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Determining the Efficacy of Polyphenols in Inhibiting the Aggregation of Amyloid Beta Proteins

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ABSTRACT

Alzheimer's Disease is caused by an aggregation of amyloid beta and tau proteins in the brain. Polyphenols, a broad class of naturally-existing compounds, have been shown to inhibit the aggregation of those proteins. This project aims to focus on expressing different combinations of those proteins, as well as assaying those proteins for aggregation inhibition using polyphenols such as curcumin, caffeic acid, epigallocatechin gallate (EGCG), and more to determine which polyphenol is most effective in doing so. We chose to use these polyphenols because of their past precedence in other work, along with their widespread prevalence. However, this project focused more on the biological and in-vitro aspect of polyphenols inhibiting amyloid beta, such as conducting multiple assays including Congo Red, Avoidance, and Dynamic Light Scattering in order to receive tangible results. Through our studies, we found that polyphenols do produce an inhibitory effect on the aggregation of amyloid-beta.

Introduction

Polyphenols are naturally occurring micronutrients found in most plant-based foods. Due to there being a wide variety of polyphenols they are divided into four subgroups of phenolic compounds: phenolic acids, flavonoids, stilbenes and lignans. Polyphenols are an important factor in plants as their purpose is to protect the plant from dangers caused by ultraviolet radiation, insects, and diseases. They are part of an average person's everyday diet, as they are found in a wide variety of foods such as fruits, vegetables, teas, spices, coffee, etc. Half a cup, or 100 mL, of coffee contains around 200 mg of polyphenols while half a cup of soy milk contains 36 mg of polyphenols [1]. Studies show polyphenols have a positive effect on the human body [1]. Consuming polyphenols could help fight diabetes by reducing blood sugar levels, improve digestion, could also lower the risk of heart diseases, and slow down Alzheimer's [2].

Alzheimer's is a disease which most commonly occurs in adults and causes memory loss and lowers cognitive abilities. Alzheimer's is caused by an unnatural buildup of two proteins, amyloid beta and tau. In a healthy brain, amyloid beta protein aids in the process of neural growth and repair. The brain is made of neurons which are connected by a branch-like structure called dendrites. Neurons receive information through the electric currents sent by other brain cells; however, when the large amounts of amyloid beta begin to clump together forming amyloid plaques known as Aβ fibrils. These plaques excessively damage neurons which block neuronal activity. This disrupts communication with other cells and halts the cells' ability to repair. This is what leads to the effects of the chronic disease in Alzheimer's patients [3]. Tau is a phosphoprotein found in neurons and binds to microtubules to influence stability. However when it is unable to do this, the microtubules become unstable and the tau phosphoprotein begins to aggregate causing neuronal damage and later Alzheimer's [4].

Although currently four medications have already been approved for Alzheimer's Disease, they have little impact and no effect on long-term disease progression [5]. Cholinesterase inhibitors are one class of these medications that act by increasing the amount of acetylcholine in the brain to overstimulate nerves in the body, increasing communication between nerve cells and thus temporarily improving neuronal functions [6]. However, because all four current medications have little effect towards long-term disease progression, to target Aβ, small organic molecules, peptide derivatives, antibodies, and many other compounds have also been used to reduce Aβ aggregation [5]. Recent research has revealed that polyphenols can play a role in reducing the aggregation of amyloid beta [5, 7]. More specifically, several in vitro studies have shown that polyphenols inhibit Aβ's nucleation phase. This is seen when amyloid beta monomers aggregate into a polymer, amyloid beta polymers aggregate into a fibril, or both, turning Aβ oligomers to a less-toxic aggregation pathway [5].

For example, one commonly known polyphenol is curcumin derived from turmeric and is recognized for its anti-inflammatory in many Asian countries [7, 8]. Curcumin has shown qualities in inhibiting amyloid beta successfully and breaking down the large plaques of Aβ fibrils. Furthermore it has shown success in reducing the development of Aβ fibrils [7]. Epigallocatechin gallate (EGCG) is another commonly found polyphenol in green tea, and it has been found to prevent amyloid beta aggregation by inhibiting amyloid beta fibrillation and turning oligomerization towards a less-toxic aggregation pathway [5]. Gallic acid, a polyphenol present in tea, wine, fruits, and other plants, has also been shown to reduce amyloid beta aggregation in a similar manner as EGCG [5]. Moreover, tannic acid, fisetin, and resveratrol, polyphenols present in many fruits and vegetables, have reduced amyloid beta aggregation by inhibiting fibrillation [9, 10, 11].

The aim of this project is to determine the efficiency of different polyphenols' ability to inhibit amyloid beta. This was conducted through in vivo assays using different strains of Caenorhabditis elegans, C.elegans: CL2006 and CL2355. The CL2006 strain with the genotype of dvIs2, is mostly used in neurodegenerative diseases due to its muscle-specific expression of Aβ, leading to a progressive paralysis that starts in adulthood. Consequently, CL2355 strains with the genotype of dvIs50, express Aβ in the neurons, which may more accurately represent toxicity of Aβ seen in Alzheimer's Disease [12].

Of the many available polyphenols, a few the project mainly focused on were: curcumin from turmeric; gallic acid and epigallocatechin gallate (EGCG) most commonly found in tea; and tannic acid found in many herbaceous or woody plant tissues [5, 7, 8, 9, 13].

Organism

We tested all our experiments using different strains of C. Elegans. C. Elegans are microscopic transparent roundworms part of the Nematoda phylum. They are used to study human diseases, specifically mitochondrial diseases. A unique feature of C. elegans is that their development is very specific, cells divide and specialize in a characteristic way, and therefore we were able to observe changes in their development more effectively [14].

C. Elegans are the perfect candidate for our research because they are inexpensive, clear, and easy to culture. They can reproduce within 24 hours, and their clearness allows us to view their cells and organelles through a microscope. They also come in different varieties which allow us to conduct various types of experiments. Specifically for Alzheimer's Disease, they possess the ability to develop muscle-associated deposits reactive to amyloid-specific dyes and the concomitant progressive paralysis phenotype, making them commonly used in bioassays [17]. C. Elegans' previous usage in various bioassays provided us with a solid background to troubleshoot any errors in culturing.

The worms lived on Nematode Growth Media (NGM) plates. Before transferring worms to the NGM plates, the plates were seeded with about 100 µL of OP-50, which acted as the bacterial diet for the C. Elegans [15]. Afterward, we would transfer the CL2006 worms, the specific strain we used, from the stock plate, onto our NGM plates and wait for them to reproduce. We had a weekly cycle to make NGM plates and transfer worms onto them, to ensure we had a fresh plate of worms ready to be used.

To survive, the C. Elegans require a rich food source, which is a media. This media was made using the following protocol [15]. 15 g agar, 2 g Tryptone soya broth, and 2.72 g KH_2PO_4 were added to a 1 L Erlenmeyer flask. Deionized water filled the flask until the 1 L mark, and the solution was stirred until it combined. This solution was then poured into agar plates, which were previously rigorously sanitized beforehand. After five minutes, or until the agar cooled and set, 70 µL centrifuged LB Broth mixture was used to inoculate each agar plate and was spread with a L spreader.

For the LB Broth mixture, 5 g tryptone, 2.5 g yeast, and 2.5 g NaCl were added to a 1 L Erlenmeyer flask. The flask was then filled up to 500 mL with deionized water. This solution was then autoclaved, inoculated with E. coli OP50, and placed in the incubator overnight in order to let the culture grow [16].

Methods

Synchronizing Worms

To ensure only the strongest worms are being used in the assays, they go through a process called synchronization. This process is conducted via the following protocol [15].

Each seeded NGM plate was washed with a pipette with an M9 buffer about four to five times to fill a 15 mL falcon tube. This mixture was then centrifuged until a solid pellet of worms formed, with the supernatant being discarded and filled halfway with fresh M9 three times. After the third round of centrifuging, all supernatant was removed, and a bleach solution was added to the tube. The bleach solution was made by mixing 5 mL 1 M NaOH, 2 mL sodium hypochlorite, 1 mL deionized water, and 8 mL M9 buffer. The falcon tube was then shaked for 6 minutes by hand; the reaction was then stopped by adding an M9 buffer to fill the falcon tube. This was then centrifuged for four minutes, and supernatant was then discarded so that only a small layer covered the pellet of worms. All falcon tubes were then placed in a 20˚C incubator overnight and then resuspended and pipetted onto an agar plate.

Synchronization of the worms is used to find the strongest worms within the plate and eliminate any larvae or eggs, so the experiment can go smoothly without any excessive worm deaths and results remain unbiased.

Preparing Polyphenols

Before we began any of our experiments, we had to prepare the polyphenol solutions, which were all 1 µM solutions. This concentration was chosen as past studies have found that when concentrations were <25 µM, polyphenols like EGCG were able to positively affect C. elegans under stress only conditions; otherwise, when concentrations went above 800 µM, they produced toxic effects [18]. Thus, in order to produce the best results, we hypothesized that a concentration of 1 µM would be safest to use. Each 1 µM stock solution was dissolved in 2 mL of Dimethyl Sulfoxide solution (DMSO) and 8 mL of Phosphate-Buffered Saline (PBS) and lasted up to 1 month. In order to test which polyphenols was most effective when inhibiting the Amyloid Beta Protein, we conducted the following assays:

Chemotaxis Assay

The chemotaxis assay, an in vivo assay, was used to determine worms' memory based on their responses to each polyphenol [19]. Worms first underwent pre-conditioning starvation after their age synchronization. Each 10 cm petri dish was then prepared via pipetting the worms with M9 buffer on a labeled agar petri dish that was spotted with 1 µL each of 95% EtOH and 10% butanone. The plate was then incubated for 1 hour at room temperature.

The plate was prepared with two-quadrants. These types of chemotaxis assays have been used to explore the roles that various neurons, receptors, and signal transduction molecules played when C. elegans was exposed to various compounds. Between 20-50 washed worms were placed near the center of the plate with an attractant and a control at polar ends along with the anesthetic, sodium azide (NaN_3). After 60 min, a chemotactic index with values from -1.0 to +1.0 was generated based on the difference between how many worms were affixed to the attractant or the control, which will be discussed more in results. Ideally, worms would be affected by the polyphenols and be more attracted in test quadrants with polyphenols [20].

The chemotaxis index was then calculated according to Equation 1.

Equation 1: Chemotaxis Index [20].

$$Chemotaxis\ Index\ =\ \frac{\#\ Worms\ in\ Both\ Test\ Quadrants\ -\ \#\ Worms\ in\ Both\ Control\ Quadrants}{Total\ Worms\ Tested}$$

Congo Red Assay

The Congo Red Assay is an in vitro assay used to quantify the levels of aggregation of the amyloid beta protein under different conditions using spectroscopy [21]. The Congo Red dye stains the amyloid beta as it is stuck between the β pleated sheets of the amyloid beta protein. The dye is used to track the level of aggregation which determines the effectiveness of the different polyphenols.

Before we ran this assay, we made an amyloid-beta HFIP solution by dissolving the peptide in HFIP (1,1,1,3,3,3-hexafluoro-2-propanol), mixing it, and aliquoting it. We stored the solution by freezing it in -20 ˚C temperature. This was done to ensure maximum usage of the amyloid-beta peptide. 20 µM Congo Red solution was also prepared by dissolving 1.3933 mg of Congo Red in 100 mL of deionized water [22].

The Assay is conducted by pouring the Congo Red solution into nine cuvettes with different concentrations of amyloid beta and polyphenol solutions. Of the nine cuvettes, two were control groups with 500 µL of Congo Red solution only and phosphate-buffered saline (PBS) only. Cuvettes 3-9 held 30 µL of Congo Red solution, with cuvette 3 having 250 µL of PBS and cuvettes 4-9 having 250 µL of respective polyphenol each. Immediately afterwards, 250 µL of amyloid-beta solution was also added to cuvettes 3-9.

The cuvettes were then put through the spectrophotometer at different wavelength frequencies - we ran this assay at 477 and 540 nm. The reading process using the spectrophotometer was conducted again after 24 hours and the results were sorted into a graph based on curette number and wavelength frequency.

Equation 2: Absorption levels of the Congo Red Dye on the amyloid beta, C_b equation [22].

$$C_b\ =\ \frac{A_{540}}{25{,}295}\ -\ \frac{A_{477}}{46{,}360}$$

DLS Assay

The Dynamic Light Scattering Assay, also known as the DLS assay, is used to determine the aggregation levels of amyloid beta proteins. We used a DynaPro-99-E-50 dynamic light scattering instrument for all of our trials.

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The DLS assay is conducted using the following protocol [22]. First, the DLS instrument is loaded for 15 minutes to warm up. Cuvettes are then washed and prepared with 12 µL of desired polyphenol and 5 µL of amyloid-beta HFIP solution. The control is a cuvette without any polyphenol and instead 17 µL of the amyloid-beta HFIP solution. For each run, the intensity of the laser power was set to a value of 90%, the temperature was set to 25.0 ˚C, and then the cuvettes were placed inside the instrument and run. From there, data was outputted in various graphs and charts through the software, recorded in results. Mean intensity (cnt/s), R (nm), and %Pd were analyzed from five successful acquisitions.

Equation 3: Hydrodynamic radius, R_h, equation.

$$Rh = \frac{kT}{6\pi\eta D}$$

Given from the Stokes-Einstein equation, k is Boltzmann's constant, T is the temperature in K, h is the solvent viscosity, and D_t is the diffusion coefficient. The diffusion coefficient tends to decrease as concentration increases and is calculated through the equation above.

Results

Chemotaxis Assay

The chemotaxis index of each polyphenol or control was calculated using [Equation 2], and it is mediated by the activation of sensory neurons and interneurons [23]. A +1.0 score indicated maximal attraction towards the target, or that 100% of the nematodes arrived in quadrants containing the chemical target; and a -1.0 score would have indicated maximal repulsion [Margie 13, doi.org/10.1016/0092-8674(93)80053-H]. A highly positive chemotaxis index would show that the polyphenol in the quadrant stimulates the worms' sensory neurons and interneurons best; while a negative response indicates the opposite [23]. Moreover, since the worm strain used in this assay is the transgenic mutant CL2355 in which Aβ is expressed, chemotaxis indices may also help in determining the effect of polyphenols on neurons with Aβ, as greater chemotaxis responses would be proof that the worms' sensory neurons and interneurons are more sensitive. Previous literature has reported significant increases in chemotaxis at micromolar concentrations with the addition of the polyphenols protocatechuic, gallic, and vanillic acid [24]. Our chemotaxis assay's results have supported this hypothesis that polyphenols would improve nematode chemotaxis, as certain polyphenols yielded positive chemotaxis indices.

As Table 1 and Figure 1 show, we tested 13 compounds (donepezil HCl, kaempferol, apigenin, hesperidin, resveratrol, quercetin, tannic acid, salicylaldehyde, curcumin, EGCG, fisetin, rutin hydrate, and genistein). Donepezil HCl is a clinically prescribed cholinesterase inhibitor used to treat Alzheimer's, so it was used as a control to test how well polyphenols would perform compared to current medications.

Because the chemotaxis indices indicate the effectiveness of a compound in attracting nematodes, the higher a chemotaxis index is the more effective its target compound is [20].

As such, the fact that donepezil HCl, with an average chemotaxis index of -0.01767, performed worse than polyphenols, with all polyphenols except for kaempferol, hesperidin, and salicylaldehyde having greater indices, appears to prove that polyphenols are more impactful on chemotaxis. Tannic acid in particular appears to be most effective, an index of 0.838, indicating that the most number of worms were attracted to this compound. Previous literature has shown that because of tannic acid's antioxidant and anti-inflammatory properties that implicate neuroprotective effects, other chemotaxis indices for CL2355 worms at 16˚C for this compound was 0.73 ± 0.08363 [25]. Meanwhile, kaempferol, apigenin, and resveratrol all had negative chemotaxis indices, indicating that they were least effective. Results from the table in Table 1 correspond to the average chemotaxis index of three trials for each compound. Chemotaxis index was calculated from Equation 1 for each test compound, and the control average index was calculated from Equation 1 for control quadrants to provide a more accurate representation of the data.

Results from Table 1 are put into a graph (Figure 1) to give a clearer view of chemotaxis indices, with 10% error bars.

Table 1. Chemotaxis Assay Results.

Compound	Chemotaxis Index
Donepezil HCl	-0.01767
Kaempferol	-0.533
Apigenin	0
Hesperidin	-0.067
Resveratrol	0.188
Quercetin	0.233
Tannic Acid	0.838
Salicylaldehyde	-0.333
Curcumin	0.2
EGCG	0.491
Fisetin	0.4467
Rutin hydrate	0.68
Genistein	0.483

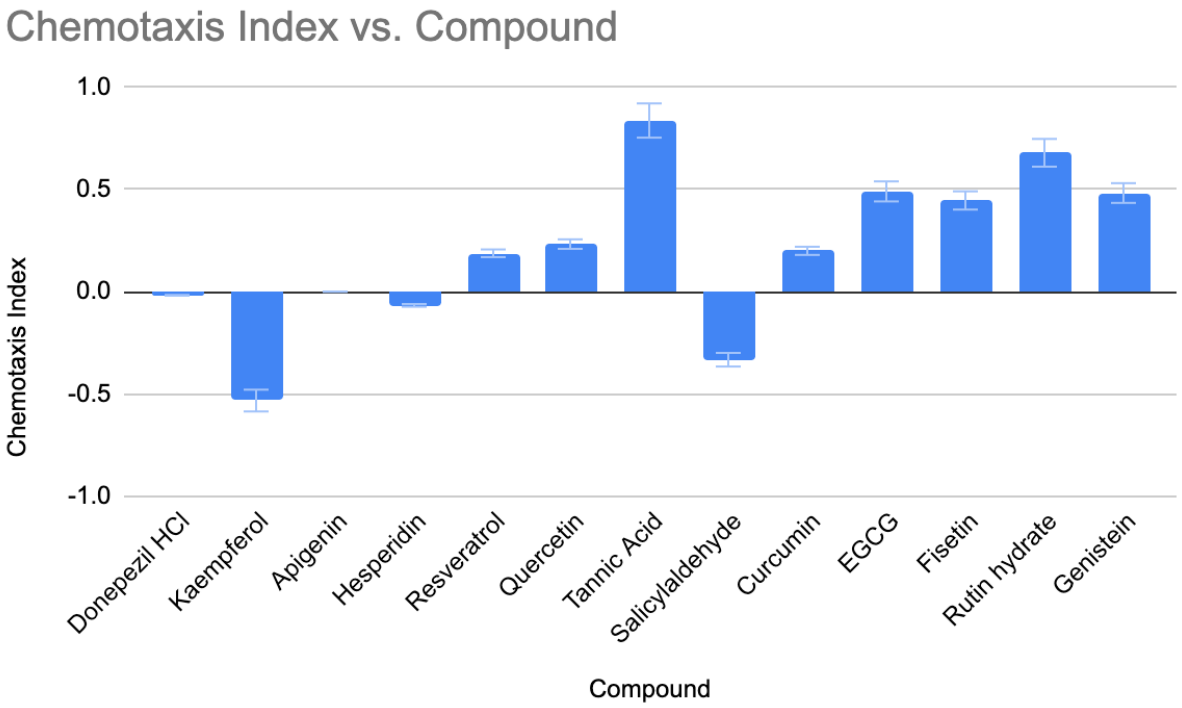

Figure 1. Chemotaxis Indices of Polyphenols and Donepezil HCl.

Congo Red Assay

Categorized as an in vitro dye-binding assay, Congo Red is used to quantify amyloid beta aggregation levels - fibril content - and to gauge the efficacy of small molecule inhibitors of that aggregation [21]. Because Congo Red, a histologic dye, binds to amyloid-beta and has a changed absorbance spectrum, the Congo Red spectrophotometric assay was an optimal method for a variety of factors. As the results are objective, they are less prone to misinterpretation.

The aggregation of the amyloid beta can be calculated using the C_b equation shown in [Equation 2]. The equation calculates the amount of Congo Red dye bound to the aggregated Aβ fibrils. The results, after calculating the absorption rates, show that the amount of Congo Red dye is bound to the experimental groups where polyphenols are present. This amount is smaller than the amount present in the control group where polyphenols are not present. We can conclude that when polyphenols are present, the aggregation of Aβ fibrils is occurring slowly, resulting in lower absorption rates of Congo Red onto the Aβ fibrils.

Looking at the table below [Graph 2], the five polyphenols had relatively similar absorption rates, all with rates around the same range. We had tested curcumin, meperidine, resveratrol, tannic acid, and epigallocatechin gallate (EGCG). If we take a closer look at the absorption rates in the C_b column of [Table 2], the highest performing polyphenols or the polyphenols with the lowest absorption rates, were curcumin and resveratrol while the lowest performing polyphenol was EGCG.

Our results had significant relation to a study conducted at the Texas Christian University in which the highest performing polyphenols included curcumin and resveratrol. The study found that cucumin inhibited both Aβ1–40 and Aβ1–41 sequences allowing it to weaken the Aβ fibrils. Similar results were observed with the polyphenol resveratrol as it inhibited the structure of Aβ1–42 and stopped it from growing any further.

Table 2. Congo Red Assay Results.

	Absorbance at 477 nm (au)	Absorbance at 540 nm (au)	C_b (overall absorption rate) (au)
PBS Buffer	1.197	0.795	$5.579354307 \times 10^{-6}$
Congo Red Dye	1.449	1.02	$9.032333508 \times 10^{-6}$
Control (Congo red dye + PBS buffer + amyloid-beta)	1.217	0.817	$6.017181938 \times 10^{-6}$
Curcumin	1.314	0.854	$5.385160671 \times 10^{-6}$
Meperidine	1.253	0.822	$5.437412412 \times 10^{-6}$
Resveratrol	1.34	0.866	$5.298080414 \times 10^{-6}$
Tannic Acid	1.251	0.823	$5.520136863 \times 10^{-6}$
EGCG	1.327	0.865	$5.539288066 \times 10^{-6}$

Data points correspond to five polyphenols and two control groups undergoing different wavelengths to find absorption levels, measured in au. Results from the table in table 2 are put into a graph (figure 2) to give a clear view of differences in absorption rates.

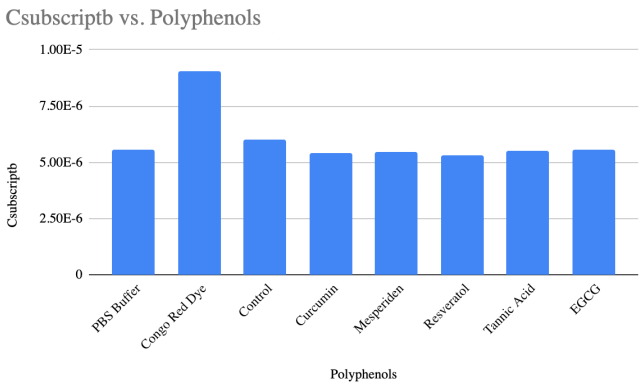

Figure 2. Congo Red Assay Absorption Levels.

DLS Assay

Dynamic light scattering (DLS) technique is a laser scattering technique that functions by shining a light into a sample and recording the intensity of scattered light at a certain angle over time. Through measuring the size of the given molecules, its results can determine the scope of aggregation through analyzing the size of fibril formation, as has been previously reported in lysozyme aggregation, amyloid-beta aggregation, and polyglutamine aggregation [26].

For the DLS assay, the mean intensity, mean R, and %Pd values were all recorded. The mean intensity value provides a quantifiable number as to the intensity of the scattered light from moving macromolecules in cnt/s. Mean intensity can be used to calculate particle size, since smaller particles diffuse faster and will thus have more rapid fluctuations in scattered light intensity than larger particles. The mean R_h value, or the hydrodynamic radius value, was also reported for polyphenol groups in nm. More specifically, this value is the radius of a hard sphere with the same diffusion coefficient as its sample; it gives further insight to the size of each particle. Lastly, we also reported the %Pd values, or the percent of polydispersity values, of each polyphenol or control; this value is a parameter that is used to describe the width of the particle size distribution. In general, samples with %Pd < ~20% are considered to be monodisperse [27]. Ideally, larger mean intensity values and smaller R values, or the radii of particles, would indicate less aggregation in a sample.

From our assay, we concluded that the R values, which refer to the radii of particles, of gallic acid, tannic acid, EGCG, and fisetin were observed to be lower than those for the amyloid-beta control solution. This would suggest that adding these polyphenols to the amyloid-beta solution successfully reduced amyloid-beta aggregation, since radius size of each particle decreased. Mean intensity for each polyphenol solution, however, was not always higher than the control's. Only gallic acid and tannic acid showed higher mean intensities of scattered light than the control. Moreover, since %Pd values for gallic acid, tannic acid, and fisetin were less than 20%, these trials were considered to be monodisperse.

Because of overall lower R values for each polyphenol compared to the control and %Pd values of <20%, the DLS assay appears to suggest that polyphenols are able to inhibit or at least slow down the aggregation of amyloid-beta. Furthermore, this also supports previous data using DLS assays, which have found that the larger the measured radius, the lower antioxidant activities were obtained [28].

Table 3. Dynamic Light Scattering Assay Results.

Polyphenol Added	Mean Intensity (Cnt/s)	Mean R (nm)	%Pd
None	818671	291.6	19.0
Gallic Acid	1246460	193.2	9.2
Tannic Acid	1012630	267.9	7.6
EGCG	187341	269.9	22.78
Fisetin	619245	274.34	14.72

Measured the mean intensity (cnt/s, or the count rate per second), mean R (nm), and %Pd for each cuvette, with control being amyloid-beta HFIP solution (no polyphenol added). Each polyphenol added was plotted on a line graph (figure 3) for the mean R (nm) and %Pd for each cuvette with different polyphenols added.

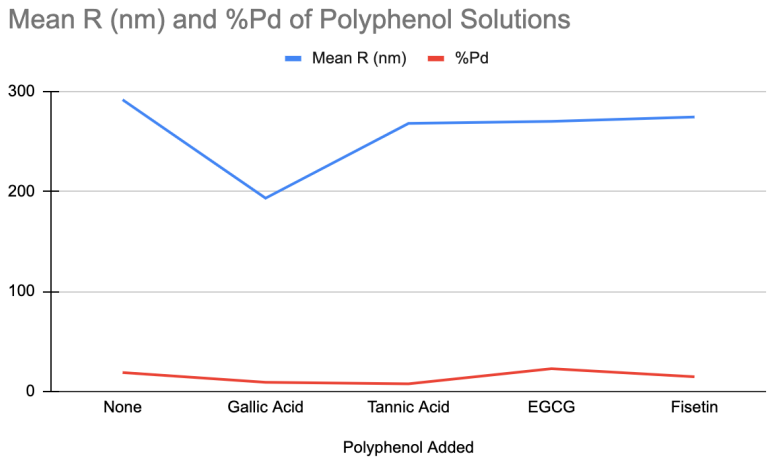

Figure 3. Polyphenol Added vs %Pd.

Discussion

Overall, we hypothesized that polyphenols would enable C. elegans to learn to avoid the avoidance chemical in the avoidance assay. The avoidance assay was conducted twice, and results did support our hypothesis. It established that curcumin, epigallocatechin gallate, and tannic acid were all conducive towards improving the worms' memory. This is most likely because all have been proven to have antioxidant effects [2]. However, further trials of this assay are needed to improve accuracy rates. Moreover, more polyphenols can be tested to better determine our hypothesis.

The chemotaxis assay was conducted three times, and results for most polyphenols tested did support our hypothesis. Since chemotaxis is the worms' ability to locate food, it is a marker for neurological function; and because the CL2355 strain used in these experiments show amyloid-beta, chemotaxis indices correspond to how each polyphenol impacted sensory neuron and interneuron function with amyloid-beta [29]. Thus, because our results showed positive chemotaxis indices for resveratrol, quercetin, tannic acid, curcumin, EGCG, fisetin, rutin hydrate, and genistein, it appears that these polyphenols improved the nematodes' neurological function by improving their sensory neurons and interneurons. Furthermore, the above polyphenols actually performed better than donepezil HCl, which is a current medication used for Alzheimer's, suggesting that polyphenols could potentially be more effective than cholinesterase inhibitors like donepezil HCl at improving neurological function. Since polyphenols have been shown to have antioxidant effects, this would support our findings that polyphenols are neuroprotective [2].

The Congo Red Assay was conducted to determine whether the cluster of amyloid beta was aggregated to a lesser degree with the presence of polyphenols. Our results proved our hypothesis correct and polyphenols showed to be effective. Looking at the control group where there were no polyphenols being added to a cuvette solution, there was a C_b absorption of 0.000006017181938 au, while the absorption rate for experimental groups with polyphenols was between 0.0000052 - 0.0000056 au. A lower absorption rate indicates that there is less aggregation of the amyloid beta. The most effective polyphenol tested in this assay was resveratrol, which is found in many fruits and plants, and had an absorption rate of 0.000005298080414 au. The least effective polyphenol, meanwhile, was EGCG. Resveratrol has been a high performer in many previous studies and is capable of disallowing the development of Aβ fibrils. This supports our prediction that polyphenols are effective inhibitors of Aβ fibrils.

The DLS assay was run three times. We hypothesized that polyphenols would decrease the mean radius size (R) values of each polyphenol-amyloid beta solution, and results from the DLS assay confirmed our hypothesis, as gallic acid, tannic acid, fisetin, and epigallocatechin gallate trials all showed mean R, radius, values of amyloid-beta being lower than amyloid beta's R value alone. Gallic acid appeared to be most effective at inhibiting amyloid beta aggregation in the DLS instrument, while fisetin appeared to be least effective. As previous literature has proven that

all four of these polyphenols have been able to inhibit amyloid beta aggregation, our results correspond with past studies. More specifically, gallic acid's mechanism of action towards amyloid beta has been to inhibit amyloid beta elongation and lead oligomerization towards a less toxic pathway [5]. However, further trials to test more polyphenols are needed. An analysis of each polyphenol's efficacy in reducing amyloid-beta's R-value compared to a pharmaceutical drug's efficacy would also be of use - donepezil HCl, for example, is a compound that could be compared to polyphenols.

Conclusion

The results of this study indicate that polyphenols inhibit amyloid-beta aggregation, supporting the observations of numerous previous studies. Notably, this relationship appeared in both the in-vitro and in-vivo assays conducted. In terms of effectiveness, curcumin often stood out in comparison to other polyphenols. In particular, it performed the best in every trial of the congo red assay, along with supportive results in the avoidance assays. This seems to fall in line with the current literature, as curcumin stands as one of the most investigated and well-respected polyphenols. Epigallocatechin gallate (EGCG) was also an exceptional performer throughout the majority of trials. It has been previously noted to be quite effective against cancer, but this remains a relatively novel discovery. These findings hold implications in terms of the treatment of Alzheimer's disease, as the most efficacious polyphenols could be used as a form of preventative medicine. Additionally, elucidating the mechanism that allows for such potent inhibition could lead to the development of novel drugs. Further research is warranted.

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