

# Exploring the Effect of Metal Chelating Properties of *Hylocereus undatus*, *Artocarpus heterophyllus*, and *Rubus idaeus* on the Lifespan of *Caenorhabditis elegans*: Potential ACE Inhibitors

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Based on the World Health Organization in 2019, over one billion people worldwide have hypertension<sup>10</sup>. Currently, angiotensin-converting enzyme inhibitors like captopril are used to lower blood pressure. However, these medicines produce side effects and are not fully natural. A potential method of lowering ACE levels, protecting against oxidative stress, removing heavy metals, and increasing lifespan was proposed using *Hylocereus undatus*, *Rubus idaeus*, and *Artocarpus heterophyllus*. It was hypothesized that if the metal chelating properties, ACE inhibitory properties, and the lifespan of the N2 *C. elegans* were tested using *Hylocereus undatus*, *Artocarpus heterophyllus*, and *Rubus idaeus*, then the *Hylocereus undatus* would produce the highest metal chelation activity, ACE inhibition activity, and the longest lifespan for the N2 *C. elegans* due to the high amount of phytochemicals and the health benefits of the fruit<sup>3</sup>. The fruit extracts were created using a 50-50 ethanol water solution. For the metal chelation test, a spectrophotometer was used to measure the absorbance of the mixture of ferrozine, FeSO<sub>4</sub> and the extracts at 562 nm. For the ACE inhibition test, a spectrophotometer was used to measure the absorbance of the mixture of FAPGG substrate, rabbit lung ACE, captopril, and the extracts at 340 nm. The days each individual worm lived was counted until all the worms perished. Three one-way ANOVA tests were performed for the three different methods. Statistically significant results ( $F(3,116) = 129.48835$ ,  $p < 0.00001$ ;  $F(3,116) = 21.7582$ ,  $p < 0.00001$ ;  $F(3,116) = 5.83881$ ,  $p = 0.00946$ ) were found overall between the groups. The *Hylocereus undatus* extract produced the highest metal chelating activity, the highest ACE inhibition activity, and had the longest lifespan for the *C. elegans*, supporting the hypothesis. Although, there was an insignificant difference between the *Rubus idaeus* and *Hylocereus undatus*. This study opens up the possibility of using natural substances especially *Hylocereus undatus* and *Rubus idaeus* as ACE inhibitors, metal chelating agents, and a source to potentially increase the human life expectancy in a long-term situation.

## Introduction

Angiotensin converting-enzyme (ACE), an enzyme located in the body of organisms, produces angiotensin I and angiotensin II. This is a substance that causes the narrowing of the blood vessels, prompting the heart to work harder than it should. In other words, angiotensin II releases hormones, producing an increase in the blood pressure. Having high ACE levels is an important issue among multiple people in the world. Based on the World Health Organization in 2019, over one billion people worldwide have hypertension, and this rate is increasing due to the pandemic<sup>10</sup>. A high ACE level could lead to a disorder or disease such as cirrhosis, diabetes, amyloidosis, HIV, and even COVID-19. Through the analysis of multiple research sources on ACE and ACE inhibitors, a significant gap was identified in current research to potentially reduce ACE naturally as this would bring various health benefits towards humans.

There are various methods of ACE inhibition for humans. Currently, the use of medicines such as captopril and lisinopril are used to help reduce the ACE present<sup>6</sup>. This is a class of medication used to treat high blood pressure and heart failure. This works by allowing the blood vessels to relax and decreasing the blood volume leading to lower blood pressure and decreased oxygen demands<sup>9</sup>. Although these types of medicine are effective, they can produce numerous negative effects. For example, according to Ratan-NM's article, "ACE Inhibitors: Mechanism of Action, Side Effects and Precautions," ACE inhibitors can cause side effects such as dry persistent cough, diarrhea, nausea, tiredness, chest pains, and fever (2021)<sup>16</sup>. Other effects include paroxysmal cough, angioedema, hypotension, and hyperkalemia, which are significant adverse effects of these angiotensin converting-enzyme inhibitors (ACEI). Due to this, the guidelines and recommendations should be considered when taking ACEI. Using this product can be harmful to the health and worsen one's conditions if an individual is allergic or over-consumes. These medicines can only be taken with a physician's recommendation, which could be inconvenient. Moreover, the current ACE inhibitors cannot be taken if an individual wants to prevent the rise of blood pressure<sup>16</sup>. Although the inhibitors are functional in reducing hypertension and other diseases, the expenses of these medicines can be difficult to afford. This is a major disadvantage to the low- and middle-income families which make up over 2/3 of the population of people who have hypertension<sup>10</sup>. Through the analysis of multiple research sources on ACE and ACE inhibitors, a significant gap has been identified in current research to potentially reduce ACE naturally as this would bring multiple health benefits towards humans.

Metals are vital for human survival and functionality. For typical biological function in the body, metals such as sodium, potassium, magnesium, copper, iron, and zinc are essential. These types of metals are beneficial to the body; although, metal toxicity can occur due to metal overload or exposure to heavy metals<sup>12</sup>. This is considered detrimental to one's health and body. To rid these toxic metals, the use of chelating agents can bind the toxic/heavy metal ions to form complexes which can easily be excreted from the body<sup>12</sup>. Metal chelation is a type of bonding of ions and molecules to metal ions. This process helps move heavy metals out of the body and cleanse it from any toxicity. Various forms of metals are present which can potentially have negative effects on the body. For example, based on Flora and Pachauri's article (2010), "Chelation in Metal Intoxication," chelation therapy is the preferred treatment for reducing the toxic effects of metals<sup>8</sup>. Common chelating agents that are used in therapies are calcium disodium ethylenediaminetetraacetic acid, dimercaprol, edetate disodium, 2,3-dimercaptosuccinic acid (DMSA), and penicillamine. The use of these therapies does not necessarily always produce positive results. Multiple side effects can occur including fevers, headaches, nausea, vomiting, burning at the IV site, and abnormally low blood-calcium levels. Moreover, combination therapies are reported as newer strategies to address the drawbacks for chelation therapies for metal toxicity. These therapies are effective and produce fewer side effects, but it is not completely natural. Based on Paul M. Wax's article, "Current Use of Chelation in American Health Care," more than 100,000 Americans receive chelation each year, but majority of these cases are not supervised by medical professionals<sup>18</sup>. Because of the expense, individuals are performing these risky therapies without medical experience. Chelation should be used carefully as there are many risks like copper deficiency<sup>18</sup>. Currently, natural substances are now being considered as potential metal chelating agents.

The use of natural substances may reduce the toxic metals and high blood pressure in the body. Specifically, in this study, *Hylocereus undatus* (dragon fruit), *Artocarpus heterophyllus* (jackfruit), and *Rubus idaeus* (raspberry) will be used to possibly reduce ACE and the oxidative stress in the body. *Hycoleracus undatus* is an oval-shaped fruit that has a bright red-purple-yellow outside, a white-flesh inside, and prominent scales.

*Artocarpus heterophyllus*, a banana-tasting fruit, has a hard and spiky outer covering with a yellow inside. *Rubus idaeus* is a common fruit that contains a red bumpy outer covering which has a sweet, tart taste. For example, based on Ranasinghe, R. A. S. N., Maduwanthi, S. D. T., & Marapana, R. A. U. J.'s article, "Nutritional and Health Benefits of Jackfruit (*Artocarpus heterophyllus* Lam.): A Review," the nutritional and health benefits of jackfruit are anticarcinogenic, antimicrobial, antifungal, anti-inflammatory, wound healing, and hypergolic effects (2019)<sup>15</sup>. Moreover, jackfruit contains the phytochemicals (chemical compounds produced by plants): carotenoids, flavonoids, volatile acids, sterols, and tannins. Additionally, based on Miho Hatanaka's article, "Health Benefits of Raspberries," raspberries have a high antioxidant content which include vitamin C, vitamin E, beta carotene, etc (n.d.)<sup>11</sup>. These fruits have phytochemicals and can reduce free radicals due to the high antioxidant activity. This fruit also has good fiber, can improve brain power, reduce blood pressure, suppress inflammation, and reduce the chance of attaining heart disease. Furthermore, based on Burlando et al.'s article (2020), "Emerging Exotic Fruits: New Functional Foods in the European Market," dragon fruits have anticancer, antimicrobial, anti-inflammatory, and neuroprotective effects, as well as cardiovascular and skin protection<sup>2</sup>. These fruits could possibly be used in drug discovery, help prevent diseases, and become potential treatments for various health problems. These three fruits have not been tested for the ACE inhibition activity or metal chelation activity. In addition, *C. elegans* will be used as the model organism to test the effects of the potential, natural ACE inhibitors on lifespan. This is because they are relatively simple organisms with molecular signals controlling its development like humans, they have a short lifespan, and they are easy to handle<sup>14</sup>. It is also used in various studies due to their transparency and fast growth<sup>14</sup>. Only a microscope needs to be used to view them, making it simple for the researcher to observe the fate of the *C. elegans*<sup>14</sup>. Due to the large amounts of benefits of the fruits, the lifespan of the *C. elegans* will likely increase. Solutions were made from the three test substances, dragon fruit, jackfruit, and raspberry. Four treatments were tested creating extracts with a 50-50 ethanol-water solution of the three substances.

Previous research shows how angiotensin converting enzymes extend the lifespan of *C. elegans*<sup>13</sup>. Kumar, Dietrich, and Kornfield's paper, "Angiotensin Converting Enzyme (ACE) Inhibitor Extends *Caenorhabditis elegans* Life Span" discusses how animal aging produces multiple changes for an individual's body system<sup>13</sup>. Aging is a well-known issue towards disability and death in humans, so treatments for this are desirable. Currently, no medicines have been discovered to reduce human aging. Some interventions have been made to delay age-related degeneration in animals rather than humans. These include dietary modifications, caloric restriction, genetic changes, and the use of certain drugs. FDA-approved compounds were screened, and it was found that captopril, which is a hypertension drug, had significantly extended the lifespan of *C. elegans*. *C. elegans* are known to be a good model for observing lifespan due to their short average lifespan. To identify the drugs that influence aging, 15 compounds were selected which are FDA approved for human use based upon their effects on the body. The compounds were added to the agar in 3 different concentrations and the lifespan of the *C. elegans* was cultured with *Escherichia coli* OP40 as a food source. 2.5 mM was known to cause a significant 23% extension of the mean lifespan and an 18% extension of maximum life span. The purpose of captopril is to reduce ACE in human bodies to maintain an even blood pressure. In this study, the *C. elegans* homolog of ACE is encoded by the *acn-1* gene. Reducing the activity of this gene had also extended lifespan in *C. elegans*. This is because captopril and *acn-1* are similar through their mechanism of action. Although, it was found that the combination of the two did not have a synergistic effect on the increase of lifespan. The results present a new drug or gene that influences ACE and could potentially be a base for therapeutic interventions. Withal, according to Borowska, S., Tomczyk, M., Strawa, J. W., & Brzóska, M. M. article, "Estimation of the Chelating Ability of an Extract from *Aronia melanocarpa* L. Berries and Its Main Polyphenolic Ingredients Towards Ions of Zinc and Copper," chokeberries are found to have a beneficial impact on health. Based on data, it can be supported that the protective action of the polyphenols and the rich products in it against heavy-metal toxicity relates to the chelating abilities and antioxidative properties (2020)<sup>1</sup>. Polyphenols due to the presence of the PH groups are able to form complexes with ions of toxic metals. Chokeberry extract may influence the body status of zinc and copper, which includes their absorption from the digestive tract<sup>1</sup>. The overarching message of the article was to express how chokeberry has a metal chelating ability towards zinc and copper. The introduction of the natural substances brings way to other fruits which could be used to improve metal toxicity -- a gap of this research study. Natural substances are slowly being used more as an ACEI. For instance, See et al.'s article, "ACE (Angiotensin Converting Enzyme) Inhibition Activity of Oven – Dried and Air – Dried *Sambong Blumea balsamifera* L.(dc.) Tea" discusses the effects of ACE inhibition on the body<sup>17</sup>. This resource describes how the current ACE inhibitors like captopril and enalapril contain side effects like cough, angioedema, and many more. In addition, it is a major challenge to find new drugs that will be more selective and have less side effects. Therefore, in this study the *Sambong Blumea balsamifera* is abundant in the Philippines which is why it was chosen. In See et al.'s study, phytochemicals like the flavonoids and terpenoids were assumed to be the reason for the inhibition of the ACE. It further explains the methodology of testing for ACE inhibition activity of the tea leaves in comparison to captopril. The study leads to testing other natural substances to test for an ACE inhibition effect -- another gap of the study.

## Methods

For safety precautions, hair was tied back, gloves were worn, and goggles were worn to prevent the spreading of the chemicals onto the skin throughout experimentation. Extracts of *Artocarpus heterophyllus*, *Rubus idaeus*, and *Hylocereus undatus* were created. The fruits were placed in a food processor and blended for 30 seconds. After this, a 50% ethanol-water solution was created with the addition of a 1:4 ratio of the pureed fruits. The fruits were set in the refrigerator for half an hour and then transferred to tubes to centrifuge for 15 minutes. After the centrifugation, the supernatant was added into new containers and were heated to remove the alcohol. Then a Furanacroyl-Phe-Gly-Gly (FAPGG) assay was used for the ACE inhibitory properties test. A tablet that has 25 mg of captopril was crushed and was filled with water to reach 25 mL<sup>17</sup>. The Tris-HCl was used as a buffer for the substrate FAPGG. 1.21 grams of Tris was dissolved in water to reach 50 mL<sup>17</sup>. The resulting solution was added with 21.9 mL of 0.2M HCL to obtain a volume of 200 mL. The ACE from rabbit lung 25 mg was used as the enzyme for the assay<sup>17</sup>. This was made into a solution with 25 mL of Tris-HCl. The extract was tested by mixing the 100 microliters of fruit extracts, 50 microliters of ACE solution, and 1 mL of FAPGG substrate were mixed in a test tube and incubated for 60 minutes at 37 degrees Celsius. At the end of 60 minutes, the solution was transferred to a cuvette and diluted with distilled water to reach the brim<sup>17</sup>. The absorbance of the samples was measured at 340 nm using UV-Vis spectrophotometer. This was done with the blank and positive control. A sample containing ACE of 50 microliters and FAPGG of 1 mL was used as the blank while captopril of 100 microliters was used as the positive control<sup>17</sup>. Then, the equation used for this assay was ACE inhibitory activity (%) =  $\left[ \frac{1 - (A_{\text{sample}} - A_{\text{sample blank}})}{(A_{\text{control}} - A_{\text{control blank}})} \right] * 100$ <sup>5</sup>. This was repeated 30 times with each fruit. Additionally, for the metal chelating activities, 0.25 mM ferrozine (0.4 mL) and 0.1 mM iron (II) sulfate (0.2 mL) were mixed in with each extract (0.2 mL)<sup>3</sup>. The mixture was strenuously shaken and left at room temperature for 10 minutes. The ferrozine was able to bind to ferrous ions in a complexation reaction to form a complex which has a unique absorbance at 562 nm. Each mixture was placed in the spectrophotometer and the absorbance was measured at 562 nm to calculate the chelation activity. The following equation was used: metal chelating activity =  $\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} * 100$  (A=absorbance)<sup>4</sup>. This was repeated 30 times with each fruit. Substances were used on the *C. elegans* to determine their effect on lifespan. This was done by boiling nematode growth medium agar in an erlenmeyer flask. After cooling to approximately 50 degrees Celsius, the agar was poured into 30 petri dishes. Then, the agar was set to cool for half an hour. After this, *E. coli* was added by an inoculation loop and left to grow for 8 hours at a temperature of 37 degrees Celsius in an incubator<sup>7</sup> as this was the food source for the *C. elegans*. Then, 100 microliters of the fruit substances and FUDR (prevents

spawning) were added and spread around the agar with an inoculation loop in the petri dishes. After, the *C. elegans* were added by cutting a section of the original culture and placing it face down on the new petri dishes. The plates were incubated at room temperature of 22 degrees Celsius. After the *C. elegans* reached the L4 phase, 30 *C. elegans* from each group were moved to a new set of petri dishes. Every other day the new group of *C. elegans* were moved to new petri dishes to ensure no contamination. Each day, the *C. elegans* were counted until none remained to determine their lifespan. To identify if the *C. elegans* perished, the nematodes that did not display spontaneous movements or respond to shaking from the platinum wire were counted as dead. After all the *C. elegans* perished, a 20% isopropyl alcohol and 70% bleach solution was added in to ensure the death of the *C. elegans*. Three one-way ANOVA tests were performed for all three different data tables.

## Results

A metal chelation test, an ACE inhibition test, and a lifespan assay on *C. elegans* were completed using *Hylocereus undatus*, *Rubus idaeus*, and *Artocarpus heterophyllus*. In each section of the experiment, differences among the substances were seen along with differences in the absorbance and lifespan rates. Based on the data, there were various percentages of increase in all three of the tests. Each substance produced a different appearance. For example, the jackfruit had the smoothest consistency and formed a pastel yellow color. The raspberry had a thicker consistency with a bright magenta color. The dragon fruit had a thin consistency with a light purple color. Although, all the extracts created from the substances formed a similar thin consistency.

According to the summary data table (Table 2) for metal chelation activity (raw table in appendix A), the mean for the *Hylocereus undatus* extract is the highest with 82.17%. The mean of the *Rubus idaeus* extract is the second highest with 73.93%. The *Artocarpus heterophyllus* extract has the mean of 49.47%, while the control has the mean of 0%. Among the three fruits, the standard deviation of the *Rubus idaeus* extract is the highest with 28.33, while the *Hylocereus undatus* extract had the lowest standard deviation of 13.06 meaning that the raspberry extract had the higher variation than the dragon fruit extract. Table 3 represents the metal chelation test for the three fruits. According to the trial for the metal chelation, statistically significant results were found at  $F(3,116) = 129.48835$ ,  $p < 0.00001$ . Figure 4 represents the metal chelation percentages of the three fruits using a graph. Based on Figure 4, the dragon fruit extract produced the highest metal chelation activity, while the jackfruit extract produced the least metal chelating activity out of the three substances. Since the results were statistically significant based upon the F-ratio, a post hoc tukey test was performed. There were statistically significant results between the *Artocarpus heterophyllus* extract and *Rubus idaeus* extract, *Artocarpus heterophyllus* extract and *Hylocereus undatus* extract, the control and *Hylocereus undatus*, the control and *Rubus idaeus* extract, and the control and *Artocarpus heterophyllus*. Although, there were no statistically significant results between *Hylocereus undatus* extract and *Rubus idaeus* extract meaning they have similar metal chelation activities.

According to the summary data table for the ACE inhibitory activity (raw table in appendix A), the mean for the *Hylocereus undatus* extract is the highest with 163.67%. The mean of the *Rubus idaeus* extract is the second highest with 157.30%. The *Artocarpus heterophyllus* extract has the mean of 91.53%, while the control has the mean of 0%. Among the three fruits, the standard deviation of the *Rubus idaeus* extract is the highest with 139.47, while the *Artocarpus heterophyllus* extract had the lowest standard deviation of 37.62 meaning that the raspberry extract had the higher variation than the jack fruit extract. According to the trial for the metal chelation, statistically significant results were found at  $F(3,116) = 21.7582$ ,  $p < 0.00001$ . Based on the bar graph, the dragon fruit extract produced the highest ACE inhibition activity, while the jackfruit extract produced the least ACE inhibition activity out of the three substances. Because the results were statistically significant based upon the F-ratio, a post hoc tukey test was performed. There were statistically significant results between the *Artocarpus heterophyllus* extract and *Rubus idaeus* extract; *Artocarpus heterophyllus* extract and *Hylocereus undatus* extract; the control and *Hylocereus undatus*; the control and *Rubus idaeus* extract; and the control and *Artocarpus heterophyllus*. Although, there were no statistically significant results between *Hylocereus undatus* extract and *Rubus idaeus* extract meaning they have similar ACE inhibition activities.

According to the summary data table for the *C. elegans* life span (raw table in appendix A), the mean for the *Hylocereus undatus* extract is the highest with 15.87 lives. The mean of the *Rubus idaeus* extract is the second highest with 14.03 lives. The *Artocarpus heterophyllus* extract has the mean of 12.43 lives, while the control has the mean of 9.43 lives. The standard deviation of the *Hylocereus undatus* extract is the highest with 7.17, while the control had the lowest standard deviation of 5.12 meaning that the dragon fruit extract had the highest variation compared to the rest of the extracts. According to the trial for the metal chelation, statistically significant results were found at  $F(3,116) = 5.83881$ ,  $p = 0.00946$ . Based on the bar graph, the dragon fruit had caused the *C. elegans* to have the longest lifespan, while the control caused the *C. elegans* to have the shortest lifespan. Because the results were statistically significant based upon the F-ratio, a post hoc tukey test was performed. There were statistically significant results between the control and *Rubus idaeus* extract & the control and *Hylocereus undatus* extract. Although, there were no statistically significant results between the control and *Artocarpus heterophyllus* extract; *Artocarpus heterophyllus* extract and *Hylocereus undatus* extract; and *Rubus idaeus* extract and *Artocarpus heterophyllus* extract.

## Discussion

*Hylocereus undatus* extract, *Rubus idaeus* extract, and *Artocarpus heterophyllus* extract were tested to determine their metal chelation activity, ACE inhibition activity, and their effect on the lifespan of *C. elegans*. The study's importance was to discover a natural substance to reduce ACE (blood pressure), oxidative stress, and heavy metals within the body. Moreover, the fruit extracts were tested on the *C. elegans* to determine if the lifespan would increase to compare with the human lifespan. *Hylocereus undatus* has properties including anticancer, antimicrobial, anti-inflammatory, and neuroprotective effects, as well as cardiovascular and skin protection. These fruits could possibly be used in drug discovery, help prevent diseases, and become potential treatments for health problems. Dragon fruit contains wide ranges of antioxidant activity and phytochemicals of betalains<sup>3</sup>, which are discovered to protect against oxidative stress in the body<sup>2</sup>. *Rubus idaeus* contains a high antioxidant content including vitamin C, vitamin E, beta carotene, and many more<sup>11</sup>. Moreover, raspberries have phytochemicals including gallotannins, coumaric acid, flavonoids, quercetin, anthocyanins, ferulic acid, and ellagic acid. These fruits can also reduce free radicals and protect against oxidative stress. Also, this fruit has good fiber, can improve brain power, reduce blood pressure, suppress inflammation, and reduce the chance of attaining heart disease<sup>11</sup>. *Artocarpus heterophyllus* has anticarcinogenic, antimicrobial, antifungal, anti-inflammatory, wound healing, and hypergolic effects. Moreover, jackfruit contains the phytochemicals of carotenoid, flavonoids, volatile acids, sterols, and tannins<sup>15</sup>. Due to the high number of antioxidants and phytochemicals in each of the fruits, they potentially can reduce blood pressure, increase longevity, and fight against toxic metals in the body. Based on the information found, it was hypothesized that if the metal chelating and ACE inhibitory properties are tested for *Hylocereus undatus*, *Artocarpus heterophyllus*, and *Rubus idaeus*, then the *Hylocereus undatus* would have the highest metal chelation and ACE inhibition activity due to the high amount of phytochemicals and health benefits of the fruit<sup>3</sup>. Moreover, it was also hypothesized that if *Hylocereus undatus*, *Artocarpus heterophyllus*, and *Rubus idaeus* extracts are added to the N2 *C. elegans*, then the *Hylocereus undatus* extract would cause the N2 *C.*

*elegans* to have the longest lifespan due to the high amount of phytochemicals and the health benefits of the fruit<sup>3</sup>. Previous results showed that ACE inhibitors help increase the lifespan of *C. elegans*<sup>13</sup>. Not only this, another study showed that chokeberries have metal-chelating effects which gives way into using natural substances as a metal-chelating agent<sup>1</sup>.

The *Hylocereus undatus* extract produced the highest percentage effect for the ACE inhibition activity and the metal chelation activity. It also produced the longest lifespan for the *C. elegans*. Although, in all the tests, the difference between the raspberry extract and dragon fruit extract are not statistically significant, meaning they have similar values for metal chelating activity, ACE inhibition activity, and the *C. elegans* lifespan. Based on the results, it can be concluded that the hypothesis was supported, but due to the insignificant difference between the raspberry and dragon fruit, both are effective in increasing *C. elegans* lifespan, and acting as metal chelating agents and ACE inhibitors. Also, all the groups except the control had a significant increase in all three tests showing their effectiveness. The reason for these conclusions could be attributed to the phytochemical content. Dragon fruit contains betanin which has a similar structure to the ACE inhibitor of captopril which is most likely why it performed the best. The other two fruits potentially did not have as much of a phytochemical content compared to the dragon fruit. Further research would need to be conducted to clarify this.

Limitations are present due to the lack of available equipment. Serial dilutions were created rather than using lab equipment to measure the concentrations of the iron sulfate and the ferrozine due to the small amounts which could have produced a source of error. Also, various methodologies are present for calculating ACE inhibition and metal chelation; although because certain equipment was unavailable, only one type of test was calculated for each activity. This means that only one metal, which was iron, was tested for the metal chelation. Due to the time limitation and COVID-19 restrictions, a test for another metal like copper could not be completed. Because of this, there is a limitation placed on the evidence regarding which metals the fruits could chelate. Also, some solutions were potentially not fully concentrated, as using heat to rid extra solution from the extracts could have not removed everything. Therefore, a rotary evaporator could have improved the methods as this would have removed the ethanol in a more efficient way, but this was not affordable.

Real-life applications include using the fruits in an every-day diet to improve one's body to help reduce or maintain blood pressure, to reduce the build up of excessive metals, and to lengthen the lifespan in the long term. The fruits, especially dragon fruit and raspberry, can be used in a raw manner, in smoothies, in jams, or as supplements. This new understanding can allow humans to include these fruits in their diet to improve blood pressure and reduce the chance of attaining various diseases. Especially since the fruits are available worldwide, this is convenient in a global setting.

For future research, testing other fruits or plants such as kumquat, peppermint, pomegranate, and lemon balm for ACE inhibition, metal chelation activity, and lifespan would be beneficial to determine if they also have better or similar effects as the fruits tested in this study. This is because each of these fruits have various health benefits and contains high phytochemicals. Also, using another model organism such as *Daphnia magna* could produce different results. Moreover, using new tests to perform ACE inhibition activity and metal chelation activity can potentially produce different or more precise results. Overall, procedural improvements and the use of new tests, substances, and measuring instruments could produce more favorable results.

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## Tables/Figures

### Experimental Design Diagram

<b>Title of the Experiment:</b> Exploring the Effect of Metal Chelating Properties of <i>Hylocereus undatus</i> , <i>Artocarpus heterophyllus</i> , and <i>Rubus idaeus</i> on the Mortality Rate of <i>Caenorhabditis elegans</i> : Potential ACE Inhibitors				
<b>Hypothesis</b> If the metal chelating and ACE inhibitory properties are tested for <i>Hylocereus undatus</i> , <i>Artocarpus heterophyllus</i> , and <i>Rubus idaeus</i> , then the <i>Hylocereus undatus</i> would have the highest metal chelation and ACE inhibition activity due to the high amount of phytochemicals and health benefits of the fruit <sup>3</sup> . If <i>Hylocereus undatus</i> , <i>Artocarpus heterophyllus</i> , and <i>Rubus idaeus</i> extracts are added to the N2 <i>C. elegans</i> , then the <i>Hylocereus undatus</i> extract would cause the N2 <i>C. elegans</i> to have the longest lifespan due to the high amount of phytochemicals and health benefits of the fruit <sup>3</sup> .				
<b>Independent Variable</b>				
<b>Independent Variable</b>	<i>Hylocereus undatus</i> extract (mL)	<i>Artocarpus heterophyllus</i> extract (mL)	<i>Rubus idaeus</i> extract (mL)	No extract - Distilled water
<b># of Repeated Trials for Metal Chelation Tests</b>	30	30	30	30
<b># of Repeated Trials for ACE Inhibitory Property Tests</b>	30	30	30	30
<b># of Repeated Trials for <i>C. elegans</i></b>	30	30	30	30
<b>Dependent Variable</b> The days that the <i>C. elegans</i> lived (lifespan) and the percentage for metal chelation tests and ACE inhibitory property tests based on the results from the spectrophotometer (absorbance of the mixture) (method found through research).				
<b>Control Group</b> The control group for the metal chelation test and the ACE inhibitory property test is with no extract, but rather distilled water. For the <i>C. elegans</i> , no extract will be used, and it will simply be the <i>C. elegans</i> with the <i>E. coli</i> .				
<b>Constants</b> Type of <i>C. elegans</i> strain (Wild type N2), type of <i>E. coli</i> (OP50), the amount of extract used, the number of trials, the spectrophotometer used, the petri dishes, the amount of ferrozine used, the amount of FeSO <sub>4</sub> used, the amount of FAPGG used, and the containers being used.				

Figure 1 - Images of dragon fruit, raspberry, and jackfruit: puree and extract

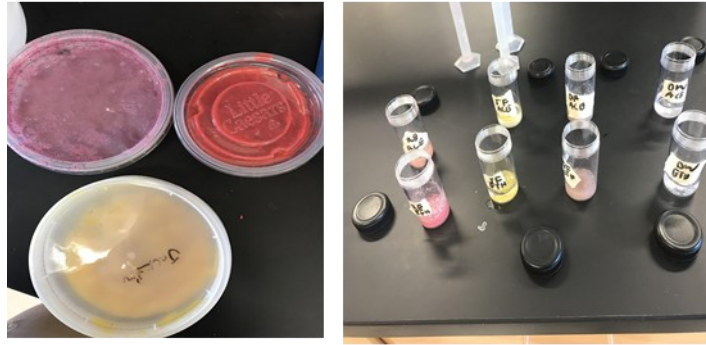


Figure 1 - The picture to the left presents the different shades of the puree of three fruits. The picture to the right presents extracts created from ethanol, distilled water, and the puree.

Table 2 - Summary of Data for Metal Chelation tests

Metal Chelation Activity (%)	<i>Artocarpus heterophyllus</i>	<i>Rubus idaeus</i>	<i>Hylocereus undatus</i>	Control
N	30	30	30	30
Mean	49.47	73.93	82.17	0
St. Dev	17.12	28.33	13.06	0

Table 2 - This presents the descriptive statistics of the metal chelation activity of *Hylocereus undatus* extract, *Rubus idaeus* extract, *Artocarpus heterophyllus* extract, and the control. The number, mean, and standard deviation of all 4 groups are listed.

Table 3 - Result Details of the Metal Chelation Test

Result Details				
Source	SS	df	MS	
Between-treatments	123001.1	3	41000.4	F=129.5
Within-treatments	36729.5	116	316.6	
Total	159730.6	119		

Table 3 - This presents the results from an ANOVA one-way test of the metal chelation activity of *Hylocereus undatus* extract, *Rubus idaeus* extract, *Artocarpus heterophyllus* extract, and the control.

Figure 4 - Bar Graph of Metal Chelation Tests

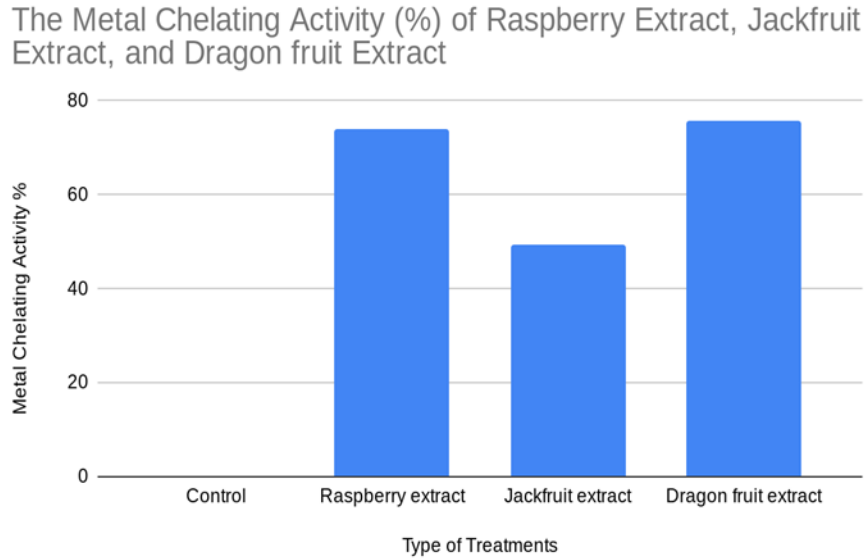


Figure 4 - This provides a bar graph of each extract from the order of control, *Artocarpus heterophyllus* extract, *Rubus idaeus* extract, and *Hylocereus undatus* extract for the metal chelation activity.

Table 5 - Post Hoc Tukey Test for Metal Chelation Tests

Pairwise Comparisons		HSD <sub>.05</sub> = 11.9762 HSD <sub>.01</sub> = 14.6204	Q <sub>.05</sub> = 3.6864    Q <sub>.01</sub> = 4.5003
T <sub>1</sub> :T <sub>2</sub>	M <sub>1</sub> = 49.47 M <sub>2</sub> = 73.93	24.47	Q = 7.53 (p = .00000)
T <sub>1</sub> :T <sub>3</sub>	M <sub>1</sub> = 49.47 M <sub>3</sub> = 82.17	32.70	Q = 10.07 (p = .00000)
T <sub>1</sub> :T <sub>4</sub>	M <sub>1</sub> = 49.47 M <sub>4</sub> = 0.00	49.47	Q = 15.23 (p = .00000)
T <sub>2</sub> :T <sub>3</sub>	M <sub>2</sub> = 73.93 M <sub>3</sub> = 82.17	8.23	Q = 2.53 (p = .28239)
T <sub>2</sub> :T <sub>4</sub>	M <sub>2</sub> = 73.93 M <sub>4</sub> = 0.00	73.93	Q = 22.76 (p = .00000)
T <sub>3</sub> :T <sub>4</sub>	M <sub>3</sub> = 82.17 M <sub>4</sub> = 0.00	82.17	Q = 25.29 (p = .00000)

Table 5 - This provides the post hoc tukey test for metal chelation activity of control (T<sub>4</sub>), *Artocarpus heterophyllus* extract (T<sub>1</sub>), *Rubus idaeus* extract (T<sub>2</sub>), and *Hylocereus undatus* extract (T<sub>3</sub>). It shows the statistical significance between each group.

Table 6 - Result Details of the ACE Inhibition Tests

Result Details				
Source	SS	df	MS	
Between-treatments	521080.7	3	173693.6	F=21.8
Within-treatments	926016.4	116	7982.9	
Total	1447097.1	119		

Table 6 - This presents the results from an ANOVA one-way test of the ACE inhibition activity of *Hylocereus undatus* extract, *Rubus idaeus* extract, *Artocarpus heterophyllus* extract, and the control.

Figure 8 - Bar Graph of ACE Inhibition Tests

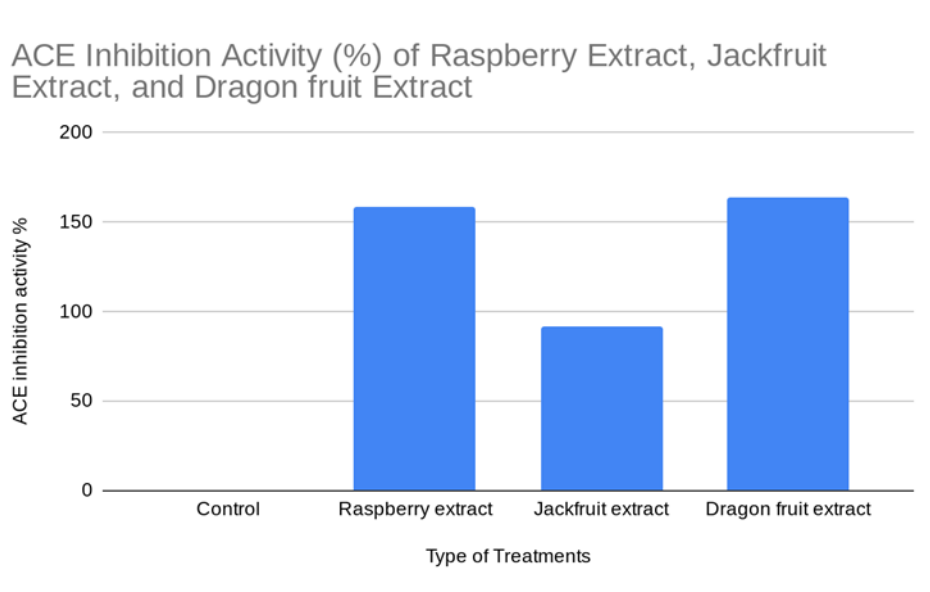


Figure 8 - This provides a bar graph of each extract from the order of control, *Artocarpus heterophyllus* extract, *Rubus idaeus* extract, and *Hylocereus undatus* extract for the ACE inhibition activity.



Table 9 - Post Hoc Tukey Test for ACE Inhibition Tests

Pairwise Comparisons		HSD <sub>.05</sub> = 60.1343 HSD <sub>.01</sub> = 73.4110	Q <sub>.05</sub> = 3.6864 Q <sub>.01</sub> = 4.5003
T <sub>1</sub> :T <sub>2</sub>	M <sub>1</sub> = 91.53 M <sub>2</sub> = 157.30	65.77	Q = 4.03 (p = .02619)
T <sub>1</sub> :T <sub>3</sub>	M <sub>1</sub> = 91.53 M <sub>3</sub> = 163.67	72.13	Q = 4.42 (p = .01182)
T <sub>1</sub> :T <sub>4</sub>	M <sub>1</sub> = 91.53 M <sub>4</sub> = 0.00	91.53	Q = 5.61 (p = .00072)
T <sub>2</sub> :T <sub>3</sub>	M <sub>2</sub> = 157.30 M <sub>3</sub> = 163.67	6.37	Q = 0.39 (p = .99262)
T <sub>2</sub> :T <sub>4</sub>	M <sub>2</sub> = 157.30 M <sub>4</sub> = 0.00	157.30	Q = 9.64 (p = .00000)
T <sub>3</sub> :T <sub>4</sub>	M <sub>3</sub> = 163.67 M <sub>4</sub> = 0.00	163.67	Q = 10.03 (p = .00000)

Table 9 - This provides the post hoc tukey test for ACE inhibition activity of control (T<sub>4</sub>), *Artocarpus heterophyllus* extract (T<sub>1</sub>), *Rubus idaeus* extract (T<sub>2</sub>), and *Hylocereus undatus* extract (T<sub>3</sub>). It shows the statistical significance between each group.

Table 10 - Summary of Data for *C. elegans* Lifespan Tests

<i>C. elegans</i> Lifespan (%)	<i>Artocarpus heterophyllus</i>	<i>Rubus idaeus</i>	<i>Hylocereus undatus</i>	Control
N	30	30	30	30
Mean	12.43	14.03	15.87	9.43
St. Dev	5.83	6.42	7.17	5.12

Table 10 - This presents the descriptive statistics of the *C. elegans* life span for the *Hylocereus undatus* extract, *Rubus idaeus* extract, *Artocarpus heterophyllus* extract, and the control. The number, mean, and standard deviation of all 4 groups are listed.

Table 11 - Result Details of the *C. elegans* Lifespan Tests

Result Details				
Source	SS	df	MS	
Between-treatments	669.4	3	223.1	F=5.8
Within-treatments	4433.2	116	38.2	
Total	5102.6	119		

Table 11 - This presents the results from an ANOVA one-way test of the *C. elegans* lifespan for *Hylocereus undatus* extract, *Rubus idaeus* extract, *Artocarpus heterophyllus* extract, and the control.

Figure 12 – Bar Graph of *C. elegans* Lifespan Tests

The *C. elegans* Mean Life Span of Raspberry Extract, Jackfruit Extract, and Dragon fruit Extract

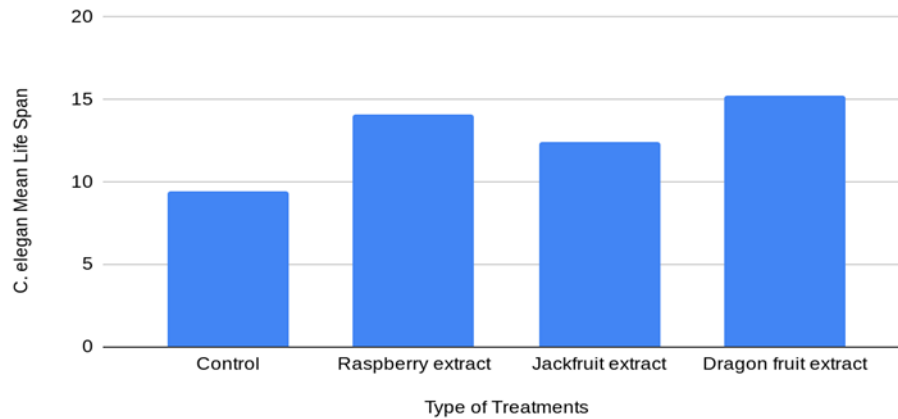


Figure 12 – This provides a bar graph of each extract from the order of control, *Artocarpus heterophyllus* extract, *Rubus idaeus* extract, and *Hylocereus undatus* extract for the *C. elegans* life span.

Table 13 - Post Hoc Tukey Test for *C. elegans* Lifespan Tests

Pairwise Comparisons		HSD <sub>.05</sub> = 4.1607 HSD <sub>.01</sub> = 5.0794	Q <sub>.05</sub> = 3.6864   Q <sub>.01</sub> = 4.5003
T <sub>1</sub> :T <sub>2</sub>	M <sub>1</sub> = 9.43 M <sub>2</sub> = 12.43	3.00	Q = 2.66 (p = .24253)
T <sub>1</sub> :T <sub>3</sub>	M <sub>1</sub> = 9.43 M <sub>3</sub> = 14.03	4.60	Q = 4.08 (p = .02402)
T <sub>1</sub> :T <sub>4</sub>	M <sub>1</sub> = 9.43 M <sub>4</sub> = 15.87	6.43	Q = 5.70 (p = .00057)
T <sub>2</sub> :T <sub>3</sub>	M <sub>2</sub> = 12.43 M <sub>3</sub> = 14.03	1.60	Q = 1.42 (p = .74830)
T <sub>2</sub> :T <sub>4</sub>	M <sub>2</sub> = 12.43 M <sub>4</sub> = 15.87	3.43	Q = 3.04 (p = .14338)
T <sub>3</sub> :T <sub>4</sub>	M <sub>3</sub> = 14.03 M <sub>4</sub> = 15.87	1.83	Q = 1.62 (p = .66027)

Table 13 - This provides the post hoc tukey test for the *C. elegans* lifespan for control (T<sub>1</sub>), *Artocarpus heterophyllus* extract (T<sub>2</sub>), *Rubus idaeus* extract (T<sub>3</sub>), and *Hylocereus undatus* extract (T<sub>4</sub>). It shows the statistical significance between each group.

Appendix A

<i>C. elegans</i> Lifespan Count - 30 for each group			
Control	<i>Artocarpus heterophyllus</i>	<i>Rubus idaeus</i>	<i>Hylocereus undatus</i>
30	30	30	30
28	28	29	29
25	27	29	28
22	27	29	28
21	25	28	28
21	23	27	28
20	22	27	26
16	22	25	26
15	22	24	25
13	21	20	22
9	17	19	21
9	15	17	20
7	12	16	19
5	11	16	18
4	9	15	18
4	6	13	16
3	4	12	16
1	4	11	16
0	3	9	14
-	0	8	12
-	-	6	10
-	-	6	9
-	-	3	7
-	-	1	4
-	-	0	3
-	-	-	1
-	-	-	0
19 days	21 days	24 days	26 days

Metal Chelation Test - Measured in % - based upon absorbance of mixture			
Control	<i>Artocarpus heterophyllus</i>	<i>Rubus idaeus</i>	<i>Hylocereus undatus</i>
0	26.09	77.17	79.35
0	42.86	105	76.09
0	104.2	82.35	83.66
0	43.33	104.2	81.51
0	47.69	82.5	73.5
0	41.5	104.17	81.67
0	55.3	83.85	62.92
0	57.9	103.85	83.08
0	61.3	16.67	82.5
0	40.45	85.5	64.67
0	42.95	89.73	71.86
0	47.98	91.65	84.29
0	43.68	73.56	80
0	79.12	89.71	78
0	58.73	86.34	83.33
0	43.65	90.50	52.5
0	42.91	30.12	82.68
0	53.77	21.58	94.5
0	59.65	84.39	98.7
0	62.87	92.44	105.8
0	69.85	101.3	89.63
0	41.63	28.6	91.5
0	43.45	55.78	93.5
0	46.63	59.63	91.7
0	48.93	42.98	95.89
0	23.04	35.67	76.3
0	20.54	20.18	68.99
0	67.65	98.59	97.23
0	24.57	88.63	54.3
0	38.66	87.59	101.22

ACE Inhibition Test - Measured in % - based upon absorbance of mixture			
Control	<i>Artocarpus heterophyllus</i>	<i>Rubus idaeus</i>	<i>Hylocereus undatus</i>
0	100	500	320
0	44.17	245	254.5
0	89	305	200.73
0	95	405.5	177.98
0	105	256.3	456.3
0	130	109.55	320
0	75	38.7	200
0	230	79.66	320.4
0	93	100.5	100.98
0	88.34	87.35	110.33
0	105.75	110.98	124.54
0	120.32	95.78	380.39
0	35.89	345.6	98.66
0	99.15	475	55.78
0	83.68	376.33	111.55
0	90.99	54.55	45.34
0	140.5	23.46	78.34
0	49.6	35.79	101.77
0	48.7	58.6	111.3
0	52.89	90.32	95
0	77.32	95.77	105
0	81.5	86.31	111.7
0	87.77	90.55	99.3
0	21.5	44.76	205
0	91.5	107.54	82.6
0	89.33	220	58.5
0	99.5	104.5	95.7
0	110.3	32.98	98.3
0	102.32	99.4	240.5
0	105.54	39.8	147.7
Avg: 0	Avg: 91.452	Avg:158.476	Avg:163.61