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USAID/HBCU RESEARCH PROJECT ENTITLED "STUDIES ON THE BITTERNESS FACTOR OF DIOSCOREA DUMETORUM FLOUR"

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FINAL REPORT

Submitted

by

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SUMMARY/ACCOMPLISHMENTS

The first phase of the project was successfully carried out. This involved the establishment of a formal collaborative linkage agreement with the Ministry of Higher Education, Computer Services and Scientific Research (MESIRES), Cameroon, with specific involvement in the project of its Institute of Medical Research and Studies of Medicinal Plants (IMPM); the establishment of field experiments in the host country from which the yam samples were to be generated; and the procurement and delivery of laboratory chemicals and other supplies to the collaborating host country scientists.

Before the harvesting period was reached, political problems erupted in the country. Civil servants whose wages or salaries could not be paid went on a strike which lasted for a very long time. At the beginning, the host country scientists were able to carry out a few of the scheduled yam harvests. However, for fear of what the other strikers would do to them for going to work at the experiment station, the collaborating scientists were unable to stay the course or be consistent with the harvesting schedule nor could they complete the schedule. The project could not be terminated because the U.S. collaborators were led to believe that the strike would soon be settled. There was an indication that although work was sporadic, workers would fully return to their regular work schedules so that those involved with the project would complete the scheduled harvesting and processing of the yams for analysis.

The research objectives were:

1. To determine the effect of tuber growth changes on the nutrient composition of D. dumetorum.

2. To identify and quantify the bitterness factor of D. dumetorum.

3. To develop a processing method for reducing or eliminating the bitterness factor in D. dumetorum.

Obviously, none of these could be effectively and accurately carried out unless the yam samples were consistently and regularly harvested according to the

experimental protocol and harvesting schedule without any omission. As indicated above, many of the scheduled periods of sample collection (yam harvest) were omitted due to absences from work of the host country collaborators who were participating in a general strike called by the civil service unions or government workers.

In those cases where yam samples were collected from the field, it did not appear that the processing procedures were properly followed due perhaps to inadequate supervision of technicians by the host country scientists. Furthermore, the host country collaborators had responsibility for certain aspects of the sample analysis in their laboratory. None of the samples was analyzed by them. It took quite a lot of effort to get them to send samples to the U.S. scientists to carry out their part of the analysis.

The data presented in this report do not lead to any definitive conclusions because of incompleteness and processing inaccuracies. Therefore, the reported analytical results can only be viewed as important preliminary information to guide or assist in any continuation or future effort that may be undertaken.

It is to be pointed out that the closing of the USAID Mission in Cameroon further exacerbated the problem, making any attempt of extending or restarting the research in that country unattainable or unallowable.

EXPERIMENTAL REPORT

INTRODUCTION

Yams from Dioscorea species are a major food crop grown in West Africa, Southeast Asia and the Caribbean. These yams provide up to half the total caloric intake in the diet of the West African population (Jadhav et al., 1981). Cultivation of Dioscorea dumetorum (trifoliate yam) is widespread in Western Cameroon. Yields obtained from these species are three to seven times higher than those of other species being cultivated (Treche and Guion, 1979). Furthermore, the shape of the tuber makes it conducive to harvesting by mechanical means.

The nutritive value of Dioscorea dumetorum yams is well documented. (Agbor Egbe & Treche, 1983; Treche & Guion, 1980; Lyonga & Ayuk-Takem, 1982). Treche et al. (1984) reported that these yams possess a relatively high crude protein content of 9-10.2% and a favorable nitrogen/energy balance. The calcium content in raw yam was 42.9 mg/100 g and 41.1 mg/100 g in boiled yam. Other yam species have much lower values. These studies also show that of all the yams of the Dioscorea species, D. dumetorum has the highest yield of starch, 88.03% on a dry basis.

D. dumetorum and its Asian analogue D. hispida have been found to contain the highly toxic alkaloids Dihydrodioscorine and Dioscorine respectively (Manske, 1960, Watt and Breyer-Brandwijk, 1962). More recently, Corley et al. (1985) have isolated and elucidated the structure of a new alkaloid called Dumetorine as well as that of Dihydrodioscorine.

Some evidence exists concerning cases of yam related poisoning in man and animals (Corkill, 1948). Dioscorine is bitter and poisonous; it produces paralysis of the central nervous system, and in general, behaves like picrotoxin. This action appears to be correlated with the -CO-C=C- group, since on the suppression of this, as in the reduced product Dihydroscorine, the picrotoxin-like action disappears (Henry,

1949). Studies with mice revealed the LD50 value for intraperitoneal administration to be approximately 65 mg/kg (Bevan et al., 1956).

Over the years the indigenous population has devised traditional methods such as baking, boiling, or leaching of sliced tubers in running water overnight to reduce or eliminate these toxic alkaloids. In spite of all these processing techniques, residual alkaloids still resulted in a number of deaths (Jadhav et al., 1981). Also, the bitter substances lend characteristic flavors that are rarely appreciated and more often avoided, and, therefore, limit the acceptance and consumption of products made from D. dumetorum yam.

Several reviews have been reported on extraction and isolation procedures of these alkaloids (Undie and Akube, 1986; Corley et al., 1985; Webster et al., 1984). To date, there have been no reports on quantification of the levels of these alkaloids (bitterness factors) during various stages of tuber development. The purpose of the study was to verify the identity of the bitterness factor at different stages of tuberization, to quantify it, and to develop an appropriate processing technique for eliminating it.

MATERIALS AND METHODS

Two hundred Dioscorea dumetorum cv. Jakiri yam sets were purchased in Cameroon. These were planted in holes 30 cm wide, 20 cm in depth and 1 m apart. The plants were identified with numbered pegs (1-100) randomly distributed in seven sets to represent the harvesting periods of the yams. Plants were harvested at equal intervals of one month starting at the beginning of the third month (thought to be the onset of tuberization) and terminating at the end of the ninth month giving a total of seven harvests in the experimental plan. There were only five harvests, each representing a different stage of tuber development which were not all in sequence due to the unfortunate circumstances in the host country that affected the research activities.

The harvested tubers were washed peeled and sliced about 3 mm thick and

divided into six portions. One portion was freeze-dried immediately. The dried samples were stored at 4°C and were used as the control samples. Each of the other portions was subjected to a different treatment. Yam slices were subjected to soaking at 6, 12, 18, and 24 hours, boiling at 15, 30, and 45 minutes, and baking for 45 and 90 minutes at 150°C in an oven. Samples were also acid washed and base washed at concentrations of 0.1 M, 0.3 M and 0.5 M. The length of time for washing was 45 and 90 minutes for each concentration. Samples were also washed with 0.5 M, 1.0 M and 1.5 M lactic acid for 45 and 90 minutes for each concentration. The samples were backwashed with distilled water for six hours and the wash liquid pooled, measured and stored for analysis. The washed or treated slices were then freeze-dried and then ground into flour and stored at 4°C until needed.

Solanidine standard was purchased from Sigma (St. Louis, MO). All chemicals and solvents used were of HPLC grade. These were purchased from Fisher Scientific (Norcross, GA) and Aldrich Chemical Company (Milwaukee, WI). Silica gel 60 was purchased from Fisher Scientific.

EXTRACTION

Powdered Tuber

Our extraction procedures for alkaloids were adapted from Undie and Akube (1986), but with modifications in sample size, volume of solvent, and extraction time to match that of the Soxtec procedure. The sample size recommended for the Soxtec procedure is 3 grams for solid samples. However, this quantity could not be standardized for our experimental conditions as the amount of sample needed for detection of alkaloids varied for the different harvesting periods. The sample size used for Harvests 2, 3, 4, and 5 were 6, 12, 8 and 8 grams respectively.

Freeze-dried tuber flour was first extracted with 50 ml petroleum ether for one hour. The ether extract was discarded and the marc was dried re-extracted in 40 ml of ethanol for one hour. The ethanol extract was concentrated to 4 ml. Undie and Akube (1986) reported their final volume of extract to be 10% of the original weight of the sample. The ethanol extract was next diluted 1:1 with distilled water

and the pH adjusted to 8.5. The sample was then subjecated to 8 ml chloroform extraction four times. The aqueous layer was discarded and the chloroform layer was concentrated to 0.2 ml using a rotary evaporator. The concentrate was next filtered with a Whatman 0.45 um disposable filter and then subjected to thin layer chromatography (TLC).

Liquid Residue (LR)

The extraction procedure for the liquid residue was based on the one proposed by Undie and Akube (1986) as the volume of solvents used were in the same proportion. Optimization of the extraction methods was achieved by subjecting 20, 30, 40, 50 and 60 ml of the liquid residue to this extraction procedure and then resolving them on TLC. Since the two latter volumes were positive for alkaloids, 50 ml was selected as the suitable volume for all liquid residue extractions.

In a separatory funnel, 50 ml of petroleum ether was added to equal volume of liquid residue. The contents were shaken and the petroleum ether layer (top) was discarded. The procedure was repeated with 75 ml of petroleum ether. The LR was pooled and then extracted with 150 ml ethanol. The ethanol extract was concentrated to 15 ml and mixed 1:1 with distilled water. The pH of this solution was adjusted to 8.5 using NH_4OH or HC1. The sample was then subjected to 30 ml $CHC1_3$ extraction four times. The chloroform layer was collected, pooled and then concentrated to 0.2 ml. Concentrate was next filtered with a Whatman 0.45 um disposable filter and then subjected to TLC and HPLC under the same conditions as the powdered tuber.

THIN LAYER CHROMATOGRAPHY (TLC)

The chloroform extract and standard were spotted on a 20 x 20 cm pre-coated silica gel 60 plate. The plate was activated by heating at 110°C for 30 minutes. The compounds were separated by an ascending method with a solvent mixture of methanol:NH_4OH:$CHC1_3$ (200:3:2 v/v/v) for 50 minutes. This solvent system was developed in our laboratory. Harborne (1973) recommended methanol:NH_4OH

(200:3) but this solvent mixture was too polar for our extracts, so we added 2 ml of $CHCl_3$. After each run, the plates were air dried. Bands were detected using Dragendorff and then eluted with MeOH for further quantitive analysis using HPLC.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The methanol was evaporated from TLC isolates and then reconstituted in 200 ml of the mobile phase hexane:isopropanol (91:1). Solanidine was then added to this solution. About 20 ml was injected into a Beckman system gold module HPLC system (Beckman Instruments, Fullerton, CA), fitted with a Versapack NH2 pre-column and a 250x4.1 mm analytical column (Alltech Associates, Inc., Deerfield, IL). Sample components were quantified on-line at 231 nm by means of a Beckman 167 UV-visible scanning detector (Beckman). The mobile phase was hexane: isopropanol (90:10 v/v) run isocratically. Flowrate was 1 ml/minute. Quantifications were made by comparing the peak areas with those for known amounts of the authentic standard (Solanidine).

Formula used for quantification is:

$$\text{Concentration} = \frac{\text{Peak area of extract}}{\text{Peak area of STD}} \times \text{Conc. STD.} \times \text{Detector Response of extract}$$

RESULTS AND DISCUSSION

The information presented in the following tables represents the results from the laboratory analysis of the few samples that were received from the host country counterparts. The inconsistency and incompleteness of the sample results are difficult to explain rather than to say that they are attributable to the uncertainty surrounding the sample collection, preparation and storage by the host country collaborators which were greatly affected by the long strikes that took place by the government workers in the country during most of the period of the research.

Several repetitions of the analysis as a way of verifying the results, did not overcome obvious inconsistencies.

Whereas the results confirm the presence of the bitterness factors at different levels during various stages of tuberization, the circumstances surrounding the sample collection and preparation make them unreliable and, therefore, it is inappropriate to make any definitive deductions or conclusions from these results. Hence one can only regard the information as useful preliminary information for a continued investigation of the problem.

It would be advisable to continue this work in view of the importance of developing an appropriate processing method for the crop to eliminate the bitterness factor. However, since the USAID Mission in Cameroon is closed, it would be more appropriate to consider another West African country such as Ghana, Togo or Benin as a host country for the work.

Table 1: Alkaloid content of D. dumetorum soaked in water at different growth stages or harvest periods. [1]

Treatment Time (min)	ALKALOIDS - Rf 0.83[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	0.24	X	0.25	X	0.24	X	1.04
6	X	T	T	1.14	a	1.8	T	1.03
12	a	0.0087	0.0012	0.73	a	0.31	T	0.09
18	a	X	0.079	0.23	0.0013	4.64	0.0043	0.008
24	a	0.17	0.1	0.004	0.02	2.36	0.012	X

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 2: Alkaloid contents of D. dumetorum slaked in water at different selected growth stages or harvest periods. [1]

Treatment Time (min)	ALKALOIDS - Rf 0.23[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	1.2	X	0.54	X	0.41	X	1.56
6	X	0.023	a	0.17	a	0.56	a	1.046
12	X	0.015	a	0.29	a	0.05	a	0.007
18	X	X	a	0.12	a	0.1	5.5	0.003
24	X	X	X	0.02	a	0.26	0.46	X

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2] Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 3: Alkaloid contents of D. dumetorum boiled in water at different selected growth stages or harvest periods. [1]

Treatment Time (min)	ALKALOIDS - Rf 0.83[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	0.24	X	0.25	X	0.24	X	1.04
15	T	0.018	T	1.21	0.002	0.31	a	0.09
30	0.033	0.015	0.03	2.25	0.31	4.64	0.0043	T
45	0.037	0.04	1.14	0.45	0.21	2.36	0.012	T

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 4: Alkaloid content of D. dumetorum boiled in water at different selected growth stages or harvest periods. [1]

Treatment Time (min)	ALKALOIDS - Rf 0.23[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	1.2	X	0.54	X	0.41	X	1.56
15	a	0.09	a	0.022	0.035	X	a	0.0053
30	a	0.22	a	0.032	a	0.31	0.064	0.006
45	a	0.08	0.004	0.17	T	0.09	0.073	0.004

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/mg of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 5: Alkaloid content of D. dumetorum baked at 150° C at different selected growth stages or harvest periods.[1]

Treatment Time (min)	ALKALOIDS at Rf 0.83 & Rf 0.23[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	Rf 0.83	Rf 0.23	Rf 0.83	Rf 0.23	Rf 0.83	Rf 0.23	Rf 0. 83	Rf 0.23
Control	0.24	1.2	0.25	0.54	0.24	0.41	1.04	1.56
45	0.032	0.064	0.001	0.28	0.016	0.128	0.026	0.079
90	0.06	0.05	0.0003	0.06	a	2.3	0.0069	0.0028

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/gm of flour.
a: denotes absence of alkaloid.

Table 6: Alkaloid contents of D. dumetorum washed in HCl for 45 min at different growth stages or harvest periods. [1]

Conc HCl	ALKALOIDS - Rf 0.83[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	1.24	X	0.25	X	0.24	X	1.04
0.1M	0.03X	X	0.36	4.28	a	1.01	a	0.11
0.3M	0.19	0.0012	0.83	X	a	2.43	a	0.05
0.5M	0.44	X	2.7	X	0.04	X	X	X

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 7: Alkaloid contents of D. dumetorum washed in HCl for 45 min at different growth stages or harvest periods. [1]

Conc HCl	ALKALOIDS - Rf 0.23[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	X	X	0.54	X	X	X	1.56
0.1M	0.0103	X	a	0.049	a	0.053	a	0.009
0.3M	a	0.13	0.018	X	a	0.22	a	0.007
0.5M	0.002	X	0.16	X	a	X	a	a

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 8: Alkaloid contents of D. dumetorum washed in HCl for 90 min. at different selected growth stages or harvest periods. [1]

Conc HCl	ALKALOIDS - Rf 0.83[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	0.24	X	X	X	0.24	X	1.04
0.1M	0.12	0.17	a	a	a	X	X	X
0.3M	0.013	X	1.068	a	a	0.048	X	X
0.5M	0.061	X	a	0.11	0.11	X	0.023	T

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 9: Alkaloid contents of D. dumetorum washed in HCl for 90 min. at selected growth stages or harvest periods. [1]

Treatment Time (min)	ALKALOIDS - Rf 0.83[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	1.2	X	0.54	X	0.41	X	1.56
0.1M	a	0.21	X	X	X	X	X	X
0.3M	a	X	0.29	X	a	0.007	a	0.002
0.5M	a	X	0.37	X	X	X	0.046	a

[1] Harvest 1 yielded little or insufficient materials for analysis.

[1]Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 10: Alkaloid contents of D. dumetorum treated with $Ca(OH)_2$ for 45 min. at selected growth stages or harvest periods. [1]

Conc $Ca(OH)_2$	ALKALOIDS - Rf 0.83[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	0.24	X	0.25	X	0.24	X	1.04
0.1M	a	0.001	X	0.32	X	0.02	X	0.056
0.3M	0.095	0.003	X	0.56	X	0.25	X	X
0.5M	0.048	0.001	X	0.087	X	0.78	X	X

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
a - denotes absence of alkaloid
T - denotes quantity in trace amounts

Table 11: Alkaloid contents of D.dumetorum treated with $Ca(OH)_2$ for 45 min at selected different growth stages or harvest periods. [1]

Conc $Ca(OH)_2$	ALKALOIDS - Rf 0.23[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	1.2	X	0.54	X	0.41	X	1.56
0.1M	X	0.023	X	0.27	X	0.018	X	0.013
0.3M	X	0.001	X	0.26	X	0.101	X	0.0035
0.5M	X	0.0062	X	0.017	X	0.48	X	0.0029

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/mg of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
F - denotes Flour
X - denotes No Sample provided for analysis

Table 12: Alkaloid contents in D. dumetorum treated with $Ca(OH)_2$ for 90 min. at selected different growth stages or harvest periods. [1]

Conc $Ca(OH)_2$	ALKALOIDS - Rf 0.83 ug[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	0.24	X	0.25	X	0.24	X	1.04
0-1M	0.028	T	X	X	X	0.11	X	0.056
0-3M	0.005	0.008	X	X	X	0.13	X	0.0021
0-5M	0.055	T	X	X	X	0.23	X	X

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2] Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
T - denotes quantity in trace amounts

Table 13: Alkaloid content of D. dumetorum treated with $Ca(OH)_2$ for 90 min at selected growth stages or harvest periods.[1]

Conc $Ca(OH)_2$	ALKALOIDS - Rf 0.23[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	1.2	X	0.54	X	0.41	X	1.56
0-1M	X	0.11	X	0.24	X	0.05	X	0.004
0-3M	X	0.025	X	X	X	0.59	X	0.0001
0-5M	X	0.001	X	0.18	X	0.22	X	X

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2] Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
F - denotes Flour
X - denotes No Sample provided for analysis

Table 14: Alkaloid levels in D. dumetorum washed in lactic acid for 45 min at different growth stages or harvest period.[1]

Conc Lactic Acid	ALKALOIDS - Rf 0.83[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	0.24	X	0.25	X	0.24	X	1.04
6	X	X	T	1.15	X	0.35	X	1.04
12	X	X	T	0.54	X	X	X	0.008
18	X	X	X	X	X	0.11	0.75	0.005

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2] Results are the mean of duplicate samples expressed in ug/mg of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
T - denotes quantity in trace amounts

Table 15: Alkaloid content of D. dumetorum washed in lactic acid for 45 min at different growth stages or harvest periods. [1]

Conc Lactic Acid	ALKALOIDS - Rf 0.23[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	1.2	X	0.54	X	0.41	X	1.56
0-5M	X	0.027	X	0.06	X	0.15	X	1.019
1-0M	a	0.086	X	0.004	X	X	X	0.008
1-5M	a	0.028	X	0.002	X	0.14	X	0.007

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2]Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
T - denotes quantity in trace amounts

Table 16: Alkaloid content of D. dumetorum washed in lactic acid for 90 min. at different growth stages or harvesting periods. [1]

Conc Lactic Acid	ALKALOIDS - Rf 0.83[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	0.24	X	0.25	X	0.24	X	1.04
0.5M	0.011	0.009	T	0.24	X	X	0.006	1.003
1.0M	0.02	0.002	0.02	0.21	X	X	0.024	0.004
1.5M	0.02	0.012	0.0034	0.2	X	X	0.14	0.02

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2] Results are the mean of duplicate samples expressed in ug/gm of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
X - denotes No Sample provided for analysis
F - denotes Flour
T - denotes quantity in trace amounts

Table 17: Alkaloid content of D. dumetorum washed in lactic acid for 90 min. at different growth stages or harvesting periods. [1]

Conc Lactic Acid	ALKALOIDS - Rf 0.23[2]							
	Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	LR	F	LR	F	LR	F	LR	F
Control	X	1.2	X	0.54	X	0.41	X	1.56
0.5M	X	0.073	X	1.8	X	0.24	0.015	0.01
1.0M	X	0.082	X	6	X	0.17	0.22	0.33
1.5M	X	0.22	X	5.1	X	0.13	0.28	0.18

[1] Harvest 1 yielded little or insufficient materials for analysis.

[2] Results are the mean of duplicate samples expressed in ug/mg of flour and ug/ml of liquid residue.
LR - denotes Liquid Residue
F - denotes Flour
X - denotes No Sample provided for analysis

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