Investigating the Effect of *Siraitia grosvenorii* and *Angelica sinensis* against Alzheimer's Disease using *Caenorhabditis elegans* as a Model of Study

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Abstract

This research paper studies how the *Siraitia grosvenorii* and *Angelica sinensis* affects Alzheimer's disease, by using *Caenorhabditis elegans* as a model of study. There are 3 parts to this study, the plant extract preparation, DPPH test and the testing of the extracts on the *C. elegans.* It has been found that both extracts can indeed help to reduce paralysis and lengthen the lifespan of the *C. elegans*, with *A. sinensis* having a greater observable effect than *S. grosvenorii*. These results show promise in the two plant extracts having a positive effect in the treatment of Alzheimer's disease in humans..

Introduction

Alzheimer's disease (AD), has been a prevalent problem in our society. Statistics suggest that nearly 50 million people globally suffer from AD, or some form of related dementia. Alzheimer's disease is a neurodegenerative disorder, characterised clinically by premature progressive dementia, and two abnormal brain structures. One is plaques, which are pieces of a protein fragment amyloid-β that builds up in the spaces between nerve cells. The other is tangles, which are twisted fibers of another protein, tau that build up inside neuron cells. People who suffer from Alzheimer's disease exhibit the presence of both proteins which have been associated with neuronal damage and death.

One possible mechanism of amyloid-β related damage is oxidative stress. The lipophilic free-radical scavenger vitamin E, a potent inhibitor of lipid peroxidation reactions, can protect neurons against amyloid-β toxicity (Behl et al. 1992). Membrane damage due to exposure of neurons to amyloid-β was also found in an electron-microscopy study, showing that high concentrations of amyloid-β added to rat pheochromocytoma PC12 cells or to rat primary cortical neurons can induce rapid membrane disintegration and the breakdown of membranous structures (Behl et al. 1994a).

A prior study found that a high dietary intake of vitamin C and vitamin E may lower the risk of Alzheimer disease. (Engelhart, 2002) In vitro studies also suggest that exogenous antioxidants reduce the toxicity of β-amyloid in the brains of Alzheimer patients. A previous randomized controlled trial found that patients taking vitamin E supplement had a slower progression of Alzheimer disease than patients taking placebo. (Sano, Ernesto, Thomas, 1997) It is thus possible that high intake of antioxidants may also prevent the onset of dementia, because antioxidants may reduce neuronal loss due to oxidative damage. (Behl et al. 1997)

There is much research conducted on the use of herbal remedies to slow down the effects of Alzhimer's disease. In 2012, Jin and Lee found out that *S. grosvenorii* extracts and purified mogrosides exhibit antidiabetic, anticarcinogenic, antibacterial, antioxidant, and antiallergic effects. An extract of *S. grosvenorii* could potentially serve as an important source of pharmaceutical treatment to delay the onset of Alzhimer's disease.

Another study by Zhuang et.al (2016), showed that *A. sinensis* had antioxidant properties. Their results indicated that ASP (*Angelica sinensis* polysaccharide) protected chondrocytes from hydrogen peroxide-induced oxidative stress and subsequent cell injury

through its antioxidant, antiapoptotic and anti-inflammatory effects in vitro. (Zhuang, C., et al 2016).

In research involving Alzhiner's disease, the organism *C. elegans*, is a useful model as they can be genetically modified to produce the causative neurotoxic peptides amyloid-β and tau, which can translate to AD in humans. (Griffin, Caldwell, & Caldwell, 2017). *C. elegans* has many advantages as a model for the study of AD, of which includes the fact that 38% of the genes in *C. elegans* have a human ortholog and that its life span is only 2-3 weeks long. Its transparency allows for observation using simple microscopy. Hence, our group chose *C. elegans* as our research tool.

This study aims to investigate if the antioxidant properties, amongst other properties in Siraitia grosvenorii and Angelica sinensis, in varied concentrations, can help to reduce the chances and damage of Alzheimer's disease in *C. elegans*.

Objectives and Hypotheses

Objective:

The objective of the experiment is to investigate the antioxidative properties of *S. grosvenorii* and *A. sinensis,* and study the extent that they can reduce paralysis and lengthen the lifespan of the *C. elegans* that produce amyloid-β.

Hypotheses:

S. grosvenorii and *A. sinensis* will demonstrate antioxidative properties.

The extract with more antioxidative properties will reduce the damage caused by oxidative stress and amyloid-β to a greater extent.

The extract with more antioxidative properties will reduce paralysis and lengthen the lifespan of *C. elegans* by a greater extent.

Methods and Materials

Part I (Preparation of Extracts)

Materials:

1. Blender
2. Weighing scale
3. Conical flasks
4. Heater
5. Magnetic Stirrer
6. Funnel
7. Filter Paper
8. Conical Centrifuge Tubes
9. Centrifuge
10. Beaker
11. Micropipette
12. Microcentrifuge Tubes
13. Sterile Syringe
14. Centrifuge Tube Racks
15. *S. grosvenorii*
16. *A. sinensis*
17. Water

Methods:

10g of *A. sinensis* was blended and added to 100ml of deionised water in a conical flask. The solution was heated and stirred at a speed of 2.7 at approximately 140°C for 40 minutes, and poured into a conical flask via a funnel and filter paper. The extracts were poured into conical centrifuge tubes and centrifuged at 7000rpm for 10 minutes. Once completed, the tubes were emptied into a beak. A micropipette was used to measure 1ml x 2 of supernatant and to transfer them to microcentrifuge tubes. The microcentrifuge tubes were centrifuged at 13000rpm for 20 minutes, and a sterile syringe with a 0.45μm pore size was used to remove bacteria. The supernatant was then poured into conical centrifuge tubes. The same process was repeated with *S. grosvenorii.*

Part II (DPPH Test)

Materials:

1. Beakers
2. Filter paper
3. Funnel
4. Dropper
5. Micropipette
6. Blender
7. Erlenmeyer flask
8. Electronic balance
9. Autoclave
10. Centrifuge
11. UV-vis Spectrophotometer
12. Magnetic Stirrer
13. Conical Flask
14. Conical Centrifuge Tubes
15. Sterile Syringe
16. Centrifuge Tube Rack
17. Methanol
18. DPPH Solution
19. Sterile water
20. MF extract
21. DG extract

Methods:

For the control set-up, 1 blank control was prepared by adding 2.9ml of methanol and 0.1ml of sterile water and 3 DPPH controls was prepared by adding 1ml of DPPH, 1.9ml of methanol and 0.1ml of sterile water. These steps were repeated with the 1 blank extract and 3 DPPH extracts for both *Siraitia grosvenorii* and *Angelica sinensis* set-ups, but using the extracts instead of sterile water. All the volumes were measured using a micropipette and added into conical centrifuge tubes, to ensure that the total volumes in all test tubes amounted to 3ml. All the conical centrifuge tubes then left in the dark for 15 minutes. Once completed, the antioxidative property level of the extracts were measured with a UV-vis spectrophotometer with a wavelength of 517nm, using the blank control and extracts against the DPPH control and extracts, and interpreted from there.

Part III (Testing Plant Extracts on C Elegans)

Materials:

1. Erlenmeyer flask
2. Aluminium Foil
3. Autoclave
4. Petri Plates
5. Peristaltic Pump
6. Air-Tight Container
7. Pipette Tip
8. Glass Rod
9. Sodium Chloride
10. Sterile Water
11. Calcium Chloride
12. Cholesterol
13. Ethanol
14. Magnesium Sulfate
15. Potassium Phosphate
16. Agar
17. Peptone
18. E. Coli OP50
19. *S. grosvenorii*
20. *A. sinensis*
21. *C. elegans*

Methods:

NGM gel and OP50 were prepared and applied to petri dishes. *C. elegans* were transferred to petri dishes and incubated for 1 day at 20°C. If needed, 100microlitres of *S. grosvenorii* or *A. sinensis* extract was added to the petri dishes. The *C. elegans* were allowed to absorb extracts over a period of 24h at 20 degrees celsius. Once completed, the *C. elegans* were observed under the microscope to count the number of paralysed and non-paralysed worms. The average percentage of worms that are paralysed was then calculated. This was done for *S. grosvenorii*, *A. sinensis* and a control set-up without any herbal extracts, with each having 3 plates.

Results and Discussion

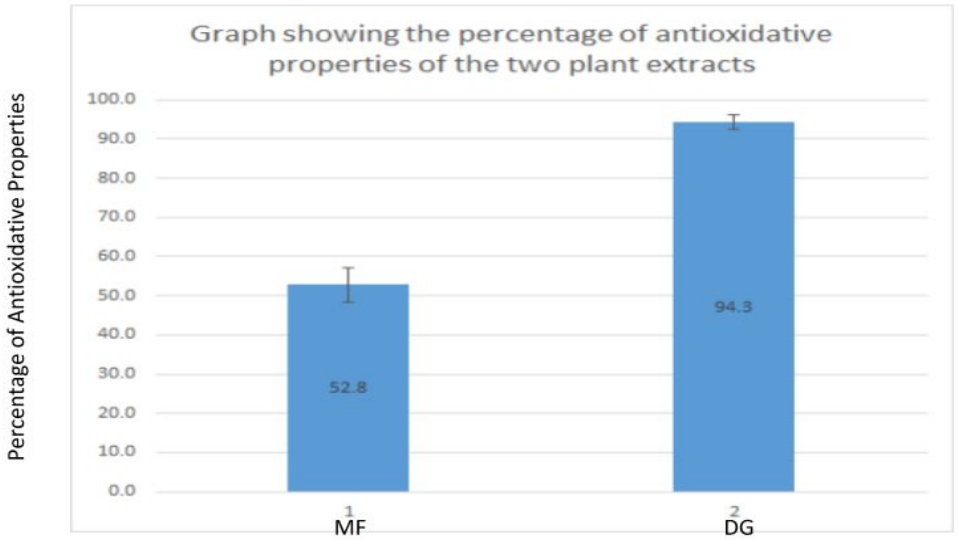

Figure 1 Graph showing the percentage of antioxidative properties in the two extracts compared to control, interpreted from the DPPH Test. The results showed that both plant extracts contained substances with antioxidative properties. In comparison, *A. sinensis* extract showed an observable higher concentration (94.3%) of antioxidative substance when compared to the *S. grosvenorii* extract (52.8%).

	P1S1	P1S2	P1S3	P2S1	P2S2	P2S3	P3S1	P3S2	P3S3	Average
Control	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
MF	17%	30%	31%	24%	20%	43%	23%	50%	42%	31.1%
DG	11%	24%	21%	20%	0%	11%	0%	17%	10%	12.7%

Legend
Percentage of Paralysed Worms
P: Plate
S: Spot

Figure 2.1 Table showing percentage of paralysed worms

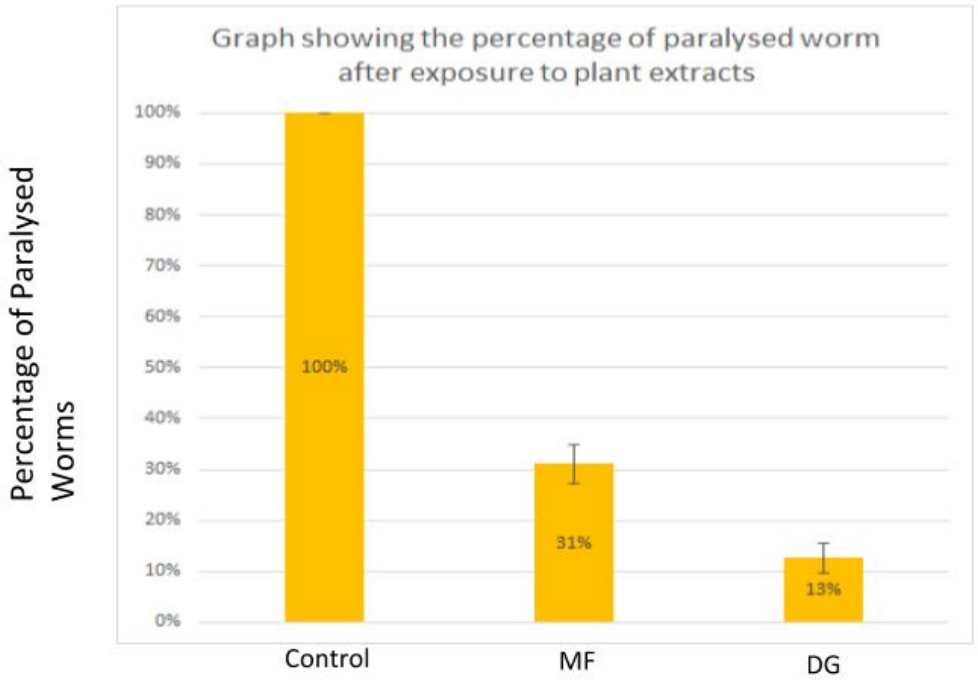

Fig 2.2 Graph showing average percentage of paralysed worms in each extract.

Both *A. sinensis* and *S. grosvenorii* demonstrate antioxidative properties. There is an observable difference in the outcomes of the worms treated with the two plant extracts. The worms treated with *A. sinensis* extract showed 13% paralysis as compared to the worm treated with *S. grosvenorii* extract which showed 31% paralysis. Both of these results were observably much lower than the worms that were treated with sterile deionised water.

Both plant extracts can reduce paralysis in *C. elegans* as compared to the control with *A. sinensis* being **1.26 times** more effective in reducing paralysis as compared to *S. grosvenorii*.

Other experiments, such as the one that was testing for antioxidative effects in *A. sinensis*, had also shown similar results.

Although the results appear promising, a more rigorous level of testing would increase the reliability of the studies as statistically tests can be conducted. We were however hindered from doing this due to time constraints during the COVID-19 situation as the delivery of the *C. elegans* from the companies in the US took way longer than expected.

In the future, we also plan to study the effect of the different concentrations and the

duration of incubation in extracts of the two plant extracts so that we can optimise the dosage used to reduce paralysis in the *C. elegans*. As our studies focused mainly on behavioural patterns of *C. elegans* that had been exposed to the plant extracts, we could not provide a conclusive insight on how the amyloid-b had been affected after the treatment. We had wanted to carry out Western blotting which would help us quantify the amount of amyloid-β in the control worms and the worms subjected to the different plant extracts.

Conclusion and Recommendations for future work

In conclusion, both herbal extracts possess antioxidative properties, with *A. sinensis* having a higher level of antioxidative properties and being able to reduce the rate of paralysis to a greater extent. These findings have implications in the field of neurodegenerative studies, particularly in slowing down or reversing the effect of neural degeneration of patients suffering from Alzhimer's disease. However, more studies in the area of pharmacological assays, immunohistochemistry and transmission electron microscopy needs to be carried out before conclusive findings can be ascertained.

If successfully shown to reduce paralysis in humans, our study has many implications and impacts. The treatment will be widely sought after by the 50 million people worldwide who suffer from Alzheimer's disease, and thus will contribute significantly to the economy of Singapore. We will be able to improve the lives of many who suffer from Alzheimer's disease, and will lay groundwork for future advancements in the field of neurology.

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