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COMPARISON OF MEASUREMENTS OF ANTIOXIDANT ACTIVITY IN THE SELECTED LEAFY VEGETABLES DEPENDING ON EXTRACTION SOLVENT

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ABSTRACT

Four leafy vegetable species, spinach (*Spinacia oleracea)*, amaranthus (*Amaranthus viridis*), fenugreek (*Trigonella foenum-graecum*) and bathua (*Chenopodium album*), were extracted with three different solvents (80% ethanol, 80% acetone and water) for maximum recovery of phenol and antioxidant compounds in the extract. The results of extraction were compared with extraction from moringa (*Moringa oleifera*) leaves that is known as a very rich source of antioxidants. The study showed that, it is very difficult to justify a single solvent for extraction of antioxidant compounds from different plants. Results from different solvents used for extracting the bioactive compounds mostly depend on the type of compound extracted (polar/medium polar/non-polar) present in leafy matrices. Here, 80% acetone extract showed highest total phenol content in moringa leaves but the overall antioxidant activity in the leaves of four vegetables was observed to be better after extraction with 80% ethanol. Nevertheless, in most assays, independent of solvent used for extraction, the moringa leaves were the richest source of polyphenols and antioxidants.

Key words: antioxidant activity; effect of extraction; leafy vegetables; polyphenols; solvent extraction

INTRODUCTION

The highly unstable free radicals (reactive oxygen species – ROS) are released into human body during different metabolic processes. These free radicals are responsible for the disruption of stability of other molecules by releasing electrons from their molecular structure. Overproduction of ROS, such as superoxide anion, hydroxyl radical, hydrogen peroxide and reactive oxygen, is associated with cellular and metabolic injury leading to aging, cancer, cardiovascular diseases, neurodegenerative disease and inflammation (Ames 1983; Sun 1990; Stadtman 1992). These harmful effects of free radicals can be minimized by the addition of sufficient amounts of natural antioxidants to human diet. These natural antioxidants play an important role in the reduction of the risk of chronic diseases such as cardiovascular diseases and cancers (Gerber et al. 2002; Kris-Etherton et al. 2002; Serafini et al. 2002). Involvement of different plant based nutrition rich in carotenoids, vitamin C, vitamin E, flavonoids, tannins and polyphenols is the only way to meet the requirement of healthy antioxidant rich diet in our daily routine nutrition.

Green leafy vegetables are an important part of human diet since ancient time. They provide us with essential nutrients like protein, carbohydrates, vitamins, minerals, essential amino acids and fibers. Direct involvement of photosynthesis process in leafy part of vegetables plays an important role in the production of more vitamin K than that of other fruits and vegetables (Bhat & Al-Daihan 2014). Moreover, it also possesses a range of bioactive phytoconstituents helpful in the management of oxidative stress and age-related ailments (Gacche et al. 2010).

The present study is designed to find out the total phenol content and antioxidant activity of the common leafy vegetables – spinach (*Spinacia oleracea*), amaranths or chaulai (*Amaranthus viridis*),

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fenugreek or methi (*Trigonella foenum-graecum*) and bathua (*Chenopodium album*) obtained using different solvents and to compare results with content of the above extracted from leaves of *Moringa oleifera,* which has been reported to be a rich source of nutrition and antioxidants (Misra & Misra 2014; Oluduro 2012; Ramachandran et al. 1980; Nweze & Nwafor 2014; Otu et al. 2013). The earlier study showed that the selection of proper extraction solvent plays an important role in the extraction of specific group of polyphenols and antioxidant compounds from different plant materials. Here, the selected vegetables were extracted with three different solvents to find out the most suitable extraction medium for each of them. Solvents were selected on the basis of earlier reports (Sulaiman et al. 2011).

MATERIALS AND METHODS

Sample preparation

Leafy vegetables namely, spinach, amaranthus, fenugreek and bathua were produced in Haryana state (northern part of India) and procured from local market of Delhi during February. Leaves of moringa were collected from the tree garden of Indian Institute of Technology Delhi. All the samples were cleaned and washed in potable water to remove dust, dirt and any other type of sticky material. Leaves were kept on sieves to drain out excess water, lyophilized (Labconco Corporation, Kansas City, Missouri) and stored at −40 °C till extraction. 1 g powder of each leaf sample was extracted with 25 ml of extraction solvent in a shaking incubator (LabTech, LSI-2005RL, Kurukshetra, India) by keeping the extraction temperature at 30 °C and extraction time 4 hours. Extraction procedures were repeated thrice for each vegetable species. Extracts were centrifuged (Remi Centrifuge R-23, Mumbai, India) at 3000 rpm for 15 minutes and the clear supernatant was stored at −4 °C till analysis.

Chemicals and reagents

All the required analytical grade chemicals and solvents (ethanol, acetone, hydrochloric acid and acetic acid) were purchased from Qualigens, Merck, India. Phenol and antioxidant reagents namely Folin–Ciocalteau (for total phenol content), 1,1-diphenyl-2-picrylhydrazyl radical, 2,4,6-tri-(2-pyridyl)-striozine (TPTZ), and ferric chloride hexa-hydrate were obtained from Sigma-Aldrich. The standard reagents for PHOTOCHEM Antioxidant Analyzer were supplied by Analytik Jena, Germany and phenol standard, gallic acid, was obtained from Sigma chemicals. Powdered chemicals, namely potassium acetate, anhydrous sodium carbonate and aluminium chloride were purchased from Fluka. Distilled grade water was used for all the testing procedures.

Total phenols content (TPC)

All the five leafy vegetable extracts were subjected to analyses for their total phenol content by using Folin–Ciocalteu reagent (diluted up to 10-fold). 200 µl vegetable extract or standard (gallic acid) was added in a test tube containing 1 ml freshly prepared Folin–Ciocalteu reagent. After an incubation of 8 minutes, 3 ml sodium carbonate (7.5%) was added to a test tube by shaking the mixture manually. The prepared mixture was allowed for 1-hour incubation in a test tube at room temperature (Jaiswal et al. 2014). All the readings were recorded in triplicates at 765 nm in UV-Vis Spectrophotometer (Shimadzu UV-2600, Kyoto, Japan). The results were expressed in mg gallic acid equivalent (GAE)·g^{-1} dry weight basis.

Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

A method of Jaiswal et al. (2014) was used to measure the DPPH radical scavenging activity of the leaf extract samples. 3900 µl of DPPH solution (0.004%) prepared in 80% methanol was added into the test tube containing 100 µl of leaf extract sample or standard. After a 60-minute incubation of this reaction mixture, an absorbance was measured at 515 nm. The values of DPPH activity were calculated in terms of percent reduction (% reduction). Values of DPPH activity for each vegetable species extract were measured in triplicates.

2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity

ABTS radical scavenging activity for extracts of five plant species was measured using the method described by Jaiswal et al. (2015). ABTS stock solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulphate in equal quantity.

The prepared stock solution was allowed to incubate in dark for 16 hours. After incubation, it was diluted with 80% ethanol in order to obtain absorbance of working stock solution of 0.700 ± 0.005 at 734 nm. 3900 µl ABTS working stock solution was added into test tube containing 100 µl extract and the mixture was allowed to incubate for 5 min to measure absorbance. ABTS radical scavenging activity results of extracts were measured against the standard catechin and the results were expressed in terms of mg catechin equivalent (CE)·dm^{-3} on dry weight basis. All the readings of vegetable extracts were recorded in triplicates. A standard catechin curve was prepared in the concentration range of 0.01–0.1 mg·ml^{-1}.

Ferric reducing antioxidant potential (FRAP) Assay

As described by Firuzi et al. (2005), the following chemical reagents were included to prepare stock solution of FRAP: 300 mM acetate buffer (which was adjusted to pH of 3.6 by the addition of acetic acid), 20 mM ferric chloride hexahydrate (dissolved in distilled water) and 10 mM TPTZ (dissolved in HCl 40 mM). The required fresh working solution for FRAP analysis was prepared from stock solutions by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml ferric chloride hexahydrate. Ferrous sulphate standard graph was prepared by taking different concentrations of ferrous sulphate (0.1–1 mM). 120 µl distilled water per standard or sample was added to 4 ml of the FRAP solution and absorbance was taken in triplicate at 593 nm after 4 min. Ferrous sulphate equivalent concentration in mM was calculated from the standard graph and expressed as mM ferrous sulphate equivalent ($FeSO_4$)·dm^{-3} dry weight basis.

Photochemluminiscence (PCL) antioxidant capacity

Standard operating protocol described by Analytik Jena, 2004 with a photochem apparatus (Analytik Jena, Leipzig, Germany) was used for PCL analysis to determine the antioxidant capacities in leafy extracts. The antioxidant activity of leaves extracted with different solvents was studied using a kit known as anti-oxidative capacity of water soluble substances (ACW). This kit was provided by the manufacturer to measure the antioxidant activity of the hydrophilic compounds.

A volume of 490 µl of reagent 1 provided in the kit and 10 µl of H_2SO_4 (95–97%) was added to the vial containing reagent 4 and vortexed for 20–30 seconds to get a standard concentration of (10 mmol·dm^{-3}) ascorbic acid. Reagent 4 working solution (0.1 nmol·μl^{-1}) was prepared by diluting the stock solution in 1:100 with reagent 1. Calibration curve was prepared by taking 0.5, 1, 2 and 3 nmol of ascorbic acid corresponding to 5–30 µl of reagents 4 working solution. Appropriately diluted samples (10 µl) were added with 1490 µl of reagent 1, 1000 µl of reagent 2 and 25 µl of reagent 3. It was vortexed for 10 seconds and subjected to analysis. Readings of each sample were taken in triplicates and mean values of results were reported. The antioxidant activity of the sample was expressed as mg ascorbic acid (AA)·g^{-1} dry weight basis.

Statistical analysis

SPSS Version 16 software was used for statistical analysis. One-way Anova with Tukey multiple range test was used to analyze the results. All the reported data were recorded in triplicates and the results were expressed as means ± standard deviation (SD). Means were accepted as significantly different at 95% confidence interval (p = 0.05).

RESULTS AND DISCUSSION

Total phenols content

In this study, four leafy vegetable species spinach, amaranthus, fenugreek and bathua were analyzed and compared with moringa for total phenol content. The leaf samples were extracted with 80% ethanol, 80% acetone and distilled water to find out an appropriate solvent system for optimum recovery of total phenols and other antioxidant compounds (Table 1). In the present study, 80% ethanol was found to be the best solvent for spinach, fenugreek and bathua. However, 80% acetone was found to be the best solvent for amaranthus and moringa. The lowest value of total phenol was obtained with 80% acetone for spinach (6.16 ± 1.08 mg GAE·g^{-1}). It shows that the extraction of total phenol depends on the solubility of different phenolic constituents in a chosen solvent. In the present study, distilled water was proved to be the least effective extraction

Table 1. Total phenol content (TPC) of leafy vegetables gallic acid equivalent in mg·g^{-1} d.w.

Vegetable species	Solvent used for extraction		
	80% ethanol	80% acetone	water
Spinach	52.61 ± 1.69^{c}	6.16 ± 1.08^{a}	9.58 ± 1.05^{b}
Fenugreek	73.56 ± 1.59^{c}	56.90 ± 0.80^{a}	68.56 ± 0.85^{b}
Bathua	164.37 ± 0.80^{c}	74.82 ± 0.61^{b}	69.51 ± 0.69^{a}
Amaranthus	33.75 ± 0.59^{b}	36.47 ± 0.91^{c}	27.89 ± 0.64^{a}
Moringa	155.90 ± 1.62^{b}	179.75 ± 0.44^{c}	83.28 ± 0.43^{a}

All values reported in rows for each vegetable with different superscript are significantly different (p = 0.05). Values reported are the means ± SD.

Table 2. DPPH radical scavenging activity (% reduction)

Vegetable species	Solvent used for extraction		
	80% ethanol	80% acetone	water
Spinach	54.88 ± 0.72^{c}	38.90 ± 0.70^{b}	34.63 ± 0.58^{a}
Fenugreek	76.53 ± 0.82^{c}	74.75 ± 0.77^{b}	69.33 ± 0.60^{a}
Bathua	87.33 ± 0.56^{c}	73.34 ± 0.60^{a}	77.88 ± 0.59^{b}
Amaranthus	81.14 ± 0.33^{c}	56.49 ± 0.83^{b}	40.32 ± 0.56^{a}
Moringa	80.44 ± 0.70^{b}	91.83 ± 0.57^{c}	57.42 ± 0.54^{a}

Note: See Table 1

Table 3. ABTS radical scavenging activity (catechin equivalent mg·dm^{-3})

Vegetable species	Solvent used for extraction		
	80% ethanol	80% acetone	water
Spinach	92.96 ± 0.24^{b}	53.20 ± 0.61^{a}	94.86 ± 0.67^{c}
Fenugreek	144.92 ± 0.87^{a}	176.20 ± 0.25^{b}	183.48 ± 0.55^{c}
Bathua	266.25 ± 0.49^{b}	190.67 ± 1.33^{a}	316.40 ± 1.31^{c}
Amaranthus	121.07 ± 0.75^{c}	111.69 ± 0.98^{b}	104.54 ± 0.90^{a}
Moringa	433.83 ± 0.65^{b}	615.36 ± 0.72^{c}	274.74 ± 0.45^{a}

Note: see Table 1

Table 4. FRAP assay (mM $FeSO_4$·dm^{-3})

Vegetable species	Solvent used for extraction		
	80% ethanol	80% acetone	water
Spinach	21.88 ± 0.68^{c}	2.47 ± 0.36^{b}	0.89 ± 0.08^{a}
Fenugreek	23.32 ± 0.52^{c}	3.42 ± 0.22^{b}	2.21 ± 0.13^{a}
Bathua	24.08 ± 0.79^{c}	4.20 ± 0.20^{b}	2.08 ± 0.07^{a}
Amaranthus	22.40 ± 0.36^{c}	3.33 ± 0.22^{b}	0.86 ± 0.08^{a}
Moringa	28.65 ± 0.47^{c}	5.89 ± 0.19^{b}	2.60 ± 0.11^{a}

Note: see Table 1

study were in accordance with the previous one, medium, with the exception of ABTS assay in spinach, fenugreek and bathua. The results of the present where the extraction of thirty-seven raw vegetables was carried out by using mixture of four different solvents (Sulaiman et al. 2011).

According to Dasgupta & De (2007), *Moringa oleifera* was the second most important source of total phenol from 11 leafy vegetables with 184 µg GAE·mg^{-1} concentration on dry weight basis. Verma et al. (2009) have reported that the total phenol content in moringa leaves to vary from 33.82 to 336.95 mg GAE·g^{-1} in different fractions of same sample, using different solvents. In another study, the value of total phenol content for moringa leaves was observed to be 216.45 mg·GAE·g^{-1} in methanol extract, while 100.12 mg GAE·g^{-1} in dichloromethane extract of the residue (Charoensin 2014). These variations in results can be attributed not only to many biotic and abiotic factors like genetic variability, age of the leaf, post-harvest handling, geographical and environmental factors (Anjorin et al. 2010), but also to the procedure of sample preparation.

From the present study, it can be inferred that the total phenol content of a plant material is dependent on the nature of extraction solvent. Another major finding endorses moringa leaf to be a better source of polyphenols as compared to the other here studied vegetables.

Antioxidant activities

The antioxidant activities of the extracts were measured by using four antioxidant analyzing assays: DPPH, ABTS, FRAP and PCL (Table 2–5). Water was the worst extraction medium in showing DPPH activity. The highest values for vegetables were obtained using 80% alcohol and only for moringa the highest values produced 80% acetone. For spinach, fenugreek and bathua, the results obtained with 80% ethanol was in accordance with results obtained for total phenols.

Also in measurement results of ABTS activity were significant differences depending on solvent used (Table 3). Water was most effective for spinach, fenugreek and bathua, whereas ethanol for amaranthus, and acetone for moringa. 80% acetone extract of moringa leaves exhibiting the highest ABTS

radical scavenging activity is in accordance with the results obtained for total phenol content and DPPH. However, for amaranthus, 80% ethanol extracts showed maximum ABTS reduction, which is in contrast to the total phenol content and in accordance to the DPPH reducing activity. For spinach, fenugreek and bathua, values obtained with water extraction are not in line with total phenol content. Reported values of Sulaiman et al. (2011) for the highest total phenol content of an extract may not show the highest radical scavenging activity, which signifies the importance of using more than one extraction solvent for such a study.

In FRAP assay, results for all plant species were the same. The highest values were obtained using ethanol and the lowest using water as extract medium (Table 4). These results are not comparable with trends observed in the DPPH and ABTS radical scavenging assays.

In PCL assay, 80% ethanol was the most effective as an extract medium for all the plant species with the exception for fenugreek, where water produced the highest assay values (Table 5). The solvent trend was 80% ethanol > 80% acetone > water and was similar as for FRAP assay.

Leaves of *Moringa oleifera* were found to be the best source of polyphenols of the five plants studied having the highest antioxidant activity in all the methods used in the present study. Anjorin et al. (2010) reported that the high antioxidant value of moringa leaf can be appreciated due to the high availability of gallic acid, ellagic acid, ferulic acid, chlorogenic acid and flavonoids (rutin, quercetin and kaempferol). The leaves of moringa are also reported to be a rich source of α and γ-tocopherol (Sánchez-Machado et al. 2006). Results of all four antioxidant assays and earlier reports on nutritional and anti-nutritional properties reported by Awodele et al. (2012) give positive references for introducing moringa leaves in human diet.

Pearson correlation coefficient

Non-significant correlation was observed between extract values (except for moringa) of TPC-PCL and TPC-ABTS (Table 6). But a TPC value of moringa extracts was significantly correlated with PCL and ABTS. Besides TPC values of spinach and bathua extracts were strongly correlated with DPPH and FRAP activities. TPC values of moringa extracts exhibited strong significant correlation in all the methods except FRAP and PCL. Spinach and amaranthus gave lower antioxidant activities with poor correlation between TPC and antioxidant activities ($p = 0.05$).

Table 5. PCL assay (ascorbic acid μmol·ml^{-1})

Vegetable species	Solvent used for extraction		
	80% ethanol	80% acetone	water
Spinach	2.78 ± 0.18^{b}	2.75 ± 0.15^{b}	0.07 ± 0.01^{a}
Fenugreek	3.73 ± 0.25^{b}	1.22 ± 0.14^{a}	4.13 ± 0.11^{c}
Bathua	5.43 ± 0.28^{c}	1.03 ± 0.10^{a}	4.90 ± 0.20^{b}
Amaranthus	4.40 ± 0.32^{b}	0.57 ± 0.10^{a}	0.46 ± 0.09^{a}
Moringa	9.51 ± 0.37^{c}	5.47 ± 0.37^{b}	0.16 ± 0.05^{a}

Note: see Table 1

Table 6. Pearson correlation coefficient (r) between total phenol and antioxidant activities

Vegetable species	DPPH	ABTS	FRAP	PCL
Spinach	0.964**	0.522	0.990**	0.448
Fenugreek	0.030	−0.590	0.688*	0.550
Bathua	0.929**	0.066	0.999**	0.551
Amaranthus	0.570	0.601	0.306	0.228
Moringa	0.995**	0.948**	0.390	0.772*

*Significant at $p = 0.05$, **Significant at $p = 0.01$

CONCLUSION

The study showed that, it is very difficult to justify a single solvent for extraction of antioxidant compounds from leafy vegetables. Choosing the right solvent for extracting the bioactive compounds mostly depends on the type of compounds (polar/medium polar/non-polar) present in leafy matrices. Here, 80% acetone extraction of moringa showed the highest total phenol content, DPPH and ABTS activity but the overall antioxidant activity of all the raw materials was better with 80% ethanol. Nevertheless, in most assays, independent of solvent used for extraction, the moringa leaves were the richest source of polyphenol and antioxidant active compounds.

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REFERENCES

Ames B.N. 1983. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. Science 221: 1256–1264. DOI: 10.1126/science.6351251.

Anjorin T.S., Ikokoh P., Okolo S. 2010. Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. International Journal of Agriculture and Biology 12: 431–434.

Awodele O., Oreagba I.A., Odoma S., Teixeira da Silva J.A., Osunkalu V.O. 2012. Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). Journal of Ethnopharmacology 139: 330–336. DOI: 10.1016/j.jep.2011.10.008.

Bhat R.S., Al-Daihan S. 2014. Phytochemical constituents and antibacterial activity of some green leafy vegetables. Asian Pacific Journal of Tropical Biomedicine 4:189–193. DOI: 10.1016/S2221-1691(14)60230-6.

Charoensin S. 2014. Antioxidant and anticancer activities of *Moringa oleifera* leaves. Journal of Medicinal Plants Research 8: 318–325. DOI: 10.5897/JMPR2013.5353.

Dasgupta N., De B. 2007. Antioxidant activity of some leafy vegetables of India: A comparative study. Food Chemistry 101: 471–474. DOI: 10.1016/j.foodchem.2006.02.003.

Firuzi O., Lacanna A., Petrucci R., Marrosu G., Saso L. 2005. Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. Biochimica et Biophysica Acta 1721: 174–184. DOI: 10.1016/j.bbagen.2004.11.001.

Gacche R.N., Kabaliye V.N., Dhole N.A., Jadhav A.D. 2010. Antioxidant potential of selected vegetables commonly used in diet in Asian subcontinent. Indian Journal of Natural Products and Resources 1: 306–313.

Gerber M., Boutron-Ruault M.C., Hercberg S., Riboli E., Scalbert A., Siess M.H. 2002. Food and cancer: state of the art about the protective effect of fruits and vegetables. Bulletin du Cancer 89: 293–312.

Jaiswal S.G., Patel M., Naik S.N. 2015. Physico-chemical properties of *Syzygium cuminii* (L.) Skeels jam and comparative antioxidant study with other fruit jams. Indian Journal of Natural Products and Resources 6: 9–15.

Jaiswal S.G., Patel M., Saxena D.K., Naik S.N. 2014. Antioxidant properties of *Piper betel* (L) leaf extracts from six different geographical domain of India. Journal of Bioresource Engineering and Technology 2: 12–20.

Kris-Etherton P.M., Hecker K.D., Bonanome A., Coval S.M., Binkoski A.E., Hilpert K.F. et al. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular diseases and cancer. American Journal of Medicine 113: 71–88. DOI: 10.1016/S0002-9343(01)00995-0.

Misra S., Misra M.K. 2014. Nutritional evaluation of some leafy vegetable used by the tribal and rural people of south Odisha, India. Journal of Natural Product and Plant Resources 4: 23–28.

Nweze N.O. Nwafor F.I. 2014. Phytochemical, proximate and mineral composition of leaf extracts of *Moringa oleifera* Lam. from Nsukka, South-Eastern Nigeria. IOSR Journal of Pharmacy and Biological Sciences 9: 99–103. DOI: 10.9790/3008-091699103.

Oluduro A.O. 2012. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria. Malaysian Journal of Microbiology 8: 59–67. DOI: 10.21161/mjm.02912.

Out P.N.Y., Saalia F.K., Amankwah E.A. 2013. Characterization of fresh *Moringa oleifera* beverage. Food Science and Quality Management 21: 26–33.

Ramachandran C., Peter K.V., Gopalakrishnan P.K. 1980. Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. Economic Botany 34: 276–283. DOI: 10.1007/BF02858648.

Sánchez-Machado D.I., López-Cervantes J., Ríos Vázquez N.J. 2006. High-performance liquid chromatography method to measure α- and γ-tocopherol in leaves, flowers and fresh beans from *Moringa oleifera*. Journal of Chromatography A 1105: 111–114. DOI: 10.1016/j.chroma.2005.07.048.

Serafini M., Bellocco R., Wolk A., Ekström A.M. 2002. Total antioxidant potential of fruit and vegetables and risk of gastric cancer. Gastroenterology 123: 985–991. DOI: 10.1053/gast.2002.35957.

Stadtman E.R. 1992. Protein oxidation and aging. Science 257: 1220–1224. DOI: 10.1126/science.1355616.

Sulaiman S.F., Sajak A.A.B., Ooi K.L., Supriatno, Seow E.M. 2011. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. Journal of Food Composition and Analysis 24: 506–515. DOI: 10.1016/j.jfca.2011.01.020.

Sun Y. 1990. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radical Biology and Medicine 8: 583–599. DOI: 10.1016/0891-5849(90)90156-D.

Verma A.R., Vijayakumar M., Mathela C.S., Rao C.V. 2009. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food and Chemical Toxicology 47: 2196–2201. DOI: 10.1016/j.fct.2009.06.005.