WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 17, 1534-1553. Research Article ISSN 2277– 7105

ORGANIC GREEN LEAFY VEGETABLES IN LOCAL MARKETS- ARE WE BENEFITTING FROM IT; A COMPARATIVE ANALYSIS OF THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF ORGANIC AND INORGANIC LEAFY GREEN VEGETABLES

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Article Received on
20 August 2018,

Revised on 07 Sept. 2018,
Accepted on 30 Sept. 2018

DOI: 10.20959/wjpr201817-13498

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ABSTRACT

Today, more than ever before, organic food is in demand as people are more aware of their beneficial properties, including the antioxidant activity and antimicrobial activity. Organic food is known to have more phytochemical compounds which are known to have antioxidant properties. The following study aims to evaluate the antioxidant activity and antimicrobial capacity of several organic and inorganic green leafy vegetables which are sold in local markets within Dehiwala, which is located in the suburb of Colombo, Sri Lanka. Five organic and inorganic vegetables were chosen for this study: centella (*Centella asiatica*), drumstick leaves (*Moringa olifera*), dwarfcopper leaf (*Alternanthera sessilis*), leeks (*Allium ampeloprasum L*) and water spinach (*Ipomoea aquatica*). To find out the phenolic, flavonoid, and antioxidant capacity of the vegetable samples, the total phenolic content, total flavonoid content, total antioxidant capacity, ferric ion reducing antioxidant power (FRAP) assay, and ABTS assay were performed. The results clearly depicted that there were no significant correlation between the phenolic and antioxidant content between organic and inorganic vegetables. However, there was a significant outcome with regards to the antioxidant content in both the organic and inorganic vegetables. The antimicrobial activity was determined by the well diffusion method which portrayed the *Staphylococcus aureus* bacterial inhibition of organic and inorganic vegetables.

KEYWORDS: Antioxidant, Antimicrobial, Organic, Inorganic, leafy green vegetables.

INTRODUCTION

Organic food is widely consumed by people who have become more health conscious of their daily intake of nutrition and are more aware of the beneficial properties of food. Green leafy vegetables contribute greatly to a healthy human diet. Subsequently, they have also played a major role in medicine since ancient times. They are a rich source of plant secondary metabolites like phytochemical compounds such as vitamin C, phenolics, flavonoids and minerals such as calcium, iron and phosphorus.[1-3]

Plant nutrition is a vital factor in organic foods, and the key elements essential for plant nutrition is preserved by soil. This acts as a natural reservoir for the essential elements that alters the composition of chemicals within the plant. For the healthy growth of plants and to obtain an optimal yield, nutrition must be available to plants in the correct quantity and proportion. Since consumer demand for high quality plant foods such as crops, fruits and vegetables keep on increasing in commercial markets, farmers have started using chemical fertilisers and organic manures to gain a higher yield in plants.[4] For this purpose, organic fertilisers are often considered functional as they are made up of animal or vegetable derived fertilisers including manure, slurry worm castings, peat and seaweed.[5] Also, recent studies have shown that the use of fertilisers influence the phyto-nutritional quality of crops. It further states that the application of inorganic fertilisers on plants reduce their antioxidant content.[4]

Plant growth is largely dependent on nitrogen, which is obtained from soil in the form of inorganic nitrogen compounds (NH_4^+, NO_2^- and NO_3^-).[6] However, this accounts for less than 5% of the total nitrogen in soil, thus justifying the use of fertilisers which increases the nitrogen, phosphorus, potassium, calcium and magnesium content in soil.[4,6] Once inorganic fertilisers are applied, a process known as mineralization occurs, releasing inorganic nitrogen into the soil, which is then taken up by plants. This increases the nitrate content present in the soil, and thereby increases the nitrate content in plants. Interestingly, green leafy vegetables are known to have the highest nitrate content in comparison to other plants.[6] When plants take up nitrogen, either as inorganic or organic forms, it increases the quantity of the fruits and leaves of plants. Accordingly, based on the carbon/nutrient (C/N) balance hypothesis, when nitrogen is readily available in plants they will primarily produce compounds with a high nitrogen content, such as proteins for growth.[4] On the other hand, when nitrogen is limited, plant metabolism changes more towards carbon that contains compounds such as

cellulose, starch, and non-nitrogen containing secondary metabolites such as phenolics.[4] This indicates that the difference in C/N ratios between organic and inorganic plants could lead to a difference in production of phenolics and other secondary metabolites.

Phenolic compounds are one of the most important groups of plant secondary metabolites, due to their association with morphological development, physiological processes and reproduction.[6,7] They are synthesized through the pentose phosphate, shikimate and phenylpropanoid pathways.[7] These compounds have a wide range of biological functions, including antimicrobial and antioxidant activity, which can be helpful in the management of oxidative stress and age related human pathologies.[3,8,9,10] The broad spectrum of biological activities of phenolics are due to their molecular structure, which is made up of one core phenol ring, in which the hydrogen is commonly replaced by a more active residue such as hydroxyl acetyl or methyl.[7] The pattern and degree of these substitutes are responsible for the variable degree of biological properties. Moreover, plants contain more phenolic rings, and are called polyphenols. Polyphenols are a family of hundreds of natural antioxidant compounds, which are made up of two subclasses; flavonoid and non-flavonoid compounds.[7] Flavonoid compounds are based on two aromatic rings connected by a bridge consisting of three carbons (C_6-C_3-C_6) and are divided into six subclasses: flavones, isoflavones, flavanones, flavonols, flavan-3-ols and anthocyanins.[7,8] On the other hand, non-flavonoid metabolites are classified into the subgroups: phenolic acids (hydroxybenzoates C6-C1, hydroxycinnamates C6-C3), lignans (C6-C3), and stilbenes (C6-C2-C6).[7,8] They are also accompanied by tannins and lignins, which only occur as complicated biopolymers; hence lack a defined carbon base.[7]

It should be noted that one of the most important property of plant phytochemicals is their antioxidant activity. Oxygen is one of the most important elements essential for all living organisms, but free radicals or reactive oxygen species (ROS) are extremely toxic and mutagenic.[8] These are generated as a result of various metabolic processes and other activities taking place in the body.[2,11] The excessive production of ROS can generate oxidative stress.

Oxidative stress caused by excessive ROS trigger damage in cell structures including DNA, lipids and proteins, which in turn could lead to cancer, inflammation, hypertension, cardiovascular diseases, cataract and neurodegenerative diseases.[2,7,8,11] The antioxidant activities of phenols are associated with their annular structure, conjugated double bonds and

the presence of functional groups in the ring.[8] They can inhibit the ROS formation and ROS trapping and interrupt the cascade of free radical reactions in lipid peroxidation and protect other compounds with their antioxidant activity.[2,7,8]

Furthermore, polyphenols are also known to possess potent antibacterial, antiviral and antifungal properties.[7,8,12] Antimicrobial resistance is currently one of the growing medical complications. The study of polyphenols for their antimicrobial activity could greatly help in understanding antimicrobial resistance. Consequently, studies have shown that several strains of gram positive and gram-negative bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella* and *Listeria monocytogenes* were sensitive to many plant polyphenols.[7,8] The polyphenols show a number of microbial virulence factors including, neutralization of bacterial toxins, inhibition of biofilm formation, reduction of host ligands adhesion and showed synergism with antibiotics.[8] Therefore, the study of polyphenols could call for the development of innovative therapies in order to treat microbial infections.

Local sellers tend to market inorganic vegetables as organic to attract consumers thereby misleading the general population. This is a common malpractice found in the local markets in Sri Lanka. This research aims to analyze the antioxidant and antimicrobial activity of both organic and inorganic green leafy vegetables to determine whether the organic products that are sold in the local markets are of genuine content. This will create awareness among consumers to opt for products that are labelled and clarified as organic than to buy from unscrupulous sellers from local markets. For this purpose, the study aims to analyze the total phenolic content, total flavonoid content, total antioxidant content, and total antimicrobial activity of organic plants and inorganic plants grown in Sri Lanka.

Green leafy vegetables were chosen due to their natural high phenolic activity, availability, and high local consumption. Five common organic and inorganic green leafy vegetables found in Sri Lanka chosen were; centella (*Centella asiatica*), drumstick leaves (*Moringa olifera*), dwarfcopper leaf (*Alternanthera sessilis*), leeks (*Allium ampeloprasum* L) and water spinach (*Ipomoea aquatica*). The samples were obtained from local markets in the Dehiwala area within Colombo, Sri Lanka. The total phenolics and total flavonoids were examined by the Folin-Ciocalteu method, and the aluminum chloride spectrophotometric method. The scavenging activity was measured by ABTS and FRAP assay. The antimicrobial activity was

measured against *Staphylococcus aureus* and *Escherichia coli* by well diffusion tests. The relationship between the parameters were also statistically analyzed.

EXPERIMENTAL PROCEDURES

Instrumentation

Analytical balance (SPU 602), fume hood (BIOBASE FH1000), hot air oven (meditry DHA-9053A), micropipettes, UV-visible spectrophotometer (JENWAY 6305), Centrifuge (80-2B), magnetic stirrer, vortex, Heating dry oven, Mechanical grinder, Fridge (LG), vortex (VM-300).

Chemicals and reagents

2, 2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) ($C_{18}H_{18}N_4O_6S_4$), aluminium chloride ($AlCl_3$), ammonium molybdate ($[NH_4]_6Mo_7O_{24}.4H_2O$), ammonium persulphate ($(NH_4)_2S_2O_8$), ascorbic acid ($C_6H_8O_6$), sulphuric acid (H_2SO_4), Folin-Ciocalteu phenol reagent, gallic acid ($C_7H_6O_5$), methanol (CH_3OH), quercetin ($C_{15}H_{10}O_7.2H_2O$), sodium carbonate (Na_2CO_3), sodium sulphate (Na_2SO_4), hydrochloric acid (HCl), TPTZ (2,4,6 tripydyl-s-triazine), glacial acetic acid (CH_3COOH), iron (III) chloride ($FeCl_3$), ammonium molybdate ($(NH_4)_6Mo_7O_{24}$).

Sample Collection and Preparation

Fresh samples of both organic and inorganic centella, drumstick leaves, dwarf copperleaf, leeks and water spinach were purchased from local shops and markets in Dehiwala, a suburban locality in Colombo, Sri Lanka. The leaves were washed thoroughly under running tap water and shade dried for one week in open air and crushed reduced to powder form using a blender. Five grams of the powdered samples were used for extraction.

Bacterial cultures of *Staphylococcus aureus* (ATCC25923-3) and *Escherichia coli* (ATCC25922-3) were obtained from the Medical Faculty, University of Colombo in Sri Lanka. The strains were maintained at 4 °C and activated at 37 °C before any susceptibility tests.

Preparation of leaf extracts

The extraction was done using a modified maceration method. 5 g of the leaf samples were crushed using a mortar and pestle with 5 ml of methanol. 35 ml of methanol was added to this mixture and dissolved in a 300 ml conical flask, sealed with aluminum foil and kept on a

magnetic stirrer at 150 rpm for 2 hours. The supernatant was collected. Another 35 ml of methanol was added into the residue and was placed on the magnetic stirrer at 150 rpm for 2 hours. The second supernatant was collected, and both the supernatants were centrifuged and filtered using the Whatman No.1 filter paper and kept in the dry oven at 40 °C overnight. The dry extract was further dissolved in 50 ml of methanol. For analysis, 5 ml of the extract was dissolved with 45 ml of methanol.

Determination of Antioxidant activity

Determination of total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method as substantiated in Shabbir *et al.*[13] Initially, 50 µL of the sample and 1 ml of deionised water was added to 0.5 ml of 1:10 diluted Folin-Ciocalteu reagent. 3 minutes later, 2.5 ml of sodium carbonate was added. After an hour of incubation in room temperature, the absorbance was measured at 765 nm in triplicates. Gallic acid (5-40 mg/L) was used for calibration of the standard curve. The results were expressed as gram gallic acid equivalent (g GAE)/g dry weight of sample.

Total flavonoid content assay

The total flavonoid content was determined using the assay shown in Shabbir *et al.*[13] Initially, 0.3 ml of extract was mixed with 3.4 ml of 30% methanol, 150 µL sodium nitrite, 150 µL aluminum chloride and 1 ml sodium hydroxide. Absorbance was measured at 506 nm for triplicates. The total flavonoid content was determined using a standard curve with quercetin (10-100 mg/L) as the standard. The results were expressed as gram quercetin acid equivalent (g QAE)/g dry weight of sample.

Total antioxidant capacity assay

The total antioxidant content was determined using phosphomolybdate assay as shown in Shabbir *et al.*[13] Initially, the reagent solution was prepared using 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate, from which 1 ml was mixed with 3 ml of sample extract. After incubation at 90 °C for 90 minutes, absorbance was measured at 695 nm for triplicates. The total antioxidant content was determined using a standard curve with ascorbic acid (2-14 mg/L) as standard. The results were expressed as gram ascorbic acid equivalent (g AAE)/g dry weight of sample.

Ferric reducing antioxidant power assay

The FRAP assay as described by Shabbir *et al*[13] was followed. The FRAP reagent was prepared by mixing TPTZ (10 mmol/L in HCl; 40 mmol/L), $FeCL_3$ (20 mmol/ L) and acetate buffer (0.3 mmol/L at pH 3.6) in the ratio 1:1:10. The working solution was prepared by mixing 1.5 ml FRAP reagent, 100 µL of distilled water and 100 µL of sample extract. After a 4 minute incubation, the absorbance was measured at 593 nm. The antioxidant activity was determined using a standard curve with ascorbic acid (0-100 mg/L) as standard. The results were expressed as gram ascorbic acid equivalent (g AAE)/g dry weight of sample.

ABTS radical scavenging assay

The ABTS assay described by Shabbir *et al*[13] was followed, albeit with slight modifications. The stock solution included ABTS reagent and ammonium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. The working solution was diluted by mixing 3 ml of ABTS in 97 ml methanol. 150 µL of sample extract was allowed to react with 2850 µL of ABTS in a clean cuvette. Since the reaction was quick, the absorbance was measured every 30 seconds at 734 nm against a methanol blank. The results were expressed as gram ascorbic acid equivalent (g AAE)/g dry weight of sample. The percentage inhibition of ABTS was calculated using the following equation.

Inhibition% = [(control – sample) / control] × 100%

Determination of Antimicrobial activity

Well diffusion test

The antimicrobial activity of the sample extracts was tested against two pathogenic bacterial strains *Escherichia coli and Staphylococcus aureus* using well diffusion. Muller-Hinton agar was prepared and poured into petri plates. Once the agar solidified, three wells were cut on each agar plate after dividing it into four quadrants. One quadrant was kept for the positive control for a gentamicin disc. Another well was used as the negative control for distilled water, and for the remaining two wells samples were added. The plates were incubated at 37 °C in an incubator for 24 hours. The inhibition zones were measured using a ruler.

Statistical analysis

The results were expressed as means ± SD, and the TPC, TFC and TAC were performed in triplicates. The results were submitted to analysis of variance (ANOVA) using Windows Excel. Moreover, the results were tested for correlation using IBM SPSS software.

RESULTS AND DISCUSSION

Over the last few years, the application of organic fertilisers have been preferred over inorganic fertilisers due to their ability to improve the antioxidant properties of plants.[14] The connection between plant secondary metabolites, which contain antioxidant properties, and organic and inorganic farming have been analyzed over the years.[14-16] Aslam *et al* (2013)[16], researched the antioxidant activity of organic and inorganic spinach, and found noticeable differences in the chemical characteristics between the organic and inorganic cultivation of vegetables. They were able to conclude that the antioxidant activity of organic spinach was higher, than their inorganic counterparts. However, they also pointed out that the antioxidant activity increased with the use of fertilisers (organic and inorganic) compared to plants grown without the addition of fertilisers. Supporting this study, a study conducted by Pereira *et al*[15] on several vegetables, showed greater antioxidant activity and phenolic compounds in organic vegetables compared to inorganic vegetables. Moreover, they also discussed how environmental factors, such as climate could alter the chemical composition of the plants. These studies support the carbon-nutrient balance hypothesis, which was formulated to address the differences in concentration of carbon and nutrients among individuals, within a species.[17] The hypothesis states that the imbalance in nutrients and carbon will allow the plants to invest excess resources in what is more available. This suggests that, plants which are grown using inorganic fertilizers, will have a higher concentration of nitrogen and a lower concentration of carbon, which are used to produce secondary metabolites such as phenols. Therefore, inorganic plants theoretically should have lower phenolic content and antioxidant activity compared to organic plants.[16]

Hence, the purpose of this study was to compare the polyphenol content, antioxidant activity and antimicrobial activity of five organic and inorganic leafy vegetables. The selected leafy green vegetables have been analyzed in countless studies for their antioxidant properties, but no study has yet revealed the changes and the effect of the application of organic or inorganic fertilizers on leafy greens. The vegetables chosen in this study were centella (*Centella asiatica*), drumstick leaves (*Moringa olifera*), dwarfcopper leaf (*Alternanthera sessilis*), leeks (*Allium ampeloprasum* L) and water spinach (*Ipomoea aquatica*).

Total Phenolic Content (TPC) assay was performed to measure the total phenolic content in the vegetable samples and was expressed in terms of equivalents of ascorbic acid (AAE). The results of the TPC assay showed no significant difference between organic and inorganic

vegetables. However, the TPC levels of inorganic centella, drumstick leaves and water spinach were the highest among all the vegetables (Figure 1). This was expected as multiple studies done on centella, drumstick leaves and water spinach showed high TPC levels.[18-24] In addition, it was noted that the TPC content of dwarf copperleaf was the lowest. This suggests that the TPC levels of the vegetables vary from one another. This variation could be attributed to the changes in management of chemicals or fertilizers which would have a direct effect on the chemical composition of the plant.[15] Moreover, the chemical composition of the plant could also vary as a result of the method of cultivation, and the weather conditions it is subjected to.[15] Therefore, comparing the TPC levels in plants which were grown at different conditions could give inaccurate results. Furthermore, the Folin-Ciocalteu reagent used in the TPC assay is not specific to all phenolic compounds and react to some phenolic compounds such as ascorbic acid.[25] Therefore, to obtain results of more accuracy, it would have been better to subtract the ascorbic acid content from the TPC level.

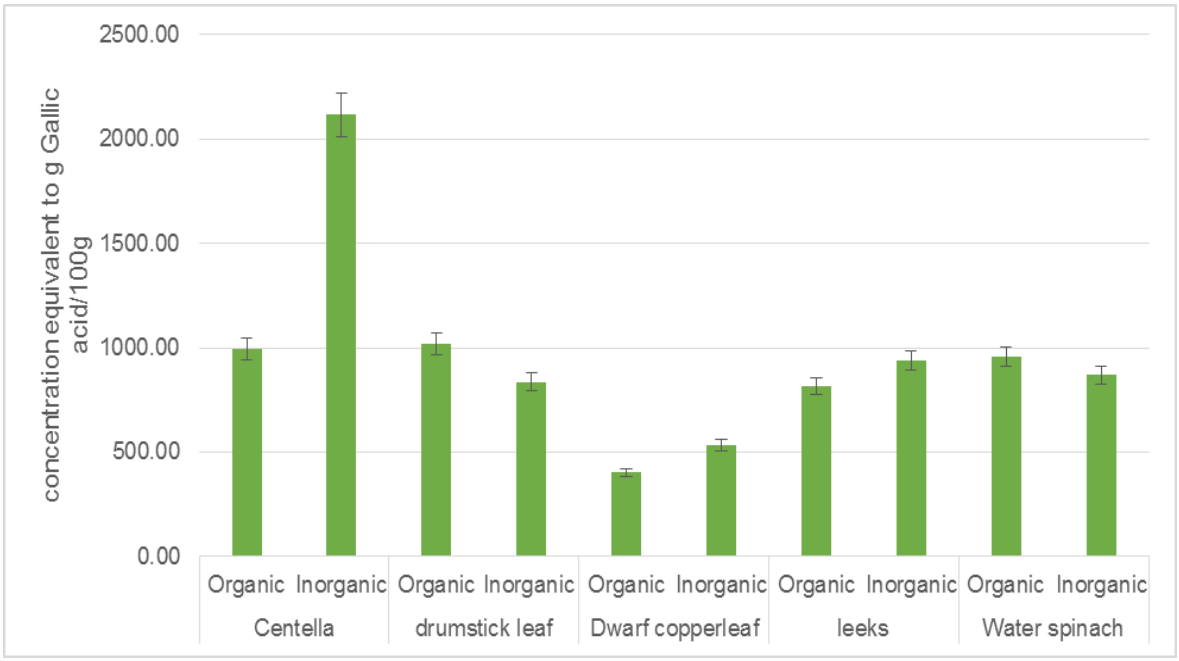

Figure 1: TPC (concentration). Inorganic centella shows the highest phenolic content.

Total Flavonoid Content (TFC) assay was performed to measure the total flavonoid content in the vegetable samples and was expressed in terms of equivalents of quercetin (QAE). The results of the TFC assay showed no significance (p value > 0.05) between TFC levels of organic and inorganic leafy green vegetables (Figure 2). Interestingly, the inorganic centella, drumstick leaves and water spinach extracts showed the highest amount of flavonoid content compared to other organic and inorganic vegetables. The high TFC levels of these samples

were justified due to the fact that the TPC levels of the same samples were high too. The two results were congruent as flavonoids are derived from polyphenols.[25] Similarly, the flavonoid content was lowest in dwarf copperleaf, which also showed lowest TPC levels. Moreover, there was no significance between TFC of organic and inorganic vegetables. Since similar results, of no significance, were observed in the TPC levels, the results were justified. However, TFC levels can be altered due to extraction methods, temperature, climate, geography and the agricultural practices. In addition, environmental factors like sunlight could also affect flavonoid content in plants, as increased amount of light could increase the total flavonoid synthesis.[19,25]

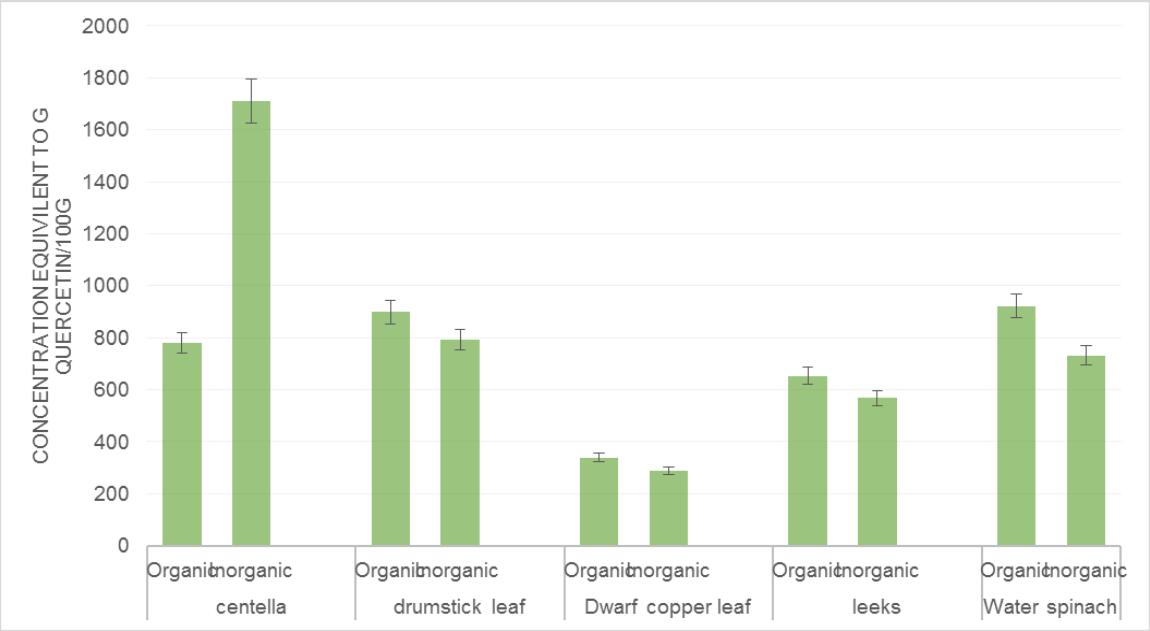

Figure 2: TFC (concentration). Inorganic centella shows the highest TFC content.

The Total Antioxidant Capacity (TAC) was quantified using phosphomolybdate assay, which is the most commonly used assay to determine the TAC of samples.[13, 26, 27] TAC assay was performed to measure the total antioxidant content in the vegetable samples and was expressed in terms of equivalents of ascorbic acid (AAE). The antioxidants (reducing agent) present within the samples reduce molybdate (VI) to molybdate (V), therefore, as the antioxidant capacity increases, more molybdate (VI) are reduced to molybdate (V).[13, 26] The resultant molybdate (V) is a green colour complex which can be spectrophotometrically evaluated at 765 nm. This reaction is usually triggered by the presence of phenols and flavonoids. The highest TAC levels were observed by inorganic centella, drumstick leaves and water spinach, which reflect the TPC and TFC levels. Similarly, organic dwarf

copperleaf and leeks showed the lowest TAC levels (Figure 3). Unlike the TPC and TFC levels, there was a clear significance (P value<0.05 = 0.0483) of TAC levels between the organic and inorganic vegetables. However, unlike most studies, the inorganic leafy green vegetables had higher overall TAC values compared to organic leafy green vegetables. Moreover, there was no significant correlation between TAC and TPC (Figure 4) and between TAC and TFC (Figure 5). Interestingly, a study done by Unal *et al*[25] showed similar results. They evaluated the polyphenol content and antioxidant capacity of several organic and inorganic leafy green vegetables. Their studies showed that all inorganic vegetables had a higher antioxidant activity compared to their organic counterparts.

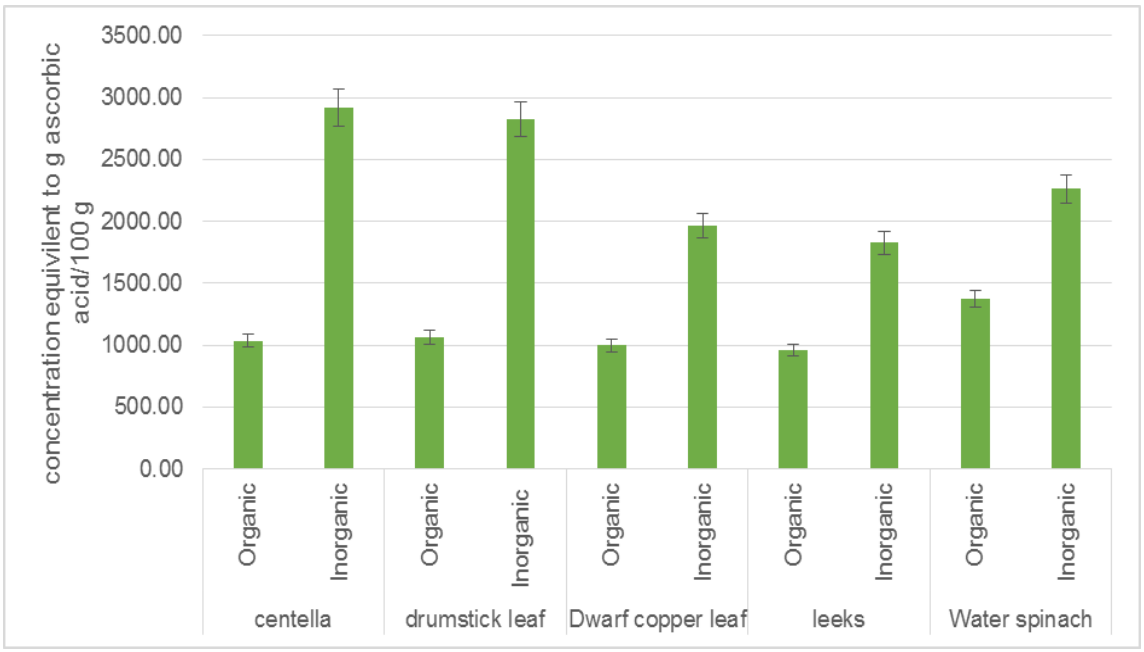

Figure 3: **TAC (concentration). Inorganic centella and water spinach show the highest TAC content, and a clear difference of TAC of organic and inorganic vegetables are seen.**

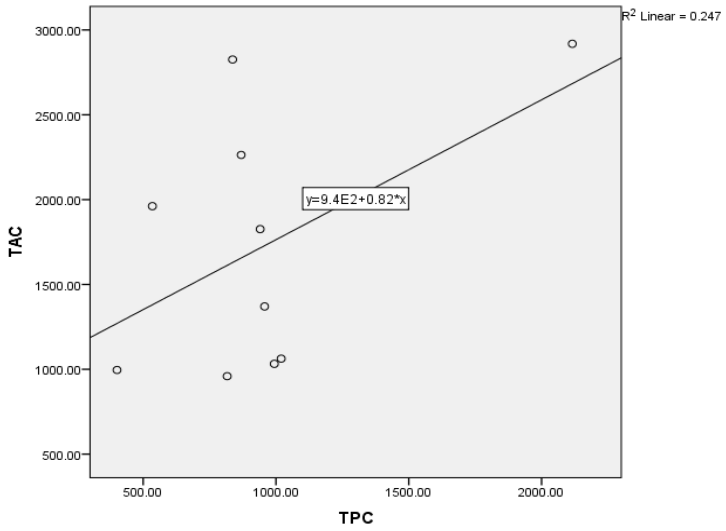

Figure 4: **Graph of correlation between TAC and TPC at 95% confidence interval.**

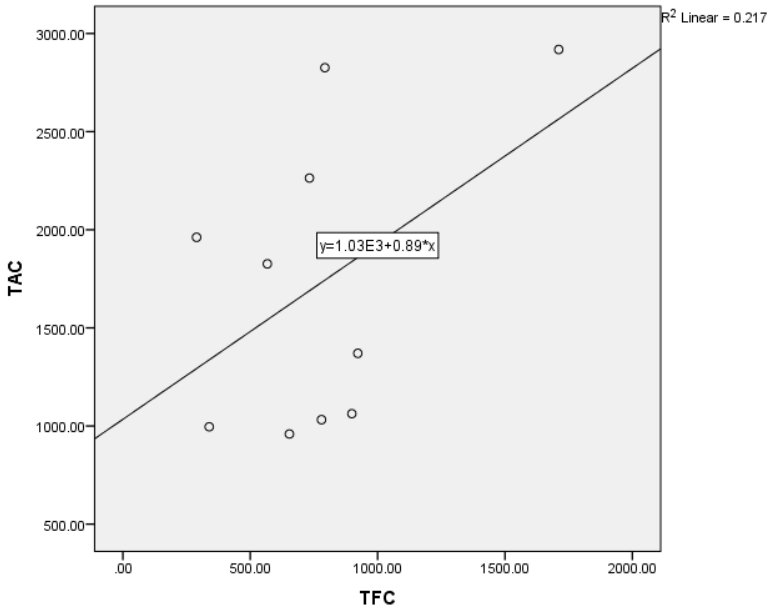

Figure 5: **Graph of correlation between TAC and TFC at 95% confidence interval.**

The ABTS radical scavenging activity assay was performed to analyze the antioxidant capacity of the vegetable extracts and was expressed in terms of equivalents of ascorbic acid. The ABTS radical scavenging activity assay measures the ability of the antioxidant present in the sample extract to scavenge the ABTS generated in aqueous phase, as compared with an ascorbic acid standard.[28] The ABTS is generated by reacting with ammonium persulfate, which is a strong oxidizing agent, with the ABTS salt. After the sample is added, reduction of the dark blue-green ABTS radical solution by the antioxidant, which donates hydrogen, is

measured by its characteristic long wave absorption spectrum (734 nm).[28-29] The scavenging activity is calculated using the formula.

Inhibition% = [(control – sample) / control] × 100%

The results show the increase of scavenging activity over a period of time. The lightening of colour of the ABTS and sample mixture, over time, indicated the rise in hydrogen donating ability of the antioxidants (Figure 6). Corroborating the results from TPC, TFC and TAC, inorganic centella, organic drumstick leaves and organic water spinach showed the highest initial scavenging activity. The shortest time was taken by inorganic drumstick, and the longest time was taken by organic spade leaf. Moreover, organic drumstick had the highest increment of scavenging activity over time. Inorganic drumstick and organic and inorganic water spinach had the highest scavenging activities at 98%, 98% and 97% respectively. The absorbance for all samples were recorded every minute. However, this was not done for the first samples analyzed (organic and inorganic centella) where the absorbance was recorded up to 0.197 and 0.194 respectively. This might have been the reason for their low to highest scavenging activities which were at 82% and 83% respectively.

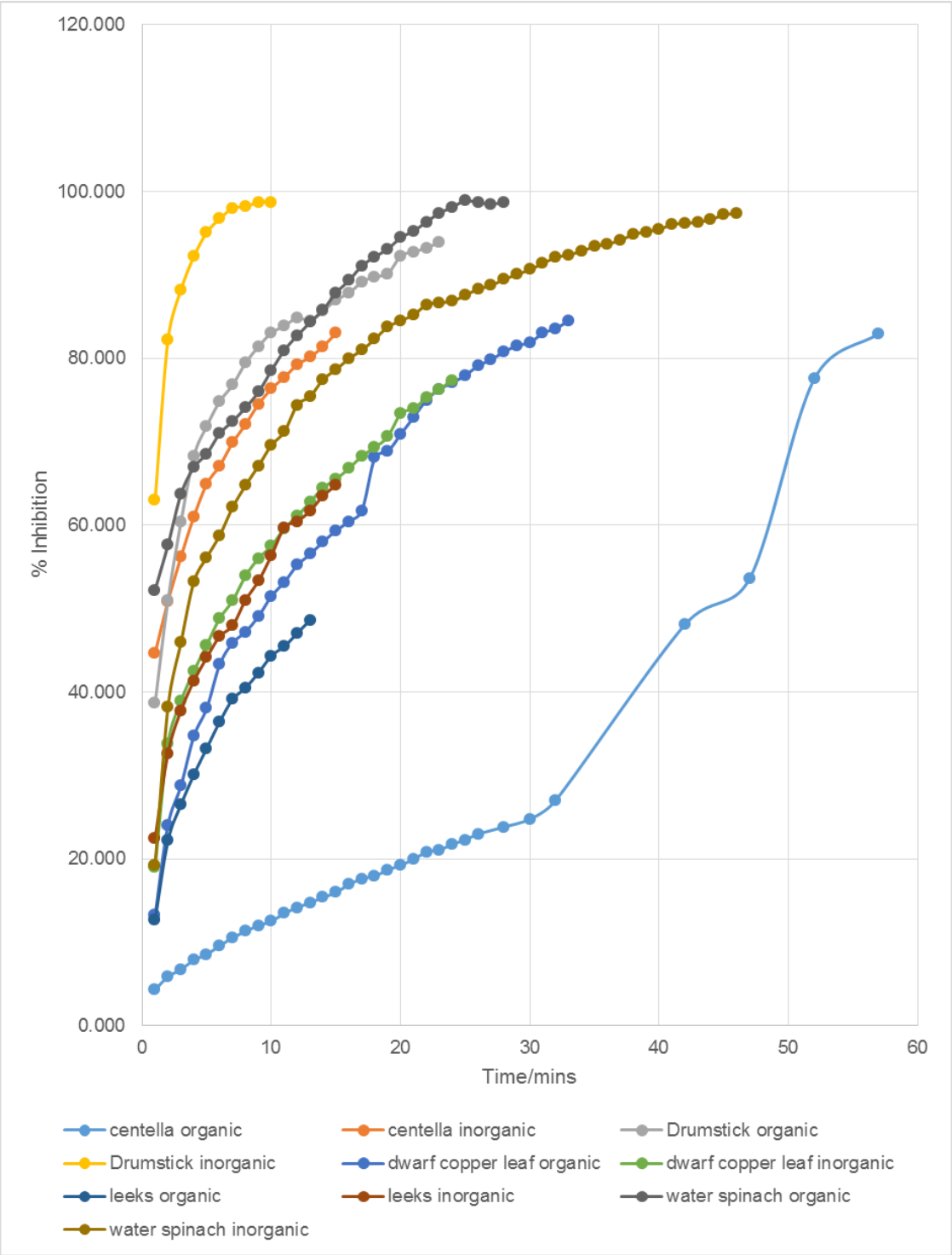

Figure 6: **Graph of ABTS- % inhibition vs Time.**

Ferric reducing-antioxidant power (FRAP) assay was performed to analyze the antioxidant capacity of the organic and inorganic vegetable extracts. FRAP is based on the reduction of the 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) and ferric iron complex to the ferrous form at low pH levels. The reduction was observed by monitoring the change in absorption in the sample at 593 nm using a UV spectrophotometer, as compared

with an ascorbic acid standard (Figure 7). Inorganic water spinach showed the highest initial reading at 2295.455 g AAE/100g of dry sample, which went on to increase up to a peak value of 4545.455 g AAE/100g of dry sample. This was followed by inorganic centella which showed an initial reading of 2038.961 g AAE/100g of dry sample and a peak value of 2709.299 g AAE/100g of dry sample. Moreover, organic water spinach had a reading of 1836.777 g AAE/100g of dry sample and a peak value of 3787.190 g AAE/100g of dry sample. Contradicting to the ABTS which showed a high reading for organic drumstick leaves, they had an initial reading of 400.606 g AAE/100g of dry sample and a peak reading of 764.242 g AAE/100g of dry sample. Moreover, organic drumstick took the longest time to reach the peak value.

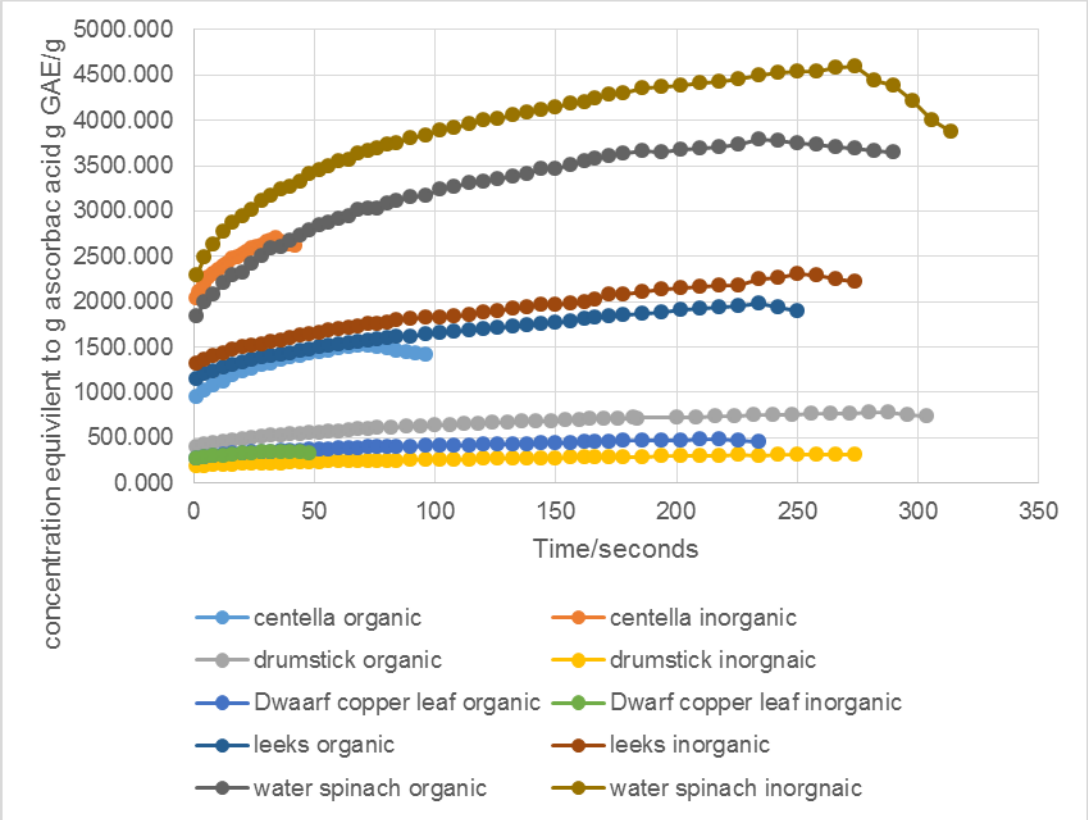

Figure 7: **Graph of FRAP- Concentration vs Time.**

While a number of studies support the association between consumption of vegetables and low incidence of chronic diseases, they also emphasize on the relationship between vegetables and their inhibitory effects on microorganisms.[30-34] Though the antimicrobial effects of plants is a commonly studied subject, not many studies have been conducted on the application of organic and inorganic fertilizers and its the concurrent effect on the antimicrobial activity of plants. In the present study, the antimicrobial activity was analyzed

using well diffusion method. Two strains of bacteria, gram positive *Staphylococcus aureus* and gram negative *Escherichia coli*, were used. There was a significant correlation between the inhibitory effects of organic and inorganic leafy green vegetables against *Staphylococcus aureus*, ($p < 0.05$) (Figure 8).

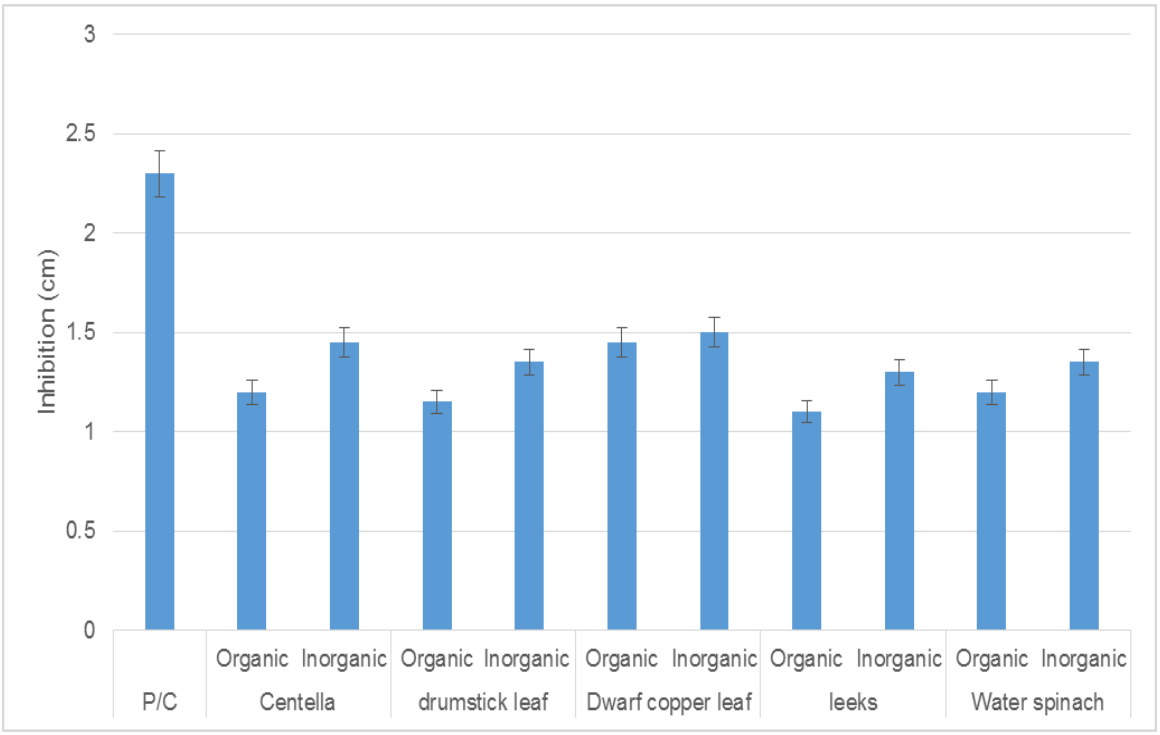

Figure 8: **Bar chart for antimicrobial activity against S.A. Positive control is given as P/C.**

Moreover, the highest inhibitory effects were observed by organic and inorganic dwarf copperleaf, which had inhibitory zones of 1.45 cm and 1.5 cm respectively. This was surprising because dwarf copperleaf showed the lowest phenolic content, flavonoid content and antioxidant capacity compared to the other samples. A possible reason for this might be that dwarf copperleaf contains more phytochemical compounds that can inhibit bacterial growth, compared to other vegetables analyzed during this present study. Indeed, other factors such as extraction methods and solvents also play a role. Therefore, to better comprehend the antimicrobial activity of green vegetables, the sample extracts should be subjected to different solvents during extraction. Similar results were obtained by other studies performed on dwarf copperleaf.[35-37] The second highest inhibitory effect for *Staphylococcus aureus* was observed by inorganic centella. This was plausible because inorganic centella expressed high TPC and TFC values. Similar studies analysing the antimicrobial activity of centella also showed inhibition of *Staphylococcus aureus* against the

plant.[38-41] On the other hand, the antimicrobial activity of the organic and inorganic vegetables against *Escherichia coli* did not show any correlation ($p > 0.05$) (Figure 9). However, similar inhibitory trends of the vegetables were observed against both *Staphylococcus aureus* and *Escherichia coli* as inorganic centella, organic dwarf copperleaf and inorganic dwarf copperleaf showed high antimicrobial activity.

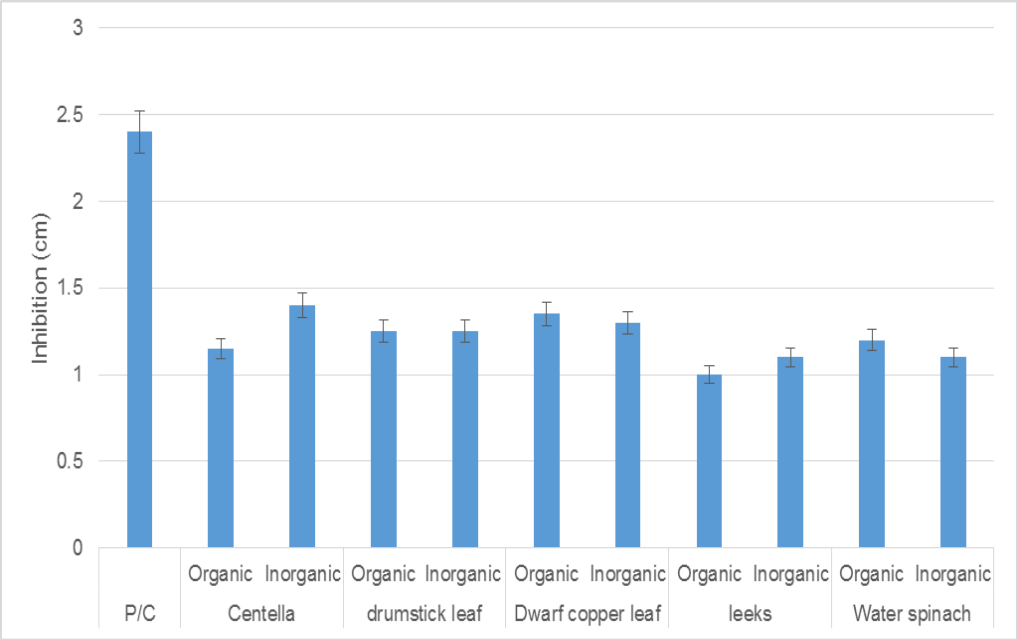

Figure 9: **Bar chart for antimicrobial activity against E.C. Positive control is given as P/C.**

CONCLUSION

In conclusion, differences were observed in the phytochemical composition, antioxidant capacity and antimicrobial activity of the selected organic and inorganic vegetables. Research conducted on similar subjects mostly conclude on the fact that organic green leafy vegetables express a higher antioxidant activity compared to inorganic green leafy vegetables. This conclusion is contrasting to the present study which clearly depicted that the inorganic vegetables had a higher antioxidant activity. Since external and environmental factors could have affected the phytochemical composition of the vegetables, further studies are required with controlled samples at different locations which would be vital to explain certain conclusions. On the other hand, since organic farming is still a developing agricultural procedure, and is expansive, the reliability of the organic vegetables available in Sri Lanka is questionable. Therefore, a possible solution is to educate consumers and agricultural

practitioners regarding the advantages and disadvantages of organic and inorganic food products.

ACKNOWLEDGMENT

Authors would like to thank BMS for financial support.

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