Quantitative Estimation of Phenolic and Flavonoids Contents and Antioxidant Activity of Aerial Parts of *Morina logifolia*

[*]D. P. Pandey
Department of Chemistry, Govt. Degree College Dehradun Shahar, Dehradun-248007, Uttarakhand, India.
[*]E.mail: pandeydp_123@rediffmail.com

DOI 10.51129/ujpah-2026-40-1(3)

Received –May 07, 2026
Revised – May 09, 2026
Accepted –May 15, 2026
Published – June 20, 2026

Abstract-Since ancient times plants have served as vital components of traditional, folk medicine, and fulfilling essential nutritional and dietary needs. The extracts of various plant species have been reported to possess anti-oxidant activities to scavenge free radicals. Therefore, the present study was aimed to determine the total phenolic, flavonoid content, free radical scavenging potential, and reducing power of chloroform extract (MCF), ethyl acetate extract (MEA), acetone extract (MAT) and ethanol extract (MAL) of aerial parts of *Morina longifolia*. The results of the present study showed that different extracts of aerial parts of *M. longifolia* showed significant antioxidant activity. The acetone extract of aerial parts showed maximum total phenolic contents (MAT 15.47 ± 0.187 mg GAE/100mg) while ethyl acetate showed maximum flavonoid contents (MEA 4.27 ± 0.086 mg quercetin/100mg). Maximum reducing power was observed in ethyl acetate extract (MEA 0.556 ± 0.003, at 500µg/ml) and highest free radical scavenging activity was observed in acetone extract (MAT 57.40 ± 0.592 at 500µg/ml).

Keywords: *Morina longifolia*, Falvonoid, Phenolic, Antioxidant Activity, DPPH, Reducing Power Assay.

Introduction

Plants are important source of medicines because they contain a wide range of secondary metabolites. The crude extracts of plants are used in traditional medical system while in modern and herbal medical systems plants have been exploited for the isolation of specific bioactive compounds[1,2]. Plants rich in phytochemicals like flavonoids, phenols, alkaloids, tannins, terpenoids shows many biological activity including antioxidant activity without any side effects[3,4]. Several studies have shown that the plant extracts obtained using different solvents such as methanol, ethanol and aqueous media exhibit potent antioxidant activities. The antioxidant activity is correlated with total phenolic and flavonoid content of the extracts. The extracts of various medicinal plant shows strong free radical scavenging activities comparable to synthetic antioxidant[5-9].

The genus *Morina* belongs to the family *Caprifoliaceae* formerly *Dipsacaceae* is

a genus of flowering plants. It includes 14 species natives to Eurasia ranging from southern Europe through Western and Central Asia. Morina species, namely *M. chinensis, M. chlorantha, M. kokonorica, M. longifolia, M. lorifolia and M. nepalensis* have been traditionally used in Tibetan, Himalayan and Central Asian systems of medicine. Phytochemical studies reveal the presence of iridoids, phenolic acids, flavonoids, terpenoids, aromatic glycosides, essential oils and other bioactive compounds which contribute to their wide-ranging pharmacological potential including anti-oxidant, anti-inflammatory, analgesic, anti-cancer, anti-microbial, antidiabetic and wound healing activities[10-15]. *M. longifolia* commonly known as 'Whorlflowers' is a small perennial herb, found in temperate and alpine regions of Himalayas from Kashmir to Bhutan at an altitude of 2400-4200 meters[16]. The antioxidant activity of *M. longifolia* of Uttarkashi region have not been carried out previously therefore the present study was aimed to determine the free radical scavenging activity and reducing potential of different extracts of aerial parts of *M. longifolia.*

Material and Methods

Preparation of extracts- The aerial parts of *M. longifolia* were collected from Dayara Bugyal (3300-3500 m asl), District Uttarkashi, Uttarakhand, India in August 2024. Plant species were identified by Dr. Pratibha Baluni, Department of Botany, Govt. Degree College Dehradun Shahar, Dehradun, Uttarakhand. The air dried and powdered aerial parts of *M. longifolia* was packed in a Soxhlet apparatus and extracted sequentially with chloroform, ethyl acetate, acetone and ethanol. The organic extracts were dried in a vacuum evaporator to obtain the chloroform extract (MCF), Ethyl acetate extract (MEA), Acetone Extract (MAT) and ethanol extract (MAL). The extracts were dissolved in dimethyl sulfoxide (DMSO) or ethanol prior to analysis depending upon their solubility. The extracts were subjected for further analysis and all the assays were done in triplicate

Determination of Total Phenolics and Total Flavonoid Contents- The total phenolic contents (TPC) of the different extracts of areal parts of *M. longifolia* was determined by modified Folin-Ciocalteu method [17] using gallic acid as standard. Folin-Ciocalteu reagent and Na_2CO_3 was added to the various extracts and after 20 min of incubation at room temperature, the mixture was centrifuged and the absorbance of the supernatant was measured at 730 nm. Total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg/g) (Table-1 and Figure-1).

The total flavonoid content (TFC) was determined by slightly modified colorimetric method [18]. The extracts were dissolved with suitable solvent and then potassium acetate and aluminium nitrate was added to it. After 40 min of incubation at room temperature, the absorbance was measured at 415 nm using quercetin as standard and results are presented in (Table-1 and Figure-1).

Table-1 Total phenolic and flavonoid contents of different extracts of aerial parts of *M. longifolia*

Extract	Total flavonoids mg Quercetin/100mg	Total phenolics mg GAE/100mg
MCF	0.70 ± 0.015	10.22 ± 0.095
MEA	4.27 ± 0.086	14.68 ± 0.124
(MAT)	3.70 ± 0.031	15.47 ± 0.187
(MAL)	3.59 ± 0.014	12.72 ± 0.280

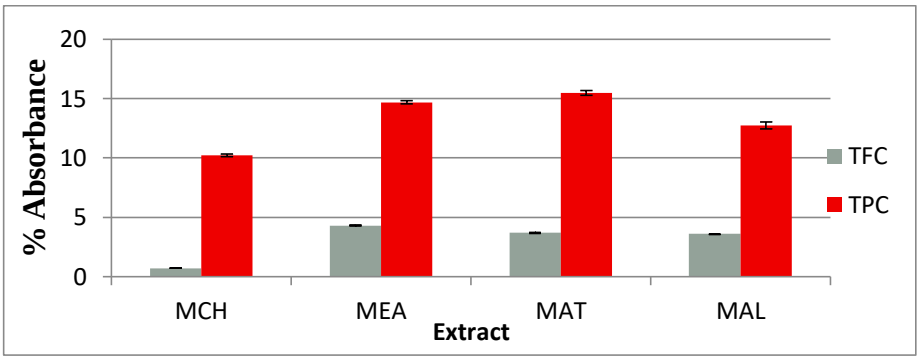

Figure- 1 Total Flavonoid and Phenolic Contents of different extracts of aerial parts of *M. longifolia*

Antioxidant Activity

Determination of reducing potential (RP)- In this method, the reduction potential of the extracts was carried out by reduction of potassium ferricyanide to potassium ferrocyanide using ascorbic acid as standard. Five concentrations (100, 200, 300, 400 and 500 µg/ml) of each extract were prepared by dissolving with suitable solvent and the reducing potential each extract was determined by the method described by Oyaizu, 1986 [19]. In one ml of each concentration of each extract 2.5 ml phosphate buffer and 2.5ml of potassium ferricyanide (10mg/ml) solution was added. The content was heated at 50^0C for 30 min and then 2.5 ml of 10% trichloroacetic acid was added to it and the mixture was centrifuged for 10 min at 3000-4000 rpm. In 2.5 ml of supernatant was diluted with 2.5 ml double distilled water and 0.5 ml of 0.1% $FeCl_3$ solution was added to it and the absorbance was recorded at 700 nm. The results were compared with ascorbic acid taken as positive control and represented as ascorbate equivalents (Table-2, Figure- 2).

Table-2 Reducing potential of different extracts of aerial parts of *M. longifolia*

CONC	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
STD	0.23	0.43	0.53	0.63	0.66
MCF	0.033 ± 0.000	0.070 ± 0.001	0.093 ± 0.000	0.105 ± 0.001	0.124 ± 0.002
MEA	0.116 ± 0.002	0.240 ± 0.003	0.336 ± 0.002	0.438 ± 0.004	0.556 ± 0.003
MAT	0.076 ± 0.002	0.162 ± 0.003	0.256 ± 0.002	0.339 ± 0.004	0.381 ± 0.006
MAL	0.108 ± 0.002	0.239 ± 0.004	0.309 ± 0.004	0.347 ± 0.003	0.367 ± 0.003

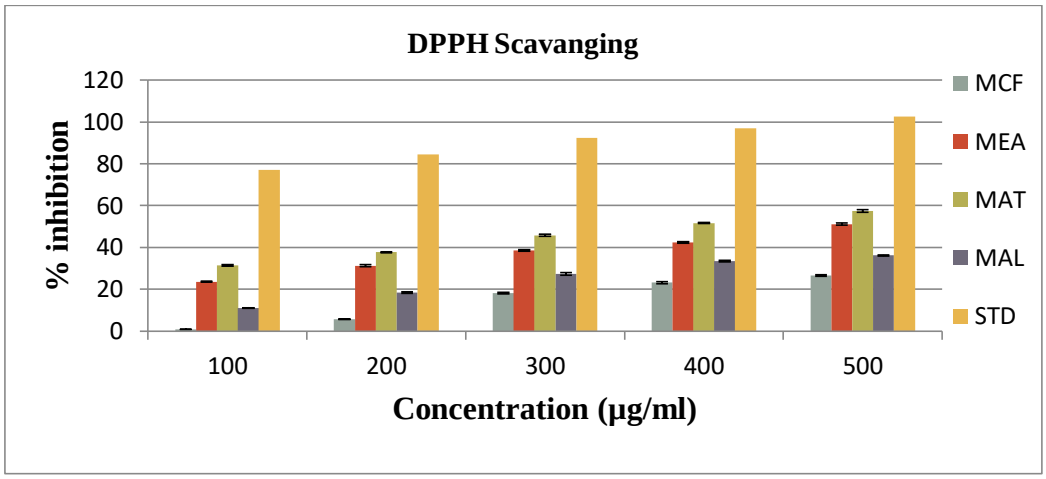

Figure-2 DPPH Assay of different extracts of aerial parts of *M. longifolia*

DPPH Radical Scavenging Activity (DPPH)- 2,2-Diphenyl-1-picrylhydrazyl commonly known as DPPH is composed of stable free radical molecules used as indicator for evaluation antioxidant activity of various compounds and extracts. It is deep blue coloured dyes changes to light yellow to colorless on reduction depending on anti-oxidant activity of the material under investigation. The redical scavenging activity was determined by the method described by Cuendet [20] with slight modifications. Five concentrations (100, 200, 300, 400 and 500 µg/ml) of each extract were prepared with suitable solvent and the antioxidant activity of

each plant extracts was assessed using the stable DPPH radical scavenging with ascorbic acid as standard. Two ml of freshly prepared ethanolic solution DPPH (0.1 M) solution was added to the different concentrations of the extracts. After 30 min of incubation in dark, the absorbance was recorded at 517 nm. The results were expressed as percentage inhibition of DPPH.

$$\% \text{ Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right] \times 100$$

The results were compared with ascorbic acid as the positive control and are presented in (Table-3, Figure-3).

Table-3 DPPH assay of different extracts obtained from aerial parts of *M. longifolia*

CONC.	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
STD	77.03	84.56	92.33	96.86	102.6
MCF	0.817 ± 0.014	5.747 ± 0.142	18.16 ± 0.463	23.22 ± 0.506	26.59 ± 0.292
MEA	23.55 ± 0.231	31.26 ± 0.421	38.49 ± 0.387	42.36 ± 0.364	51.17 ± 0.616
MAT	31.37 ± 0.360	37.76 ± 0.289	45.78 ± 0.506	51.67 ± 0.341	57.40 ± 0.592
MAL	11.10 ± 0.105	18.35 ± 0.409	27.33 ± 0.660	33.46 ± 0.336	36.17 ± 0.355

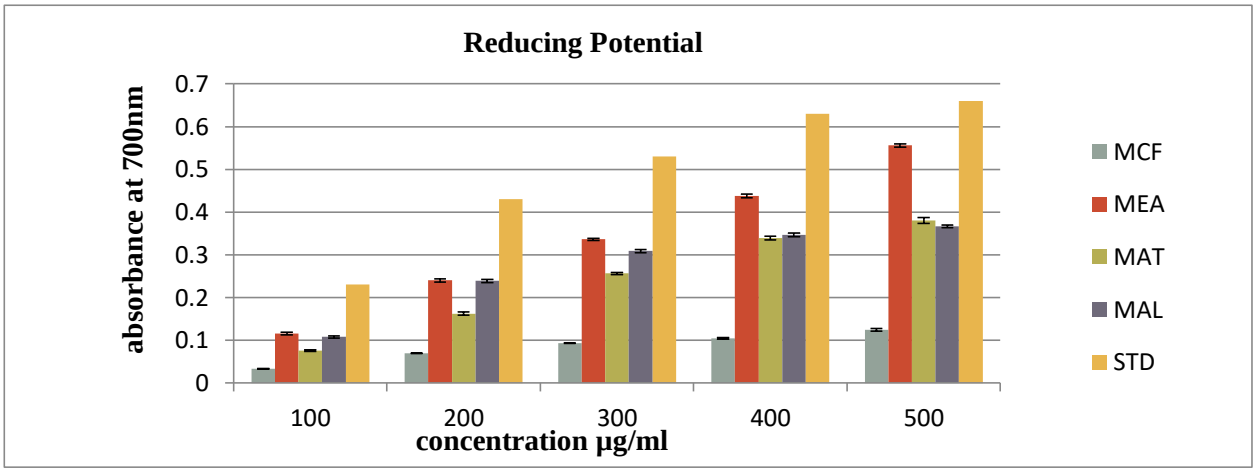

Figure-3 Reducing potential of different extracts of aerial parts of *M. longifolia.*

Results and Discussion

Total phenolic and flavonoids contents- The Phenolic substances and flavonoids have been shown to be responsible for the antioxidant activity of many of the plant species (Geetha et al., 2005) [21]. Therefore, the amount of total phenolics and total flavonoids present in the different extracts were investigated. The content of total phenolics is expressed as mg Gallic acid/100 mg of extract (GAE) and the content of total flavonoids as mg quercetin/100 mg of extract (QE) as shown in Table 1. The acetone extract (MAT) of *M. longifolia* was found to have the maximum content of total phenolics contents, 15.47 ± 0.187 mg (GAE/100 mg extract) and total flavonoids content was found in ethyl acetate extract (EA) 4.27 ± 0.086 mg QE/100 mg extract). *M.* Chloroform (MCF) extract had the lowest content of total phenolics content (10.22 ± 0.095 mg GAE/100 mg extract) as well as total flavonoids content (0.70 ± 0.015 mg QE/100 mg extract).

Reducing Power- The reducing potential of different extracts of *M. longifolia* was determine by using five different concentration of each extract and was compared with ascorbic acid as positive control. The reducing potential was found to be concentration dependent and the order of RP was found MCF<MAL<MAT<MEA. The MEA extract shows maximum reducing potential at the conc. of 500 µg/ml (**0.556 ± 0.003)** which is slightly less than the

standard (0.63). Minimum reducing potential was found in MCF extracts (0.033±0.001) at the concentration of 100 µg/ml.

DPPH Radical Scavenging Activity- Five different concentrations of each extract of *M. longifolia* were used to determine the DPPH scavenging activity using ascorbic acid as positive control. The free radical scavenging activity of MAT extracts of *M. longifolia* showed maximum activity whereas MCF showed minimum activity. It was found that the free radical scavenging activity of all extracts increases with the increase in concentrations. The order of DPPH scavenging activity of different extracts was found to be MCF<MAL<<MEA<MAT.

The result of present study showed that acetone and ethyl acetate extracts shows more reducing potential and DPPH scavenging activity then the other extracts which might be due to presence of polyphenolic and flavonoid compounds in these extracts. Our previous study on different extracts of *M. longifolia* revealed positive test for polyphenolic compounds in the acetone and ethyl extracts [22]. Previous study on antioxidant activity shows that extracts rich in polyphenolic compounds showed antioxidant activity. A study by Matheus et al. (2025) on antioxidant activity of *M. oleifera* showed that the leaf and seed oil extracts exhibited high antioxidant activity due to rich concentration of polyphenolic and flavonoid compounds [23].

Polyphenolic compounds and flavonoids represent one of the largest groups of secondary metabolites in plants which play a major role in reducing the oxidative stress. The antioxidant activity of these compounds is due to their ability to neutralize the free radicals, to coordinate with pro-oxidant metals and to modulates the antioxidant enzyme systems [24]. Based on the clinical evidences, diets rich in polyphenols and flavonoids are suggested to confer protective effects against chronic diseases, particularly cancer, diabetes, inflammatory disorders, nervous system diseases and cardiovascular diseases etc. [25-26].

The present study revealed the scavenging property of *M. longifolia* may be due to the presence of polyphenolic and flavonoid compounds that can scavenge the free radicals and chelate the pro-oxidant metals. The result of present study showed that acetone and ethyl acetate extracts which shows more reducing potential and DPPH scavenging activity then the other extracts.

Conclusion

From the above study it can be concluded that all the extracts of aerial parts of *M. longifolia* exhibited antioxidant activity but the extracts which are rich in phenolic and flavonoid contents shows more activity than the other extracts.

Acknowledgement

The author is thankful to Professor Surendra H. Bodkhe, Head Pharma-ceutical Division, Guru Gashidas Vishwavidyalay (CG), India, for his valuable suggestions.

Disclaimer Statement

Author declares that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Parveen, A.; Parveen, R.; Akhatar, A.; Parveen, B.; Siddiqui, K.M. and Iqbal M. Concepts and quality considerations in Unani system of medicine. *Journal of AOAC International* 2020; 103: 609-33

2. Maher, PA. Plant-based Medicine and Pharmacology: Using Plants as a Source of Potential Therapeutics for the Treatment of Alzheimer's Disease. *The Yale J of Biology and Medicine* 2020; 93(2): 365.

3. Forni, C.; Facchiano, F.; Bartoli, M.; Pieretti, S.; Facchiano, A.; D'Arcan-gelo, D.; Norelli, S.; Valle, G.; Nisini, R.; Beninati, S. and Tabolacci, C. Ben-eficial role of phytochemicals on oxi-dative stress and age-related diseases. *Bio. Med. Research International*; 2019; doi. Org/10.1155/2019/8748253.

4. Tran, N.; Pham, B. and Le, L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology*; 2020; 9(9): 252.

5. Oyeyinka, B.O. and Afolayan, AJ. Comparative and Correlational Eva-luation of the phytochemical consti-tuents and antioxidant activity of Musa sinensis L. and Musa paradisiaca L. fruit compartments (Musaceae). *The Scientific World Journal;* 2020; doi. Org /10.1155/2020/4503824.

6. Lesjak, M.; Beara, I.; Simin, N.; Pintac, D.; Majkic, T.; Bekvalac, K.; Orcic, D. and Mimica, D.N. Antioxidant and anti-inflammatory activities of quer-cetin and its derivaties. *J. of Functional Food*; 2018; 40:68-75.

7. Beatrice, M.G.; Alex, K.M.; Stephen, K.M. and Mathew, P.N. In-vitro anti-oxidant activities of methanolic ex-tracts of *Caesalpinia volkensii* Harms., *Vernonia lasiopus* and *Acacia hockii*. *Evidence Base Complementary and Alternative medicine;* **2020**; Article-ID 3586268, 10 (2020). Doi.org/10.1155/ 2020/3586268.

8. Mortada, M.El.S.; Maher Mel-H, Heba R.M. and Ezzar, El-SA.L. Phyto-chemical investigation and in-vitro antioxidant activity of different leaf extracts of *Salix thumb*., *Journal of Applied Pharmaceutical Science*., 2015, 5(12); 80-85.

9. Nwozo, O.S.; Effiong, E.M.; Aja P.M. and Awuchi C.G. Antioxidant phyto-chemical and therapeutic properties of medicinal plants. A review. *Inter-national Journal of Food Properties*; 2023; 26(1); 359-388.

10. Kumar, A.; Varshney, V.K.; Rawat, M.S.M.; Martinez, J.R. and Stashenko, E.E. Chemical composition of the essential oils of *M. longifolia* Wall. Leaves. *Journal of Herbs, Spices and Medicinal Plants*.; 2013; 19(4):348-356.

11. Bodkhe, S.H.; Ram, A. and Pandey, D.P. A new aromatic glycoside from *M. longifolia*, *Asian Journal of Chemistry;* 2010; 22(4):2789-2793.

12. Teng, R.W.; Xie, H.; Liu, X.; Wang, D. Z. and Yang, C.R. A novel acylated flavonol glycoside from *M. nepalensis*. *Fitoterapia*.; 2002; 73: 95-96.

13. Teng, R.W.; Xie, H.Z.; Liu, X.; Wang, D.Z. and Yang, C.R.; Two new acy-lated flavonoid glycosides from *M. nepalensis*. *Magnetic Resonance in Chemistry*; 2002; 40(6):415-420.

14. Teng, R.W.; Xie, H.Z.; Wang, D.Z. and Yang, C.R. Four new ursane type

saponins from *M. nepalensis*. *Magnetic Resonance in Chemistry*; 2002; 40(6): 603-608.

15. Fuderaro, T.A. and Stemitz, F.R. (5αH)-6-Epidihydrocornin, the first known iridoid glycoside with a trans-fused system. *Tetrahedron Letters*.; 1992; 33(21):2953-2954.

16. Pratham, S.; Kharazi, A.Z.; Bakhs-heshi, R.H.R.; Nur, H.; Ismail, A.F.; Sharif, S.; Krishna, S.R.R. and Berto, F. Anti-oxidant, Anti-microbial and anti-viral properties of herbal mate-rials. *Antioxidants*; 2020; 9:1309-1321.

17. Nurmi, K.; Ossipov, V.; Haukioja, E. and Pihlaja, K. Variation of total phe-nolic content and individual low-mole-cular-weight phenolics in foliage of mountain birch trees (Betula pubescens sp. Tortuosa). *J. Chem. Ecol*.; 1996; 22: 2023-2040.

18. Zhishen, J.; Mengcheng, T. and Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*; 1999; 64: 555-559.

19. Oyaizu, M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japan Journal of Nutrition;* 1986; 44: 307-15.

20. Cuendet, M.; Hostettmann, K; Potterat, O and Dyatmiko, W. Iridoid glucosides with free radical scavenging properties from Fagraeablumei. *Helvetica. Chim-ica. Acta.;* 1997; 80(4): 1144-52.

21. Geetha, T.; Malhotra, V.; Chopra K. and Kaur, IP. Anti-mutagenic and antioxidant/ proxidant activity of quercetin. *Indian J. Exp. Biol.;* 2005; 43:61-67.

22. Pandey, D. P. Anti-Microbial Activity of Aerial Parts of *Morina longifolia. UJPAH;* 2025; 39(2):32-37.

23. Matheus, C. de B.; Victoria, C. de S.S.; Ana GBS., Jacinto da CSN; Julliano, M. de, L.M.; Patryck, E.M dos S.; Wellington, de A.O.; Luana, C.B.B.C.; Mariana, P.F.; Alisson, M de O.; Thamarah de, A.L.; Thiago, H.N. and Patricia, M.G.P. Antioxidant activity and chemoprotective effect of M. oleifera Lam. Leaf infusion against methotrexate-induced damage in mice. *Toxicology Reports*; 2025; 14:102000.

24. Yordi, E.G.; Perez, E. M.; Matos M.J. and Villares, U.V. Antioxidant and pro-oxidant effects of polyphenolic com-pounds and structure activity relation-ship evidence. In Nutrition, Well-Being and Health. Bouvaed Intech-open; London; U.K.; 2012; pp. 24-41.

25. Huang, D.; Ou, B. and Prior, R.L. The chemistry behind antioxidant capacity assays. *J. of Agri. and food Chemistry*; 2005; 53(6): 1841-18.

26. Sanchez, M.C. Review: Method used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International;* 2002; 8(3):121-137.