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Exposure Assessment of Oxalate from the Native Vegetables Among the Dusun Ethnic Group in Sabah, Malaysia.

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ABSTRACT

Background: Determination of the potentially toxic native vegetables was done through interviews of plant informants. Quantification of oxalate were done by using picrate oxalate kit. There were nine species of potentially toxic native vegetables from the Poaceae and Araceae family identified by the plant informants in Ranau, Tambunan and Kuala Penyu. **Objective:** The objectives of this study were to determine the potentially toxic native vegetables consumed by the Dusun ethnic group in Sabah and to assess the exposure level to oxalates found in the native vegetables among the consumers. **Results:** The total oxalate content ranged from 19.65 – 64.06 mg/100g for raw samples which decreased to 2.33 – 5.40 mg/100g after boiling. Exposure assessment calculation showed that the exposure to oxalate from native vegetables consumption among the Dusun ethnic in Ranau, Tambunan and Kuala Penyu are considered below the safe level. The highest dose of exposure calculated for male respondents was 0.0270% from the reported fatal dose and 0.058% from the reported fatal dose among female respondents.. **Conclusion:** In conclusion, all nine species of native vegetables in this study contain oxalate. However, proper processing method before consumption may reduce the potential toxicity of the native vegetables and reduce the exposure to a safer level.

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INTRODUCTION

Vegetables are necessary and highly recommended for daily life as it provides the human body with the essential nutrients (Mohamed *et al.*, 2003). According to Knudsen *et al.* (2008), 30 types of plants delivered 95% of human daily intake of plant food calories and the other 5% are delivered by another 300 other plants species. There are foods traditional and well known in one country or region but maybe unknown by others, hence known as the country's or region's novel food. Many native or wild edible plants are nutritionally rich especially vitamins and nutrients which are perceived to have health benefits (Ali-Shtayeh *et al.*, 2008; Herforth, 2010).

Oxalate is involved in several metabolic processes in plants which occur as insoluble oxalate crystals or water-soluble oxalate such as sodium hydrogen oxalate and free oxalic acid (Siener *et al.*, 2006). High-capacity calcium regulation and protection against herbivores are two major functions of calcium oxalate crystals formation in plants (Franceschi and Nakata, 2005). Oxalate is known to be present in relatively small amounts in many plants (Savage *et al.*, 2000). Among foods known to contain oxalate are spinach, broccoli, rhubarb, carrot, beets, nuts, chocolate, wheat bran and strawberries (Noonan and Savage, 1999; Savage *et al.*, 2000). Large amount of consumption of oxalate may cause oxalosis and hyperoxaluria (Honow and Hesse, 2002). Oxalate forms strong chelates with dietary calcium which precipitates as insoluble salts and accumulate in the renal glomeruli (Judprasong *et al.*, 2006). Renal failure occurs when accumulation of oxalate in the proximal tubule cells cause cell necrosis as well as physical blockage of the flow and haemorrhage (Hughes and Norman, 1992; Guo and McMartin, 2005). Furthermore, calcium oxalate will precipitate in the urine and eventually form kidney stone (Hughes and Norman, 1992). Excessively high or insufficiently low amounts of exposure to toxic components in food plants may create risk. Therefore, reliable methods to estimate the amounts ingested are important (Kroes *et al.*, 2002). Scientific data and history of safe consumption for most plant foods are very difficult to be obtained even though they may have been eaten for several hundreds of years (Knudsen *et al.*,

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2008). There are also few initiatives and scientific data to establish the safety of botanical products when used in food (Schilter *et al*., 2003). Some food was assumed safe because no evidence of adverse effects has been reported over time. Moreover, the community has acquired experiences in proper processing or preparation method to safely consume the food (Knudsen *et al*., 2008).

In Sabah, there are still lack of data which represent the range of dose consumption and toxicity effect of native vegetables, despite several studies and surveys done on native plants used for food and medicine by the local people of Sabah. It is proposed that the input from this research can be used as a starting point to further investigate the potential toxicity of wild native vegetables to its consumers in Sabah. An exposure level of various toxic compounds due to consumption of native vegetables mostly used by the people in Sabah and the range of safe dose consumption can be determined for safer short and long term usage. Therefore, this study aims to, determine the potentially toxic native vegetables still consumed by the Dusun ethnic group in Sabah and to assess the exposure level to oxalates found in the native vegetables among the consumers.

2. Methodology:

2.1 Determination of Food Types and Respondents:

Primary and secondary plant informants were identified in each district to aid in identification of the potentially toxic native vegetables which are still widely consumed in their respective area.

2.2 Determination of Survey Location:

Three locations were picked for the study namely Kampung Bundu Tuhan in Ranau district, Kampung Tikolod in Tambunan district, and Kampung Kiambor in Kuala Penyu district. Ranau, Tambunan and Kuala Penyu are three of the districts where the Dusun ethnic are concentrated with a total population of 81,592, 31,043 and 6,183 respectively (Department of Statistics, Malaysia 2010).

2.3 Determination of potentially toxic wild native vegetables:

Key informants among the elderly were selected based on their knowledge and experience in native plants usage as well as their nature of occupation whom are mostly farmers. The technique involves a deliberate choice of an informant based on their qualities as informants (Tongco, 2007). Informants were also chosen based on their willingness and ability to provide information based on their knowledge and experience (Bernard, 2002; Lewis & Shephard, 2006).

2.4 Determination of total oxalate content in native vegetables:

Oxalate concentration in the samples was determined by Oxalate Kit by Trinity Biotech. Oxalate reagents were warmed to 37°C. Tubes were labeled for Blank, Standard, and food Sample. 1 mL of Reagent A was added into each tube. 50µl of sample were added in respective sample tubes. 50µl of deionized water were added in Blank tube and 50µl of standard were added in Standard tube. 0.1 mL of oxalate Reagent B was added to all tubes and immediately mixed by gentle inversion. All tubes were incubated at 37°C for 5 mins. Then, absorbances of blank, control, standard and sample were determined at 590 nm. Calculations to determine oxalate concentration in miligrams/100g were done.

2.5 Survey of Food Consumption Frequency:

The food consumption data was obtained by using forms which were created based on the Malaysian Adult Nutrition Survey Methodology by Ministry of Health (2003). The food intake measurement for each selected vegetables were calculated based on the serving sizes according to Food Portion Sizes of Malaysian Foods Album (2002/2003). The food consumption forms were administered at the household level (FAO, 2007). The forms were divided into two sections; Section A and Section B. Section A consisted of four demographic and anthropometric questions such as the age, weight and height of the respondents. Section B consisted of six main questions on the consumption patterns of the selected native vegetables from their respective districts.

2.6 Exposure Assessment Analysis:

The intake of a chemical in a food item are expressed as the concentration in food item times the amount of food item consumed (O'brien et al., 2006). The Food Ingestion Exposure Dose equation ATSDR (2005) :

D = (CL x CRi x EF)/ BW , where
D = exposure dose (mg/kg/day)
Cl = contaminant concentration (mg/g)
CRi = consumption rate of food (g/day)
EF = exposure factor
BW = body weight (kg)

Exposure duration for Malaysian male is 71.7 years and female 76.5 years (Ministry of Health, 2007). The exposure doses were calculated based on gender and age groups.

3.Results:

3.1 Sosio-demografic Data:

Results on the sosio-demographic data of respondents in three districts is shown in Table 1. A total of 303 households in Ranau district were selected to be surveyed with a total number of 1342 respondents. In Tambunan and Kuala Penyu districts, a total of 78 and 66 households were surveyed, respectively. The total numbers of respondents were 409 in Tambunan district and 271 in Kuala Penyu district.

3.2 Dietary Intake Measurement of Native Vegetables:

Mean consumption (gram/day) for all native vegetables were calculated and differentiated between male and female. The results are shown in Table 2. It can be seen that generally male had a higher mean of consumption compared to female respondents. Table 6.8, Table 6.9 and Table 6.10 show the mean consumption of wild native vegetables according to different age groups. For overall mean consumption, the highest consumption mean recorded was Poring (*Gigantochloa levis*) consumption of 156.21 ± 109.80 g/day among respondents in Tambunan. In Ranau, Poring (*Gigantochloa levis*) was the most consumed native vegetable with a consumption mean of 149 ± 107.01 g/day. In Kuala Penyu both Poring (*Gigantochloa levis*) and Tamalang (*Bambusa vulgaris*) recorded an overall consumption mean of 138.46 ± 96.27 g/day and 120.66 ± 80.83 g/day, respectively.

3.3 Level of Oxalate in native vegetables:

As shown in Table 3, in Ranau, Lolondu (*Colocasia gigantea*) showed the highest amount of oxalate which is 56.41 mg/100g which was reduced to 3.46 mg/100g after boiling. In Tambunan, Dungkalang (*Schismatglottis motleyana*) showed the highest oxalate content which was 64.06 mg/100g in raw samples and reduced to 2.66 mg/100g after boiling. Both raw Poring (*Gigantochloa levis*) and Tamalang (*Bambusa vulgaris*) in Kuala Penyu showed a total oxalate content of 35.42 mg/100g and 33.74 mg/100g, respectively. Mean total oxalate content of all raw Poring (Gigantochloa levis) samples from three districts showed no significant difference ($p > 0.05$). Mean total oxalate of raw Tulu / Wuluh (*Schizostachyum brachycladum*) from Ranau and Tambunan showed no significant difference ($p > 0.05$). In the Araceae samples, the highest value recorded was 64.06 ± 10.77 mg/100g.

3.4 Intake measurement of selected wild native vegetables:

Mean consumption (gram/day) for all native vegetables were calculated and differentiated between male and female. The results are shown in Table 4. It can be seen that generally male had a higher mean of consumption compared to female respondents.

3.5 Exposure Assessment of Oxalate in Native Vegetables:

As shown in Table 5, the overall dose of exposure to oxalate among male respondents in all three districts can be considered to be below the safe level. The highest exposure doses for raw sample were 0.027 mg/kg/day for raw Poring from Kuala Penyu which was reduced to 0.0032 mg/kg/day (0.000063% fatal dose) after boiling. The highest exposure dose of oxalate for female respondents calculated was for raw Lolondu and Poring from Ranau as shown in Table 6. The initial raw samples dose of exposure was reduced from 0.028 mg/kg/day to 0.003 mg/kg for lolondu (0.0000363% fatal dose) and 0.004 mg/kg (0.0000772% fatal dose) for Poring.

Table 1: Socio-demographic characteristics of respondents in all location.

Characteristics	Ranau	Tambunan	Kuala Penyu
Number of households surveyed	303	78	66
Number of respondents	1342	409	271
Male (%)	700 (52.2)	231 (56.5)	132 (48.7)
Female (%)	642 (47.8)	178 (43.5)	139 (51.3)
Age (years old) (SD)			
Mean	31.58 (21.42)	27.04 (19.56)	33.69 (22.27)
Median	25.00	20.00	30.00
Mode	20.00	18.00	15.00
Mean Age (Male)	31.45 (21.63)	26.20 (19.26)	33.80 (22.19)
Mean Age (Female)	31.63 (21.20)	28.12 (19.70)	33.59 (22.44)
Weight (kg) (SD)			
Mean	49.28 (17.24)	46.52 (17.05)	49.85 (18.01)
Median	51.00	50.00	51.00
Mode	45.00	60.00	45.00
Mean Weight (Male)	52.95 (18.99)	47.74 (18.65)	54.85 (19.43)
Mean Weight (Female)	45.30 (14.08)	44.94 (14.60)	45.09 (15.15)

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Height (cm) (SD)			
Mean	148.43 (22.17)	146.06 (22.69)	147.63 (25.72)
Median	154.00	153.00	153.00
Mode	156.00	150.00	145.00
Mean Height (Male)	152.04 (23.08)	147.25 (24.31)	152.25 (28.24)
Mean Height (Female)	144.76 (20.52)	144.52 (20.35)	143.24 (22.31)

Table 2: Mean consumption data (g/day ± SD) of native vegetables according to gender.

Characteristics	Ranau	Tambunan	Kuala Penyu
Number of households surveyed	303	78	66
Number of respondents	1342	409	271
Male (%)	700 (52.2)	231 (56.5)	132 (48.7)
Female (%)	642 (47.8)	178 (43.5)	139 (51.3)
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Weight (kg) (SD)			
Mean	49.28 (17.24)	46.52 (17.05)	49.85 (18.01)
Median	51.00	50.00	51.00
Mode	45.00	60.00	45.00
Mean Weight (Male)	52.95 (18.99)	47.74 (18.65)	54.85 (19.43)
Mean Weight (Female)	45.30 (14.08)	44.94 (14.60)	45.09 (15.15)
Height (cm) (SD)			
Mean	148.43 (22.17)	146.06 (22.69)	147.63 (25.72)
Median	154.00	153.00	153.00
Mode	156.00	150.00	145.00
Mean Height (Male)	152.04 (23.08)	147.25 (24.31)	152.25 (28.24)
Mean Height (Female)	144.76 (20.52)	144.52 (20.35)	143.24 (22.31)

Table 3: Mean total oxalate content (mean ± SD, g/100g) in raw and cooked samples of wild native vegetables.

Location (District)	Species	Raw sample (mg/100g)	Cooked samples (mg/100g)	Percent of reduction (%)
Ranau	*G. levis*	37.46 ± 6.15^{aA}	5.04 ± 1.24^{B}	86.55
	S. brachycladum	38.26 ± 5.57^{aA}	4.27 ± 2.61^{B}	88.84
	Schismatoglottis ahmadii	45.20 ± 21.41^{A}	4.56 ± 2.37^{B}	89.91
	Colocasia gigantea	56.41 ± 19.79^{A}	3.46 ± 0.80^{B}	96.96
Tambunan	*G. levis*	41.60 ± 4.23^{aA}	4.19 ± 3.87^{B}	89.92
	S. brachycladum	21.37 ± 11.21^{aA}	2.33 ± 1.63^{B}	89.10
	S. latifolium	37.42 ± 18.28^{A}	3.31 ± 1.01^{B}	91.15
	Schismatglottis motleyana	19.65 ± 4.70^{A}	5.40 ± 2.34^{B}	72.52
	Colocasia esculanta	64.06 ± 10.77^{A}	2.66 ± 0.63^{B}	95.85
Kuala Penyu	*G. levis*	35.42 ± 12.66^{aA}	4.15 ± 1.93^{B}	88.28
	B. vulgaris	33.74 ± 12.18^{A}	3.73 ± 3.27^{B}	88.94

Note: *Gigantochloa levis* = Poring, *Schizostachyum brachycladum* = Tulu (Ranau) / Wuluh (Tambunan), *Schizostachyum latifolium* = Tombotuon, *Schismatoglottis ahmadii* = Dukaruk, *Schismatoglottis motleyana* = Dungkalang, *Colocasia gigantea* = Lolondu, *Colocasia esculanta* = Dar and *Bambusa vulgaris* = Tamalang.
Means denoted with the same letters in the same column and same species are not significantly different at $p>0.05$
Means denoted with the similar capital letter within the same row are not significantly different at $p>0.05$

Table 4: Mean consumption data (g/day ± SD) of native vegetables according to gender.

Location	Native vegetable	Mean consumption (g/day ± SD)		
		Overall	Male	Female
Ranau	*Poring*	149.00 (107.01)	154.12 (111.10)	143.13 (101.90)
	Tulu	120.02 (90.67)	124.62 (94.80)	114.97 (85.70)
	Dukaruk	135.90 (100.79)	140.58 (102.69)	130.85 (98.55)
	Lolondu	120.91 (79.76)	122.07 (81.74)	119.65 (79.76)
Tambunan	*Poring*	156.21 (109.80)	162.61 (114.30)	147.94 (103.44)
	Tombotuon	98.97 (81.32)	104.75 (87.12)	91.84 (73.17)
	Wuluh	141.52 (114.41)	146.22 (118.39)	135.52 (109.17)
	Dungkalang	109.34 (82.82)	113.16 (83.77)	104.85 (81.89)
	Dar	126.55 (86.70)	127.66 (87.96)	125.13 (85.31)
Kuala Penyu	*Poring*	138.45 (96.27)	144.87 (99.64)	131.91 (92.70)
	Tamalang	120.66 (80.83)	124.23 (87.30)	117.19 (74.16)

Table 5: Dose of exposure (mg/kg/day) of oxalate in raw and cooked native vegetable samples among male respondents.

Location	Native Vegetables	Dose of Exposure (mg/kg/day)		% Fatal dose (5000 mg/kg)
		Raw	Cooked	
Ranau	*Poring*	0.0230	0.0031	0.000062
	Tulu	0.0016	0.0002	0.000004

	Dukaruk	0.0209	0.0021	0.000042
	Lolondu	0.0221	0.0014	0.000027
Tambunan	*Poring*	0.0123	0.0012	0.000025
	Wuluh	0.0065	0.0007	0.000014
	Tombotuon	0.0053	0.0005	0.000009
	Dungkalang	0.0020	0.0005	0.000011
	Dar	0.0221	0.0009	0.000018
Kuala Penyu	*Poring*	0.0270	0.0032	0.000063
	Tamalang	0.0203	0.0022	0.000045

Table 6: Dose of exposure (mg/kg/day) of oxalate in raw and cooked native vegetable samples among female respondents.

Location	Samples	Dose of Exposure (mg/kg/day)		% Fatal dose (5000 mg/kg)
		Raw	Cooked	
Ranau	*Poring*	0.025	0.006	0.0000718
	Tulu	0.016	0.002	0.0000436
	Dukaruk	0.022	0.004	0.0000582
	Lolondu	0.028	0.003	0.0000363
Tambunan	*Poring*	0.012	0.002	0.0000298
	Wuluh	0.007	0.001	0.0000137
	Tombotuon	0.005	0.000	0.0000100
	Dungkalang	0.002	0.001	0.0000109
	Dar	0.020	0.001	0.0000249
Kuala Penyu	*Poring*	0.028	0.004	0.0000772
	Tamalang	0.011	0.002	0.0000487

4. Discussion:

In a study done by Judraprasong *et al.*, (2006), Thailand bamboo shoots samples oxalate content was 23 ± 10 mg/100g for raw samples and was reduced to 10 ± 4 mg/100g after boiling. A study done by Juajun *et al.* (2012) reported a higher value of oxalate content in Dar (Colocasia esculenta) which is 424.4 mg/100g in raw samples and 331.7 mg/100g in boiled samples. Boiling of *C. esculenta* for 30 minutes reduced the oxalate content by 34.2%. The variations of total oxalate content may vary due to the genetic differences between plant groups as wells as cultivation conditions (Wilson *et al.*, 1982). Other causes of variations in oxalate content are different sources of plants, soil quality, climate or different state of vegetables growth (Austenfeld & Leder, 1978; Libert & Franceschi, 1987; Honow & Hesse, 2002).

There had been assumptions that the oxalate absorption in humans is insignificant because calcium oxalate is virtually insoluble in aqueous solutions (Al-Wahsh *et al.*, 2005). Among the factors of oxalate absorption is the amount and form of oxalate in the food consumed, the amount of calcium and magnesium in the food, and the presence of oxalate-degrading bacteria in the intestines (Massey, 2007). The ingestion of 4-5 g of oxalate is the minimum dose capable of causing death in an adult (Fasset, 1973; Gontzea, 1973; Noonan and Savage, 1999). However there are reports showing that 10 – 15 g is the usual amount which may lead to fatalities (Gontzea, 1973). However, the effect of ingestion of different food with oxalate content at different doses is still currently unknown (Siener *et al.*, 2006). In order to reduce the exposure to oxalate, foods containing high in oxalates should be consumed in moderation as the occasional consumption of high oxalate foods will not pose health problems (Noonan & Savage, 1999).

Most plants containing calcium oxalate crystals are members of the arum family (Osweiler et al., 1985). The potential toxicity of the calcium oxalate in plants when being consumed also might be reduced as it was reported that boiling decrease the plant total oxalate content 30% to 87% (Chai and Leibman, 2005; Savage et al., 2000; Judprasong et al., 2006). It was found that the most effective way to reduce the soluble oxalate in taro up to 94.5% reduction is by cooking at 100°C for 60 minutes while boiling for 60 minutes reduced the soluble oxalates by 73% (Hang et al., 2012). Savage & Catherwood (2007) reported that baked taro corms at 180°C for 40 minutes still contain a significantly high levels of oxalate compared to boiled taro corms content which suggest that the cooking method also influence the reduction of the oxalate content in plants. In order to reduce the exposure to oxalate, foods containing high in oxalates should be consumed in moderation as the occasional consumption of high oxalate foods will not pose health problems (Noonan & Savage, 1999). However, individuals may have different tolerance on the same food; hence no food can be considered to be absolutely safe under all circumstances (Constable, 2007). The secondary metabolites in plants have an upper safety level as well as some health benefits in lower levels (Knudsen et al., 2008). Furthermore, processing of these edible plants before consumption is important to ensure the safety of native vegetables consumption.

5. Conclusion:

As showed in the results, it can be concluded that the exposure to oxalate from native vegetables consumption among the Dusun ethnic in Ranau, Tambunan and Kuala Penyu is considered below the safe level. For oxalate exposure, the highest dose of exposure calculated for male respondents was 0.0000270% from the reported fatal dose and 0.000058% from the reported fatal dose among female respondents. The exposure dose calculated for each age group also showed that the dose was below the safe level. Therefore, the results of the acute dietary exposure assessment indicated that all population groups had estimated acute dietary exposures under the fatal dose for all native vegetables.

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