Research Article

# Effect of *Colocasia esculenta* (Taro) Extract in the Reduction of Vulval Development in Let-60 Gene of *Caenorhabditis elegans*

**Laarni Hannah Lacorte*, Michaela Gabrielle Amador, Hanz Kristen Carmona, Nicole De Vera, Ricah Mae Gabriel, Alphonsus Rey Manigbas, Diozelle Pascua, Azilana Yddet Leyble**

Department of Medical Technology, Far Eastern University Manila, Nicanor Reyes St, Sampaloc, Manila, Metro Manila, PHILIPPINES.

Submission Date: 15-01-2020; Revision Date: 14-03-2020; Accepted Date: 11-04-2020

## ABSTRACT

**Introduction:** Different changes in the environment drives the body to adapt for survival. Exposure to toxic agents can release free radicals from cellular metabolism that serve as a medium for a healthy cell to mutate. Let-60 gene or let-23 EGFR (epidermal growth factor receptor) regulated epithelial tissue development and homeostasis. However, inappropriate activation of EGFR is driver of tumorigenesis. Taro, root vegetable whose corm is often consumed as food. **Aim:** Taro's optimal protein content is investigated for its reduction properties against the development of vulva in *Caenorhabditis elegans*. *Caenorhabditis elegans* is an auxotrophic nematode that is often utilized as a representative of a mammal due to its genotypic similarities. **Methods:** Three concentrations, 0.5 mg/mL, 10 mg/mL and 50 mg/mL were subjected to the synchronized-growth *Caenorhabditis elegans*. Neither concentration utilized was toxic to the initial development of the nematode. The synchronized L1 stage *C. elegans* worms, which were cultivated from the nematode growth medium at 20°C, were utilized and transferred into 4 NGM plates for a more synchronized growth. The worms of L1 stage were obtained and flooded with *E. coli* OP50 as its food source. The worms of L1 stage were then grouped according to the concentration. The number of worms, those with and without vulva, are quantified. Results will be tabulated and Two-way mixed ANOVA was used to compare the effectiveness of the concentrations used and to analyze the significant difference between the data collected. **Results:** The 10 mg/mL concentration of *Caenorhabditis elegans* extract yielded the greatest number of nematode sans their vulva. **Conclusion:** No concentration of the extract was detrimental to the viability to the nematode after 24 hr of treatment. The taro extract may have reduced the vulva in *Caenorhabditis elegans* at the 10 mg/mL and 50 mg/mL concentration. Among the three concentrations, the 10 mg/mL had the most significant reducing effect.

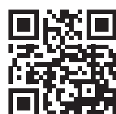
Key words: *Caenorhabditis elegans*, *Colocasia esculenta*, corm, vulva, Let-60 gene, let-23 EGFR.

**Correspondence:**
***Laarni Hannah Lacorte,***
Department of Medical Technology, Far Eastern University-Manila, Nicanor Reyes Street, Sampaloc Manila-1008, PHILIPPINES.
Phone no: +63 02 88777738
Email: llacorte@feu.edu.ph

## INTRODUCTION

Taro (*Colocasia esculenta*) is a starchy edible plant wealthy of vitamins and nutrients and traditionally been used as a medical plant for curative purposes. A wide variety of bioactive compounds can be extracted from all plant parts of taro and these compounds were shown to have important pharmacological activities including wound healing, antioxidant, antimicrobial and anti-inflammatory properties. Also, Brown[1] established that the Taro corm exhibited an anti-proliferative characteristic on a colon cancer cell line of a laboratory rat. Likewise, the crude taro extract inflicted *in vivo* proliferation of B220$^+$ splenocyte and distinct decrease in the immature B220$^+$ B cells in the bone marrow of BALB/c mice Pereira.[2] Due to its title of being a standard model organism for a variety of genetic investigations, *Caenorhabditis elegans* is used to demonstrate the reducing aptitude of Taro

SCAN QR CODE TO VIEW ONLINE



www.ajbls.org

DOI : 10.5530/ajbls.2020.9.9

at its let-60 gene. The focal problem of this research study is to discern the effect of taro extract (TE) to the vulval development in *C. elegans*. Specifically, this research will discern the concentration of taro that will and will not exhibit toxicity on the *C. elegans*. It will also focus on the concentration of the taro extract that will it affect the vulval development. Lastly, the concentration of the taro extract that will be most effective in reducing the vulva of the nematode. This study will focus mainly on the effect of *Colocasia esculenta* extract in reducing the vulval development in let-60 *Caenorhabditis elegans*. Other effects of the plant on the said model are not covered by this study. Also, the researchers will select only one market to purchase taro corm to avoid any disturbances and ensure the same quality of the plant.

## METHODOLOGY

### Case Study Research Design

This research entitled "Effect of *Colocasia esculenta* (Taro) extract in the reduction of Vulval development in let-60 gene of *Caenorhabditis elegans*" will utilize quantitative experimental research. This research was formulated to determine the effectivity of the Taro corm extract towards vulval development as a model for its anti-meta static activity. Such effectivity will be determined through the different concentrations of the said extract needed to affect the formation of vulva in *C. elegans*.

### Study Setting

The extraction of the taro corm was conducted at University of the Philippines Research Laboratory and freeze dried at University of Santo Tomas Research Center for the Natural Sciences and Applied Sciences. Testing the effectivity of taro was administered at Far Eastern University Research Laboratory, Sampaloc Manila.

### Data Collection

The plant used for this study was taro. Also, the nematode, *C. elegans*, was utilized. Taro was purchased from Iriga Local Market, Camarines Sur, Bicol Region and was transported and stored at Far Eastern University (see Figure 1). It was presented to the Bureau of Plant Industry for verification and authentication. The nematode, *C. elegans* was ordered from United States of America, transported to University of the Philippines, Manila campus. Eventually, it was transported and stored in Far Eastern University, Manila.

### Data Gathering Procedure

### Taro extract preparation and lyophilization

The 3 kilograms of taro corm was prepared and extracted in University of the Philippines Manila Natural Products Laboratory using ammonium sulfate protein precipitation (see Sig 2). According to Wingfield,[3] ammonium sulfate precipitation is commonly utilized method in the precipitation of protein. At principle, higher concentration of the salt would mean lower the solubility of the protein in the solution. Hence, more are lead to precipitate. After the extract was obtained, it was lyophilized at the University of Santo Tomas Research Center for the Natural Sciences and Applied Sciences (see Figure 3 and 4).

### Protein Estimation of Taro

According to He,[4] the process of total protein content was initiated through crude extract with a working standard solution (0-100mg/mL) of bovine serum albumin (BSA) or test substance (1mL) was introduced into each tube. To which, 1.5 mL of Bradford reagent was added. The tubes were mixed thoroughly and could stand at room temperature for 5 min. The absorbance of the reaction mixture was read at 595nm.

### Preparation of LB Broth

Approximately, 20 g of Luria-Bertani (LB) broth powder was measured and suspended in 1 liter of distilled water. Afterwards, the solution was thoroughly mixed and dissolved through heating with frequent agitation. The solution was let boil for 1 min until complete dissolution was achieved. Meanwhile, screw-cap test tubes were sterilized and autoclaved at 121°C for 15 min. The solution was transferred and were set-aside to cool down. Right after, the solution was poured in screw capped tubes.

### Preparation of LB Agar

Through an analytical balance, 35 g of Luria-Bertani (LB) agar powder was measured. The powder was dissolved in 1 liter of distilled water. Further dissolving was completed through heating with frequent agitation. The solution was let boil for 1 min until complete dissolution was achieved. The containers, encompassing the solution, were sterilized and autoclaved at 121°C for 15 min. As such in the preparation of LB broth, the Luria-Bertani (LB) agar solutions were allowed to cool down. Concurrently, the prepared solution was poured in plates.

### Preparation of Nematode Growth Medium

Researchers utilized a pre-wielded nematode growth medium, specifically designed for *C. elegans*, from Carolina Biological Supply Company. The bottle of NGM, which contained 135mL of the medium, was placed in a beaker filled with water to ensure proper submersion. Afterwards, it was heated on a hot plate until the medium fully liquefied. Consequently, it was let to cool down before. The liquefied NGM were transferred to approximately 30 plates per bottle. The medium was stored at 20°C for 48 hr before using. The medium was sealed to prevent contamination and drying.

### Seeding NGM plates

Approximately, 0.01 mL of *Escherichia coli* OP50 liquid culture was transferred using a pipette and were spread to the center of the NGM plates – creating a bacterial lawn. Afterwards, desired number of *C. elegans* was introduced to the media. The plates, containing the *C. elegans* flooded with *Escherichia coli* OP50, was stored under 20°C according to Costasince it is easy to maintain and has a very fast life- cycle. Its genome is small and a virtually complete physical map in the form of cosmids and YAC clones exists. Thus it was chosen as a model system by the Genome Project for sequencing, and it is expected that by 1998 the complete sequence (100 million bp.[5] Plates are pulled-out from its environment whenever necessary.

### Synchronization

According to Ceróntransparency and short life cycle that have made it a suitable experimental system for fundamental biological studies for many years 2. Discoveries in this nematode have broad implications because many cellular and molecular processes that control animal development are evolutionary conserved 3. *C. elegans* life cycle goes through an embryonic stage and four larval stages before animals reach adulthood. Development can take 2 to 4 days depending on the temperature. In each of the stages several characteristic traits can be observed. The knowledge of its complete cell lineage 4,5 together with the deep annotation of its genome turn this nematode into a great model in fields as diverse as the neurobiology 6, aging 7,8, stem cell biology 9 and germ line biology 10. An additional feature that makes *C. elegans* an attractive model to work with is the possibility of obtaining populations of worms synchronized at a specific stage through a relatively easy protocol. The ease of maintaining and propagating this nematode added to the possibility of synchronization provide a powerful tool to obtain large amounts of worms, which can be used for a wide variety of small or high-throughput experiments such as RNAi screens, microarrays, massive sequencing, immunoblot or in situ hybridization, among others. Because of its transparency, *C. elegans* structures can be distinguished under the microscope using Differential Interference Contrast microscopy, also known as Nomarski microscopy. The use of a fluorescent DNA binder, DAPI (4',6-diamidino-2-phenylindole,[6] synchronization is permitted through granting the nematode to grow until adult stage. 90 gravid adult worms were picked and transferred to 3 NGM plates, each containing 30 worms. Initial step of synchronization allowed the researchers to harvest gravid adults through dousing the plates with M9 buffer. Afterwards, the solution from the doused plates were transferred to 15 mL screw-cap test tubes. The tubes were then centrifuged for 10 min at 1500 rpm at room temperature. The supernatant was discarded through a Pasteur pipette. Right after, it was washed, three times, until the test tube appeared clear to the naked eye.

Then, the desired bleaching solution, an alkaline hypochlorite, was added to the test tubes containing the solution. Alkaline hypochlorite is utilized to disintegrate the worms sans the eggs. The eggs are not affected due to its shell's insensitivity to bleach.[7] The tubes were agitated for 3 to 9 min through a centrifuge. To stop the reaction of the bleaching solution, the M9 buffer was added to fill the tube. The tubes were centrifuged once more for 10 min at 1500 rpm to completely cease the reaction. Afterwards, the supernatant was discarded. Washing of the tubes, through the M9 buffer, were performed three more times to completely wash the eggs. Agitation, through manual mixing, was performed to conclude the synchronization. The solution was transferred to a plate for final microscopic observation (See Figure 5).

#### *In vitro* toxicity testing on *Caenorhabditis elegans*

The L4 stage *C. elegans* worms grown in NGM were used for toxicity testing. It was gathered and transferred into four plates containing 15 worms each. The first group which was the control along with the second, third and fourth group were treated with the food source, *E. coli* OP50 before it was flooded with the extract in different concentrations, 0.5 mg/mL, 10 mg/mL and 50 mg/mL respectively. Growth and development of the nematode was monitored after 24 hr. Then numbers of alive and dead worms were determined under a microscope.[8]

### Testing for the effect of the Taro extract to *C. elegans*

The synchronized L1 stage *C. elegans* worms, which were cultivated from the nematode growth medium at 20°C, were utilized and transferred into 4 NGM plates for a more synchronized growth. The worms of L1 stage were obtained and flooded with *E. coli* OP50 as its food source.

The worms of L1 stage were then grouped into four according to the concentration – the control, 0.5 mg/mL, 10 mg/mL and 50 mg/mL. The first group was the control. The nematodes were only treated with *E. coli* OP50. The second, third and fourth groups were flooded with the extract in different concentrations – 0.5 mg/mL, 10 mg/mL and 50 mg/mL, respectively. The worms were monitored after 48 hr upon introduction of the extract. Three (3) trials were performed by the researchers. Results were monitored and determined under a stereomicroscope. This was done to supervise a more accurate and precise quantification of vulval reduction.

### Mode of Analysis

To evaluate the research, the number of worms, those with and without vulva, are quantified. Afterwards, results will be tabulated and Two-way mixed ANOVA will be used to compare the effectivity of the concentrations used and to analyze the significant difference between the data collected.

## RESULTS

### Protein estimation of *Colocasia esculenta* (Taro) extract

Table 1 shows the protein content of the sample in different trials with their corresponding corrected absorbance. Trial 1 contains 200.01 mg of protein in 1 g of sample. Trial 2 contains 197.35 mg of protein in 1 g of sample. Trial 3 contains 205.35 mg of protein in 1 g of sample. The mean total protein of the trials 1 to 3 yields 200.90 mg of protein in 1 g of sample.

### *In vitro* toxicity testing of *C. elegans*

Table 2 shows the result of toxicity testing of *Colocasia esculenta* extract in 0.5 mg/mL, 10 mg/mL and 50 mg/mL to fifteen (15) L4 *C. elegans*.

## DISCUSSION

From the Table 2, the control exhibited a ratio of 15:0 (alive: dead) from trial 1 to trial 3. At 0.5 mg/mL concentration there were no dead *C. elegans* found in trials 1 and 3, however trial 2 yielded a ratio of 13:2. At 10 mg/mL, all trials yielded a ratio of 15:0. At 50 mg/mL yielded a ratio of 15:0 in trials 1 and 3 but 12:3 in trial 2 (see Sig 6 and 7).

As seen in Table 3, all *C. elegans* present in the negative control contained vulva – from Trial 1 to Trial 3. On the other hand, few *C. elegans* found in 0.5 mg/mL had no vulva upon introduction of the extract. In trial 1, 4 nematodes out of 15 had no vulva. Trial 2 showed that 3 out of 15 had no vulva. Furthermore, 6 out of 15 nematodes showed no vulva at trial 3. At the 10 mg/mL concentration, trial 1 showed 6 out of 15 *C. elegans* lost its vulva. 8 out of 15 nematodes without vulva was seen in both trial 2 and 3. Additionally, at the 50 mg/mL, 5 nematodes showed no vulva at trial 1 while 6 and 7 nematodes showed no vulva at trial 2 and 3, respectively (see Figure 8 and 9).

Testing for the simple main effects for treatment group means testing for differences in number of *C. elegans* with vulva between treatment groups at each category of the within-subjects factor, time periods. As there are two categories of the within-subjects factor in this study, there will be two separate tests required, pre-treatment and post-treatment.

As shown in Table 4, there was no statistically significant difference in number of *C. elegans* with vulva between treatment groups at the pre-treatment of taro extract, $F(3, 8) = 0.692$, $p = .582$. However, results for post-treatment show different effects. There was statistically significant difference in number of *C. elegans* with vulva between treatment groups at the post-treatment of taro extract, $F(3, 8) = 25.33$, $p < .001$.

As seen in Table 5, the number of *C. elegans* with vulva was statistically significantly less than the number of *C. elegans* in each of the three concentrations of taro extract as compared to the negative control group: $M = 4.0$, $SE = .943$, $p = .012$ for 0.5 mg/mL taro extract; $M = 7.0$, $SE = .943$, $p < .001$ for 10 mg/mL of taro extract; $M = 6.0$, $SE = .943$, $p = .001$ for 50 mg/mL. Also, number of *C. elegans* with vulva of 10 mg/mL of taro extract ($M = 7.67$, $SD = 1.53$) was also significantly

**Table 1: Concentration of total protein in samples.**

| Sample | Corrected Absorbance | | | mg protein/g sample | | | Mean | Standard Deviation | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 3 | | | |
| Taro | 0.15 | 0.148 | 0.154 | 200.01 | 197.35 | 205.35 | 200.90 | 4.07 | 2.96 |

**Table 2: Toxicity testing of control, 0.5 mg/mL, 10 mg/mL, 50 mg/mL of taro extract in Fifteen (15) L4 *C. Elegans*.**

| Plates | Trial 1 | | Trial 2 | | Trial 3 | |
|---|---|---|---|---|---|---|
| | Alive | Dead | Alive | Dead | Alive | Dead |
| Control<br>15 L4 *C. elegans* + *E.coli* + D.$H_2O$ | 15 | 0 | 15 | 0 | 15 | 0 |
| 0.5 mg/mL<br>15 L4 *C. elegans* + 0.5 mg/mL + *E.coli* | 15 | 0 | 13 | 2 | 15 | 0 |
| 10 mg/mL<br>15 L4 *C. elegans* + 10 mg/mL + *E.coli* | 15 | 0 | 15 | 0 | 15 | 0 |
| 50 mg/mL<br>15 L4 *C. elegans* + 50 mg/mL + *E.coli* | 15 | 0 | 12 | 3 | 15 | 0 |

**Table 3: Testing of Taro extract on the vulval development of *C. elegans* in Fifteen (15) L4 C. *elegans* in control, 0.5 mg/mL, 10 mg/mL and 50 mg/mL.**

| Plates | Trial 1 | | Trial 2 | | Trial 3 | |
|---|---|---|---|---|---|---|
| | With vulva | Without vulva | With vulva | Without vulva | With vulva | Without vulva |
| Control<br>15 L4 *C. elegans* + (*E.coli* + D.$H_2O$) | 15 | 0 | 15 | 0 | 15 | 0 |
| 0.5 mg/mL<br>15 L4 *C. elegans* + 0.5 mg/mL of extract + *E.coli* | 11 | 4 | 12 | 3 | 9 | 6 |
| 10 mg/mL<br>15 L4 *C. elegans* + 10 mg/mL of extract + *E.coli* | 9 | 6 | 7 | 8 | 7 | 6 |
| 50 mg/mL<br>15 L4 *C. elegans* + 50 mg/mL of extract + *E.coli* | 10 | 5 | 8 | 6 | 8 | 7 |

**Table 4: Tests of Simple Main Effects for Treatment Groups for Taro Extract Concentrations and Control Group.**

| Treatment Group | Type III Sum of Squares | df | Mean Square | F | Sig. | Remarks |
|---|---|---|---|---|---|---|
| Pre-Treatment | 2.25 | (3, 8) | .75 | .692 | .582 | NS |
| Post-Treatment | 95.0 | (3, 8) | 31.67 | 25.33 | <.001 | ** |

*NS. Not significant at $p < 0.05$, $**p < .01$*

less than the number of *C. elegans* with vulva of 0.5 mg/mL of taro extract ($M = 10.67$, $SD = 1.53$). The number of *C. elegans* of any other pair of treatment groups in the post reduction was not statistically different from each other. This means that the taro extracts with doses 0.5 mg/mL, 10 mg/mL and 50 mg/mL are effective in reducing or eliminating vulva in *C. elegans*.

Further scrutiny of the results in Table 4 and Table 5 and using Table 6 and Figure 1, the number of *C. elegans* with vulva at 10 mg/mL taro extract concentration group at the pre-treatment ($M = 15$, $SD = 0.0$) largely decreased after hours of treatment ($M = 7.67$, $SD = 1.16$). Also, the number of *C. elegans* with vulva at 0.5 mg/mL taro extract concentration at the pre-treatment or before the vulva reduction ($M = 14.33$, $SD = 1.16$) largely decreased after hours of taro extract treatment ($M = 10.67$, $SD = 1.53$). Moreover, the number of *C. elegans* with vulva at 50 mg/mL of taro extract ($M = 14$, $SD = 1.73$) has also significantly decreased

**Table 5: Pairwise Comparisons Using Bonferroni Adjustments between Treatment Groups for Taro Extract Concentrations and Control Group in the number of *C. elegans* with vulva at Post-Treatment**

| Treatment Group Comparisons | Mean Difference | *p*-value | Decision |
|---|---|---|---|
| Group 1 vs. Group 2 | 4.33 | 0.006** | DS > TE (0.5 mg/mL) |
| Group 1 vs. Group 3 | 7.33 | <0.001** | DS >TE (10 mg/mL) |
| Group 1 vs. Group 4 | 6.33 | 0.001** | DS > TE (50 mg/mL) |
| Group 2 vs. Group 3 | 3.00 | 0.044* | TE ( 0.5 mg/mL) >TE (10mg/mL) |
| Group 2 vs. Group 4 | 2.00 | $0.205^{NS}$ | - |
| Group 3 vs. Group 4 | −1.00 | $0.702^{NS}$ | - |

Group 1- Control (DS), Group 2- Taro extract (0.5 mg/mL), Group 3-Taro extract (10 mg/mL), Group 4 - Taro extract (50 mg/mL)
*NS. Not significant at $p < 0.05$, *. $p < 0.05$, **.$p < 0.01$*

**Table 6: Descriptive Statistics of Number of *C. elegans* with Vulva of Three Varying Taro Extract Concentrations and One Control Group in Two Time Periods.**

| | Treatment Groups | Mean | Std. Deviation | N |
|---|---|---|---|---|
| Pre-Treatment | Control | 15.0 | .00 | 3 |
| | 0.5 mg/mL | 14.33 | 1.155 | 3 |
| | 10 mg/mL | 15.00 | .000 | 3 |
| | 50 mg/mL | 14.00 | 1.732 | 3 |
| | Total | 14.58 | .996 | 12 |
| Post-Treatment | Control | 15.00 | .000 | 3 |
| | 0.5 mg/mL | 10.67 | 1.528 | 3 |
| | 10 mg/mL | 7.67 | 1.155 | 3 |
| | 50 mg/mL | 8.67 | 1.155 | 3 |
| | Total | 10.50 | 3.090 | 12 |

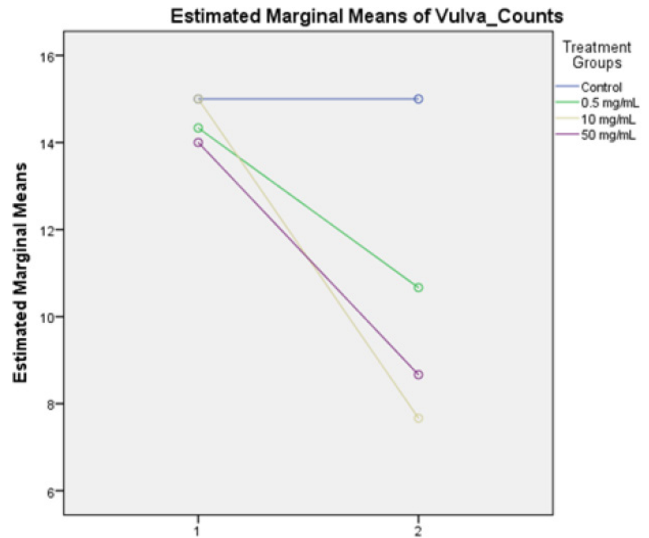



**Figure 1: Profile plots of number of *C. elegans* with vulva of three varying taro extract concentrations and one control group in two time periods.**

**Table 7: Tests of Simple Main Effects for Time Periods for Taro Extract Concentrations and Control Group.**

| Treatment Group | Type III Sum of Squares | df | Mean Square | F | Sig. | Remarks |
|---|---|---|---|---|---|---|
| Distilled Water | 1350.00 | (1, 2) | 1350.00 | . | . | NS |
| Taro extract (0.5 mg/mL) | 20.17 | (1, 2) | 20.17 | 6.37 | .128 | NS |
| Taro extract (10 mg/mL) | 80.67 | (1, 2) | 80.67 | 121.0 | .008 | ** |
| Taro (50 mg/mL) | 42.67 | (1, 2) | 42.67 | 36.57 | .026 | * |

*NS. Not significant at p>0.05, *. p<0.05, **. p<0.01*

after hours of taro extract treatment ($M$ = 8.67, $SD$ = 1.16). In other words, the treatment of taro extract was significantly effective in eliminating or reducing vulva in *C. elegans*.

Testing for the simple main effects for time periods means testing for differences in number of *C. elegans* with vulva between time points at each category (group) of the between-subjects x' factor, treatment groups. As there are four categories of the between-subjects factor in this study, there will be four separate tests required. The summary of the results is shown in Table 7.

As displayed in Table 7, there was no statistically significant effect of time on number of *C. elegans* with vulva for the negative control. There was also no statistically significant effect of time on number of *C. elegans* with vulva for the 0.5 mg/mL taro extract concentration, $F(1, 2) = 6.37, p = .128$. In other words, this taro extract concentration failed to eliminate or reduce vulva in *C. elegans*. Meanwhile, each of the other treatment groups: 10 mg/mL taro extract, $F(1, 2) = 121.0, p = .008$ and 50 mg/mL taro extract, $F(1, 2) = 42.67, p = .026$ was significantly effective in eliminating vulva in *C. elegans*.

## CONCLUSION

According to the data gathered in acute toxicity assay, no concentration of the extract was detrimental to the viability to the nematode after 24 hr of treatment. Through the performed experiment and the data gathered, the taro extract may have reduced the vulva in *Caenorhabditis elegans* at the 10 mg/mL and 50 mg/mL concentration. On the test of its reducibility characteristic, the number of *C. elegans* with vulva were significantly less in each of the three concentrations of taro extract as compared to the negative control group. Among the three concentrations, the 10 mg/mL had the most significant reducing effect. Albeit, the significance of the 50 mg/mL is at close-range with that of the 10 mg/mL. This could also mean that the concentration of the taro extract which will reduce the vulva development of the *C. elegans* is between these

values. However, even with the gathered data, the results were still inconclusive due to different external factors such as starvation, temperature and the increased population in the prepared mediums. These conditions may lead to the reduction of the vulva disregarding the treatment performed on the model.

## ACKNOWLEDGEMENT

The researchers would like to express their heartfelt gratitude to everyone behind the success of this research paper.

The researchers would also like to acknowledge to Ms.Goldilucks I. Sampilo, RMT, LPT for the personal and hands-on guidance towards finishing this research paper.

Our outmost gratitude to Sir Frederick Gella, Program head of the Department of Mathematics of Far Eastern University – Manila, for guiding us through the statistical part of this research. Without whom, this paper will lose its essence.

Also, the researchers give their sincerest thanks to Dr. Paul Mark Medina for the insightful training on handling *Caenorhabditis elegans*. To Professor John Sylvester Nas for introducing the wonders of *Caenorhabditis elegans* in Far Eastern University through one of his symposiums inside the university.

Lastly, the researchers would like to thank Professor Paolo Robert Bueno of the University of the Philippines – Manila and the University of Santo Tomas Research Center for the Natural Sciences and Applied Sciencesfor sharing their laboratory services as part of the experimentation.

## CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

### Authors' Contributions

Ms. Laarni Hannah Lacorte presented the idea, proof-read the paper and supervised the findings of this study. Hanz Kristen Carmona, Nicole De Vera, Ricah Mae Gabriel, Alphonsus Rey Manigbas and Diozelle Pascua designed the study and wrote the first draft of the manuscript. Nicole De Vera and Diozelle Pascua had the taro plant authenticated and lyophilized. Michaela Gabrielle Amador and Alphonsus Manigbas submitted the taro corm to proper institution for the extraction and protein content estimation. Author Gabriel and author Carmona performed the toxicity testing of taro extract on *C.elegans*. Alphonsus Rey Manigbas, Michaela Gabrielle Amador and AzilanaYddetLeyble constructed the concentration utilized in this research. Hanz Kristen Carmona and Azilana Yddet Leyble managed the introduction of this paper. Author Amador managed the literature searches. Ricah Mae Gabriel, Diozelle Pascua and Nicole De Vera managed the procedural part of the study. Lastly, author Alphonsus Rey Manigbas interpreted the results and discussion. All authors read and approved the final manuscript.

## ABBREVIATIONS

**EGFR:** Epidermal growth factor receptor; **BSA:** Bovine Serum Albumin; **TE:** Taro Extract ; **LB:** Luria-Bertani; **NGM:** Nematode Growth Medium.

## SUMMARY

Let-60 gene or let-23 EGFR (epidermal growth factor receptor) regulated epithelial tissue development and homeostasis. Inappropriate activation of EGFR may lead to production of tumor. Optimal protein content in taro extract is investigated for its reduction properties against the development of vulva in *Caenorhabditis elegans*. *Caenorhabditis elegans* is an auxotrophic nematode with similar genotype as with mammals. Three concentrations of taro extract, 0.5 mg/mL, 10 mg/mL, and 50 mg/mL were subjected to the synchronized-growth *Caenorhabditis elegans*. Neither concentration utilized was toxic to the initial development of the nematode. The synchronized L1 stage *C. elegans* worms, which were cultivated from the nematode growth medium at 20°C. The *C. elegans* of L1 stage were obtained and flooded with *E. coli* OP50 as its food source. The worms of L1 stage were then grouped according to the concentration. The number of worms, those with and without vulva, are quantified. Among the differecnt concentrations, the 10 mg/mL concentration of *Colocasia esculenta* extract yielded the greatest number of nematode sans their vulva.

## REFERENCES

1. Brown AC, *et al*. The anti-cancer effects of poi (*Colocasia esculenta*) on colonic adenocarcinoma cells *in vitro*. Phytotherapy Research. 2005;19(9):767-71. 10.1002/ptr.1712.
2. Pereira PR, *et al*. Crude extract from taro (*Colocasia esculenta*) as a natural source of bioactive proteins able to stimulate haematopoietic cells in two murine models. Journal of Functional Foods. 2015;18:333-43. 10.1016/j.jff.2015.07.014.
3. Wingfield PT. Protein Precipitation using Ammonium Sulfate HHS Public Access. Curr Protoc Protein Sci. 2010;3(1):3. 10.1002/0471140864.psa03fs13.
4. He F. Brad ford Protein Assay. Bio Protoc. 2011;101:e45.
5. Costa JC, Lilley CJ, Urwin PE. Caenorhabditis elegans as a model for plant-parasitic nematodes. Nematology. 2007;9(1):3-16. 10.1163/156854107779969664.

6. Porta-de-la-Riva M, *et al*. Basic <em>Caenorhabditis elegans</em> Methods: Synchronization and Observation. Journal of Visualized Experiments. 2012;10(64):1-9. 10.3791/4019.
7. Cold Spring Harbor Laboratories. The Nematode Caenorhabditis elegans. Cold Spring Harbor. 2007. [Online] http://groups.molbiosci.northwestern.edu/morimoto/research/Protocols/IX. C. elegans/A. General/3. Bleaching Protocol.pdf.
8. Himri I, *et al*. Toxicity testing of tartrazine using the nematode caenorhabditis elegans, brine shrimp larvae (*Artemia salina*) and KGN granulosa cell line. Journal of Applied Pharmaceutical Science. 2013;3(11):51-8. 10.7324/JAPS.2013.31110.


**Cite this article:** Lacorte LHC, Amador MGT, Carmona HKB, Vera NSD, Gabriel RMG, Manigbas ARB, Pascua DM, Leyble AYA. Effect of *Colocasia esculenta* (Taro) Extract in the Reduction of Vulval Development in Let-60 Gene of *Caenorhabditiselegans*. Asian J Biol Life Sci. 2020;9(1):60-7.