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Research Article
Antioxidant Activity of *Cinnamomum iners* Leaves Standardized Extract

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Abstract
In this study the antioxidant activity of *C. iners* leaves standardized extract was evaluated. *Cinnamomum iners* leaves (methanol extract, chloroform fraction and subfraction 1) was standardized using cinnamic aldehyde. The phenolic and flavonoid content of *C. iners* leaves and cinnamic aldehyde were determined in this study. In addition, the antioxidant potential of *C. iners* leaves was evaluated using DPPH (2,2-Diphenyl-2-picryl-hydrazyl) free radical scavenging method, hydrogen peroxide decomposition study and reducing power assay. This study showed that *C. iners* leaves standardized extract showed promising antioxidant property.

Key words: Active portion, scavenging, antidiabetic, *Cinnamomum iners*, subfraction, chloroform fraction

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reactive Oxygen Species (ROS) is closely related to serious health effects such as cancer, coronary heart disease and aging (Gulcin, 2012). These free radicals are being derived from normal respiration process or exogenous factor such as pollution (Madhavi *et al*., 1996). Human body had developed defence system to completely remove ROS by the means of enzymes (Diplock, 1994). However, the imbalance between free radicals and antioxidants will lead to oxidative stress, resulting in protein damage and lipid per oxidation (Mustaffa *et al*., 2010a). Natural antioxidants present in plants are closely linked to their ability to combat reactive oxygen species. Since ancient time people has been using various plants, which possess antioxidant properties as medicine (Kumaran and Karunakaran, 2007). Antioxidants may also enhance immune defense system, thus reducing risk of cancer and infection (Wu *et al*., 2006). Moreover, it has been revealed that antioxidant potential of plant products against various diseases is mainly due to the presence of phenolic compounds such as phenolic diterpenes, phenolic acids and flavonoids (Chua *et al*., 2008).

Cinnamomum iners plant grows wildly along the road side (Choi, 2003). It appears as dense, bushy with dull green in color. There is numerous report on medicinal benefit of this plant such as antidiabetic, antimicrobial, anticancer and analgesic (Mustaffa *et al*., 2010a, 2011; Pang *et al*., 2009). This study is the extension of our previous research on antidiabetic activity of *C. iners* leaves. Current study was undertaken to correlate between antidiabetic activity of *C. iners* leaves active portion (methanol extract, chloroform fraction and subfraction 1) and the antioxidant activity. Several studies have shown that diabetes mellitus (type 1 and 2) is accompanied by increased formation of free radicals and decreased antioxidant capacity, leading to oxidative damage of cell components (Mustaffa *et al*., 2014a). The inhibition of intracellular free radical formation would provide a therapeutic strategy to prevent oxidative stress and the related diabetic vascular complications (Mustaffa *et al*., 2014b). This study aimed to search for a natural antioxidant source to combat oxidative stress and to prevent diabetic vascular complications.

MATERIALS AND METHODS

Plant material: *Cinnamomum iners* leaf were collected at USM (University Sains Malaysia). The authentication work was carried out by a botanist from School of Biological Sciences, USM where, the plant material was deposited. The voucher specimen number is 11014.

Preparation of herb extracts: The plant leaf were washed with water to remove dirt prior to the drying process. The leaf were then crushed into fine powder. Powdered dried leaf (500 g) of the plant were serially macerated in petroleum ether (60-80, 2500 mL), chloroform (2500 mL) and methanol (2500 mL) for 3 days each. The residue after methanolic extraction was macerated in water for 24 h to obtain water extract. Then, the leaf extract was filtered and concentrated under reduced pressure at 55°C in a rotary evaporator. The concentrated extract obtained was placed in the oven at 60°C for 3 days to remove the remaining solvent. The aqueous extract was placed in freeze drier instead of oven.

Fractionation of the active extract (methanol extract): Methanol extract (2 g) was suspended in distilled water (500 mL). Then, the suspension obtained was placed into a 1 L separator funnel. Firstly, the solution was extracted with chloroform (3×250 mL). Next, the aqueous layer was extracted with ethyl acetate and n-butanol (3×250 mL) to obtain three respective fractions. All fractions obtained were concentrated using the rotary evaporator. Chloroform fraction, ethyl acetate fraction and n-butanol fraction were kept in oven at 60°C to remove the remaining solvents. The aqueous fraction was placed in freeze drier instead of oven.

Subfractionation of the active fraction (chloroform fraction): Chloroform fraction was further extracted in hexane-chloroform mixture (1:3). The supernatant formed was collected, filtered and concentrated using rotary evaporator and dried in oven at 60°C to obtain subfraction 1 (SF 1). The residue was dried and then similarly washed with chloroform until no color was formed. Again, this supernatant was filtered, concentrated using rotary evaporator and freeze dried to obtain subfraction 2 (SF 2).

Standardization of active extract, active fraction and active subfraction using cinnamic aldehyde: Sample solution of methanol extract, chloroform fraction and subfraction 1 (1 mg mL^{-1}) was prepared by dissolving in ethanol. Sample (1 µL) was injected and the profile was acquired using the GC-MS. The peak identification and quantification was carried out using the retention time and mass spectrum provided by the MS library.

Limit of quantification and detection: The ICH Harmonised Tripartite Guideline procedure was employed for the determination of Limit of Quantification (LOQ) and Limit of Detection (LOD). Signal-to-Noise (S/N) method was used for the determination of LOD and LOQ of cinnamic aldehyde. The

LOD is the minimum concentration of the analyte that can be differentiated from assay background. The LOD is calculated in S/N ratio of 3:1. The LOQ is the minimum concentration of the analyze that can be quantified and determined by peak-to-peak noise, in ratio of 10:1 (ICH Q2B, 1996).

Precision: The ICH Harmonised Tripartite Guideline procedure was used for the inter-day and intra-day precision determination. Inter-day and intra-day precision were determined by calculating the percentage of relative standard deviation (RSD %) (ICH Q2B, 1996). Percentage of RSD of calculated using the following formula:

$$\text{RSD (\%)} = \frac{\text{SD}}{\text{M}} \times 100$$

where, M is mean of experimentally determined concentration and SD is standard deviation of M.

Determination of total phenolic content: The Total Phenolic Content (TPC) was determined by spectrophotometric method (Wolfe *et al*., 2003). Firstly, 1 mL of sample (1 mg mL^{-1}) was mixed with 1 mL of Folin-Ciocalteu's phenol Reagent followed by addition of 10 mL of a 7% Na_2CO_3 solution. Next, 13 mL of deionized distilled water was added and the mixture was kept in the dark for 90 min at 23°C, Finally, the absorbance was determined at 750 nm using spectrophotometer. The Mean±SD results of triplicate analyses were expressed as milligram gallic acid equivalents per gram plant material.

Estimation of total flavonoid content: Total flavonoid content was determined according to a method by Sakanaka *et al*. (2005). Firstly, 0.3 mL of extracts (1 mg mL^{-1}), 0.15 mL of $NaNO_2$ (0.5 M) and 0.15 mL of $AlCl_3.6H_2O$ (0.3 M) were mixed. Thereafter, 1 mL of NaOH (1 M) was added. Lastly, the solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using catechin standard solution and expressed as milligram catechin equivalents per gram of plant material. The data represent as the mean of triplicate analyses.

DPPH (2,2-diphenyl-2-picryl-hydrazyl) free radical scavenging method: The DPPH scavenging activity of *C. iners* standardized leaves extract was determined by the modified method of Brand-Williams *et al*. (1995) and Bursal and Gulcin (2011). *Cinnamomum iners* active portion (77 µL, 0.1-2.5 mg mL^{-1}) was added to DPPH (6×10-5 mol^{-1}) and vortex-mixed. Then, the mixture was placed in the dark for 15 min at room temperature. Thereafter, the absorbance was measured at 515 nm using spectrophotometer and DPPH scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Ac-At}}{\text{Ac}} \times 100$$

where, Ac is absorption of control (t = 0 min) and At is absorbance of tested extract or standard solution (t = 15 min). The DPPH solution without plant extract or standard served as control. Vitamin E was used as standard antioxidant.

Hydrogen peroxide (H_2O_2) scavenging activity: The H_2O_2 scavenging activity of *C. iners* leaves active portion was evaluated using the method of Ruch *et al*. (1989), Firstly, H_2O_2 solution (40 mM) was prepared in phosphate buffer (pH 7.4). Next, active portion of *C. iners* leaves or standard (0.1-4.8 mg mL^{-1}, 2.0 mL) was transferred into the test tubes containing H_2O_2 (1.2 mL). Finally, the absorbance of samples in the test tubes were determined at 230 nm using spectrophotometer. The abilities of samples to scavenge the hydrogen peroxide were calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Ac-At}}{\text{Ac}} \times 100$$

where, Ac is absorption of control and At is absorbance of tested extract or standard solution. The H_2O_2 solution without plant extract or standard served as control. Vitamin C was used as standard antioxidant.

Reducing power assay: The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the test samples were determined according to Oyaizu (1986). The formation of Fe (II) was monitored by measuring the formation of Perl's Prussian blue at 700 nm using spectrophotometer. Briefly, different concentrations of the sample (0.05-2.5 mg mL^{-1}, 2 mL) were mixed with 2 mL of phosphate buffer (0.2 M, pH 6.6) and 2 mL of potassium ferricyanide (10 mg mL^{-1}). Next, the mixture was incubatedat 50°C for 20 min followed by addition of 2 mL of trichloroacetic acid (100 mg L^{-1}). Thereafter, the mixture was centrifuged at 3000 rpm for 10 min and the supernatant was collected. Then the supernatant was diluted with 2 mL of distilled water and 0.4 mL of 0.1% (w/v) fresh ferric chloride. Finally, the

absorbance was measured after 10 min at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power.

Statistical analysis: Data was analyzed by SPSS version 12.0.1 software program. Statistically significant difference between groups was calculated by the application of analysis of variance (ANOVA) followed by Dunnett's test. A difference was considered significant at $p<0.05$. The IC_{50} values were calculated from linear regression line.

RESULTS

Standardization of active extract, fraction and subfraction: Methanol extract, chloroform fraction and subfraction 1 of *C. iners* leaf was standardized using cinnamic aldehyde as the marker compound. The GC-MS profiles of methanol extract, chloroform fraction and subfraction 1 of *C. iners* leaf were compared with standard cinnamic aldehyde. The analysis show the presence of cinnamic aldehyde in all samples, but in varying proportions. Mass spectroscopy of cinnamic aldehyde identified in the samples showed major characteristic fragmentation pattern (m/z = 45, m/z = 59, m/z = 69, m/z = 77, m/z = 89, m/z = 103, m/z = 111, m/z = 119, m/z = 131, m/z = 152, m/z = 161 and m/z = 179 M+ = 204) exactly identical to the standard cinnamic aldehyde mass fragmentation pattern. The retention time of pure cinnamic aldehyde was 10.12 min whereas, the retention times of the samples were 10.2±0.2 min. The regression curve of cinnamic aldehyde is linear with y-intercept of 814 051 and r^2 value of 0.9921. The amount of cinnamic aldehyde in methanol extract, chloroform fraction and subfraction 1 is presented in Table 1.

LOD and LOQ: The LOD and LOQ of cinnamic aldehyde were 15.6 and 70.5 ppm, respectively.

Precision: The intra-day and inter-day precision for determination of cinnamic aldehyde was presented in Table 2. The RSD (%) value for intra-day were 0.0008, 0.0028, 0.0018 and 0.00097, respectively. The RSD (%) for inter-day precision were 0.006, 0.003, 0.002 and 0.00078, respectively.

Total phenolic and flavonoid content: Total phenolic and flavonoid content of *C. iners* active portion and cinnamic aldehyde was presented in Table 3. Results showed that increasing pattern of total phenolic and flavonoid content from methanol extract, chloroform fraction to subfraction 1.

DPPH and H_2O_2 scavenging activity: *Cinnamomum iners* active portion and cinnamic aldehyde was capable of scavenging hydrogen peroxide and DPPH which is shown in Table 4. Vitamin-C which is a natural antioxidants was used as positive control.

Reducing power assay: The reducing power of active portion of *C. iners* leaves and cinnamic aldehyde was monitored by its reduction capability of Fe^{3+} ferricyanide complex to a ferrous form and the result is shown in Fig. 1. *Cinnamomum iners* active portion and cinnamic aldehyde showed statistically significant difference compared to vitamin E at the highest concentration (2.5 mg mL^{-1}).

DISCUSSION

Medicinal plants have been used throughout human history to cure various diseases. However, owing to the fact that there is lack of both qualitative and quantitative standards of herbal medications, standardization serves as a technique for authentication of herbs to assure the quality control of these materials (WHO., 1991). Hence, *C. iners* leaf active portion had been standardized using cinnamic aldehyde, which acts as its bio active compound.

Table 1: Amount of cinnamic aldehyde in methanol extract, chloroform fraction and subfraction 1 of *Cinnamomum iners* leaf

Materials	Amount of cinnamic aldehyde (ppm)	Amount of cinnamic aldehyde (%)
Methanol extract	83.2	8.32
Chloroform fraction	148	14.80
Subfraction 1	332	33.20

Table 2: Precision and recovery for determination of cinnamic aldehyde

Concentration of cinnamic aldehyde (ppm)	Intra-day			Inter-day		
	Mean±SD	Recovery (%)	Precision (RSD (%))	Mean±SD	Recovery (%)	Precision (RSD (%))
62.5	61.0±0.05	97.60	0.0008	60.8±0.38	97.28	0.006
125	124.0±0.35	99.20	0.0028	123.0±0.46	98.40	0.003
250	248.5±0.45	99.40	0.0018	249.1±0.51	99.64	0.002
1000	998.4±0.97	99.84	0.00097	996.1±0.78	99.61	0.00078

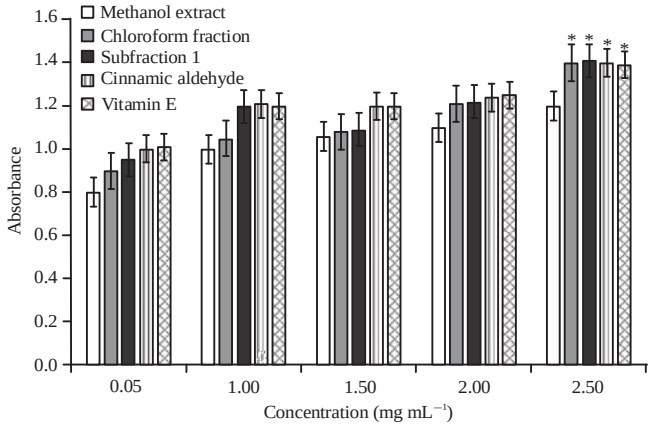

Fig. 1: Reducing power of *Cinnamomum iners* active portion, cinnamic aldehyde and vitamin E at various concentrations, values are the Means±SD of three replicates, *Significant difference with vitamin E at $p<0.05$, data was analyzed using one way ANOVA followed by *post hoc* Dunnett's test

Table 3: Total phenolic and flavonoid content of *Cinnamomum iners* active portion and cinnamic aldehyde

Materials	Total phenolic content (mg GAE/g plant material)	Total flavonoid content (mg CE/g plant material)
Methanol extract	208.25±0.21	15.42±0.10
Chloroform fraction	255.14±0.46	18.06±0.05
Subfraction 1	301.08±0.51	18.78±0.14
Cinnamic aldehyde	315.26±0.79	18.50±0.16

Values are expressed as Mean±SD, GAE: Gallic acid equivalent, CE: Catechin equivalent

Table 4: IC_{50} values of *Cinnamomum iners* active portion and cinnamic aldehyde

	IC_{50}±SD	
Materials (mg mL^{-1})	DPPH	H_2O_2
Methanol extract	0.56±0.04	1.50±0.25
Chloroform fraction	0.45±0.05	1.08±0.10
Subfraction 1	0.21±0.07	0.71±0.03
Cinnamic aldehyde	0.33±0.05	0.64±0.04
Vitamin E	0.18±0.06	0.40±0.24

Values are expressed as Mean±SD, DPPH: 2,2-diphenyl-2-picryl-hydrazyl, H_2O_2: Hydrogen peroxide

Antioxidant screening of *C. iners* active portion was evaluated using DPPH scavenging method, H_2O_2 decomposition and reducing power assay. The DPPH assay is a rapid and sensitive method which has been widely used for the screening of scavenging activity of antioxidant. The ability to scavenge DPPH free radical indicate hydrogen donating capability of plant extracts (Baumann *et al*., 1979). The H_2O_2 scavenging activity is closely related to the ability of plant extract in accelerating the conversion of H_2O_2 to H_2O to prevent accumulation of H_2O_2 in body. Accumulation of H_2O_2 in body may cause formation of harmful hydroxyl radicals that lead to oxidative DNA damage (Miller *et al*., 1993). Table 4 represent the IC_{50} value for DPPH and H_2O_2 scavenging activity of *C. iners* active portion along with its bio active compound, cinnamic aldehyde. The present results indicate that DPPH and H_2O_2 scavenging activity of *C. iners* leaves increase from methanol extract, chloroform fraction to subfraction 1 (Table 4). This might be due to increasing amount of cinnamic aldehyde which is well known for its antioxidant activity (Wondrak *et al*., 2010). The IC_{50} value of methanol extract in scavenging DPPH is in agreement with previous studies by Mustaffa *et al*. (2010b). The DPPH scavenging activity of subfraction 1 (0.21 mg mL^{-1}) is as good as other plant with good antioxidant activity (*Centella asiatica*, 0.2 mg mL^{-1}, *Pisonia alba*, 0.18 mg mL^{-1}, *Orthosiphon stamineus*, 0.21 mg mL^{-1}, *Mentha arvensis*, 0.22 mg mL^{-1}; *Ocimum basilicum*, 0.19 mg mL^{-1}) (Subhasree *et al*., 2009; Zakaria *et al*., 2008). The IC_{50} value of methanol extract to scavenge H_2O_2 is in agreement with previous study of antioxidant activity of *C. iners* leaves (Mustaffa *et al*., 2010b).

The reducing power a compound is related on its ability in donating electrons (Zou *et al*., 2004). The reducing power

of *C. iners* active portion is shown in Fig. 1. As illustrated in Fig. 1, the reducing power of *C. iners* active portion and cinnamic aldehyde increase in concentration dependent manner. At the highest concentration, (2.5 mg mL^{-1}), chloroform fraction and subfraction 1 of *C. iners* leaves showed significantly higher reducing power than standard antioxidant (Vitamin E). Furthermore, the reducing power of chloroform fraction and subfraction 1 was found to be as good as *Smilax excelsa* leaves extract (5.0 mg mL^{-1}, Absorbance, 1.6) which is used as natural antioxidant source in Turkey (Ozsoy *et al*., 2008). This goes to say that *C. iners* leaves has a good electron donating capability to scavenge free radicals. Flavonoids and phenolic compounds might contribute to the remarkable reducing activity of *C. iners* leaves as phenolic hydroxyl groups present in them act as a reducing agent by donating electron (Shahidi *et al*., 1992).

CONCLUSION

The present study clearly showed that *C. iners* leaves showed promising antioxidant activity and served as potential natural antioxidant source to prevent formation of free radicals.

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REFERENCES

Baumann, J., G. Wurn and F.V. Bruchlausen, 1979. Prostaglandin synthetase inhibiting O-2 radical scavenging properties of some flavonoids and related phenolic compounds. Arch. Pharmacol., 307: R1-R77.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.

Bursal, E. and I. Gulcin, 2011. Polyphenol contents and *in vitro* antioxidant activities of lyophilised aqueous extract of kiwi fruit (*Actinidia deliciosa*). Food Res. Int., 44: 1482-1489.

Choi, O.H., 2003. Tumbuhan Liar: Khasiat Ubatan and Kegunaan Lain. 1st Edn., Kuala Utusan Publications, Lumpur, pp: 132-133.

Chua, M.T., Y.T. Tung and S.T. Chang, 2008. Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. Bioresour. Technol., 99: 1918-1925.

Diplock, A.T., 1994. Antioxidants and disease prevention. Mol. Aspects Med., 15: 293-376.

Gulcin, I., 2012. Antioxidant activity of food constituents: An overview. Arch. Toxicol., 86: 345-391.

Kumaran, A. and R.J. Karunakaran, 2007. Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. Food Chem., 100: 356-361.

Madhavi, D.L., S.S. Deshpande and D.K. Salunkhe, 1996. Food Antioxidants: Technological, Toxicological and Health Perspectives. Marcel Decker, New York, USA., ISBN-13: 9780824793517, pp: 145-148.

Miller, M.J., H. Sadowska-Krowicka, S. Chotinaruemol, J.L. Kakkis and D.A. Clark, 1993. Amelioration of chronic ileitis by nitric oxide synthase inhibition. J. Pharmacol. Exp. Therapeut., 264: 11-16.

Mustaffa, F., J. Indurkar, S. Ismail, M.N. Mordi, S. Ramanathan and S.M. Mansor, 2010a. Analgesic activity, toxicity study and phytochemical screening of standardized *Cinnomomum iners* leaves methanolic extract. Pharmacogn. Res., 2: 76-81.

Mustaffa, F., J. Indurkar, S. Ismail, M.N. Mordi, S. Ramanathan and S.M. Mansor, 2010b. Antioxidant capacity and toxicity screening of *Cinnomomum iners* standardized leaves methanolic extract. Int. J. Pharmacol., 6: 888-895.

Mustaffa, F., J. Indurkar, S. Ismail, M. Shah and S.M. Mansor, 2011. An antimicrobial compound isolated from *Cinnamomum iners* Leaves with activity against methicillin-resistant *Staphylococcus aureus* Molecules, 16: 3037-3047.

Mustaffa, F., Z. Hassan, N.A. Yusof, K.N. Abdul Razak and M.Z. Asmawi, 2014a. Antidiabetic and antihyperlipidemic potential of standardized extract, fraction and subfraction of *Cinnamomum iners* leaves. Int. J. Pharm. Pharmaceut. Sci., 6: 220-225.

Mustaffa, F., Z. Hassan, N.A. Yusof, K.N. Abdul Razak and M.Z. Asmawi, 2014b. Antidiabetic mechanism of standardized extract, fraction and subfraction of *Cinnamomum iners* leaves. Int. J. Pharmaceut. Sci. Rev. Res., 26: 209-214.

Oyaizu, M., 1986. Studies on product of browning reactions: Antioxidant activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr., 44: 307-316.

Ozsoy, N., A. Can, R. Yanardag and N. Akev, 2008. Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem., 110: 571-583.

Pang, K.L., W.L. Thong and S.E. How, 2009. *Cinnamomum iners* as mitogen-activated protein kinase kinase (MKK1) inhibitor. Int. J. Eng. Sci. Technol., 1: 310-313.

Ruch, R.J., S.J. Cheng and J.E. Klaunig, 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10: 1003-1008.

Sakanaka, S., Y. Tachibana and Y. Okada, 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem., 89: 569-575.

Shahidi, F., P.K. Janitha and P.D. Wanasundara, 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr., 32: 67-103.

Subhasree, B., R. Baskar, R.L. Keerthana, R.L. Susan and P. Rajasekaran, 2009. Evaluation of antioxidant potential in selected green leafy vegetables. Food Chem., 115: 1213-1220.

WHO., 1991. Guidelines for the Assessment of Herbal Medicines. World Health Organization, Geneva, Switzerland.

Wolfe, K., X. Wu and R.H. Liu, 2003. Antioxidant activity of apple peels. J. Agric. Food Chem., 51: 609-614.

Wondrak, G.T., N.F. Villeneuve, S.D. Lamore, A.S. Bause, T. Jiang and D.D. Zhang, 2010. The cinnamon-derived dietary factor cinnamic aldehyde activates the Nrf2-dependent antioxidant response in human epithelial colon cells. Molecules, 15: 3338-3355.

Wu, L.C., H.W. Hsu, Y.C. Chen, C.C. Chiu, Y.I. Lin and J.A. Ho, 2006. Antioxidant and antiproliferative activities of red pitaya. Food Chem., 95: 319-327.

Zakaria, Z., R. Aziz, Y.L. Lachimanan, S. Sreenivasan and X. Rathinam, 2008. Antioxidant activity of *Coleus blumei*, *Orthosiphon stamineus*, *Ocimum basilicum* and *Mentha arvensis* from Lamiaceae family. Int. J. Nat. Eng. Sci., 2: 93-95.

Zou, Y., Y. Lu and D. Wei, 2004. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. J. Agric. Food Chem., 52: 5032-5039.