

## **Toxicity and Nutrient Testing of Local Roselle (*Hibiscus sabdariffa* L.) Fruit**

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### **ABSTRACT**

*This study of the native roselle (*Hibiscus sabdariffa*) fruit was subjected to experimental and qualitative methods of research. It determined the nutrient content of roselle seeds and the lethal dose of the extract. Focus group discussion was used in gathering the needed data for the earlier traditional usage of the plant in the local community representing the households from the three (3) municipalities in Ilocos Sur, namely San Juan, Santa Catalina and Caoayan. Tests were conducted in the Industrial Testing Division Institute, Department of Science and Technology, Bicutan, Taguig City (ITDI, DOST). Results showed that the essential food nutrients of the seeds; per 100 g from the extract of the Roselle fruit are ash (5.36), protein (24.7), fat (18.2), total carbohydrates (48.6) and food energy, kcal/100g (457). In the toxicity testing (LD50), a total of 30 mice were used and given an increasing doses of 35, 40 and 45 grams per kilogram. Acute oral toxicity test showed no adverse or abnormal signs or death within the first 24 hours up to 14-day period of observation. Institutional Animal Care and Use Committee (IACUC) certificate was secured. Results of the focus group confirmed the value of the roselle plant to the local folks as a safe food to eat. To them, it is becoming a vanishing local crop.*

**Keywords:** *Caoayan, Ilocos Sur, mice, food energy, carbohydrates, protein*

### **INTRODUCTION**

Roselle is a plant commonly found in the municipalities of Ilocos Sur particularly in Barangay Pantay Tamurong, Don Alejandro Quiroigico and Don Lorenzo Querubin, Caoayan, Ilocos Sur. The tops and the calyx are used as flavor enhancer to the Ilocano cuisine known as “Dinengdeng” while the seeds are used as beverage similar to the hot choco of cacao. The plant is slowly disappearing and very few are left among the households probably due to the advent of urbanization and introduction of a ready mixed flavor enhancers in the market. Moreover, the lack of value of the plant as a popular crop also contributed to its disappearance in the nearby municipalities. Today, the plant is categorized as a vanishing plant.

Roselle plant is part of the cuisine of the Ilocano dishes like “Sinigang” and morning beverage. The farmers considered it a tasteful morning brew. It is cheap and studies show good nutritional value of the plant. In the advent of technological advancement of agricultural world and the increased value of yellow corn and tobacco, the plant slowly disappeared among Ilocano household and rarely seen already. It is important to bring back the plant as alternative food

source to augment the escalating prices of high value crop for everyday nourishment.

Rocha, Bonnlaender, Sivers and Pischel (2016) revealed that roselle has been traditionally used as food source, herbal and beverage. Results in the study showed that extracts have antibacterial, antioxidant, nephron and hepato protective, renal or diuretic effects even on lipids and antihypertensive effects. The researchers mentioned that results could be attributed to the strong antioxidant activities of the extracts with the inhibition to  $\alpha$ -glucosidase and  $\alpha$ -amylase, likewise to the inhibition of angiotensin-converting enzymes (ACE), and direct vaso-relaxant effect or calcium channel modulation. Phenolic acids (esp. protocatechuic acid), organic acid (hydroxycitric acid and hibiscus acid) and anthocyanins (delphinidin-3-sambubioside and cyanidin-3-sambubioside).

Mahadevan, and Kamboj (2009) mentioned that in Australia, the plant roselle is known as rosella or rosella fruit. It is also well known to countries such as Malaysia, Myanmar, Indonesia and Thailand and even in the United States. Accordingly, the plant is also used and cultivated as folk medicine both as diuretic, mild laxative and as treatment of ailments such as nerve disease and even cancer. In Europe, it was documented that the plant is used as food coloring with its good value of export to other countries. The bast fiber of the roselle stem is also utilized. It is used as substitute for jute in burlap making. In the Philippines, the leaves are also used as flavoring comparable to spinach only in its spicy version. Sorrel drinks were made from the calyces of the roselle plant in the Caribbean areas. Due to the high vitamin content of the calyces, it is harvested fresh and made into drinks in Malaysia. Moreover, in Mexico, Trinidad Tobago, Jamaica and in Central America, the drinks from the calyces are very popular.

The Philippine Council for Agroforestry and Natural Resources Research and Development (2007) Los Baños, Laguna (Philippines) under the Department of Science and Technology mentioned various uses of roselle such as in the jellies and other gastronomic uses.

The study of Florendo, Bañez and Queddeng (2016) on Alternative Source of Herbal Medicine: Add-On to Buri (*Corypha elata* Roxb.) Leaf Industry confirmed the essential composition of the Buri leaves, namely sterols saponins, glycosides, and tannins.

Gaya, et al. (2014) conducted preliminary phytochemical screening of the ethanolic seed extract of roselle. Results of their study showed the presence of alkaloids, saponins, tannins, anthraquinones, steroids, cardiac glycosides, flavonoids and phlobatanins. They also conducted acute toxicity (LD50) of roselle seed extract in albino rats and was found to be above 5000 mgkg<sup>-1</sup>. The lactogenic effect of ethanolic seed extract of roselle was investigated by administering extract and metoclopramide in albino rats. The extracts were

administered at varying doses, 400, 800 and 1600 mgkg<sup>-1</sup>) and drug (5mgkg<sup>-1</sup>) for six days orally.

In Nigeria, the decoction of the seeds is traditionally used to enhance or induce lactation in cases of poor milk production, poor letdown and maternal mortality. The study of Gaya, Mohammad, Baje and Adekunle (2009) determined the Adekunle toxicological and lactogenic studies on the seeds of roselle extract on serum prolactin levels of albino wistar rats.

It is noted that in countries such as Mexico, Thailand and Africa, several parts of the plant are used for medicinal purposes, namely hypertension and urinary tract infections (UTI). Hussain, Khan, Rehman, Hamayun, Shah, Nisar, Bano, and Shinwari (2009) mentioned that the plant is cultivated in an arid region, with limited water and land resources to cultivate various crops specially vegetables. However, a few seasonal vegetables are available to the local communities in meager quantities. The ash, carbohydrate, protein, moisture, fat, fiber contents, energy values and nutrient composition of eight vegetables, namely *Abelmoschus esculentus*, *Spinacia oleracea*, *Praecitrullus fistulosus*, *Luffa acutangula*, *Allium sativum*, *Amaranthus viridus*, *Chenopodium album* and *Momordica charantia* were determined. Among these, *A. sativum*, *S. oleracea* and *C. album* have the highest micro and macronutrients.

On toxicity, a similar study was conducted by Bañez (2016) entitled, Insecticidal Effects of Arrowhead (*Syngonium podophyllum*) and Jicama (*Pachyrhizus erosus*) against Odorous House Ants (*Tapinoma sessile*). The researcher compared the toxicity efficacy of aqueous and alcoholic extracts prepared from the mature leaves of two species arrowhead and jicama against odorous house ants. Results of her study showed that there is a significant interaction effects between the treatments (aqueous and alcoholic) and the kind of plant used. Arrowhead alcoholic leaf extract when compared with aqueous extract; positive and negative controls have significant differences between and among all pairs, same is true with jicama. Jicama is more effective than arrowhead in both the treatments alcoholic and aqueous, the mean difference is significant at 0.05 level. The alcoholic extract is more effective than the aqueous. The jicama leaf extract is more effective than arrowhead.

The Philippines is known for its rich natural resources especially in plant varieties like the roselle. In the United States particularly in Florida, the calyces of the plant are being made into juice which is comparable to cranberry, a very popular commercialized drink rich in vitamin C. In the Philippines, most especially in the province of Ilocos Sur, the plant has no or little crop value to the farmers as it is only for the household use. Most of the farms were concentrated to the popular crops of high demand like rice, corn, tobacco and other high-value crops. It is a common observation that the plant is taken for granted and rapidly belonging to the category of vanishing crop. It is therefore the aim of this endeavor to conduct scientifically tested procedures to this plant to add on to its

traditional value. Moreover the study aims to re-establish the presence of roselle plant to the farmlands and to the households and eventually elevate its status to the market as a high-value crop. This may contribute to the economic status of the local community that considerably focus to corn and tobacco as major source income. Further, this study hopes to contribute to the food security and sufficiency program of the country and help mitigate the challenges of malnutrition of the country.

This study was conducted to determine the nutrient content of roselle seeds and the lethal dose (LD50) of the extract to the Imprinting Control Region (ICR) mice characterized by the decreased motor activity, respiratory rate, hyperemia, ptosis, piloerection, loss of grip strength, pinna and righting reflexes and taxia. Additionally it determined the traditional household use and survey the presence of the plants in the municipalities of Ilocos Sur.

## METHODOLOGY

The study made use of experimental and qualitative methods of research in determining the median lethal dose (LD50) as well as nutrient analysis of the native roselle seeds. Qualitative method thru focus group was employed in determining the traditional household use and presence of the plants in the municipalities of Ilocos Sur.

The study used the focus group discussion (FGD) to gather the needed data on the traditional household use of local roselle and presence of the plants in the municipalities of Ilocos Sur.

During the FGD five (5) focus groups representing the households from the three (3) municipalities in Ilocos Sur, namely San Juan, Santa Catalina and Caoayan comprise the participants. Participants in Malamin, San Juan and Santa Catalina, Ilocos Sur and some barangays in Caoayan, Ilocos Sur particularly in Pantay Tamurong, Don Lorenzo Querubin and Don Alejandro Quirolgico are included for the two-hour discussions with the use of a facilitator's guide. The participants were purposively chosen from among themselves with the assistance of the elders and local barangay officials.

### Methods and Materials

The following experimental procedures are presented to understand the processes involved in this research study.

#### Sample Preparation

**Cultivation.** The plants were cultivated and grown organically in Don Lorenzo Querubin, Caoayan, Ilocos Sur.

**Harvesting and Drying.** The fruits were gathered by hand and sundried for 3 days.



**Figure 1. Roselle fruits**

**Grinding.** Grinding of the seeds was done in Chemfree Food Company in Bacnotan, La Union

The tests were conducted in Industrial Testing Division Institute (ITDI), Department of Science and Technology (DOST), Bicutan, Taguig City.



**Figure 2. Set-up for the toxicity test**

### **Acute Oral Toxicity Test**

The concentration of sample suspension was 30mg/mL dissolved in distilled water pH of sample: 5.34. The animals used were the female ICR mice of 27-29 grams. Three (3) preliminary increasing doses (35 g/kg, 40 g/kg and 45 g/kg) in serial were given orally to determine the expected dose that will cause 50 percent death of the experimental animals. The animals were observed by the toxidrome ranges of decreased motor activity, respiratory rate, hyperemia, ptosis, and piloerection. Loss of grip strength, pinna, righting reflexes and taxia for 14-day period were keenly observed too.

The mortality ratio was computed using the formula below:

$$\text{Mortality Ratio} = \frac{\text{Number of mice administered with positive sign (death)}}{\text{Total number of animals tested}}$$



**Figure 3. Mice undergoing toxicity**

Qualitative data on the traditional household use was gathered by means of focus group discussion (FGD). The presence of the plants in the municipalities of Ilocos Sur was surveyed by the enumerators. Five (5) focus groups were held with the members of the households in the three (3) municipalities in Ilocos Sur, namely San Juan, Santa Catalina and Caoayan. Below is the outline and location of the groups in which they were convened. Ilocano was the primary dialect used.

**Table 1**  
**Summary of the Three (3) municipalities in Ilocos Sur during Focus Group Discussion**

Location	Date	Dialect
Resurrection, San Juan, Ilocos Sur	February 26, 2016	Ilocano
Cabittaogan, Santa Catalina	March 10, 2016	Ilocano
Pantay Tamurong	March 16	Ilocano
Don Lorenzo Querubin	April 10, 2016	Ilocano
Don Alejandro Quirolgico	April 15, 2016	Ilocano

### **Ethical Consideration**

Institutional Animal Care and Use Committee (IACUC) certificate from the ITDI, DOST was obtained. The animals were sacrificed and necropsied after 14 days of observation. The IACUC certificate specifically approved the protocols using animals. Permission letters from the concerned local government units were secured prior to the conduct of the survey and focus group discussion to the different municipalities.

Permission letters and consent were personally handed over to the Ilocano Elders of the different barangays included in the study. The study followed the National Commission of Indigenous Peoples (NCIP) protocol for the Ilocano ethnic group where in respondents were given informed consent to

participate in the study. All the respondents were fully informed verbally and in writing about the nature and demands of the study and were informed that they could withdraw from the study at any time. The barangay folks decided among themselves who will participate in the focus group discussion.

## RESULTS AND DISCUSSION

**Table 2**  
**Nutrient Content of Roselle (*Hibiscus sabdariffa*) Seeds**

Nutrient Content	Test Method	Result
Moisture	968.11*	3.15
Ash, %	923.03*	5.36
Protein	Block Digestion-Kjeldahl	24.7
Fat	By difference	18.2
Total Carbohydrates	By calculation	48.6
Food Energy, kcal/100g		457

Results of the proximate nutrient analysis in the 120 gram sample of roselle (*Hibiscus sabdariffa*) seeds showed the presence of protein (24.7), fat (18.2), total carbohydrates (48.6) and total food energy, kcal/100g (457). The results showed that roselle seed extract contains nutrients for the Filipinos which is approximately 18 percent of the food energy, and 38 percent protein which could augment to the malnourishment concerns of the country. Moreover, the sample could provide an alternative food source to the community.

**Table 3**  
**Behavioral Observation of Female ICR Mice After Oral Administration of Sample Suspension (*Hibiscus sabdariffa* L. Seeds)**

Dose g/kg	No. of Animals	Characteristics							
		Motor activity	Respiration	Circulation	Vision/ eyes	Hair skin	Grip strength	Pinna and righting reflexes	Taxia
35	10	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours
40	10	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours
45	10	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours	normal after 24 hours

Results significantly show three preliminary increasing doses of 35, 40, and 45 g/kg were used and given orally to the laboratory animals. No death was observed even at a high dose of 45 g/kg given orally in serial in group of ten (10). The dose continued in the next 24-48 hours and daily up to 14 days. In all the doses introduced, 35, 40, and 45 g/kg no adverse/abnormal signs or death occurred within 24 hours. No other adverse/abnormal signs or death occurred within the 14-day period of observation.

However, since the plant is traditionally being eaten by the community folks since time immemorial, the safety is more or less established and preliminary confirmed by the experiment made.

It is noted that acute toxicity refers to adverse effect occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an inhalation exposure of 4 hours (UN, 2011). Initial consideration is taken such as the identity and chemical structure of test substance, its physical chemical properties, results of in vitro or in vivo toxicity tests, toxicological data on structurally related substance and not giving substances at doses known to cause marked pain and distress. Limit test can be used efficiently to identify chemicals that are likely to have toxicity.

**Table 4**  
**Summary of Mortality Ratio of Mice Administered Orally with Sample Suspension (*Hibiscus sabdariffa* L. Seeds) to Female ICR Mice**

Group Number	Dose g/kg	Number of Animals	Mortality Ratio				
			DAY 1	DAY 2	DAY 3	DAY 7	DAY 14
I	35	10	0/10	0/10	0/10	0/10	0/10
II	40	10	0/10	0/10	0/10	0/10	0/10
III	45	10	0/10	0/10	0/10	0/10	0/10

With the three groups made at 10 each from the increasing doses of 35, 40 and to the maximum of 45, all the experiment animals survived or no mortality was observed. After the experiment, the animals were sacrificed and necropsy after 14 days of observation. Necropsy examination showed no significant abnormal findings. As a matter of fact, increased in weight was observed in all the test animals. Findings further confirmed the food safety of the sample.

**Summary of Findings of the Result of the Focus Group Discussion**

The following findings are from a series of five (5) focus groups conducted with members of the research team. Participants in San Juan and Santa Catalina and some barangays in Caoayan, Ilocos Sur particularly in Pantay Tamurong, Don Lorenzo Querubin and Don Alejandro Quirolgico were recruited for two-hour discussions with the use of a facilitator’s guide.





**Figure 4. The author and the participants during focus group discussion**

Participants in all groups have agreed upon on the household use of the plants particularly the tops, calyx in Ilocano cuisine as well as the use of the seeds as beverage. They commented that the taste was comparable to local cacao.

*“Naimas nga pagdengdeng diay ugot na ken nananam diay bukel a pagkape”*

(The tops are good for vegetable stew and the seeds are made into delicious drinks)

Participants also agreed that the roselle plant is already considered a vanishing plant of the household due to its non-high value crop category in the agriculture.

*“Isuna na laeng ta mapukpukawen ta dida met aguimulan, nu awan agtubo ay ket awanen ah”*

(It is disappearing already since it is not being cultivated)

Participants described the plant as a good alternative food source.

*“Kunada, napintas a mula dayta a pagtaudan ti taraon”*

(It is a nice plant, a good source of food.)

## **CONCLUSIONS**

Roselle seeds contain essential nutrients such as fats, protein and carbohydrates that could provide alternative food source to the Filipinos with food safety initially established as manifested with no mortality among the laboratory animals used in the experiment. In terms of usage in the community,

the plant is documented to be an important household ingredient in Ilocano cuisine and fast becoming a vanishing local crop.

### RECOMMENDATIONS

It is recommended that roselle be brought back to the Ilocano cuisine as dietary intake. Its importance should be emphasized by means of information dissemination especially the agencies concerned such the Department of Agriculture and Local Government Units and eventually making it a high value crop in the local market. Information drive with seed sample distribution must be made by the Department of Agriculture and Local Government Units to re-introduce the plant.

A further study like product development must be conducted to maximize the uses of the roselle.

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### ACKNOWLEDGEMENT

It is with great pleasure for the author to acknowledge with gratitude all who contributed in the completion of this research project. First to the Almighty and all of her loved ones above who prayed for its completion; to the administrators and colleagues of the University of Northern Philippines for the funding and other support services provided.

