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Research article

Determination of antioxidants and nutritional compositions of the flower, leaf and seed of *Moringa oleifera* Lam.

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Abstract: The tropical rainforests contain many tree species that produce edible fruits, seeds, vegetables and medicine. *Moringa oleifera* the tree species is widely cultivated in semi-arid, tropical and sub-tropical areas. The plant may be consumed as vegetable to improve nutrition, taken as medicinal plant to cure ailments and improve health, cultivated as forage for livestock, and used for live fencing. *Moringa oleifera* leaf, flower and seed were subjected under two forms (wet and dry) for antioxidant and dry form for nutritional. The nutritional compositions that were investigated are; moisture content, crude fibre, ash, crude protein and fats. The antioxidants to be investigated were: vitamin C, flavonoid, phenol, DPPH (1, 1-diphenyl picaryl hydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS assay (ABTS, 2, 2′-azinobis (3-ethylbenzothiazoline 6-sulfonic acid). Standard procedures were used in the determination of the nutritional and antioxidant of *M. oleifera* leaf, flower and seeds. The result shows that the seeds nutritional composition is significantly higher in crude fiber (29.35%) and flower nutritional composition is also significantly higher in moisture (6.29%) and ash (13.05%) but the two are low in others respectively. Specifically, based on this result, both the wet and dry samples are good source of antioxidant and they are not detrimental to health.

Keywords: Antioxidants, Nutritional compositions, *Moringa oleifera*, Flowers, Seeds.

INTRODUCTION

The tropical rainforests contain many tree species that produce edible fruits, seeds, vegetables and medicine. These tree species have been noted to have immense socio-economic, nutritional, medicinal and cultural values, especially to rural dwellers (Onyekwelu & Stimm, 2011). According to Onyekwelu *et al.* (2015), tree species serve as alternative food sources, especially at the onset of the farming season when farmers prepare their crops for planting and thus, contribute to food security. Many of the species are valuable sources of nutrition with significant health benefits against malnutrition and possible nutritional benefits conferring enhanced resilience to disease (Barany *et al*., 2001).

Moringa oleifera Lam. is one of the tropical forest tree species of immense socio-economic, nutritional and medicinal importance. It is commonly referred to as "Drumstick tree", which belongs to the family *Moringaceae* and rich in antioxidant compounds (Amaglo *et al*., 2010). The tree species is widely cultivated in semi-arid, tropical and sub-tropical areas. The plant may be consumed as a vegetable to improve nutrition, taken as a medicinal plant to cure ailments and improve health, cultivated as forage for livestock and used for live fencing (Gopalan *et al*., 1989). *M. oleifera* is an important source of antioxidants, tools in nutritional biochemistry that could be beneficial for human health; the leaves, seed and flowers are used by the population with great nutritional importance (Andrea *et al*., 2012).

Uses for the various parts of *M. oleifera* plant have been reported. The powder produced from the seed of *M. oleifera* is used as an effective primary coagulant for water treatment (Pritchard *et al*., 2010). It also possesses the potential to remove cadmium from the aqueous system (Sharma *et al*., 2006). The flower of *M. oleifera* is considered a delicacy in many places; it contains a good amount of both calcium and potassium. It is believed that *M. oleifera* leaves are good sources of vitamins C, calcium, β-carotene, potassium, as well as protein (Fakankun *et al*., 2013). It works as an effective source of natural antioxidant. Due to the presence of several sort of antioxidant compounds such as flavonoid, ascorbic acid, carotenoids and phenolics, *Moringa oleifera* is able to extend the period of food containing fats (Siddhuraju & Becker, 2003).

Antioxidants, which are essential for human health, are nature's way of protecting the body and cells from damaging free radicals (Capasso, 2013; Onyekwelu *et al*., 2015). Free radicals are unstable molecules that are generated from exposure to sun, stress and as part of the natural aging process (Hamid *et al*., 2010). Dietary antioxidants play important roles in controlling oxidative stress (Nikki, 2001). Nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies. In terms of structure and antioxidant function, each nutrient has its distinctive role.

For centuries and in many cultures around the world, different parts of Moringa have been used to treat problems

such as skin infections, anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera and many other illnesses (Khawaja *et al.*, 2010).

Fuglie (2001) reported the acceptance of *Moringa* as a source of nourishment and healthy living especially to the poor. The flower, seed and leaf of *Moringa oleifera* Lam. are essential sources of proteins, vitamins, minerals, calcium, potassium etc. They also exhibit very important nutritional value to nourish small children, pregnant women and nursing mothers as a treatment for malnutrition. The abundance of vitamin A in Moringa can contribute to the treatment of xerophthalmia (night blindness). The composition of the flower, seed and leave is essential in order to ascertain the important roles they play in humans.

Consequently, this study determines the antioxidant and nutritional values of the leaf, flower, and seed of *M. oleifera.*

MATERIALS AND METHODS

Collection and processing of seed, leaf and flower

The seed, leaf and flower of *Moringa oleifera* were collected (plucked from the tree by hand) from Forestry and Wood Technology Plantation situated in the Federal University of Technology Akure, Ondo State, Nigeria. The samples were rinsed with distilled water to remove any attached dirt. They were divided into two; the first part was oven-dried while the second part was kept in the refrigerator. Afterward, both samples were grinded using the electric blender and the quantity to be used were weighed using electronic weighing balance.

Antioxidant analysis procedure for seed, leaf and flower of Moringa oleifera

Determination of total phenolic contents

1.0 ml of extract solution (5 mg ml^{-1}) was added in a 100 ml volumetric flask that contained about 60 ml distilled water. The content was thoroughly mixed with the addition of 5.0 ml of Fiolin-Ciocalteu reagent. After 1-8 mins, 15.0 ml Na_2CO_3 (20%) was added and the volume was made up to 100 ml using distilled water. The mixture was allowed to stand for 2 hours with intermittent shaking. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). An equation obtained from the standard tannic acid calibration graph was used to determine the total phenolic content, which was denoted as mg of tannic acid equivalent (TAE).

Flavonoids determination

About 1 g is dissolved in 10 ml of distilled water and filtered after 24 hours 0.2 ml (200 ul) of the filtrate into test tubes and add 150 ul of 5% $NaNO_2$. After 6 mins, add 0.3 ml (300 ul) 10% $AlCl_3$. Add 1 ml of 1 M NaOH. Then, read the absorbance in a spectrophotometer at 510 nm (yellow colour).

DPPH determination (2, 2-diphenyl-1-picrylhydrazyl) 1,000

0.3 mM solution of DPPH in methanol was prepared. 1 g was dissolved in 10 ml of distilled water and filtered after 24 hours. 500 ul of sample filtrate was added into test tubes. 500 ul of 0.3 mM DPPH was added and incubated for 30 mins at room temperature. 30 mins later, the absorbance was measured at 516 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 18 µg ml^{-1}) was used as standard. The indication of higher free radical scavenging activities was due to the lower absorbance of the reaction mixture. The capability to scavenge the DPPH radical was calculated using equation 1 below:

$$\text{DPPH Scavenged (\%)} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100 \quad \text{..... (Equation 1)}$$

FRAP determination (Ferric Reducing Antioxidant Power)

250 ul of the sample was added to test tubes (distilled water as blank) before adding 250 ul of 0.02 M of Phosphate buffer at pH 6.9. Later, 250 ul of 1% KFeCN was incubated for 20 mins at 50^{o}C 250 ul of 10% TCA before adding 200 ul of 0.1% of $FeCl_3$ (Ferric Chloride) freshly prepared. 1 ml of distilled water was added and the absorbance was read at 700 nm. A standard curve was obtained using Trolox standard solution at various concentrations (0.5, 1.0, 1.5, 2.0 mmol L^{-1}). The absorbance of the samples was compared with that of the Trolox standard and the results were expressed in terms of micromoles Trolox equivalents per kilogram (µmol TE kg^{-1}).

ABTS assay determination (ABTS, 2, 2'-azinobis (3-ethylbenzothiazoline 6-sulfonic acid) 1,000

$ABTS^{+}$ was produced by mixing ABTS stock solution (7 mmol L^{-1} in water) with 2.45 mmol L^{-1} potassium persulfate. The solution was held at room temperature in the dark for 16 hours before use. Once the radical had formed, the absorbance at 734 nm was adjusted to 0.7 nm by dilution with 95% ethanol. 1 ml (1000 ul) of freshly prepared $ABTS^{\bullet+}$ solution was added to 0.2 ml (200 ul) sample. After allowing the reaction mixture to stand for 6 mins at room temperature, the absorbance at 734 nm was immediately recorded. Blank is absolute ethanol.

$$\text{IABTS} = [(AB - AA)/AB] \times 100 \quad \text{..... (Equation 2)}$$

$$\text{ABTS (mM g}^{-1}) = \frac{\%\ \text{sample} \times \text{conc. of standard}}{\%\ \text{standard} \times \text{concentration of sample}} \times \text{MW of standard}$$

Where,

$$\%\ \text{sample} = \frac{\text{Abs. of ref.} \times \text{Abs. of sample}}{\text{Abs. of sample}} \times 100$$

$$\%\ \text{std} = \frac{\text{Asbs. of ref.} \times \text{Abs. of std}}{\text{Abs. of ref}}$$

Vitamin C determination

The vitamin C content was determined using ascorbic acid as the reference compound. 200 µl of the extract was pipetted and mixed with 300 µl of 13.3% of TCA and 75 µl of DNPH. The mixture was incubated at 37ºC for 3 hrs and 500 µl of 65% H_2SO_4 was added before adding 500 ul of water. The absorbance was read at 520 nm. Blank was made up of all reagents without the sample.

Nutritional analysis procedure

The moisture content was determined using oven-dried method (A.O.A.C., 1990). Crude fat was also determined using the method of A.O.A.C. (1990). Pearson (1976) method was used for crude protein determination. The crude fibre was determined according to the method of Joslyn (1990). For ash determination, clean crucibles were ignited at 350ºC for about 15 mins, cooled in a desiccator and weighed. 1 g of each sample was transferred into each of the appropriately labeled crucibles and then re-weighed. Then, the crucibles with their contents were transferred into the muffle furnace at 550ºC for about 5 hrs. After complete ashing, the crucibles were allowed to cool in a desiccator and then re-weighed. The percentage of ash was then determined. The percentage ash was calculated using the equation:

$$\%\ \text{Ash} = \frac{W_2 - W_1}{1g} \times 100 \quad \text{..... (Equation 3)}$$

Where, W_1 = Weight of crucibles, W_2 = Weight of crucibles + sample.

RESULTS

The result from table 1 shows that for Moisture content, Ash, Crude fiber, Fat content and Crude protein; there is significant difference (P <0.05) among the plant parts (leaf, flower, and pod) of *Moringa oleifera*. The result of Duncan multiple range test of proximate composition of *Moringa oleifera* plant from leaf, flower and pod are summarized in table 2. This result revealed that moisture content is significantly higher in flower (6.29±0.31) and is significantly lower in seed (3.12±0.29). However the seed, flower and leaf are all significantly different from each other. Ash content is significantly higher in flower (13.05±0.00) and significantly lower in seed (6.10±0.23) but there are significant differences between leaf, seed and flower. The crude fibre in seed is significantly higher (29.35±0.42) and significantly lower in flower (17.58±0.09) but there are significant differences between the leaf, flower and seed. Fat content in leaf is significantly higher (11.08±0.32) and significantly lower in flower (8.86±0.12). However, there are significant differences across the three plant parts. Crude protein in seed is significantly higher (7.05±0.00) and significantly lower in leaf (5.96±0.16). Meanwhile, there are significant differences across the plant parts.

Table 1. ANOVA table showing nutritional content of the leaf, flower and seed of *Moringa oleifera* Lam. plant.

Variables	Source	Sum of Square	df	Mean of Square	F	Significance
MC	Plant part	15.236	2	7.618	128.92*	0
	Error	0.355	6	0.059		
	Total	15.59	8			
Ash	Plant part	60.544	2	30.272	1024.43*	0
	Error	0.177	6	0.03		
	Total	60.721	8			
Crude Fibre	Plant part	627.751	2	313.876	2837.51*	0
	Error	0.664	6	0.111		
	Total	628.415	8			
Fat	Plant part	19.455	2	9.728	95.73*	0
	Error	0.61	6	0.102		
	Total	20.065	8			
Crude protein	Plant part	14.983	2	7.491	438.52*	0
	Error	0.103	6	0.017		
	Total	15.085	8			

Note: * = significant difference (P <0.05).

Table 2. Showing the results for Duncan multiple range tests for Nutritional composition of the leaf, flower and seed of *Moringa oleifera* Lam. plant.

Variables (%)	Leaf	Flower	Pod/Seed
Moisture Content	4.42±0.06^{b}	6.29±0.31^{c}	3.12±0.29^{a}
Ash Content	11.56±0.20^{b}	13.05±0.00^{c}	6.10±0.23^{a}
Crude Fibre	8.98±0.39^{a}	17.58±0.09^{b}	29.35±0.42^{c}
Fat Content	11.08±0.32^{c}	8.86±0.12^{b}	7.52±0.44^{a}
Crude Protein	5.96±0.16^{b}	3.94±0.17^{a}	7.05±0.00^{c}

Note: Each value is a mean of three replicates ± standard error. Means within the same row and followed by different alphabet are significantly different (P <0.05).

The result from table 3 revealed that there are significant differences (P <0.05) in the antioxidant composition of the dry plant parts (leaf, flower and seed) of *Moringa oleifera*. However, phenol is not significantly different (P >0.05). The result for Duncan multiple range test in table 4, however, revealed that the leaf has the highest value of Flavonoid, DPPH and Vitamin C, while the seed has higher content in Phenol (7.33±0.01) and ABTS (10.15±0.11) but lower in Flavonoid, DPPH, Vitamin C and FRAP meanwhile the flower has its highest content value in FRAP (78.51±4.38) but lower in Flavonoid, DPPH, Vitamin C, Phenol and ABTS.

Table 3. ANOVA table showing Antioxidant content of dry leaf, flower and seed of *Moringa oleifera* Lam. plant.

Variables	Source	Sum of Squares	df	Mean Square	F	Significance
Phenol	Plant part	0.260	2	0.130	0.704	0.531
	Error	1.107	6	0.185		
	Total	1.367	8			
Flavonoid	Plant part	18.302	2	9.151	122.304*	0.000
	Error	.449	6	.075		
	Total	18.751	8			
DPPH	Plant part	1830.530	2	915.265	258.486*	0.000
	Error	21.245	6	3.541		
	Total	1851.775	8			
FRAP	Plant part	258.050	2	129.025	5.352*	0.046
	Error	144.657	6	24.110		
	Total	402.707	8			
Vitamin C	Plant part	406.723	2	203.361	18.001*	0.003
	Error	67.782	6	11.297		
	Total	474.505	8			
ABTS	Plant part	243.447	2	121.723	19.482*	0.002
	Error	37.489	6	6.248		
	Total	280.935	8			

Note: * = significant (P < 0.05) difference.

Table 4. Showing the results for Duncan multiple range tests for the Antioxidant composition of the dry leaf, flower and seed of *Moringa oleifera* Lam. plant.

Variables	Leaf	Flower	Pod/Seed
Phenol (mg g^{-1})	7.18±0.17^{a}	6.92±0.40^{a}	7.33±0.01^{a}
Flavonoid (mg g^{-1})	10.15±0.11^{b}	7.14±0.22^{a}	7.11±0.11^{a}
DPPH (%)	56.80±0.15^{b}	53.67±1.29^{b}	25.11±0.88^{a}
FRAP (mg g^{-1})	65.70±1.96^{a}	78.51±4.38^{b}	69.67±1.05^{ab}
Vitamin C (mg g^{-1})	61.09±1.38^{b}	60.64±0.98^{b}	46.61±2.90^{a}
ABTS (mg g^{-1})	40.64±1.02^{b}	31.80±2.04^{a}	44.17±1.02^{b}

Note: Each value is a mean of three replicates standard error. Means within the same row and followed by different alphabet are significantly different (P <0.05).

The result from table 5 revealed that there are significant differences (P <0.05) in the antioxidant composition of the dry plant parts (leaf, flower and seed) of *Moringa oleifera*. The result for Duncan multiple range test in table 6, however, revealed that the leaf has the highest value of Phenol, Flavonoid, FRAP, Vitamin C, and ABTS, while the flower has highest in DPPH (71.51±2.10) but lower in Phenol, Flavonoid, FRAP, Vitamin C, and ABTS. The result revealed that flavonoid content in wet sample is significantly higher (26.21±0.97) in leaf and is significantly lower (8.14±0.11) in seed. DPPH in wet flower is significantly higher (71.51±2.10) and significantly lower (33.35±1.51) in seed, while the value for ABTS is significantly higher (162.54±2.04) in leaf and significantly lower (44.17±3.06) in flower.

Table 5. ANOVA table showing Antioxidant content of wet leaf, flower and seed of *Moringa oleifera* Lam. plant.

Variables	Source	Sum of Squares	df	Mean Square	F	Significance
Phenol	Plant part	61.469	2	30.734	42.814*	0.000
	Error	4.307	6	0.718		
	Total	65.776	8			
Flavonoid	Plant part	495.798	2	247.899	248.690*	0.000
	Error	5.981	6	0.997		
	Total	501.779	8			
DPPH	Plant part	2377.930	2	1188.965	174.635*	0.000
	Error	40.850	6	6.808		
	Total	2418.779	8			
FRAP	Plant part	202.912	2	101.456	72.572*	0.000
	Error	8.388	6	1.398		
	Total	211.300	8			
Vitamin C	Plant part	5103.394	2	2551.697	24.555*	0.001
	Error	623.505	6	103.918		
	Total	5726.900	8			
ABTS	Plant part	21144.668	2	10572.334	597.902*	0.000
	Error	106.094	6	17.682		
	Total	21250.762	8			

Note: * = significant (P < 0.05) difference.

Table 6. Showing the results for Duncan multiple range tests for the Antioxidant composition of the wet leaf, flower and seed of *Moringa oleifera* plant.

Variables	Leaf	Flower	Pod/Seed
Phenol (mg g^{-1})	12.21 ± 0.28^{b}	10.55 ± 0.39^{b}	6.02 ± 0.70^{a}
Flavonoid (mg g^{-1})	26.21 ± 0.97^{c}	15.39 ± 0.19^{b}	8.14 ± 0.11^{a}
DPPH (%)	62.27 ± 0.34^{b}	71.51 ± 2.10^{c}	33.35 ± 1.51^{a}
FRAP (mg g^{-1})	133.42 ± 0.07^{b}	132.97 ± 0.25^{b}	123.13 ± 1.54^{a}
Vitamin C (mg g^{-1})	146.21 ± 8.97^{b}	88.94 ± 3.51^{a}	108.01 ± 3.34^{a}
ABTS (mg g^{-1})	162.54 ± 2.04^{c}	44.17 ± 3.06^{a}	95.41 ± 2.04^{b}

Note: Each value is a mean of three replicates ± standard error. Means within the same row and followed by different alphabet are significantly different (P <0.05).

DISCUSSION

Nutritional composition of Moringa oleifera leaf, flower and seed

The result of this study shows that the Moisture content, Ash, Crude fibre, Fat content, and Crude protein values observed among the plant parts (leaf, flower, and pod) of *Moringa oleifera* were significantly different (P <0.05). However, while the leaf is better source of fat content, the flower is better source of moisture content and ash content, the pod is a better source of crude protein and crude fibre. The moisture content of the three samples (flower leaf and seed) were lower than the value reported for *Garcinia kola* Heckel by Onyekwelu *et al*. (2015). They reported 71.99%, 92.62%, and 87.68% for the seed kernel, fruit pulp and fruit pod respectively of the species.

The *Moringa oleifera* flower had higher content in ash content. Also, the seed had higher content crude fibre while the leaf and seed had higher content in fat content and crude protein respectively. The value of crude fibre in this result is higher in seed compared to the value (7.73±0.35) reported by Abiodun *et al*. (2012) for *Moringa* seed flour. The crude fibre content was also higher than the value reported melon seed (17.36 to 25.06%) reported by Ebuehi & Avwobobe (2006) and (24.8 to 30.0%) for *Citrillus lanatus* and *C. colocynth* species respectively (Mabaleha *et al*., 2007).

Antioxidant composition of Moringa oleifera leave, flower and seed

Antioxidants are naturally occurring chemicals in foods that help to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention (CSIRO, 2007). Six antioxidant factors at different forms (wet and dry) were investigated in the leaf, flower and seed of *Moringa oleifera* which are total phenol, flavonoid, DPPH, FRAP, Vitamin C and ABTs. The result of antioxidant content in the wet sample was higher compared to the dry sample. The flavonoid and vitamin C content in the dry leaf flower and seed are high when compared to result of *C. albidum* and *G. kola* in literature. Likewise, the wet sample is higher in Phenol, flavonoid, vitamin C and DPPH when compared to result of *C. albidum* and *G. kola* in literature. Ihemeje *et al.* (2013) investigated the flavonoid content of ripe and unripe fruits of *Dennettia tripetala* Baker f. (pepper fruit) and reported the flavonoid content of 3.10 mg g^{-1} for unripe fruit and 1.2 mg g^{-1} for the ripe fruit and concluded that the unripe fruit has higher

flavonoid content compare to the ripe one which are much lower than the value reported in this study. Also, the phenol content of *M. oleifera* dry leaf flower and seed/pod in this study was lower compared to what was reported by Onyekwelu *et al*. (2015) for *Chrysophyllum albidum* G.Don and *Garcinia kola* Heckel.

However, the dry and wet leaf, flower and seed of *M. oleifera* have higher value of DPPH compare to the one reported by Onyekwelu *et al.* (2015) that investigated the DPPH content of *C. albidum* fruit pulp, seed, and fruit skin as well as that of the fruit pulp, seed kernel, and fruit pod of *G. kola*. They reported the DPPH values of 50.4%, 41.2% and 46.6% for *C. albidum* fruit pulp, seed kernel, and fruit skin respectively and 26.28%, 54.59% and 41.84% for *G. kola* fruit pulp, seed kernel and fruit pod respectively. Also, the dry and wet leaf, flower and seed of *M. oleifera* have much higher value of Vitamin C compared to the one reported by Onyekwelu *et al.* (2015). They investigated the Vitamin C content of *C. albidum* fruit pulp, seed, fruit skin and fruit pulp as well as seed kernel, and fruit pod of *G. kola* and reported a value of 0.60 mg g^{-1}, 0.60 mg g^{-1} and 0.30 mg g^{-1} for *C. albidum* fruit pulp, seed kernel and fruit skin respectively and 1.15 mg g^{-1}, 0.79 mg g^{-1} and 0.37 mg g^{-1} for *G. kola* fruit pulp, seed kernel and fruit pod respectively. Also, the vitamin C content of 17.97±0.09 mg / 100 g DW is higher than 10.83±0.25 mg / 100 g DW reported for *Berberis lyceum* Royle. (Kasmal fruit) and 10 mg / 100 g DW for *Diospyros mespiliformis* Hochst. ex A.DC. by Sood *et al*. (2010). The result of this study shows that the wet sample of leaf, flower and seed of *Moringa oleifera* composition is much higher when compared to the dry sample of leaf, flower and seed of *Moringa olefera*. Based on this result, it can be deduced that the wet sample of leaf have higher content of phenol, flavonoid, FRAP, vitamin C and ABTS compared to the dry sample of leaf.

CONCLUSION

The results of this study have shown that *Moringa oleifera* seed, leaf and flower contain nutritional and antioxidant composition necessary for good functioning of the human body. This study has also revealed the wet and dry forms of the antioxidant and dry form of nutritional compositions of *M. oleifera* leaf, flower and seed. Hence, it can be concluded that the nutritional composition of *Moringa oleifera* seed has high concentrations of crude fibre and protein while the flower has a high concentration of moisture and ash content. The leaf has a high concentration of fat.

Also, both the wet and dry samples are the good source of antioxidant and they are not detrimental to health. Accordingly, the leaf of wet samples is better source of antioxidant and it is not detrimental to health. Therefore, there is need to create awareness of the nutritional and antioxidant composition of the leaf, flower and seed of the *Moringa oleifera*, because they play important roles in human health as they serve as chemicals in foods that help to neutralize excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention.

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