Treatment Analysis for Alzheimer's Disease using *Caenorhabditis Elegans* as a Model

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Abstract: ***Alzheimer's Disease, a progressive neurodegenerative condition lacking a definitive and guaranteed treatment, prompts critical investigation for effective remedies to manage its behavioral and cognitive impact. Herbal extracts like Ginkgo Biloba, Lion's Mane, Basil, and Sage present potential options to alleviate plaque build-up caused by Alzheimer's. This study aims to identify the most efficacious herbal extract for treating Alzheimer's, using aged Caenorhabditis elegans (C. elegans) as a model organism. The hypothesis states that treated C. elegans will exhibit increased behavioral movement and altered molecular effects compared to the untreated C. elegans. The Independent Variable consists of the various extracts fed to the C. elegans. The Dependent Variables consist of the C. elegan's behavioral abilities (speed, responsiveness, foraging) and C. elegan's molecular effects measured by protein concentration. The Control Variable is the untreated aged C. elegan's behavioral movement and molecular effects. Data was collected using WormLab and molecular assays to validate and determine the treatment's effectiveness. Through ANOVA testing, statistically significant differences emerged in four out of five measured tests, rejecting the null hypothesis more often than accepting it. Results from data indicate Ginkgo Biloba extract as the best extract, due to displaying increased speed, responsiveness, and foraging ability in C. elegans compared to other extracts and untreated C. elegans. This suggests Ginkgo Biloba as a highly possible treatment option.***

Keywords: ***Alzheimer's Disease, Caenorhabditis Elegans, Ginkgo Biloba, Herbal Extracts***

I. INTRODUCTION

Alzheimer's disease is one of the most progressive neurodegenerative diseases. The disease gradually impairs memory, cognition, behavior, and movement. Humans experience the disease in stages, beginning with minor memory loss and confusion and progressing to severe cognitive impairment and behavioral abnormalities [1]. As Alzheimer's Disease is the most common cause of dementia, an individual with dementia may struggle to recognize foods in front of them [2]. With such effects that occur when an individual has Alzheimer's, treatment options must be tested in order to analyze whether the treatment is viable or not. There are many treatment options available for Alzheimer's Disease, however, there is no definite treatment option for Alzheimer's Disease [3].

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Herbal treatments have been on the rise after many failed trials of FDA-approved drugs, fewer side effects, and toxicity reduction [4].

Specifically, herbal medicines of Ginkgo Biloba, Hericium erinaceus (Lion's Mane), Salvia officinalis (Sage), and Ocimum basilicum (Basil) contain high amounts of neuroprotective properties [5]. For instance, in a clinical study out of the 20 clinical trials that were considered, 14 of them (70.0%) concluded that Ginkgo Biloba extract can help Alzheimer's patients' cognitive abilities [6]. In a comprehensive research study that analyzed the herbal extract of Lion's Mane, it was found that the extract's bioactive components might treat a variety of brain disorders, including Alzheimer's. This made Lion's Mane a viable candidate among medicinal mushrooms [7]. Additionally, Lion's Mane was able to prevent β-amyloid (Aβ) cytotoxicity, stimulate the generation of neural growth factor (NGF), and shield nerve cells from ER stress or oxidative stress-related fatalities, all of which are big targets for Alzheimer's Disease pathology [8]. Likewise, Sage extracts were shown to be effective in cognitive tests for Alzheimer's such as ADAS-cog and CDR-SB compared to a placebo group. Basil extracts were shown to restrict hippocampal accumulation of β-amyloid build-up remarkably [9]. From this dive through the literature, it can be seen that the following herbal extracts contain antioxidative, anti-amyloidogenic, and anti-inflammatory properties due to positive results in trials [10]. With these four herbal treatment extracts being neuroprotective, a treatment analysis will need to be done to determine a definite treatment from this analysis. To achieve this, model organisms are needed to test these treatments before running them in clinical trials.

To progress further research affiliated with Alzheimer's disease, model organisms are very crucial to understanding the disease. A whole series of model organisms are used to study Alzheimer's such as transgenic mice, fruit flies, zebrafish, worms, etc [11]. Out of all the model organisms, the *Caenorhabditis elegans (C. elegans)* worm model tends to be one of the most exceptional and valuable organisms to model human neurodegenerative diseases. This is because *C. elegans* have a very short lifespan of about 18-20 days and a fast reproductive cycle, making this a great model for studying aging [12]. Furthermore what sets this model apart is the striking 59% homology between human CGI genes and *C. elegans* genes (34-87% in range) [13]. Additionally, human-comparable genes account for 83% of the *C. elegans* proteome [14]. Although quite small, at a molecular scale, the nervous system of *C. elegans* is similar to that of mammals.

C. elegans are facile to maintain in the laboratory and with their simple anatomy and transparent body it is possible to see their internal organs under a stereomicroscope.

In this analysis of herbal extract treatments, behavior/locomotion and molecular experimentation of protein concentration was done. To establish the most effective herbal extract treatment for Alzheimer's in this study, the behavioral analysis compared the behavior and locomotion of aged *C. elegan* fed on these extracts to aged *C. elegan* not fed on these extracts. The aged *C. elegans* imitated Alzheimer's disease serving as the model system. The overall protein concentration of aged *C. elegans* including the control (untreated aged *C. elegans*) was quantified for the molecular experimentation. It is hypothesized that the treated aged *C. elegans* (the *C. elegans* that were fed the extract) will emit more behavioral movement and molecular effects compared to the untreated aged *C. elegans* (Control).

II. METHODS

The materials used to execute this study include living *Caenorhabditis elegans* N2, Stereomicroscope, Autofluorescence filters, 100 mm x 15mm Petri dishes, Prepared Nematode Growth Medium Agar (NGM), 1000 µm Micropipette, 1000 µm pipette tips, Plastic Pipettes, Inoculating Loops, Sterile Copper Rings, *Escherichia coli* K-12 Nutrient Broth (*E. coli* K-12), Ginkgo Biloba Liquid Extract, Lion's Mane Liquid Extract, Basil Liquid Extract, Sage Liquid Extract, Digital Microscope Camera attachment for stereomicroscope, Microcentrifuge, Microcentrifuge Tubes, distilled water, WormLab quantification application on a computer, RIPA Cell Lysis Buffer, spectrophotometer for absorbance readings, Bicinchoninic Protein Assay (BCA) Kit for quantification of overall protein concentration, and a 96-well plate. The Kit includes Working Reagents and BSA standards.

The framework of the study consisted of an Independent Variable as the type of herbal extract the aged *C. elegans* are feeding on. The Dependent Variable was the aged *C. elegan's* behavioral and molecular effects under the various treatments. The Control Variable was the aged *C. elegan's* behavioral and molecular effects under no treatment. The *C. elegan's* behavioral function was defined by three main components: Speed, External Stimuli Response, and Foraging behavior with 5 operational definitions in total. The overall speed of each *C. elegan* (mm/s), distance moved from tapping/nudging the *C. elegan* (mm), response time after tapping/nudging the *C. elegan* (s), the time it took for the *C. elegan* to locate food (s), and the distance the *C. elegan* traveled to locate food (mm) were all quantified. For the aged *C. elegans'* molecular effects, overall protein concentration was quantified for aged *C. elegans* (mg/mL).

To indicate whether a *C. elegan* has aged or not, under the microscope with the autofluorescence filters applied, the *C. elegans* contained a red color pigment that indicates that the *C. elegan* has aged. The pigment is called Lipofuscin, which is used to be a marker of aging and anatomical decline in *C. elegans* since Lipofuscin is known as "age pigment" [15][16][17][18][19].

The procedure of this experiment contains two phases of experimentation. The first experimentation phase uses distilled water to transfer *C. elegans* to plates. The second experimentation phase uses the chunking method to transfer *C. elegans* to plates. The first step was done by melting the prepared NGM and pouring 6 plates. After cooling the agar, 4 plates were labeled as per each extract, 1 plate was labeled the control, and 1 plate was labeled as "batch". For example, the worms undergoing the treatment of Lion's Mane will be named as "Lion's Mane Plate 1." Then each of the extracts and *E. coli* were mixed to create the treatment and food source solution for the *C. elegans*. This was done by gathering 4 microcentrifuge tubes and micro pipetting 1 mL of well-stirred *E. coli* into each tube. Then 1 mL of each herbal extract was pipetted into each designated microcentrifuge tube for the extract. By using an aseptic technique the extract and the *E. coli* were stirred well using inoculation loops. Before the mixing of *E. coli* and respective herbal extracts was done, a validation test was done to ensure the *E. coli* K-12 was not killed off since the herbal treatments contain antioxidant properties and may inhibit certain bacteria. An antimicrobial susceptibility test was done to ensure that the herbal treatments do not inhibit *E. coli* K-12. From the Zone of Inhibition results, there was no inhibition zone detected from the tests implying that the *E. coli* K-12 was not inhibited. This means that the food source for the *C. elegans* will not be killed off. Then for all of the plates labeled for each extract, *E. coli* and the extract solution were streaked lightly on the agar using an inoculation loop. All the plates were then incubated with the agar facing up overnight at 25 degrees Celsius. The next day, the extracts. The next day the *C. elegans* were transferred from the stock *C. elegan* plate to the plate labeled "batch" using the chunk agar method. This was done by using an inoculation loop to cut a small chunk of agar which was transferred to the plate labeled "batch." After 2 days, a population of healthy *C. elegans* started to form on the batch plate. On this day it was time to transfer the *C. elegans* to individual extract plates. 1 mL of distilled water was pipetted and poured onto the batch plate using a micropipette. The batch plate was then swirled to pick up the *C. elegans* with the distilled water. The distilled water from the plate with the *C. elegans* was pipetted using a micropipette. 1mL of this was pipetted and placed into an empty microcentrifuge tube. This step was repeated 4 times because there were four plates of extracts. Then the 4 *C. elegan* and distilled water solution tubes were placed in a microcentrifuge and were balanced. The tubes were microcentrifuged for 3 minutes at 6,000 RPM. Then using a micropipette, 1mL of the *C. elegan* and distilled water solution were placed on each extract plate and were swirled. All the plates were placed in the incubator for 7 days at 25 degrees Celsius. After 7 days, the plates were ready for data collection for phase 1 behavioral analysis.

For phase 2 behavioral analysis of the experimentation, the same process was done from phase 1 except for the transferring of *C. elegans* with distilled water.

This is because, for phase 2 experimentation, the *C. elegans* were transferred using the chunk agar method aseptically.

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For the molecular analysis, the Bicinchoninic Protein Assay (BCA) Kit was used provided with Working Reagents and 8 BSA Standards provided with instructions. The same plate setup was used from the chunk agar method for transferring. On each herbal extract *C. elegan* plate, a quadrant was randomly selected for interpretation and each *C. elegan* from the random quadrant was placed on a new plate. This was done for all 4 treatment plates and control plates so that the *E. coli* bacteria and herbal treatments would not cause interference in gathering cell lysates. 0.5 mL of RIPA Cell Lysis buffer was ejected on the newly transferred plates swirled for 2 minutes and then retrieved. After retrieval, the liquid was drawn and put into respective microcentrifuge tubes representing each plate (4 treatment plates, 1 control plate). The tubes were centrifuged at 10,000 RPM for 3 minutes. The supernatant was collected without disturbing the pellet. The supernatant included the denatured proteins. Using the instruction manual from the BCA protein assay kit, 6500 µl of Reagent A was mixed with 130 µl of Reagent B to create the working reagent. Then, a dilution series was created with the given BSA standard. The dilution series consisted of 2000, 1500, 1000, 750, 500, 250, 125, and 25 µg/mL with a dilution factor of 9. The standards, working reagents, and supernatant (denatured proteins) were placed on the 96-well plate respectively. Well-plate was incubated at 37°C for 30 minutes. This is to allow the BCA reaction to occur. The well plate was inserted into the spectrophotometer for absorbance quantification with a wavelength set at 562 nm. With the absorbance values, a standard curve was graphed and calculated to solve for protein concentration.

For retrieving the data process for behavioral analysis, videos were taken under the stereomicroscope with the Autofluorescence films using the digital microscope camera. A four-quadrant grid was drawn on each petri dish to separate each section into quadrants. To analyze a quadrant of *C. elegans*, a random number generator chose a number 1 through 4 to indicate which quadrant to analyze for each of the plates. This helps to eliminate any biases during the experiment. For measuring the speed of the *C. elegan*, the *C. elegans* which had a red-colored appearance were the ones that aged and had a loss of anatomical function. All data retrieved for every data measure were from the *C. elegans* who had a red appearance. Videos were recorded for 1 minute. The video was then uploaded to the WormLab application on a computer to retrieve the speed of the worm in mm/s. The *C. elegan* tapping data (*C. elegan's* response to stimuli) was achieved by gently tapping the worm with a needle and was recorded for a minute. This video was then uploaded to WormLab and data for the distance the *C. elegan* moved in mm and the response time in seconds. For the food foraging data, 5 plates of NGM agar plates were made. The copper rings were placed on each plate to make barriers so that the *C. elegan* would not go past the ring. *E. coli* with the designated extract was streaked on the agar of one side of the copper ring. On the opposite side of the ring, About 5 *C. elegans* were placed by picking up the *C. elegans* from a designated plate with herbal extract and the distance and time were recorded for the *C. elegans* foraging behavior. After the foraging recording, the recording was uploaded into WormLab to retrieve the time it takes for the *C. elegans* to locate food (s) and the distance the *C. elegans* traveled to locate the food (mm). This process for foraging was done for all 4 plates with each herbal extract (treatment plates) and the control plate that contains no herbal extract (control plate).

After the data collection, the data was analyzed and statistical tests were used to analyze whether each test either rejected or accepted the null hypothesis. Statistical analysis tests of ANOVA and T-tests were used with significance set at $p < 0.05$. This same process for data collection was done for both experimentation phase 1 and phase 2.

III. RESULTS

All the data shown below are averages of the raw data from both experimental phases. The total trial number collectively is 279. Each individual *C. elegan* was counted for a data point in the raw data only. The control is known to be the aged *C. elegans* undergoing no treatment.

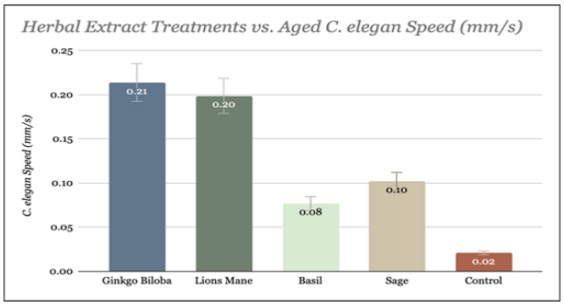

Fig. 1: Bar Graph Displaying the Average Aged *C. elegan* Speed for each Herbal Extract and no Treatment in Millimeters Per Second

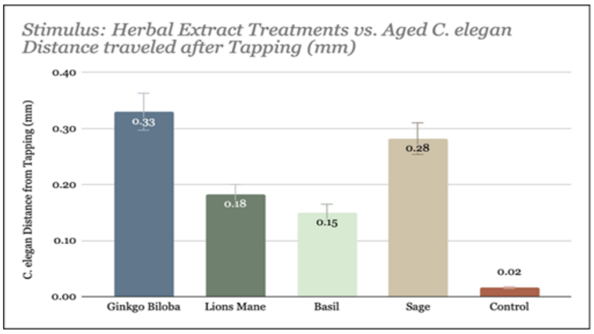

Fig. 2: Bar Graph Displaying the Average Aged *C. elegan* Distance Moved from Original Position by Tapping them in Millimeters (External Stimulus)

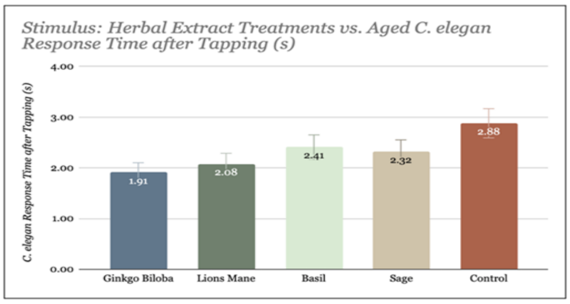

Fig. 3: Bar Graph Displaying the Average *Aged C. elegan* time when Moving from Original Position by Tapping them in Seconds (External Stimulus)

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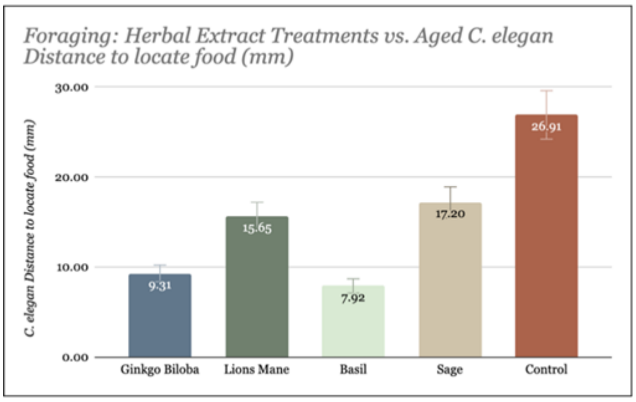

Fig. 4: Bar Graph Displaying the Average Aged *C. elegan* Distance to Locate Food in Millimeters (Foraging Method)

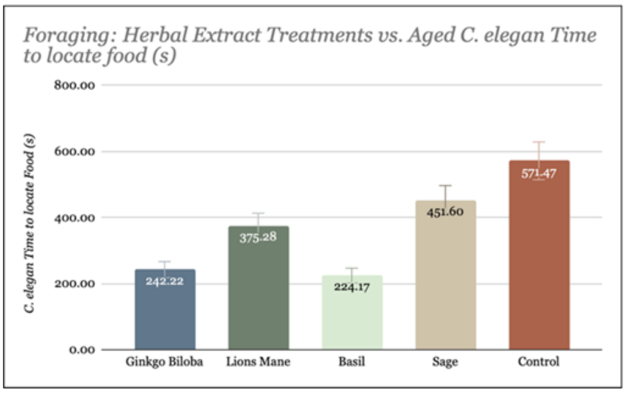

Fig. 5: Bar Graph Displaying the Average Aged *C. elegan* time to Locate Food in Seconds (Foraging Method)

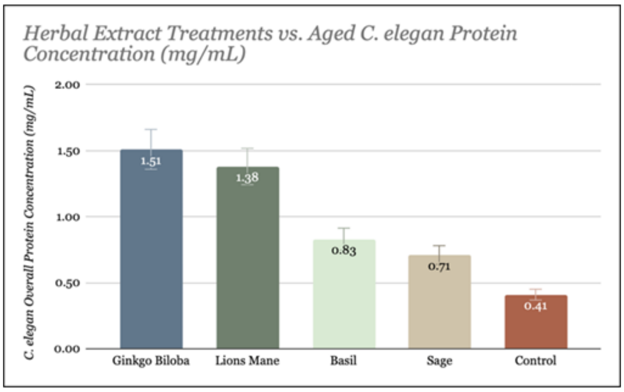

Fig. 6: Bar Graph Displaying the Average Aged *C. elegan* Protein Concentration Per Treatment in Mg/Ml (Molecular)

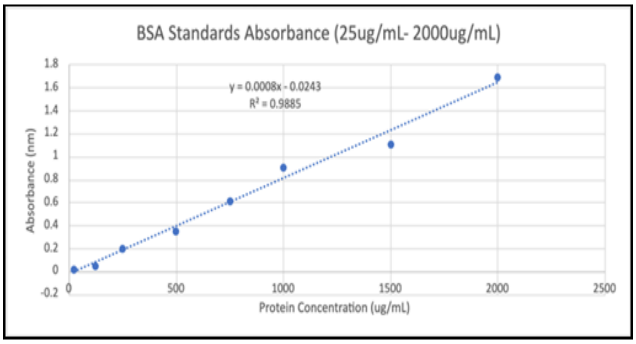

Fig. 7: BSA Standard Absorbance Values from Spectrophotometer Standard Curve Approximation for Protein Concentration Quantification

IV. DISCUSSION AND CONCLUSIONS

From the results and data above there were many instances where one extract performed better than the other in one test while another extract performed better in another test. When the results for the average speed of the aged *C. elegans* feeding on natural extracts and those not feeding on extracts were compared, the ones feeding on extracts were all faster than the *C. elegans* not feeding on extracts. From this test, the herbal extracts did work to speed up the *C. elegans*. However, in this case, Ginkgo Biloba was the best extract to promote the fastest-aged *C. elegans*. This is shown where the *C. elegans* feeding on Gingko Biloba had a speed of 0.162 mm/s compared to the others such as Lion's Mane with 0.145 mm/s, Basil with 0.076 mm/s, Sage with 0.094 mm/s, and the Control with no treatment with a speed of 0.024 mm/s.

For the second behavioral test, the Distance from Tapping, the *C. elegans* who were feeding on the extracts achieved higher distances compared to the *C. elegans* who were not feeding on the extracts. According to Figure 2, the average distance for the extracts collectively is 0.2515 mm compared to the distance of no treatment *C. elegans* (Control) of 0.012. These numbers show that the *C. elegans* undergoing treatment have higher distances moved in response to the tapping, leading to the fact that the treated *C. elegans* have higher responsiveness. Out of all the extracts, Ginkgo biloba showed more responsiveness with a distance value of 0.33.

For the third behavioral test, the Response Time after tapping, the treated *C. elegans* with the various extracts had a faster response time compared to the non-treated *C. elegans* (Control). This is evident in Figure 6 which displays that all the herbal extracts had faster times than the *C. elegans* with no treatment. Out of all the herbal extracts, Gingko performed the best and fastest with a time of 1.91 seconds.

For the fourth behavioral test, the Foraging Method of the Distance to locate food, the treated *C. elegans* did not achieve higher distances on average compared to the untreated *C. elegans*. This data is evident in Figure 8 where *C. elegans* undergoing treatment has a total average of 56.74 compared to the untreated *C. elegans* movement being 62.89. This is due to the data being spaced out for the *C. elegans* undergoing treatment. Overall, sage treatment with a distance of 75.22 was the most successful in this case out of all extracts.

The fifth and final behavioral test was the Foraging Method of the Time to locate food in seconds. The treated *C. elegans* were faster in locating the food compared to the non-treated *C. elegans*. This is evident in Figure 10 where the average time for all the extracts combined equal 323.32 compared to the *C. elegans* without the treatment of 571.47 seconds. Out of all the extracts, the fastest response was from the *C. elegans* feeding on Basil. In the molecular protein concentration test, it can be seen that the *C. elegans* who were fed the treatments all had greater protein concentrations compared to the *C. elegans* who were not treated (control). The protein concentrations were quantified by the quantified BSA Standard absorbance values with wavelengths at 562 nm shown in Figure 7. As Ginkgo Biloba had the highest amount of protein concentration of 1.51 mg/mL, it can be concluded that Ginkgo Biloba specialized in the protein concentration test for this treatment analysis.

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ANOVA Statistical Analysis tests were used to determine whether the null hypothesis was rejected or not. The null hypothesis of the experiment would be that there was no significant difference in the behavioral function (speed, response to stimuli, foraging abilities) of aged *Caenorhabditis elegans* when exposed to various herbal extract treatments (Ginkgo Biloba, Lion's Mane, Basil, Sage) compared to aged *C. elegans* not exposed to these herbal extract treatments.

Table 1: ANOVA Statistical Analysis Table Showcasing F Value and F Crit Value

C. elegan **Speed**	**External Stimuli:** ***C. elegan*** **Distance from Tapping**	**External Stimuli:** ***C. elegan*** **Response Time After Tapping**	**Foraging:** ***C. elegan*** **Distance to Locate Food**	**Foraging:** ***C. elegan*** **Time to Locate Food**	**Molecular: Protein Concentration of** ***C. elegan***
F: 6.40	F: 15.70	F: 2.15	F: 27.31	F: 2489.54	F: 11.58
F crit: 2.78	F crit: 2.84	F crit: 2.82	F crit: 2.87	F crit: 2.87	F crit: 3.48
Stat Sig.	Stat. Sig.	Not Stat Sig.	Stat. Sig.	Stat. Sig.	Stat. Sig.

For the first behavioral measure test, the speed of *C. elegans*, the P value is less than 0.05, the F value is 6.40, and the F crit value is 2.78. From these numbers, since the F value is greater than the F crit value, the speed of the *C. elegans* data has a statistically significant difference therefore the null hypothesis is rejected. This indicates that the hypothesis is accepted.

For the second behavioral measure, the External Stimuli Test for *C. elegan* distance from tapping, the P value is less than 0.05, the F value is 15.70, and the F crit value is 2.84. From these numbers, since the F value is greater than the F crit value, the *C. elegans* distance from the tapping stimuli data has a statistically significant difference therefore the null hypothesis is rejected. This means that the hypothesis is accepted.

For the third behavioral measure, the External Stimuli test for *C. elegan* time after tapping, the P value is less than 0.05, the F value is 2.15, and the F crit value is 2.82. From these numbers, since the F value is less than the F crit value since the F value is less than the F crit value, the null hypothesis is accepted and the hypothesis is rejected.

For the fourth behavioral measure, the Foraging test for *C. elegan* distance to locate food, the P value is less than 0.05, the F value is 27.31, and the F crit value is 2.87. From these numbers, since the F value is greater than the F crit value, the *C. elegans* distance to locate food has a statistically significant difference therefore the null hypothesis is rejected and the hypothesis is accepted.

For the fifth and final behavioral measure, the Foraging test for *C. elegan* time to locate food, the P value is less than 0.05, the F value is 2489.53 and the F crit value is 2.87. From these, since the F value is greater than the F crit value, the *C. elegans* time to locate food has a statistically significant difference therefore the null hypothesis is rejected and the hypothesis is accepted.

In the molecular effects of the aged *C. elegans*: Overall Protein Concentration, the P value is less than 0.05, the F value is 11.58 and the F crit value is 3.48. From these numbers, since the F value is greater than the F crit value, the *C. elegans* protein concentration is statistically significant. This implies that the null hypothesis is rejected therefore the hypothesis remains true.

From the results of the statistical tests, 5 out of the 6 statistical tests came out to be statistically significant and the null hypothesis was overall rejected around 83% of the time (⅚). Therefore, this shows that the experiment was a success. Overall, in this study, the hypothesis was supported true.

A T-test was done to assess whether there was any statistical significance between the Herbal Extract and the Control Group to support the Experimental Hypothesis. This analysis helps determine if there was a significant effect of the treatment that was administered to the *C. elegans*.

Table 2: T-Test Table Showcasing P Value for Each Herbal Extract V. Control

C. elegan **Speed**	**External Stimuli:** ***C. elegan*** **Distance from Tapping**	**External Stimuli:** ***C. elegan*** **Response Time after Tapping**	**Foraging:** ***C. elegan*** **Distance to locate food**	**Foraging:** ***C. elegan*** **Time to locate Food**	**Molecular: Protein Concentration of** ***C. elegan***
Ginkgo Biloba v. Control p value: 0.001	Ginkgo Biloba v. Control p value: 9.42	**Ginkgo Biloba v. Control p value: 0.037**	**Ginkgo Biloba v. Control p value: 0.000**	Ginkgo Biloba v. Control p value: 3.212	**Ginkgo Biloba v. Control p value: 0.007**
Lion's Mane v. Control p value: 0.005	**Lion's Mane v. Control p value: 0.012**	Lion's Mane v. Control p value: 0.073	**LM v. Control p value: 0.002**	Lion's Mane v. Control p value: 2.491	**Lion's Mane v. Control p value: 0.002**
Basil v. Control p value: 0.192	Basil v. Control p value: 1.669	**Basil v. Control p value: 0.016**	**Basil v. Control p value: 0.000**	Basil v. Control p value: 4.069	**Basil v. Control p value: 0.043**
Sage v. Control p value: 0.047	**Sage v. Control p value: 0.004**	**Sage v. Control p value: 0.032**	**Sage v. Control p value: 0.007**	Sage v. Control p value: 3.708	Sage v. Control p value: 0.106

Note: Boxes underlined and bolded indicate a statistically significant outcome (less than 0.05)

According to Table 2 in the T-test Table, the p-values that were less than 0.05 were to be determined as statistically significant. It can be interpreted that 15/24 (62.5%) Statistical Analysis T-tests were statistically significant and 9/24 (37.5%) Statistical Analysis T-tests were not statistically significant. Overall, the null hypothesis of the experiment is rejected 62.5% of the time.In determining the overall best and definitive extract for Alzheimer's treatment, Ginkgo Biloba extract shows more promising results compared to the other extracts.

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This is evident where Ginkgo Biloba was the better result when measuring the speed of the *C. elegans*, the distance from tapping, and the time it takes for the *C. elegan* to respond to the tapping. After three of the five behavioral tests were conducted, Ginkgo was shown to have superior results, and Ginkgo Biloba could specialize in the greatest total protein concentration. Ginkgo is therefore far more promising than the others.

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DECLARATION STATEMENT

I must verify the accuracy of the following information as the article's author.

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- **Funding Support:** This article has not been sponsored or funded by any organization or agency. The independence of this research is a crucial factor in affirming its impartiality, as it has been conducted without any external sway.
- **Ethical Approval and Consent to Participate:** The data provided in this article is exempt from the requirement for ethical approval or participant consent.
- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Authors Contributions:** The authorship of this article is contributed solely.

REFERENCES

1. Scott, K. R., & Barrett, A. M. (2007). Dementia syndromes: evaluation and treatment. Expert Review of Neurotherapeutics, 7(4), 407–422. https://doi.org/10.1586/14737175.7.4.407
2. Jung, D., Lee, K., De Gagne, J. C., Lee, M., Lee, H., Yoo, L., Won, S., & Choi, E. (2021). Eating Difficulties among Older Adults with Dementia in Long-Term Care Facilities: A Scoping Review. International Journal of Environmental Research and Public Health, 18(19), 10109. https://doi.org/10.3390/ijerph181910109
3. Yiannopoulou, K. G., Anastasiou, A. I., Zachariou, V., & Pelidou, S. (2019). Reasons for failed trials of Disease-Modifying Treatments for Alzheimer Disease and their contribution in recent research. Biomedicines, 7(4), 97. https://doi.org/10.3390/biomedicines7040097
4. Karimi, A., Majlesi, M., & Rafieian-Kopaei, M. (2015). Herbal versus synthetic drugs: Beliefs and facts. Journal of Nephropharmacology, 4(1), 27-30.
5. John, O. O., Amarachi, I. S., Agbo, C. P., Adaeze, E., Kale, M. B., Umare, M. D., & Upaganlawar, A. (2022). Phytotherapy: A promising approach for the treatment of Alzheimer's disease. Pharmacological Research - Modern Chinese Medicine, 2, 100030. https://doi.org/10.1016/j.prmcm.2021.100030
6. Xie, L., Zhu, Q., & Lu, J. (2022). Can We Use Ginkgo biloba Extract to Treat Alzheimer's Disease? Lessons from Preclinical and Clinical Studies. Cells, 11(3), 479. https://doi.org/10.3390/cells11030479
7. Brandalise, F., Roda, E., Ratto, D., Goppa, L., Gargano, M. L., Cirlincione, F., Priori, E. C., Venuti, M. T., Pastorelli, E., Savino, E., & Rossi, P. (2023). Hericium erinaceus in Neurodegenerative Diseases: From Bench to Bedside and Beyond, How Far from the Shoreline? Journal of Fungi, 9(5), 551. https://doi.org/10.3390/jof9050551
8. Szućko-Kociuba, I., Trzeciak-Ryczek, A., Kupnicka, P., & Chlubek, D. (2023). Neurotrophic and Neuroprotective Effects of Hericium erinaceus. International Journal of Molecular Sciences, 24(21), 15960. https://doi.org/10.3390/ijms242115960
9. Heshami, N., Mohammadali, S., Komaki, A., Tayebinia, H., Karimi, J., Oshaghi, E. A., Hashemnia, M., & Khodadadi, I. (2021). Favorable effects of dill tablets and Ocimum basilicum L. extract on learning, memory, and hippocampal fatty acid composition in hypercholesterolemic rats. DOAJ (DOAJ: Directory of Open Access Journals), 24(3), 300–311. https://doi.org/10.22038/ijbms.2021.49013.11230
10. Gregory, J., Vengalasetti, Y. V., Bredesen, D. E., & Rao, R. V. (2021). Neuroprotective herbs for the management of Alzheimer's disease. Biomolecules, 11(4), 543. https://doi.org/10.3390/biom11040543
11. Alvarez, J., Álvarez-Illera, P., Santo-Domingo, J., Fonteríz, R. I., & Montero, M. (2022). Modeling Alzheimer's Disease in Caenorhabditis elegans. Biomedicines, 10(2), 288. https://doi.org/10.3390/biomedicines10020288
12. Zhang, S., Li, F., Zhou, T., Wang, G., & Li, Z. (2020). Caenorhabditis elegans as a Useful Model for Studying Aging Mutations. Frontiers in Endocrinology, 11. https://doi.org/10.3389/fendo.2020.554994
13. Lai, C., Chou, C., Ch'ang, L., Liu, C., & Lin, W. (2000). Identification of Novel Human Genes Evolutionarily Conserved in Caenorhabditis elegans by Comparative Proteomics. Genome Research, 10(5), 703–713. https://doi.org/10.1101/gr.10.5.703f
14. Henricson, A., Sonnhammer, E. L., Baillie, D. L., & Gomes, A. V. (2004). Functional characterization in Caenorhabditis elegans of transmembrane worm-human orthologs. BMC Genomics, 5(1). https://doi.org/10.1186/1471-2164-5-85
15. Pincus, Z., Mazer, T., & Slack, F. J. (2016). Autofluorescence as a measure of senescence in C. elegans: look to red, not blue or green. Aging, 8(5), 889–898. https://doi.org/10.18632/aging.100936
16. V P, S., S, S., & George, Prof. J. (2021). Alzheimer s Disease Classification and Detection using MRI Dataset. In International Journal of Innovative Technology and Exploring Engineering (Vol. 10, Issue 5, pp. 70–72). https://doi.org/10.35940/ijitee.e8662.0310521
17. Salehi, A. W., Baglat, P., & Gupta, G. (2020). Alzheimer's Disease Diagnosis using Deep Learning Techniques. In International Journal of Engineering and Advanced Technology (Vol. 9, Issue 3, pp. 874–880). https://doi.org/10.35940/ijeat.c5345.029320
18. Kumari, B. A. S., Shetty M, C., H M, L., Jain, M. P., & S, S. (2020). Exploring the methods on early detection of Alzheimer's disease. In International Journal of Recent Technology and Engineering (IJRTE) (Vol. 9, Issue 1, pp. 1754–1758). https://doi.org/10.35940/ijrte.a2391.059120
19. Pai, R., & Wadhwa, A. (2022). Artificial Intelligence based Modern Approaches to Diagnose Alzheimer s. In Indian Journal of Artificial Intelligence and Neural Networking (Vol. 2, Issue 2, pp. 1–14). https://doi.org/10.54105/ijainn.b1045.022222

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