ensuring the safe delivery of newly industrialized traditional foods. Recently, Williams et al., (2023) highlighted the need to reform existing food regulatory frameworks to better incorporate cultural knowledge and histories. It advocates for the use of cultural authorities in food safety assessments, as well as allowing room for traditional knowledge to play a more significant role in regulatory decision-making. This shift would help address regulatory hurdles currently impeding indigenous businesses from accessing broader markets both in Australia and globally. Proposed solutions include creating new processes within regulatory frameworks that respect traditional practices while also ensuring public safety through modern food safety data.

The presented workflow is not without limitations and requires further refinement. The MRM quantitative method had several discrepancies compared to the spectrophotometric quantitative method, chiefly seen in the Australian turmeric which showed a 4-fold lower alkaloid content in the MRM quantification. In this case, the differences in the spectrophotometric results for the alkaloid content of Australian turmeric may stem from two potential factors: the absence of reference standards for identifying and quantifying alkaloids in the chromatogram and the lack of specificity in the UV–Vis method. Conversely, the total alkaloid content in the black pepper control was relatively consistent between the methods. These results indicate that future research needs to employ specific alkaloid ontologies when using reference standards for UV–Vis based protocols.

Conclusion

In conclusion, this study presents a systematic workflow for estimating alkaloid and cyanogenic glycosides in traditional foods, and subsequent quantification of targeted alkaloids. All eight Traditional foods contained no detectable toxic cyanogenic compounds based on the library databases used. Atropine was detected in the untargeted metabolic profile of native pepper but was shown to be of a trace amount when quantified. Therefore, each species is deemed nontoxic regarding cyanogenic glycosides and toxic alkaloids. These findings are consistent with the food safety of traditional foods and the traditional knowledge of indigenous Australians, from whom some of these species were sourced. While more comprehensive toxicology is likely required to secure food safety certification for newly acquired traditional foods, the present workflow allows for a rapid assertion of food safety in these wild harvested species and endorses continued investigation toward commercialization. This methodology is significant in its relatively inexpensive and timely determination of two primary food toxicity categories; however, the estimation of total alkaloids should be conducted with the most appropriate reference standard for accurate quantitation. The main benefit to the presented workflow is in a two-factor estimation of total alkaloids and cyanogen glycoside content using wet chemical techniques and chromatography. Such methods provide an economical and relatively accurate assessment of alkaloid and cyanogen toxicity risks. Follow-up metabolomics is however the most helpful in determining what compounds are expected to be present before finally applying a targeted approach for features that are of interest or concern.

Data availability

The data supporting this study are not publicly available due to confidentiality agreements established with the Traditional Owners. Access to the data is restricted under a non-disclosure agreement to protect the intellectual and cultural property of the Traditional Owners. Researchers seeking further information or

collaboration should contact the first author by email to discuss potential access by the terms set by the Traditional Owners.

Author contributions

Thomas Hay (Conceptualization [lead], Data curation [equal], Formal analysis [lead], Investigation [lead], Methodology [lead], Visualization [equal], Writing - original draft [lead], Writing - review & editing [equal]), Joseph Nastasi (Data curation [equal], Formal analysis [equal], Supervision [equal], Writing - review & editing [equal]), Gerry Turpin (Resources [equal], Writing - review & editing-Supporting), Dale Chapman (Writing - review & editing-Supporting), Bronwyn Fredricks (Writing - review & editing [equal]), Melissa Anne Fitzgerald (Project administration [equal], Supervision [equal], Writing - review & editing [equal]), Suzanne Thompson (Resources [equal]).

Funding

This study is funded by the Australian Research Council 20/21 Discovery grant: 'A Deadly Solution: Towards an Indigenous-led bush food industry'. GA ID: GA141113.

Conflicts of interest

The authors declare no conflicts of interest related to the content or publication of this article. All data, analyses, and interpretations presented herein were conducted independently, without influence from any personal, financial, or professional interests.

Acknowledgements

The authors acknowledge the traditional owners of the species and lands on which we work, the many Indigenous peoples of Australia. We are truly grateful to work in partnership in such endeavours and pay our respects to past and present elders. Specifically, we would like to thank Gerry Turpin, Cherry Turpin, Valmai Turpin, Suzanne Thompson, Dale Chapman, and Professor Bronwyn Fredericks for sharing their traditional knowledge and guidance when sourcing and investigating Australian genetic resources, along with advice on the research process, including the ethics, and methodology. Lastly, thank you to Amanda Nouwens and Peter Josh from the School of Chemistry and Molecular Biosciences, Mass Spectrometry Facility, University of Queensland for their contribution for their assistance with instrument operation and method development.

References

Adamski, Z., Blythe, L. L., Milella, L., & Bufo, S. A. (2020). Biological activities of alkaloids: From toxicology to pharmacology. *Toxins*, *12*, 210.

Alseekh, S., Aharoni, A., Brotman, Y., Contrepois, K., D'Auria, J., Ewald, J., & Fernie, A. R. (2021). Mass spectrometry-based metabolomics: A guide for annotation, quantification and best reporting practices. *Nature Methods*, *18*, 747–756. https://doi.org/10.1038/s41592-021-01197-1.

Aniszewski, T. (2007). *Alkaloids-secrets of life:: Aklaloid chemistry, biological significance, applications and ecological role*. Amsterdam, Netherlands: Elsevier.

Appenteng, M. K., Krueger, R., Johnson, M. C., Ingold, H., Bell, R., Thomas, A. L., & Greenlief, C. M. (2021). Cyanogenic glycoside analysis in American elderberry. *Molecules*, *26*, 1384. https://www.mdpi.com/1420-3049/26/5/1384. https://doi.org/10.3390/molecules26051384.

Ashihara, H., Ludwig, I. A., Katahira, R., & Crozier, A. (2015). Trigonelline and related nicotinic acid metabolites: Occurrence, biosynthesis, taxonomic considerations, and their roles in planta and in human health. *Phytochemistry Reviews*, *14*, 765–798. https://doi.org/10.1007/s11101-014-9375-z.

The Australasian Virtual Herbarium (2024a). *Antidesma spp* Retrieved 18/06/2024 from https://avh.ala.org.au/occurrences/search?taxa=Antidesma.

The Australasian Virtual Herbarium (2024b). *Piper hederaceum*. The Australasian Virtual Herbarium Retrieved 18/06 from https://avh.ala.org.au/occurrences/search?taxa=piper+hederaceum.

The Australasian Virtual Herbarium (2024c). *Syzygium fibrosum* Retrieved 18/06/2024 from https://avh.ala.org.au/occurrences/search?taxa=Syzygium+fibrosum.

Bessaire, T., Ernest, M., Christinat, N., Carreres, B., Panchaud, A., & Badoud, F. (2021). High resolution mass spectrometry workflow for the analysis of food contaminants: Application to plant toxins, mycotoxins and phytoestrogens in plant-based ingredients. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment*, *38*, 978–996. https://doi.org/10.1080/19440049.2021.1902575.

Bharucha, Z., & Pretty, J. (2010). The roles and values of wild foods in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 2913–2926. https://doi.org/10.1098/rstb.2010.0123.

Bolarinwa, I. F., Oke, M. O., Olaniyan, S. A., & Ajala, A. S. (2016). A review of cyanogenic glycosides in edible plants. *Toxicology-New Aspects to This Scientific Conundrum*, 179–191. https://doi.org/10.5772/64886.

Burns, A. E., Bradbury, J. H., Cavagnaro, T. R., & Gleadow, R. M. (2012). Total cyanide content of cassava food products in Australia. *Journal of Food Composition and Analysis*, *25*, 79–82. https://doi.org/10.1016/j.jfca.2011.06.005.

Chen, W., Gong, L., Guo, Z., Wang, W., Zhang, H., Liu, X., & Luo, J. (2013). A novel integrated method for large-scale detection, identification, and quantification of widely targeted metabolites: Application in the study of rice metabolomics. *Molecular Plant*, *6*, 1769–1780. https://doi.org/10.1093/mp/sst080.

Cyril, O. (2023). Nutrient and phytochemical compositions of aqueous extracts of black pepper (Piper guineense) seed, turmeric (Curcuma longa) and ginger (Zingiber officinale) rhizome. *Journal of Home Economics Research*, 107–108.

Drew, R., & Bailey, R. (2018). Australian tropical fruits: A source of distinctive flavours. *II international symposium on beverage crops Acta Horticulturae*, *1274*, 41–56. https://doi.org/10.17660/ActaHortic.2020.1274.5.

El Bairi, K., Ouzir, M., Agnieszka, N., & Khalki, L. (2017). Anticancer potential of Trigonella foenum graecum: Cellular and molecular targets. *Biomedicine & Pharmacotherapy*, *90*, 479–491. https://doi.org/10.1016/j.biopha.2017.03.071.

Frison, E. A., Smith, I. F., Johns, T., Cherfas, J., & Eyzaguirre, P. B. (2006). Agricultural biodiversity, nutrition, and health: Making a difference to hunger and nutrition in the developing world. *Food and Nutrition Bulletin*, *27*, 167–179. https://doi.org/10.1177/156482650602700208.

Gigliarelli, G., Pagiotti, R., Persia, D., & Marcotullio, M. C. (2017). Optimisation of a Naviglio-assisted extraction followed by determination of piperine content in Piper longum extracts. *Natural Product Research*, *31*, 214–217. https://doi.org/10.1080/14786419.2016.1217209.

Haq, I. U., Imran, M., Nadeem, M., Tufail, T., Gondal, T. A., & Mubarak, M. S. (2021). Piperine: A review of its biological effects. *Phytotherapy Research*, 35, 680–700. https://doi.org/10.1002/ptr.6872.

Hay, T., Prakash, S., Daygon, V. D., & Fitzgerald, M. (2022). Review of edible Australian flora for colour and flavour additives: Appraisal of suitability and ethicality for bushfoods as natural additives to facilitate new industry growth. *Trends in Food Science & Technology*, 129, 74–87. https://doi.org/10.1016/j.tifs.2022.09.003.

Hay, T. O., Kontogiorgos, V., Thompson, S., Nastasi, J. R., & Fitzgerald, M. (2024). A new hydrocolloid to rival gum Arabic: Characterisation of a traditional food gum from Australian Acacia cambagei. *Food Hydrocolloids*, 153, 110003. https://doi.org/10.1016/j.foodhyd.2024.110003.

Hidalgo-Mora, J. J., García-Vigara, A., Sánchez-Sánchez, M. L., García-Pérez, M.-Á., Tarín, J., & Cano, A. (2020). The Mediterranean diet: A historical perspective on food for health. *Maturitas*, 132, 65–69. https://doi.org/10.1016/j.maturitas.2019.12.002.

John, B., Sulaiman, C., George, S., & Reddy, V. (2014). Spectrophotometric estimation of total alkaloids in selected Justicia species. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6, 647–648.

Konstantinidis, N., Franke, H., Schwarz, S., & Lachenmeier, D. W. (2023). Risk assessment of trigonelline in coffee and coffee by-products. *Molecules*, 28, 3460. https://doi.org/10.3390/molecules28083460.

Kotte, S. C. B., Dubey, P., & Murali, P. (2014). Identification and characterization of stress degradation products of piperine and profiling of a black pepper (Piper nigrum L.) extract using LC/Q-TOF-dual ESI-MS. *Analytical Methods*, 6, 8022–8029. https://doi.org/10.1039/C4AY01088D.

Lakstygal, A. M., Kolesnikova, T. O., Khatsko, S. L., Zabegalov, K. N., Volgin, A. D., Demin, K. A., & Kalueff, A. V. (2018). Dark classics in chemical neuroscience: Atropine, scopolamine, and other anticholinergic deliriant hallucinogens. *ACS Chemical Neuroscience*, 10, 2144–2159.

Lee, J. G., Kim, A. Y., Kim, D. W., & Kim, J. Y. (2021). Determination and risk characterisation of bio-active piperine in black pepper and selected food containing black pepper consumed in Korea. *Food Science and Biotechnology*, 30, 209–215. https://doi.org/10.1007/s10068-020-00860-1.

Lopes, C. V. A., Mihrshahi, S., Ronto, R., & Hunter, J. (2023). Aboriginal food practices and Australian native plant-based foods: A step toward sustainable food systems. *Sustainability*, 15, 11569. https://doi.org/10.3390/su151511569.

Macedo, A. L., Boaretto, A. G., Silva, A. N. D., Maia, D. S., Siqueira, J. M. D., Silva, D. B., & Carollo, C. A. (2021). Evaluation of the effect of Brazilian savanna (Cerrado) seasons in flavonoids and alkaloids accumulation: The case of Duguetia furfuracea. *Journal of the Brazilian Chemical Society*, 32, 1840–1850.

Madariaga-Mazon, A., Hernandez-Alvarado, R. B., Noriega-Colima, K. O., Osnaya-Hernandez, A., & Martinez-Mayorga, K. (2019). Toxicity of secondary metabolites. *Physical Sciences Reviews*, 4, 11. https://doi.org/10.1515/psr-2018-0116.

Mani, D., & Dhawan, S. S. (2014). Scientific basis of therapeutic uses of opium poppy (Papaver somniferum) in Ayurveda. *Acta Horticulturae*, 1036, 175–180. https://doi.org/10.17660/ActaHortic.2014.1036.20.

Marques, G. L. M., Siqueira, A. A., Minassa, V. S., Peres, M. D., Pelição, F. S., & Sampaio, K. N. (2018). Method validation for simultaneous determination of atropine, pralidoxime and 12 organophosphorus compounds in blood samples by means of high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). *Journal of Chromatography B*, 1097-1098, 44–53. https://doi.org/10.1016/j.jchromb.2018.09.006.

Matos, M. S., Anastácio, J. D., & Nunes dos Santos, C. (2021). Sesquiterpene lactones: Promising natural compounds to fight inflammation. *Pharmaceutics*, 13, 991. https://doi.org/10.3390/pharmaceutics13070991.

Moujir, L., Callies, O., Sousa, P. M., Sharopov, F., & Seca, A. M. (2020). Applications of sesquiterpene lactones: A review of some potential success cases. *Applied Sciences*, 10, 3001. https://doi.org/10.3390/app10093001.

Nastasi, J. R., Daygon, V. D., Kontogiorgos, V., & Fitzgerald, M. A. (2023). Qualitative analysis of polyphenols in glycerol plant extracts using untargeted metabolomics. *Metabolites*, 13, 566. https://doi.org/10.3390/metabo13040566.

Patel, S. (2013). Food, pharmaceutical and industrial potential of Carissa genus: An overview. *Reviews in Environmental Science and Bio/Technology*, 12, 201–208. https://doi.org/10.1007/s11157-012-9306-7.

Rajkumari, S., & Sanatombi, K. (2017). Nutritional value, phytochemical composition, and biological activities of edible Curcuma species: A review. *International Journal of Food Properties*, 20, S2668–S2687.

Rollando, R. (2018). Hedyotis corymbosa L. and Sterculia quadrifida R.Br Ethanolic extract enhances Cisplatin's cytotoxicity on T47D breast cancer cells through cell cycle modulation. *The Journal of Pure and Applied Chemistry Research*, 7, 160–172.

Saragih, G. S., & Siswadi, S. (2019). Antioxidant activity of plant parts extracts from Sterculia quadrifida R. Br. *Asian Journal of Pharmeceutical and Clinical Research*, 12, 143–148. https://doi.org/10.22159/ajpcr.2019.v12i7.33261.

Schrenk, D., Gao, L., Lin, G., Mahony, C., Mulder, P. P., Peijnenburg, A., Pfuhler, S., Rietjens, I. M. C. M., Rutz, L., These, A., & Steinhoff, B. (2020). Pyrrolizidine alkaloids in food and phytomedicine: Occurrence, exposure, toxicity, mechanisms, and risk assessment-a review. *Food and Chemical Toxicology*, 136, 111107. https://doi.org/10.1016/j.fct.2019.111107.

Tiwari, A., Mahadik, K. R., & Gabhe, S. Y. (2020). Piperine: A comprehensive review of methods of isolation, purification, and biological properties. *Medicine in Drug Discovery*, 7, 100027.

Tong, S., Bambrick, H., Beggs, P. J., Chen, L., Hu, Y., Ma, W., Steffen, W., & Tan, J. (2022). Current and future threats to human health in the Anthropocene. *Environment International*, 158, 106892. https://doi.org/10.1016/j.envint.2021.106892.

Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., Kanazawa, M., VanderGheynst, J., Fiehn, O., & Arita, M. (2015). MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nature Methods*, 12, 523–526. https://doi.org/10.1038/nmeth.3393.

Vieira, L. V., Juvenato, M. E. M., Krause, M., Heringer, O. A., Ribeiro, J. S., Brandão, G. P., Kuster, R. M., & Carneiro, M. T. W. (2022). The effects of drying methods and harvest season on piperine, essential oil composition, and multi-elemental composition of black pepper. *Food Chemistry*, 390, 133148. https://doi.org/10.1016/j.foodchem.2022.133148.

Wang, M., Carver, J. J., Phelan, V. V., Sanchez, L. M., Garg, N., Peng, Y., & Luzzatto-Knaan, T. (2016). Sharing and community curation of mass spectrometry data with global natural products social molecular networking. *Nature Biotechnology*, 34, 828–837. https://doi.org/10.1038/nbt.3597.

Williams, L. B., Jones, M., & Wright, P. F. (2023). Decolonising food regulatory frameworks: Importance of recognising traditional culture when assessing dietary safety of traditional foods. *Proceedings of the Nutrition Society*, 82, 454–467. https://doi.org/10.1017/S0029665123003026.

Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., & Sayeeda, Z. (2018). DrugBank 5.0: A major update to the DrugBank database for 2018. *Nucleic Acids Research*, 46, D1074–D1082. https://doi.org/10.1093/nar/gkx1037.

World Health Organization (WHO). (2018). *Natural toxins in food*. Geneva, Switzerland: World Health Organization. https://www.who.int/news-room/fact-sheets/detail/natural-toxins-in-food. https://doi.org/10.14252/foodsafetyfscj.2018006s.

Wu, P.-C., Chuang, M.-N., Choi, J., Chen, H., Wu, G., Ohno-Matsui, K., & Cheung, C. M. G. (2019). Update in myopia and treatment strategy of atropine use in myopia control. *Eye*, 33, 3–13. https://doi.org/10.1038/s41433-018-0139-7.

Yang, R., Xue, Z., Zhang, M., Li, X., & Guo, Y. (2024). Non-food natural sesquiterpene lactone α-santonin: Chemistry and biology. *Industrial Crops and Products*, 220, 119169. https://doi.org/10.1016/j.indcrop.2024.119169.

International Journal of Food Science and Technology, 2025, **60(1)**, vvae013
https://doi.org/10.1093/ijfood/vvae013
Original Article