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Antioxidant Study of Garlic and Red Onion: A Comparative Study

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ABSTRACT

Garlic (*Allium sativum* L.) and red onion (*Allium cepa* L.) are among the most common ingredients in Malaysian cuisines. These two *Allium* species are believed to possess medicinal properties including antioxidants. Accordingly, the aim of this study was to compare antioxidant level and activities (i.e. at primary and secondary levels) in both the *Allium* species collected from markets around Kuantan, Pahang Darul Makmur, Malaysia. Current results of total phenolic content (TPC) assay indicate that TPC is higher in red onion (i.e. 53.43 ± 1.72 mg GAE/100g) as compared to garlic (i.e. 37.60 ± 2.31 mg GAE/100g). In addition, EC_{50} value of garlic is lower than that of the red onion, showing a higher free radical scavenging activity in garlic than in red onion. However, the primary antioxidant activities of both the samples are lower than the standard antioxidant, BHA. Therefore, there is a poor relationship between the TPCs and the primary antioxidant activities, indicating that the primary antioxidant activities of both the *Allium* species are not solely due to the phenolic compounds. For secondary antioxidant activity, FIC assay shows that at the highest sample concentration of 1.0 mg/mL**,** red onion has higher ferrous ion chelating effect (i.e. 45.00 ± 1.73%) as compared to garlic (i.e. 43.29 ± 3.89%). Furthermore, both the *Allium* samples show slightly higher ion chelating effect than BHA (i.e. 43.14 ± 1.07%) but lower than EDTA (i.e. 97.9 ± 0.07%). Overall, the findings of the present study show a negative relationship between the results of TPC assay, DPPH radical scavenging activity assay, and FIC assay. To strengthen the validity of the present results and to further assess the potential of both the *Allium* species as natural antioxidant sources, more different assays need to be considered for future work.

Keywords: *Allium cepa* L., *Allium sativum* L., total phenolic content, antioxidant activity

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INTRODUCTION

Modern consumers are becoming more health conscious and more aware of food nutritional value. Among the nutrients, antioxidants are popular due to their ability to prevent many physiological diseases or illnesses. Antioxidant is defined as any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate (Li *et al*., 2007). Antioxidants are believed to play a very important role in the body defence system against reactive oxygen species (ROS) or free radicals, which are harmful by-products generated during aerobic activity of normal cells. Increasing the intake of dietary antioxidant is believed to assist in maintaining an adequate antioxidant status and therefore, the normal physiological function of living system. According to Tepe *et al*. (2005), antioxidants have great importance in terms of preventing oxidative stress that may cause several generative diseases. Many fruits and vegetables are potentials for decreasing risk effect of several chronic diseases, such as cancer, coronary heart disease and many more.

The *Allium* family has over 700 members; each with different tastes, forms and colours; nonetheless, they are close in biochemical, phytochemical, and neutraceutical contents (Tepe *et al*., 2005). Red onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) are among the important parts of diet in many world populations, and there is also a long-held belief in their health enhancing properties. Among the oldest cultivated plants, garlic and red onion are used as food and for medicinal application as they have been proven to convey many benefits to human due to their long storage and portability. One of the advantages of these *Allium* species is that they could be dried and preserved for several months. Garlic, for instance, has been applied as culinary spice and medicinal herb, and it is an important constituent of the traditional Chinese medicine. On the other hand, onions (including red onions) are native to Eurasia but now grow all over the world. The bulb of onion is used medicinally and onion has been consumed as food for many centuries. In Malaysia, these two *Allium* species are widely used and they are becoming very important components in the preparation of almost all Malaysian cuisines and delicacies.

According to Benkeblia (2005), *Allium* species are revered to possess anti-bacterial and anti-fungal activities, and they contain the powerful antioxidants, sulphur and other numerous phenolic compounds which have aroused great interests for food industries. During the last 20 years, *Allium* spices have been among the most studied vegetables and aroused great interest. In previous studies, garlic is found to exhibit antioxidants activity (Tepe *et al*., 2005) and this fact is set as the foundation for possibilities on the presence of antioxidant activities in other *Allium* species. Apart from that, according to Li *et al*. (2006), synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) need to be replaced with natural antioxidants as several studies showed that a number of synthetic antioxidants were toxic and carcinogenic in animals. Consumers are quite sceptical on the production of any synthetic antioxidant products, and there has always been a good public acceptance when it comes to natural antioxidants. For that reason, the aim of this project was to compare the antioxidant level and activities (i.e. at primary and secondary levels) in garlic and red onion that are available in Malaysian markets in Kuantan, Pahang Darul Makmur.

MATERIALS AND METHODS

Sample Collection

Samples of garlic (*A. sativum* L.) and red onion (*A. cepa* L.) were purchased from the local markets and supermarkets in Kuantan, Pahang Darul Makmur, Malaysia. The samples were randomly selected off the shelves based on their freshness.

Sample Preparation and Extraction

The samples were cut into smaller pieces to ease the drying process. Following the suggestion by Khamsah *et al*. (2006), the drying process was done in a warm room at 45°C (not exceeding 50°C) until all the moisture was gone. However, the findings of our previous antioxidant studies suggest no significant detrimental effects on the total phenolic compounds when drying the samples at 60°C and 70°C (Norshazila *et al*., 2010; Nurliyana *et al*., 2010). Extraction was done using the Soxhlet method (Siddhuraju *et al*., 2002). The samples were weighed at 100 g and inserted into an extraction tube of Soxhlet apparatus. The extracting solvent, i.e. 70% ethanol, was then added into the round flask. The Soxhlet apparatus was then assembled, the heat was set at 60°C and left running for 12 hours. After the extraction process, the samples in the round flask were subjected to rotary evaporation to remove the extracting solvent from the extracts. Finally, the extracts were subjected to freeze drying to remove water from the extracts. The extracts were kept in the dark at 4°C until further uses.

Total Phenolic Content (TPC) Assay

TPC was determined by using Folin Ciocalteu's reagent (Lim *et al*., 2006). 0.3 mL of the extract was introduced into the test tubes, followed by 1.5 mL of Folin Ciocalteu's reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min in the dark. The absorption of the samples was taken at 765 nm using Perkin Elmer Lambda 25 UV/Vis spectrophotometer. The TPCs were expressed in gallic acid equivalents (GAE). The gallic acid calibration line has the equation of $y = 9.2402x + 0.0149$ ($R^2 = 0.9971$), where y is the absorbance at 765 nm and x is the concentration of phenolic compounds in mg/g of the sample (the graph is not shown).

1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The free radical scavenging activity of each sample was measured using Perkin Elmer Lambda 25 UV/Vis spectrophotometer, based on the decrease absorbance of ethanolic DPPH solution at 517 nm (Lim *et al*., 2006). Different dilution extracts (0.2 – 1.0 mg/mL), amounting to 1.0 mL, were added to 2.0 mL of the DPPH solution. The samples were then vortexed to thoroughly mix it. The samples were then left to stand in the dark for 30 min. The absorbance readings of the samples were taken at 528 nm using Perkin Elmer Lambda 25 UV/Vis spectrophotometer. Synthetic antioxidant, butylated hydroxyanisole (BHA), was used as a positive control for this assay. The antioxidant activity was expressed as:

$$\% \text{ disappearance} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

$A_{control}$ = Absorbance reading of the control
A_{sample} = Absorbance reading of the sample

$EC_{50,}$ effective concentration of the extract required for 50% scavenging of DPPH· radicals were calculated from the plotted graph of scavenging activity against sample concentration.

Ferrous Ion Chelating (FIC) Assay

Chelating effects of the samples were measured using the FIC assay. Serial dilutions of the samples were prepared (0.02 mg/mL - 0.1 mg/mL). Next, 50 µL of Ferum chloride ($FeCl_2$, 2 mM) and 1.65 mL of 70% ethanol were added to 500 µL of the sample. The samples were vortexed to mix it thoroughly and were left to stand for 5 min in the dark. After that, 100 µL of ferrozine (5 mM, dissolved in 70% ethanol) was added, and subjected to vortex to mix the samples thoroughly. The samples were once again left to stand in the dark for another 5 min. Finally, the absorbance readings of the samples were measured at 562 nm using Perkin Elmer

Lambda 25 UV/Vis spectrophotometer. Both ethylenediaminetetraacetic acid (EDTA) and BHA were used as the controls. The ability of each sample to chelate ferrous ion was calculated relative to the control consisting of only iron ferrozine, using the following formula:

$$\text{Chelating effect \%} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ = Absorbance reading of the control
A_{sample} = Absorbance reading of the sample

Statistical Analysis

All the samples and readings were prepared and measured in triplicate. The results were presented in mean ± standard deviation. As for the data and graphs, they were subjected to analyses using Microsoft® Office Excel 2003.

RESULTS AND DISCUSSION

TPC Assay

Phenolic compounds are the major group contributing to the antioxidant activity of vegetables, fruit, cereals and other plant-based materials. The antioxidant activity of the compounds is partly due to one electron reduction potential that is the ability to act as hydrogen or electron donors (Chan *et al*., 2007). Atoui *et al*. (2005) mention that the antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Determination of these compounds is usually performed by reacting phenolic compounds with Folin-Ciocalteu's reagent. The Folin-Ciocalteu reagent, Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic antioxidants and polyphenol antioxidants. Upon this reaction, the two classes of compounds will form a complex known as the phosphomolybdic-phosphotungstic-phenol complex which triggers the formation of a blue colour solution. According to Ajila *et al*. (2007), the more intense the formation of blue colour indicates a higher phenolic content inside the samples. The present study shows that red onion possessed higher TPC (i.e. 53.43 ±1.72 mg GAE/100 g) compared to garlic (i.e. 37.60 ±2.31 mg GAE/100 g), whereby red onion exerted an intense blue solution than the sample solution of garlic (*Fig. 1*). However, Benkeblia (2005) found out that the methanolic extract of garlic (*A. sativum* L. var. Cristo) shows higher TPC than the methanolic extract of red onion (*A. cepa* var. Rouge Amposta), and the difference could be due to the different types of species variants and extracting solvents used in both the studies.

DPPH Assay

DPPH assay is a primary antioxidant activity test that determines the free radical scavenging activity of the respective samples. Primary antioxidant involves the mechanism, whereby

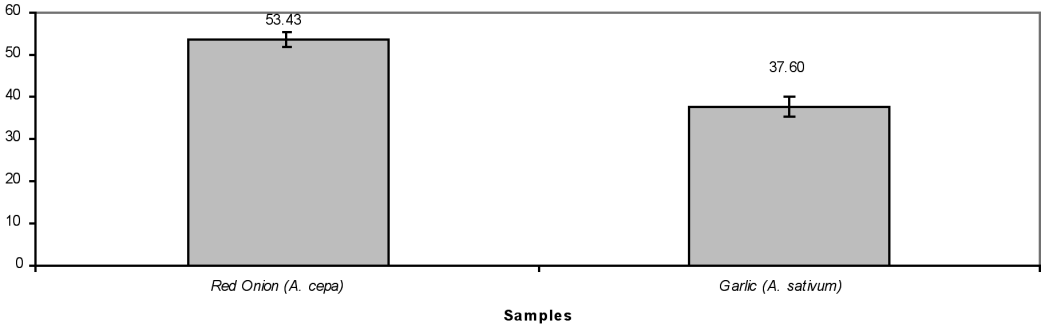

Fig. 1: Level of the total phenolic content in each sample. The results were expressed as gallic acid equivalents (GAE)

it inhibits the oxidation reaction by combining it with the free radicals or reacting hydrogen peroxides. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, it gives rise to the reduced form of the DPPH compound, leading to the reduction of the violet colour.

In the present study, the mechanism of the radical scavenging activity was observed based on the reducing purple colour of DPPH solution. *Fig. 2* shows that free radical scavenging activities of both garlic and red onion were lower than the positive control, BHA; indicating their weak free radical scavenging activities. In terms of IC_{50}, the lowest value is shown by the positive control, BHA (0.16 ±0.01 mg/mL), followed by garlic (0.95 ±0.01 mg/mL) and red onion. However, IC_{50} for red onion could not be directly determined from the graph due to the low percentage of the radical scavenging activities over the measured extract concentrations. Nonetheless, *Fig. 2* clearly suggests that their IC_{50} could be more than 1.0 mg/mL. The results clearly show that garlic has more capability to scavenge the free radicals as compared to red onion, although the primary antioxidant activities of both samples are lower than the standard antioxidant, BHA. Likewise, the findings from Benkeblia (2005), garlic shows higher free radical scavenging activity than red onion over the increasing sample concentrations.

Similar research conducted in other plants and fruit have shown that high radical scavenging activities are usually associated with high TPC. For instance, Lim *et al*. (2006) described that high radical scavenging activity was contributed by the presence of high phenolic content in guava extracts. There are several other studies that share similar results on the contribution of phenolic compounds to the high radical scavenging activity. However, the present study does not support the findings. Therefore, it is suggested that apart from phenolic compounds, there could be other organic compounds contributing to the high radical scavenging effect in garlic, even though its TPC is lower than red onion. Khamsah *et al*. (2006) suggested that free radical scavenging activity is not due to the phenolics only because they found that the antioxidant activity of methanol extract of *Orthosiphon stamineus* was not solely caused by phenolic compounds. IC_{50} data further support that garlic has higher radical scavenging activity (i.e. IC_{50} of garlic= 0.95 mg/mL) than red onion, but the IC_{50} of red onion could not be determined directly from *Fig. 2* due to the low activity over the increasing concentrations. On the contrary, *Fig. 2* clearly suggests that its IC_{50} could be more than 1.0 mg/mL. IC_{50} of both the *Allium* samples are higher than BHA (i.e. IC_{50} of BHA= 0.16 mg/mL), showing their low and weak free radical scavenging activities.

FIC Assay

FIC assay is a common test used to determine the secondary antioxidant activity by observing the reducing purple colour of the reaction solution. The assay mechanism is based on the decrease in the absorbance of iron (II)-ferrozine complex. Meanwhile, secondary antioxidants are also known as the peroxide decomposers, where it inhibits polypropylene oxidation by decomposing hydroperoxide. Secondary antioxidants are responsible for suppressing the formation of radicals and protecting against oxidative damage (Lim *et al*., 2006). Iron-ferrozine complex has the maximum absorbance at 562 nm and large decrease in absorbance indicates strong chelating power. By forming a stable iron (II) chelate, an extract with a high chelating power reduces free ferrous ion concentration, which leads to decrease the extent of Fenton reaction that are implicated in many diseases (Lim *et al*., 2006). The assay determines the effectiveness of the chemical compound in the sample extract in competing ferrous ion with ferrozine.

Iron is known to generate free radicals through the Fenton and Haber-Weiss reaction. Fenton Weiss reaction is a reaction between ferrous ion and hydrogen peroxide which produces highly reactive hydroxyl radicals implicated in many diseases (Llyod *et al*., 1997). Metal ion-chelating activity of an antioxidant molecule prevents oxy-radical generation and

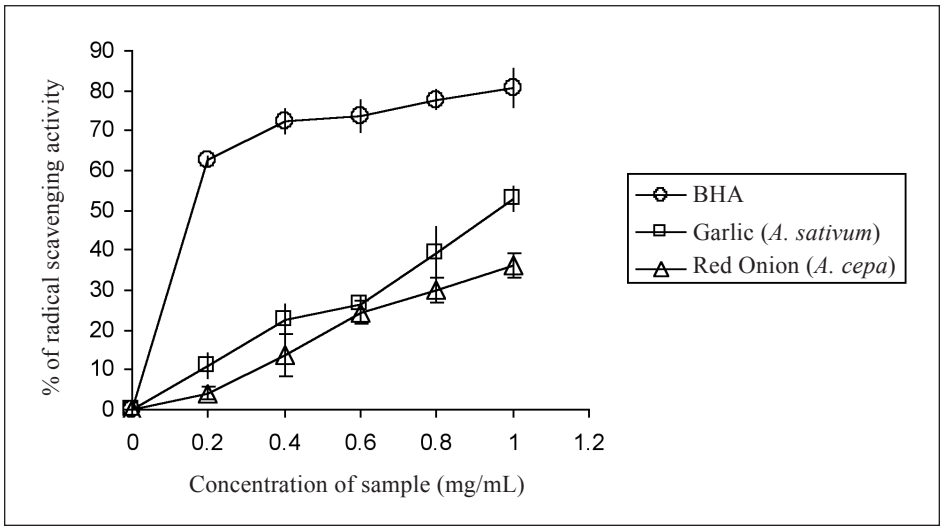

Fig. 2: Comparison of the free radical scavenging activity between the positive control - BHA and samples. IC_{50} value (in mg/mL) for each sample was derived from the graph at 50% free radical scavenging activity

the consequent oxidative damage (Kumar *et al*., 2008). Metal ion-chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the catalysing transition metal in lipid oxidation.

Fig. 3 shows that BHA, garlic and red onion have not much difference in the chelating effects, whereby all of them reached up to 43.14 ± 1.07%, 43.29 ± 3.89%, and 45.00 ± 1.73% at the highest sample concentration of 1.0 mg/mL, respectively. The results also indicate that the ferrous ion chelating effects of red onion are higher than BHA over the increasing concentrations. Furthermore, among the two *Allium* species, the chelating activity of red onion is slightly higher that that of garlic. However, EDTA which serves as the positive control shows the highest percentage of the chelating effect (97.9 ±0.07%). Besides, red onion can be considered as moderate metal chelator since its activities are approximately two times lesser than EDTA. Overall, the results suggest that both *Allium* species may be regarded incapable of strongly obstructing the generation of •OH radicals from Fenton reaction (Kosem *et al*., 2007).

The Relationship between the Results of TPC, DPPH and FIC Assays

There are positive relationships between TPC assay and DPPH radical scavenging activity assay, based on the findings of several previous studies (e.g. Ordoňez *et al*., 2005; Luther *et al*., 2007; Silva *et al*., 2007; Tawaha *et al*., 2007). Most of the researches have mentioned that high phenolic content will lead to high radical scavenging activity. Nonetheless, the present study on garlic and red onion shows a negative relationship between results of TPC assay and DPPH radical scavenging activity assay. Garlic is proven to be better radical scavenger as compared to red onion even though it expresses lower phenolic content. As for FIC assay, most of the previous findings have discovered that even though certain samples possess potent radical scavenging activity, the samples either possess moderate or weak ion chelating activity. For instance, a study conducted by Lim *et al*. (2006) on guava has shown that the samples of guava possess potent radical scavenging activity but they have weak ion chelating effects. Likewise, the current study revealed that garlic and red onion had weak (i.e. for red onion) to

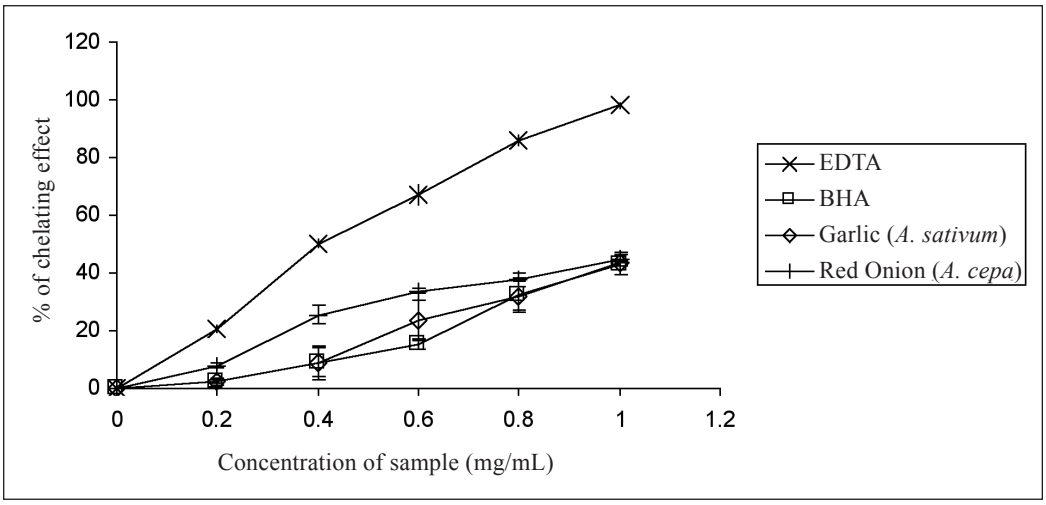

Fig. 3: Comparison of ferrous ion chelating effects between EDTA, BHA and the samples

moderate (i.e. for garlic) ion chelating activities. Overall, the results of the present study do not show good relationship between TPC assay, DPPH radical scavenging activity assay and FIC assay.

CONCLUSIONS

The findings of the current study have shown that red onion (*A. cepa* L.) possesses higher TPC than garlic (*A. sativum* L.*)*. However, garlic has expressed higher free radical scavenging effect (i.e. the primary antioxidant activity) as compared to red onion. As for the ion chelating effect (i.e. the secondary antioxidant activity) measured by the FIC assay, both *Allium* species have been found to have weak (i.e. for red onion) to moderate (i.e. for garlic) ion chelating activities compared to the controls - BHA and EDTA. Overall, the current findings reveal a negative relationship between the results of TPC assay, DPPH radical scavenging activity assay and FIC assay (Table 1). Nonetheless, in order to gain better views on the antioxidant levels and activities in red onion and garlic, further studies on purification, identification and quantification of each phenolic compound and other non-phenolic compounds are necessary in future.

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TABLE 1
Summary of the antioxidant properties for garlic (*Allium sativum* L.) and red onion (*Allium cepa* L.)

Sample	Total phenolic content	Free radical scavenging activity	Metal ion chelating effect
Garlic	37.60 ±2.31 mg GAE/100g	Lower than BHA	Slightly higher than BHA, lower than EDTA
Red onion	53.43 ±1.72 mg GAE/100g	Lower than BHA	Higher than BHA, lower than EDTA

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