Investigating the antibacterial and antioxidant effects of chia seeds

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Abstract

In recent years, antibiotic resistance has been on the rise due to the widespread use of common antimicrobials. *Salvia hispanica* (chia seeds) show potential to be an effective natural antibiotic that has not been previously used in medical applications and therefore this project aims to investigate the antibacterial effects of *Salvia hispanica* (chia seeds) and its antioxidant properties as well as cytotoxicity towards the nematode *Caenorhabditis elegans*. Antibacterial properties have been determined via the antibacterial well-diffusion test and the antibacterial colony count test. The chia seed extract showed significant inhibition of growth against all bacteria tested on, which involved both Gram-positive and Gram-negative bacteria, namely *Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Escherichia coli and Pseudomonas fluorescens*. The 1,1-diphenyl-2-picryl-hydrazil (DPPH) assay was used to determine the antioxidant effects of chia seeds. The extract displayed notable antioxidant properties in the assay. Chia seed extract also resulted in a lower survival rate of the nematode *C. elegans* than in the control, suggesting that it possesses cytotoxic effect.

Introduction

In the 21st century, chia (*Salvia hispanica*) seeds are gaining increasing interest among food producers and consumers because of their prohealth properties. *Salvia hispanica* L. is an annual plant of the Lamiaceae family. It grows in dry and semi-dry climates, and it is native to present-day Mexico and Guatemala.

Chia seeds has high nutritional value due to a high content of omega-3 fatty acids. They are also a good source of oil, protein, dietary fibre, minerals and polyphenols. According to Noshe and Al-Bayyar (2017), chia seed oil is a rich source of polyunsaturated fatty acids (PUFA). Omega-3 plays an important role in maintaining good health, including prevention of cardiovascular disease, high blood pressure and inflammatory diseases. In addition, chia seeds and oil extracted from seeds contain antioxidants such as tocopherol, phytosterol and carotene, as well as phenolic compounds (Noshe & Al-Bayyar, 2017). Other antioxidants in chia seeds include chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol which have cardiac, hepatic protective effects, anti-ageing and anti-carcinogenic effects (Ullah *et al.*, 2016). The extract of chia seeds at a concentration of 2% was also shown to inhibit lipid oxidation of the pork sausages, increasing their shelf life and stability (Scapin *et al.*, 2015).

Guru kumar *et al.* (2016) reported that the crude extract of chia seeds inhibited the growth of prostate cancer cell lines (PC-3) in a dose dependent manner. Flavonoids are known to exert anticancer activities in a variety of cells. The anticancer activity of chia seeds was attributed to the flavonoids and tannins present in the seed extract. Rodrigues *et al.* (2018) studied the effect of chia seed oil extract in lowering the lipid level in the model organism, *Caenorhabditis elegans*. They observed a reduction in triglycerides and lipid droplets (containing triglycerides and cholesterol esters) of the nematodes following exposure to chia seeds oil. Besides the antioxidant and cytotoxic activity of chia seeds, Divyapriya, Veeresh, and Yavagal (2016) studied its antibacterial property. Zones of inhibition were observed against bacteria that cause periodontal diseases. The effectiveness of chia seed extract as an antibacterial agent in tooth paste, gel or mouth rinse can be further investigated to find out the ability of *Salvia hispanica* in preventing oral infections.

Hypothesis and Objectives

Objectives

This research project aims to investigate the antibacterial property of chia seeds towards Gram-positive and Gram-negative bacteria as well as its antioxidant properties and if the extract is cytotoxic towards the nematode *Caenorhabditis elegans.*

Hypotheses

Our hypotheses are that Chia seeds show varying antibacterial activity towards Gram-positive and Gram-negative bacteria, possess antioxidant properties and is cytotoxic towards the nematode *Caenorhabditis elegans.*

Methods

Preparation of chia seed extract

1 g of chia seed was ground in 10 ml of deionised water using a mortar and pestle. The chia seed ground was then left to soak in deionised water for 24 hours at 4°C. The mixture was then placed in an ice bath and sonicated. They were manually shaken after every 10 seconds of sonication. The mixture was centrifuged at 7000 rpm for 10 min, the supernatant was collected and filter-sterilised through a sterile microfilter.

Growth of precultures of bacteria and yeast

The bacteria used were *Bacillus cereus* ATCC 11778, *Pseudomonas fluorescens* ATCC 948, *Bacillus subtilis ATCC 19659, Staphylococcus epidermidis ATCC 12228* and *Escherichia coli* ATCC 25922. Bacteria were inoculated into 10 ml LB broth and grown overnight at 30°C in a shaking incubator. The absorbance of bacterial cultures at 600 nm was standardised at 0.8.

Antibacterial well diffusion test

Test organisms were spread on the surface of Mueller-Hinton agar plates. Wells were created in the agar and filled with 80 µl of chia seed extract. The negative control was sterile water and the positive control was 10% bleach. The plates were incubated at 30°C for 1 day and observed for zones of inhibition.

Antibacterial colony count test

In the test set-up, 0.5 ml of each broth culture of test organism was added to 2.5 ml of filter-sterilised chia seed extract 2.0 ml of LB broth was added. For the control set-up, sterile water was added instead of chia seed extract. Five replicates were performed. The mixtures are then incubated in the shaking incubator overnight at 30°C. 1ml of each broth culture was placed in a cuvette. The absorbance of the culture was measured at 600 nm with a UV-vis spectrophotometer. Based on the absorbance values, serial 10-fold dilutions were done with 0.85% sodium chloride solution. 0.1 ml of the diluted culture was spread on LB agar and the plates were incubated at 30°C overnight. The number of colonies was determined.

DPPH antioxidant test

1,1-diphenyl-2-picryl-hydrazil (DPPH) is a free radical which produces a purple solution when dissolved in methanol. When it is reduced by antioxidants, a change in colouration from purple to yellow is observed. The negative control consisted of 1.0 ml of DPPH, 1.9 ml of methanol and 0.1 ml of sterile water. In the test set-ups, sterile water was replaced by chia seed extract. Five replicates were prepared. For the respective blanks for each set-up, methanol was added instead of DPPH solution. The initial absorbance was measured at 517 nm against the respective blanks and the mixtures were then left to stand in the darkness for 20 min, before the final absorbance readings were measured. The radical scavenging activity (in %) was then calculated based on the following equation:

(Final absorbance of control – Final absorbance of test)/Final absorbance of control x 100%

Cytotoxicity test

The composition of Nematode Growth Medium (NGM) is as follows: 0.9 g NaCl, 7.5 g agar, 0.75 g bacto peptone in 300 ml water. After autoclaving, 0.3 ml cholesterol (5 mg/ml), 0.3 ml $MgSO_4$ (1 M), 0.3 ml $CaCl_2$ (1 M) and 7.5 ml potassium phosphate buffer pH 6.0 (1 M) were added. *E. coli* OP50 was grown in LB broth overnight at 30°C. 0.05 ml of *E. coli* culture was added to the centre of a fresh NGM plate and grown overnight at 37°C. A block of agar containing C. elegans N2 was placed on the plate and incubated at 20°C for 2 days. *C. elegans* and *E. coli* were collected in M9 buffer and filtered through a sterile 8 µm membrane filter. *C. elegans* remains on the membrane and is suspended in M9 buffer. 100 µl of chia seed extract was spread on one NGM plate. *C. elegans* suspended in M9 buffer was added to the NGM plate. In the control setup, 100 µl of sterile water replaced the chia seed extract. Five replicates of each setup were prepared. The percentage survival of worms was determined after 1 day. Worms were considered dead if they did not move.

Risk and Safety

All bacteria used are biosafety level 1 microorganisms which may cause opportunistic infections. To avoid exposure to bacteria and corrosive chemicals, gloves and lab coats were worn. Work involving bacteria was done in the biological safety cabinet. Bacterial cultures were autoclaved at 15 psi for 15 min in biohazard bags before disposal.

Results

Well Diffusion Test

The average of the 5 readings each obtained from the tests per bacteria for both sample and positive control were plotted on their respective bar graphs as seen in Figure 1 and 2 to determine the overall effectiveness of the Chia seed extract's inhibition on bacterial growth. The chia seed extract was effective in inhibiting the growth of all 5 bacteria it was tested on which include both Gram-positive and Gram-negative bacteria (Fig. 1 to 3). It was most effective against *Escherichia coli and Staphylococcus epidermidis.* The Kruskal Wallis test was also performed for both sets of data and the p value for the first test was 0.00041 and the p value for the second test is 0.00040, both of which were significant.

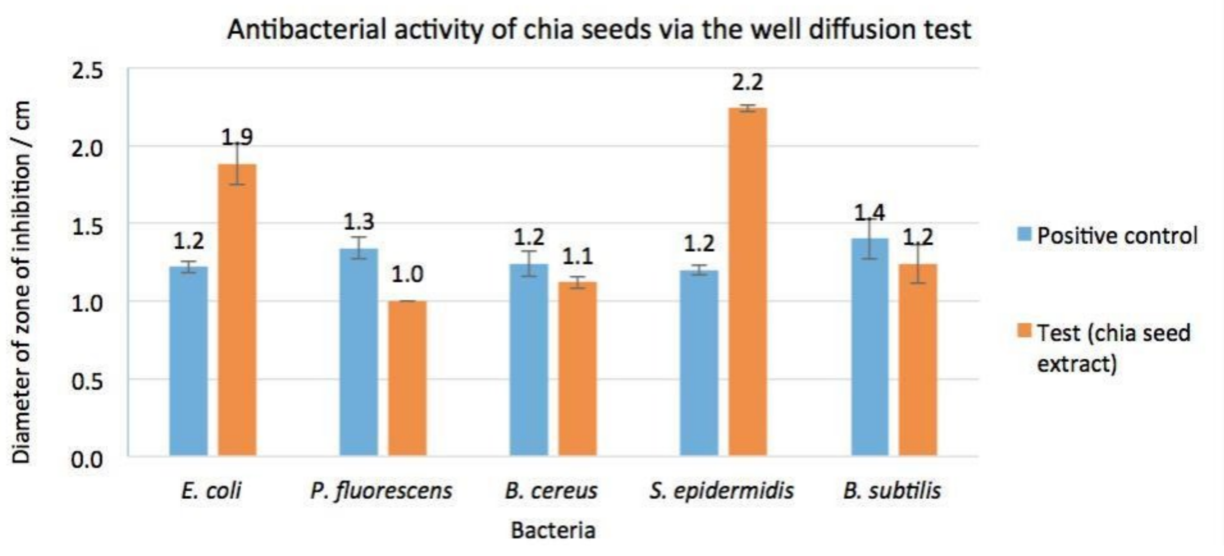

Fig. 1: Bar graphs of zones of inhibition by both positive control and chia seed extract in Well diffusion test 1.

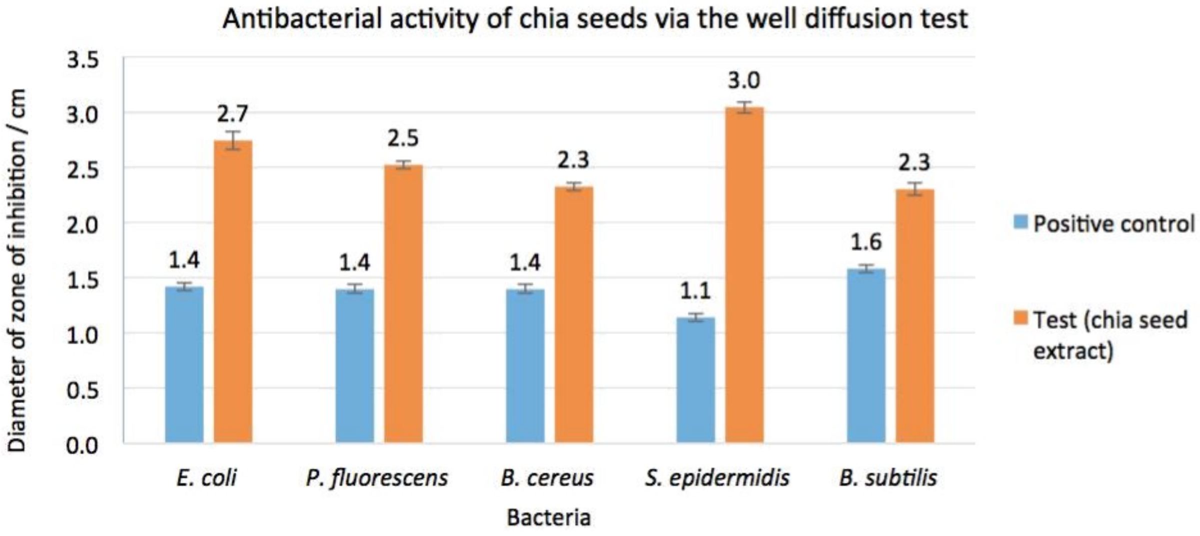

Fig. 2: Bar graphs of zones of inhibition by both positive control and chia seed extract in the well diffusion test 2.

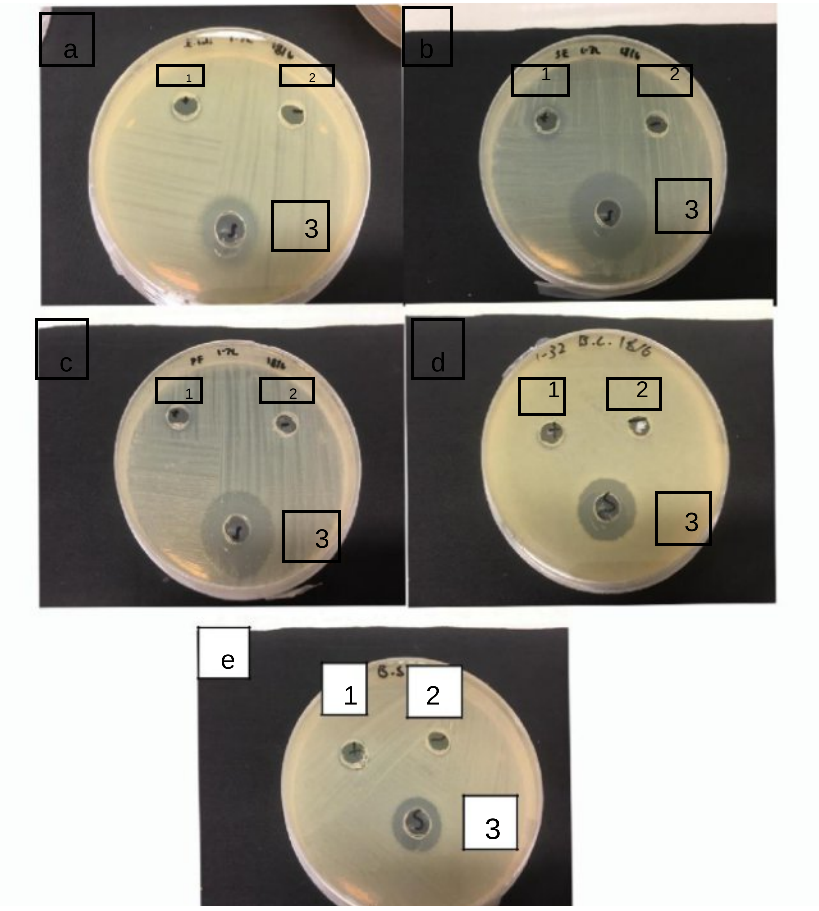

Fig. 3: Well diffusion plates for (a) *Escherichia coli,* (b) *Staphylococcus epidermidis*, (c) *Pseudomonas fluorescens,* (d) *Bacillus cereus*, and (e) *Bacillus subtilis.* Wells were filled with (1) 10% bleach (positive control), (2) sterile water (negative control), and (3) chia seed extract (test).

Colony Count Test

Chia seed extract showed strong antibacterial properties against *Staphylococcus epidermidis* and *Pseudomonas fluorescens.* The Mann-Whitney U test was performed for both bacteria and the p value for both bacteria was 0.01208, indicating significant difference between the control and test (Fig. 4 and 5).

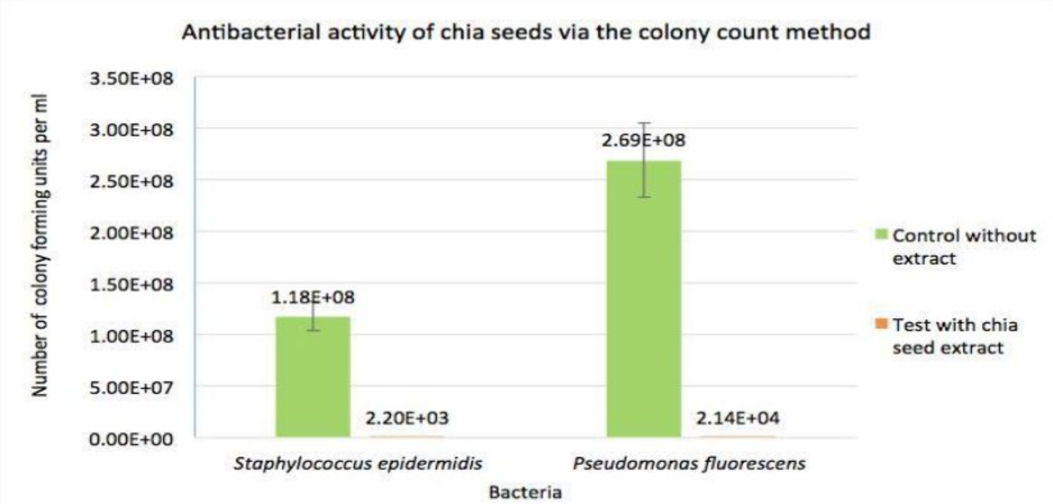

Fig. 4: Bar graphs of colony forming units (CFU) in control and test setups for both *Staphylococcus epidermidis* and *Pseudomonas fluorescens*.

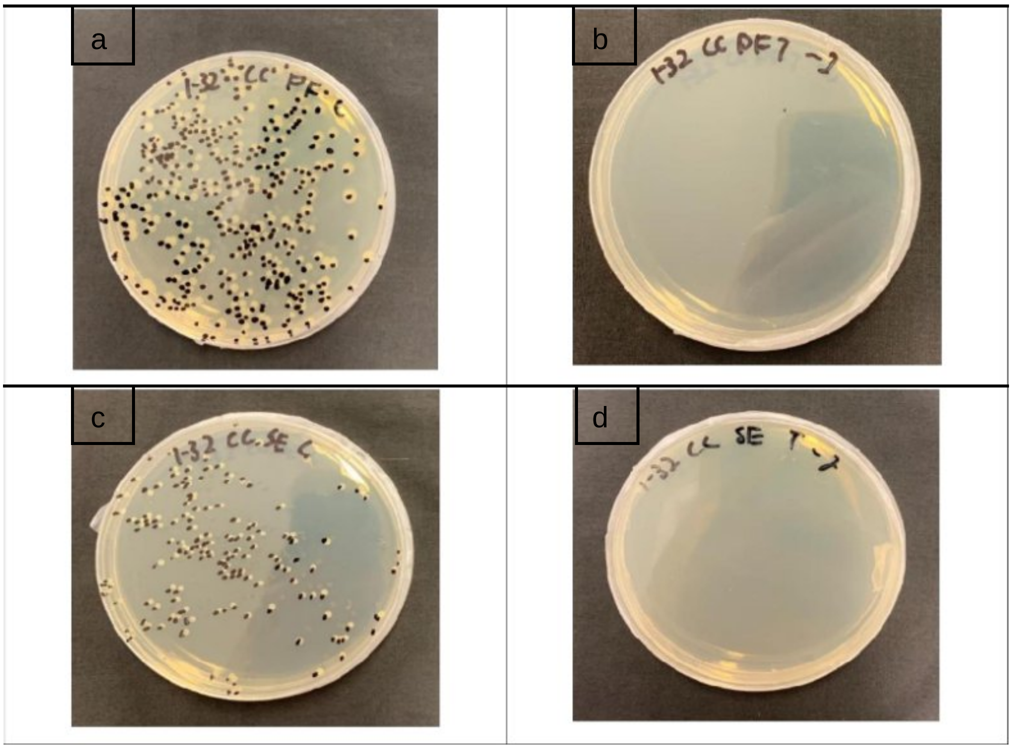

Fig. 5: Colony count plates showing (a) *P. fluorescens* without chia seed extract (control), (b) *P. fluorescens* with chia seed extract (test), (c) *S. epidermidis* without chia seed extract (control), (d) *S. epidermidis* with chia seed extract (test).

DPPH antioxidant assay

The radical scavenging activity of the extract was calculated at 43.7%. The changes in absorbance values indicate a different light intensity passing through the solution which in turn represents colour change in the solution. The Mann-Whitney U test p value was 0.0027, showing significant difference between the mean final absorbance of the control and test setups (Fig. 6).

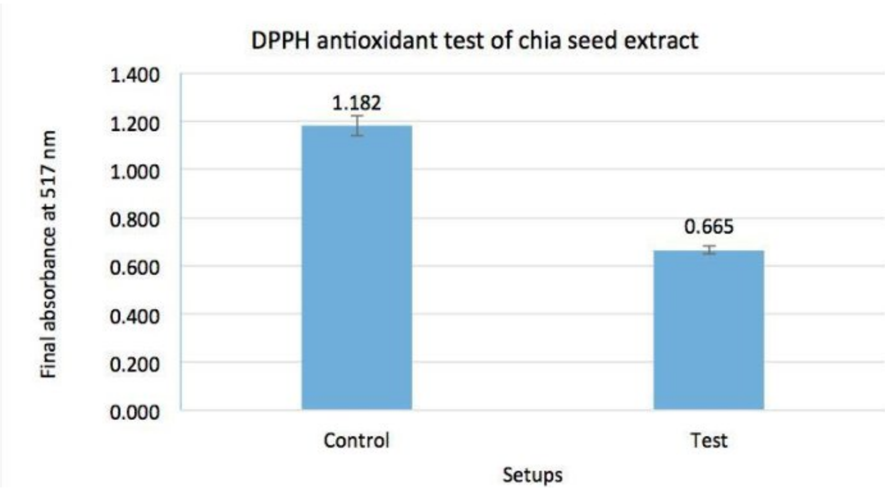

Fig. 6: Bar graphs showing the absorbance values of control and test set-ups in the DPPH assay.

Cytotoxictity Test

After incubation for 1 day with the chia seed extract and sterile water for the test and control respectively, the number of dead and living worms was determined. The average percentage survival rates for the control setup without chia seed extract and test with chia seed extract were 85.4% and 30.4%, respectively. The Mann-Whitney U test p value was 0.01208, hence the difference between the control and test was significant. This is shown in Fig. 7.

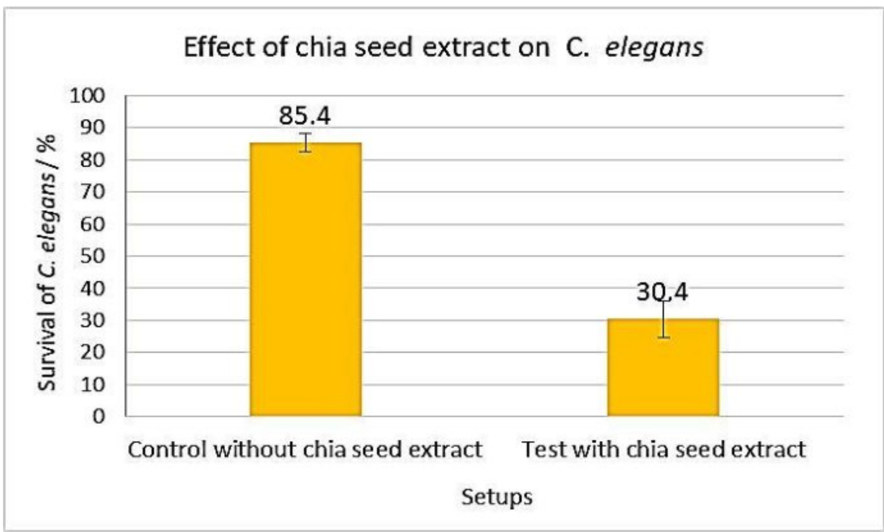

Fig. 7: Bar graphs showing the effect of chia seed extract on survival of *C. elegans*

Discussion

Divyapriya *et al.* (2016) reported that chia seeds process significant antibacterial effect against main periodontal disease pathogens which supports our results showing that chia seeds are generally effective against multiple types of bacteria. As suggested in Gómez-Favela *et al.* (2017) research using seeds that have just germinated may have an increased amount of compounds that are possibly responsible for antibacterial and antioxidant properties, therefore research conducted on freshly germinated chia seeds could be done to determine if the antibacterial and antioxidant properties of chia seeds have potential to be further enhanced by natural means. The compounds responsible for the antibacterial properties can be isolated and be further examined for other properties. However, research done by Segura-Campos, Salazar-Vega, Chel-Guerrero and Betancur-Ancona (2013) showed that none of the tested chia seed extract exhibited antimicrobial activity, which greatly differed from the results collected by this project. This could be due to different batches of chia seeds being used. Different batches of chia seeds used for the extract during the experiment had different concentrations of antibacterial and antioxidant effects. Therefore the results for each test may have varied. In addition, The 2 bacteria that chia seed extract showed the most antibacterial effect against are different in Gram properties and thus it is likely that the compounds exhibiting antibacterial properties in chia seeds are able to penetrate both the thick peptidoglycan layer of Gram-positive bacteria and the double phospholipid bilayer of Gram-negative bacteria implying that these bioactive compounds are smaller than 2nm and also lipid soluble.

Tepe *et al.* (2006) studied the antioxidant activity of ethanolic chia seed extract and reported that polyphenols of chia seed inhibited to a significant extent the free radicals in a beta carotene linoleic acid system. The DPPH test carried out in this study showed that the water extract of chia seed had radical scavenging activity of 43.7%. However the extract from Tepe *et al.* (2006) is methanol based while this project uses a water based extract thus showing that chia seed extract contains antioxidant compounds that are effective in water as well which can be easily obtained and is non-toxic for human consumption .There is also a possibility that the chia seeds used in their research are from different areas and thus even though they are of the same species, the seeds may contain varying concentrations of antioxidant compounds.

Conclusions

In conclusion, the well diffusion tests conducted have demonstrated antibacterial effects of chia seeds (*Salvia hispanica*) against Gram-positive and Gram-negative bacteria (*Staphylococcus epidermidis*, *Bacillus cereus*, *Bacillus subtilis, Escherichia coli* and *Pseudomonas fluorescens*). Similarly in the antibacterial colony count test, the extract exhibited antibacterial effect against *Staphylococcus epidermidis* and *Pseudomonas fluorescens.* The survival of the nematode *C. elegans* was lower in the presence of chia seed extract than that of the control. This suggests that the extract possesses cytotoxic activity against *C. elegans*.

A limitation was that the different batches of chia seed extracts prepared may have contained different levels of antibacterial, antioxidant and cytotoxic substances. Moreover, cancer cell lines were not available to test the cytotoxic activity of chia seed extract.

As an application and further work, the bioactive compounds in chia seeds can be extracted to be used in antibacterial solution or cream for wound dressing and disinfection. In addition, the antioxidant effects and antibacterial effects of chia seeds suggests its application as natural preservatives for food products and to extend shelf life of food products. The optimal method of chia seed extraction and extract storage can also be investigated in future projects to maximise the yield from the extraction process.

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