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Bitterness and Toxicity in Wild Yam (*Dioscorea* spp.) Tubers of Nepal

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Abstract. Wild yams make a significant contribution to diets of tribal people in Nepal. However, these wild tubers are unpalatable, taste bitter, produce inflammation and show occasional toxicity. Four wild yam species, which are eaten after primary treatment by Nepali aborigines, were analyzed for bitter and toxic principles. Bitter components were identified as furanoid norditerpenes (diosbulbins A and B). Diosbulbins A and B were found in the range of 0.023–0.046 and 0.151–0.442 g kg^{-1}, respectively. Results demonstrated that diosbulbin B, with an average value of 0.314 g kg^{-1}, was the principal bitter compound as compared to diosbulbin A (0.037 g kg^{-1}). The toxic alkaloid, dioscorine and histamine (an allergen) were not detected in these tubers, whereas cyanogens (as HCN equivalent) content were found ranging from 3.2 to 6.0 ppm. Our results revealed that Nepali wild yam tubers are not toxic varieties, as they do not contain either toxic dioscorine or histamine and cyanogens contents were satisfactorily below the safety limits. The inflammation and occasional toxicity observed could possibly be due to the presence of high level of oxalate in these tubers. Domestic cooking methods were found to be very efficient in removing bitterness, thus making the bitter yams palatable.

Key words: Bitterness, Cyanogens, Dioscorea, Diosbulbins, Toxicity, Wild yams

Introduction

Yams belong to Dioscoreacea family. They are herbaceous plants with twine. Approximately 600 *Dioscorea* species are eaten in various parts of the world [1]. Yams, the edible starchy tubers, are of cultural, economic and nutritional importance in the tropical and subtropical regions of the world [2]. In fact they are one of the principal sources of food and nutrient energy for many people in the tropics. World's estimated yam production in 2003 was 39,643,170 tones [3].

Yam has been suggested to have nutritional superiority when compared with other tropical root crops. They are reported as good sources of essential dietary nutrients [4–12]. Study reports have also pointed out that a few yam species contain some toxic compounds and can impart serious health complications [7, 13–16]. Some species of wild yams, particularly wild forms, are toxic and/or unpalatable, taste bitter and cause vomiting and diarrhea when large amount are ingested without proper processing or if eaten raw [17].

A toxic principle in some yam species has been reported as dioscorine, a toxic alkaloid [16–18]. Dioscorine triggers the fatal paralysis of the nervous system when a fragment of the tuber weighing 100 g is ingested [2]. Similarly, histamine was reported as the principal allergen, causing mild inflammation and itching, in some plants of Dioscoreaceae family [19]. On the other hand, the bitter substances in some yam species have been reported as furanoid-norditerpene groups of compounds [20–22]. The bitterness and toxicity of many yam species may be caused by high level of saponins. Such is the case with *Dioscorea tokoro* in Japan, in which saponin is responsible for its bitterness [17, 23].

Nepal has also been included in the potential regions for collection and consumption of yam [24]. Unlike other common tubers such as potatoes, cassava and sweet potatoes, yams are still in its wild forms and are not farmed in Nepal. These wild yams make a significant contribution in the diets of the tribal people (Chepang, Tharu, Manjhi, Bantar, Danuwar, Tamang etc.) of Nepal, particularly in rural areas where they are freely available [25]. The tubers were found with a high amount of protein, a good proportion of essential amino acids and appeared as a fairly good source of many dietary minerals [5]. However, their wider utilization is limited due to the presence of some toxic and antinutritional factors. Historical reports say Nepali wild yams taste bitter, show mild inflammation and itching on skin contact and cause some other toxic effects, if consumed without proper processing. Raw as well as cooked tubers are sometimes bitter, and the bitterness may be strong enough to render the tuber unpalatable. Ingestion of large amount of wild tubers has also been reported to cause caustic effects, irritation to mouth, throat or intestinal tract and absorptive poisoning. The local people prepare (Figure 1) the tubers before consumption to make them palatable.

Since no prior analyses of the bitter and toxic compounds of Nepali wild yam tubers have been carried out, the aim of the following study was to investigate on the bitter and toxic principles in wild yam tubers of Nepal.

Materials and Methods

Samples

Four varieties of wild yams (*Dioscorea bulbifra, D. versicolor, D. deltoidea and D. triphylla*), that are common as foods, were used for this study. Yam tubers were collected

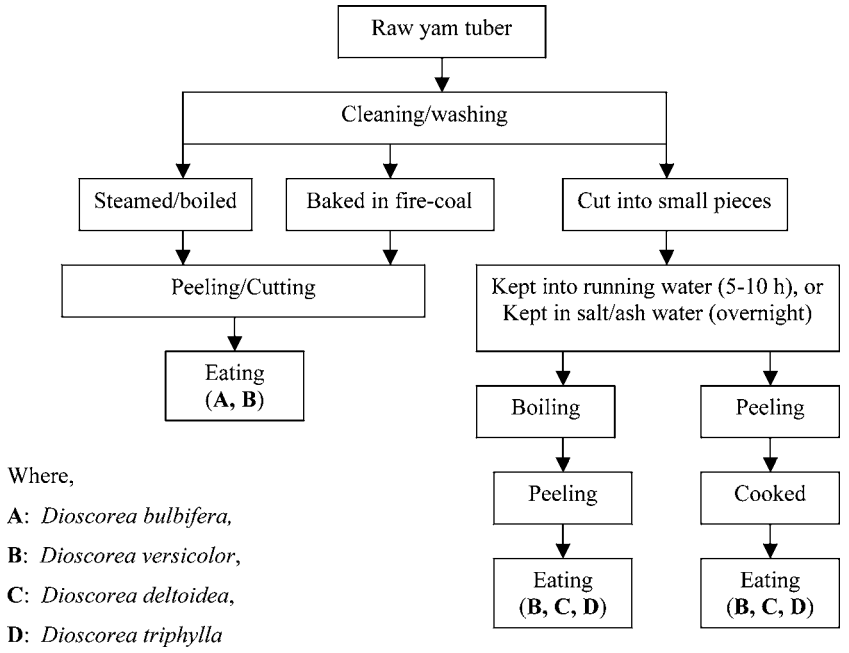

Figure 1. Consumption practices of wild yam in Nepal [39].

with the help of aboriginal people from Narayani Zone (mid-central region) of Nepal. Samples were brought to this laboratory and stored in a domestic refrigerator until analysis was done. Fresh peeled tubers were used for assay of bitterness and toxicity. All other chemicals and reagents used were of analytical grade and purchased from Wako Pure Chemicals Industries Ltd., Japan.

Methods

Extraction and Identification of Bitter Principles. Bitter compounds were extracted as described by Telek et al. [22]. One hundred grams of peeled and sliced yam tubers were extracted in an electric blender with 125 ml of methanol for 10 min. The slurry was centrifuged and clear supernatant was collected in a 500 ml flask. The sediments were washed back to the blender with 100 ml of methanol and re-extracted. After the third extraction, the pooled solution was passed through a polyamide column of 1.25 cm diameter and 15 cm length (Polyclar AT, GAF Corp., New York). The column impeded the passage of phenolic components. It was washed with methanol until the dark elute reached the lower tip of the column. Thus, slightly light yellow solution was obtained. The solution was dried over Na_2SO_4, filtered and concentrated in a rotary evaporator to about 1 ml.

The concentrated methanolic extract was spotted on a 20 $\times$ 20 cm TLC plate (Silica gel G, 60 F_{254}, 0.5 mm thickness, Merck). The compounds were separated by an ascending method with a solvent mixture of ethyl acetate:cyclohexane (96:4). After the plates were air-dried, one side (vertically) was cut off and sprayed with anisaldehyde reagent (1% anisaldehyde in glacial acetic acid containing 2% concentrated sulfuric acid). The sprayed plates were then heated to 105 °C for 10 min and examined for spots. The R_f values of different compounds separated on TLC were recorded.

The spots at the sides were used as guides to remove the silica gel containing the separated substances. The gel was scraped off from the plates and eluted with hot methanol. The solution was then concentrated to about 5 ml. Strips of filter paper were immersed in this solution and dried. These papers were tasted (sensory evaluation) for bitterness. Solutions tasted bitter were further subjected to mass spectrometry (MS) and NMR analysis. Field desorption (FD) mass spectra were obtained with a JEOL JMS-SX102A mass spectrometer and ^{1}H-NMR spectra were observed by using a Bruker AMX-500 instrument using pyridine-d_5 as a solvent. Chemical shifts were calculated from the residual solvent signal of δ7.19 ppm.

Quantification of Bitter Compounds Using HPLC. The bitter compounds (diosbulbins) were quantified by the following HPLC method developed in our laboratory. The TLC isolated substances were made to about 500 μl and this solution was filtered through a 0.45 μm millipore filter (Nihon Millipore Kogyo, Japan) before injection. HPLC analysis of the extracted sample was carried out on 250 $\times$ 6.0 mm Inertsil SIL 150A-5 column (GL Sciences, Inc., Tokyo, Japan) using hexane:2-propanol (1:9) as mobile phase at a flow rate of 1 ml/min and UV detector operating at 315 nm. Normalized peak area method was used for calculating the bitter compounds and results were expressed as g kg^{-1} fresh weight.

Table 1. Cooking treatments (boiling, pressure cooking and baking) given to yam tubers

Cooking method	Description
Boiling	Water was added to the pieces cut at the ratio of 1:1 (w/w) and cooked in a closed stainless steel vessel for exactly 30 min. Water was discarded after boiling.
Pressure cooking	Cut pieces were cooked in a pressure cooker (Hawkins, India) for exactly 15 min.
Baking	Cut pieces were wrapped in aluminum foil and baked in a hot air circulation oven at 180 °C for 45 min.

Effect of Cooking on Bitter Principles. Yam tubers were washed free of dirt, peeled, cut into pieces of about 50 g and given three different cooking treatments (Table 1), boiling, pressure cooking and baking. After cooking, each sample was extracted and investigated for diosbulbins as mentioned above.

Extraction and Identification of Toxic Alkaloid—Dioscorine. An extraction process described by Leete and Pinder [26] was used with a slight modification. Approximately 40 g of peeled and sliced yam tuber was extracted with 200 ml of 0.5 N HCl in an electric blender. After standing for 2 days, the mixture was filtered and made alkaline (pH 10–11) with K_2CO_3 and extracted with three portion (600–200 ml × 3) of ether using a separating funnel. All the extracts were combined and dried overnight with Na_2SO_4. Dried extract was filtered and concentrated under reduced pressure to a final volume of about 5 ml.

The concentrated extract was spotted on a 20 × 20 cm TLC plate (Silica gel G, 60 F_{254}, 0.5 mm thickness, Merck). The compounds were separated by an ascending method with a solvent mixture of chloroform:ethanol:ammonia (100:10:0.5). The plates were air-dried and were sprayed with Dragendorff reagent. The calculated R_f value was compared with the literature R_f value [26]. The compound having R_f value of 0.3 was isolated and further subjected for MS and NMR analysis. Field desorption (FD) mass spectra were obtained with a JEOL JMS-SX102A mass spectrometer and ^{13}C-NMR spectra were observed by a Bruker AMX-500 instrument using chloroform-*d* as a solvent.

Detection for Histamine. Histamine was detected essentially according to the method described by Cogne et al. [7]. Yam tubers (40 g) were extracted with methanol (100 ml). The extract was centrifuged at 3000 rpm for 10 min and supernatant was filtered. The extraction was repeated three times and the combined extract was evaporated to about 5 ml. The concentrated extract was tested for histamine using a TLC method. The sample extract was compared with that of reference histamine solution (1 mg/ml) on a TLC plate (Silica gel, 60 F_{254}, Merck). The compounds were separated by an ascending method with a solvent mixture of acetone:water:ammonia (90:7:3). The plates were air-dried and were sprayed with ninhydrin reagent (a solution of 30 mg of ninhydrin in 10 ml of butanol and 0.3 ml of acetic acid).

Estimation of Cyanogens. Cyanogens were determined following the method of Bradbury, Bradbury and Egan [27]. Approximately 20 g of yam tuber was cut into small pieces and blended for 2–3 min in a household blender in about 60 ml of 0.1 M H_2SO_4. The mixture was filtered by a water pump through filter paper and the residue was washed with 0.1 M H_2SO_4. The filtrate and washings were combined and made up to 100 ml with the same acid in the volumetric flask. Two milliliters of this solution and 2 ml of 4 M H_2SO_4 were heated in stoppered test tubes for 50 min in a boiling water bath. After cooling to room temperature, 5 ml of 3.6 M NaOH was added; the solution was filtered and made to stand for 5–10 min to allow the breakdown of cyanohydrins to cyanide.

To 7 ml of 0.2 M acetate buffer at pH 5.0 in a stoppered test tube, was added 1 ml of the alkaline cyanide solution and 0.4 ml of 0.5% chloramine-T solution. After 5 min at room temperature, 1.6 ml of the isonicotinic/barbituric acid reagent was added. The blue color was developed at room temperature and the absorbance was measured at 600 nm against a blank of distilled water. The absorbance was converted to microgram KCN by the use of a straight-line calibration of absorbance vs. amount of KCN in 10 ml of colored solution.

The calibration curve was obtained from the standard solutions. A stock solution was produced by dissolving 37.5 mg of dry KCN in 500 ml of 0.2 M NaOH. The amounts of 0.2, 0.4, 0.8, 1.2, and 1.6 ml were diluted to 10 ml with 0.2 M NaOH in standard flasks; 1 ml of aliquots of each dilution were then treated with 7 ml of 0.2 M acetate buffer and chloramne-T, and subsequently isonicotinic/barbituric acid reagent, using the procedure mentioned above.

Table 2. Compounds extracted from wild yam, R_f values their taste

	R_f values				
Compound numbers	*Dioscorea bulbifera*	*Dioscorea versicolor*	*Dioscorea deltoidea*	*Dioscorea triphylla*	Taste
1	–	–	–	0.13	NB
2	0.21	0.21	0.21	0.21	Bitter
3	0.39	0.39	0.39	–	Bitter
4	0.52	0.52	0.52	–	NB
5	0.58	–	–	–	NB
6	0.65	0.65	–	0.65	NB
7	0.75	–	0.75	0.75	NB
8	–	0.91	0.91	0.91	NB

Note. NB: Not bitter.

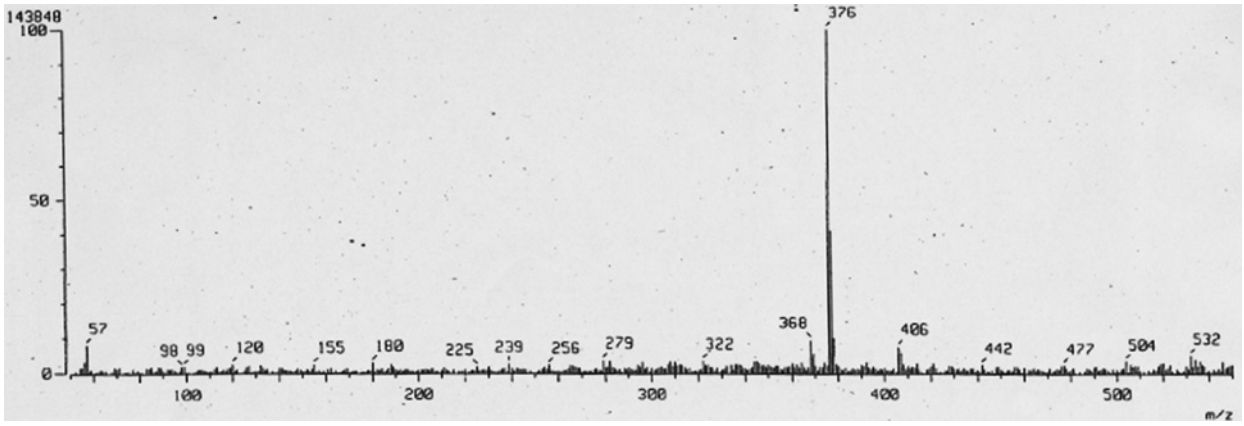

Figure 2. FD mass spectrum of diosbulbin A.

The cyanogens were calculated and presented as HCN equivalent in mg/kg fresh weight (ppm), which was calculated by using the following formula developed by Bradbury et al. [27].

HCN equivalent in milligram per kilogram of fresh yam

$$= 1.868\,(Y \times V)/W$$

where Y: amount of KCN in 10 ml solution (μg); W: weight of fresh yam (g), and V: volume of sample extract (ml).

Results and Discussions

Bitterness in Wild Yams

TLC analysis of bitter substance revealed 5–6 compounds by anisaldehyde spray method. The characteristic R_f value of these detected compounds are shown in Table 2. The compounds associated with the two spots (R_f value: 0.21 and 0.39) were bitter in taste, whereas other detected spots were not bitter as evaluated by sensory taste. There was variation in the bitter and nonbitter compounds among the species. These observations were consistent with the study reports of others [20–22]. Interestingly, all the three species contained both the bitter compounds (R_f 0.39 and 0.21), whereas *D. triphylla* contained only one bitter compound (R_f value: 0.21).

Bitter compounds detected from preparative TLC were further characterized by MS and NMR analysis. The mass spectrum of compound number 3 (R_f value: 0.39) indicated a molecular ion at *m/z* 376. Similarly the mass spectrum for another bitter compound number 2 (R_f value: 0.21) indicated a molecular ion at *m/z* 344. Both these compounds were further characterized by NMR analysis. MS and NMR spectrum are shown in Figures 2–5. A comparison of these observed results (TLC, MS and NMR) with the previous study reports [17, 20, 28–30] confirmed that detected compound with R_f value: 0.39 and 0.21 were diosbulbins A ($C_{20}H_{24}O_7$) and B ($C_{19}H_{20}O_6$), respectively.

Figure 6 shows the HPLC profiles of isolated diosbulbins. Bitter compounds, diosbulbins A and B, were found in the range of 0.023–0.046 and 0.151–0.442 g kg^{-1}, respectively (Table 3). Result demonstrated that diosbulbin B, with an average value of 0.314 g kg^{-1}, was the principal bitter compound as compared to diosbulbin A, which had an average value only 0.037 g kg^{-1}. It is very difficult to compare this result, because very scarce data on quantity of bitter compounds (diosbulbins) in yam tubers

Table 3. Bitter compounds (diosbulbin a and b) in wild yams tubers[a]

Yam species/parameters	Diosbulbin A (g kg^{-1})	Diosbulbin B (g kg^{-1})
Dioscorea bulbifera	0.043 ± 0.004	0.151 ± 0.02
Dioscorea versicolor	0.046 ± 0.009	0.316 ± 0.03
Dioscorea deltoidea	0.023 ± 0.009	0.346 ± 0.09
Dioscorea triphylla	Not detected	0.442 ± 0.05
Range	0.023–0.046	0.151–0.442
Overall mean	0.037	0.314

[a] Values are mean ± SD ($n = 3$).

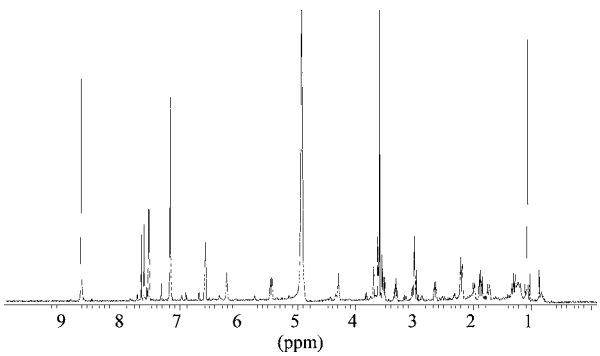

Figure 3. ^{1}H-NMR spectrum of diosbulbin A (pyridine-d_5).

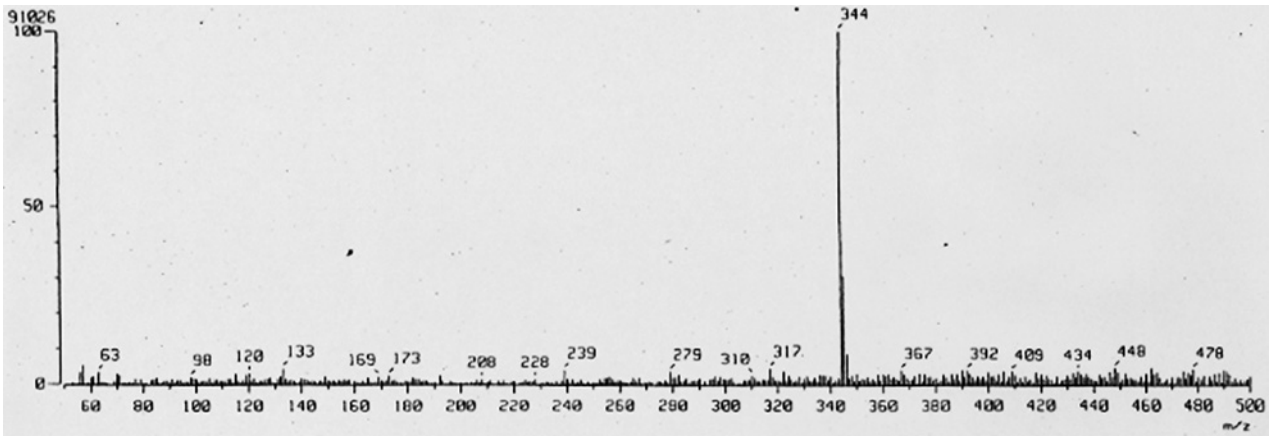

Figure 4. FD mass spectrum of diosbulbin B.

have been reported. However, the total amount of bitter compounds in Nepali wild yam tubers obtained in our study was comparatively lower than reported values for some other yam species [17, 20]. This could possibly be due to the difference in the estimation methods and yam species.

Effect of domestic cooking (boiling, pressure cooking and baking) on bitter principles was also investigated (Figure 7). Cooking showed considerable reduction of bitter principles from uncooked condition. Boiling could reduce the bitterness in the range 75–100%, whereas pressure cooking and baking reduced the bitterness in the range 50–75% only. Present investigation clearly indicated that boiling is the most suitable cooking method for these wild yams to reduce the bitterness towards the acceptable level and rendering the final food palatable.

Toxicity in Wild Yams

Preparative TLC analysis of yam samples showed that out of four, three yam samples (*D. deltoidea, D. versicolor and D. triphylla*) had a component of R_f value 0.3, a reported value for toxic alkaloid dioscorine [26]. However, the ^{13}C-NMR data revealed that the isolated compounds (with R_f value 0.3) did not show peaks of dioscorine. Therefore, our results indicate that these Nepali wild yams do not contain toxic dioscorine. Previous study reports also indicated that only few yam species (*D. hispida* and *D. dumetorum*) contained dioscorine whereas most of the yam species were free from such toxic alkaloid [8, 17].

In this study, yam tubers were found negative for histamine test. The cyanogens (as HCN equivalent) content in wild yam tubers were found ranging from 3.2 to 6.0 ppm (Table 4). The results showed that *D. versicolor* had the highest content of cyanogens (6.0 ppm) and all other species had almost half of this value; and were ranging from 3.2 to 3.3 ppm. The cyanogens content in studied yam tubers were found notably lower than reported cyanogens levels for wild cassava [31] and reported levels for various food sources [32]. The results indicated that the cyanogens levels found in these yam tubers studied were satisfactorily below the safety level for cyanide poisoning. The lethal dose range of humans for HCN taken by mouth is estimated to be only 0.5–3.5 mg/kg body weight [33]. Present study results indicated that Nepali wild yam tubers are not toxic varieties, as they do not contain either toxic dioscorine or histamine and cyanogens were found well below the

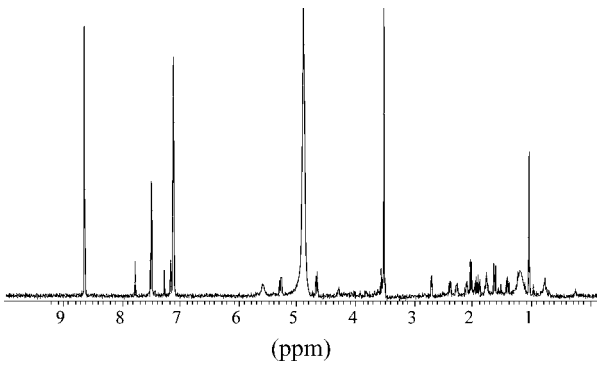

Figure 5. ^{1}H-NMR spectrum of diosbulbin B (pyridine-d_5).

Table 4. Cyanogens content (as HCN equivalent) in wild yam tubers[a]

Yam species	Cyanogens content (ppm)
Dioscorea bulbifera	3.3 ± 0.9
Dioscorea versicolor	6.0 ± 1.4
Dioscorea deltoidea	3.2 ± 0.6
Dioscorea triphylla	3.3 ± 1.3

[a] Values are mean ± SD ($n = 9$).

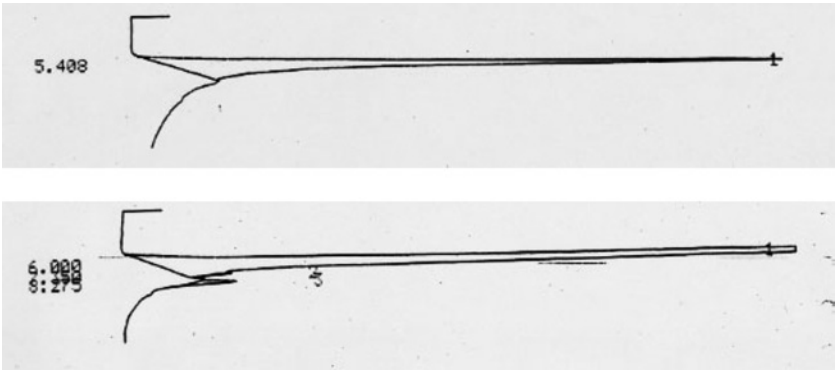

Figure 6. HPLC profiles of bitter compounds. Column: Inertsil SIL-150A-5, solvent: Hexane/2-propanol, 1/9, UV detector: 315 nm, flow rate: 1 ml/min, sample size: 10 μl. (Upper, retention time 5.4: Diosbulbin A, and lower, retention time 6.0: Diosbulbin B).

safety levels. However, the observed acrid taste, inflammation and occasional toxicity in these wild yams could possibly be due to higher levels of oxalates present in these tubers. The oxalate contents were found to be about 4–10 times higher than the reported values for tropical cultivated yam species [34]. Ekpedeme, Bassey, and Ekaete [35] reported that high levels of antinutrients, such as oxalate and HCN, are known to be very poisonous to humans. Further, it is possible that the sharp needles of calcium oxalate are themselves responsible for acridity, inflammation and swelling of tongue, mouth and throat [7, 36]. Calcium oxalate raphides cause microtraumatisms and granulomtous lesions might develop [37]. Ingestion of higher amount of oxalate (2 g at a time) is thought to be fatal dose for humans [38]. The acridity, inflammation and occasional toxicity observed in these wild yam tubers may largely be due to these reasons.

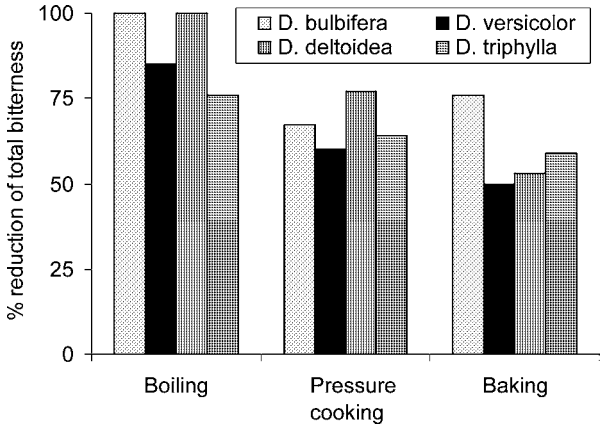

Figure 7. Average reduction percentage in total bitter principles on cooking (boiling, pressure cooking and baking) of wild yams.

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