Research Article

In vitro Free Radical Scavenging Activities of Ethanolic Extracts of Leaf and Fruit of *Trichosanthes dioica* Roxb. and Leaf of *Clitoria ternatea* L.

R. Kavitha*
*Department of Biochemistry, Vellalar College for Women, (Autonomous), Thindal, Erode, Tamil Nadu, India.
***Corresponding author's E-mail:** erokavi_vasu@yahoo.com

Accepted on: 08-05-2014; **Finalized on:** 30-06-2014.

ABSTRACT

Trichosanthes dioica and *Clitoria ternatea* are medicinal plants used in Ayurveda and Siddha medicine for the treatment of various diseases. The ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* were determined quantitatively for total phenolic content and flavonoids. And their antioxidant activity were investigated for its free radical scavenging activity by using different *in vitro* antioxidant models like DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical, hydroxyl radical and superoxide radical scavenging activity, reducing power assay and total antioxidant capacity by Phosphomolybdenum method. Maximum total phenolics and flavonoid content were present in leaf of *C.ternatea*. The extracts showed free radical scavenging activity in dose dependent manner and quantity of total phenolics and flavonoid present. *C.ternatea* leaf showed the highest scavenging activity when compared to leaf and fruit of *T.dioica*.

Keywords: Antioxidant, *Clitoria ternatea*, Free radicals, *Trichosanthes dioica*.

INTRODUCTION

Oxidative stress, an imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body, is closely associated with number of diseases.[1] Oxygen free radicals initiate peroxidation of lipids, which in turn stimulates glycation of proteins and inactivation of antioxidant enzymes. Antioxidants are substances that delay or prevent the oxidation reactions. It plays an important role to protect the human body against damage caused by ROS.[2] Antioxidants stop the free radical generation by trapping the free radicals and thus they inhibit the chain reactions which can lead to destruction of healthy cells.[3]

Certain natural antioxidants present in plants have been shown to reduce oxidative stress and the development of major diseases.[4] Numerous studies have been carried out on many plants, vegetables and fruits because they are rich sources of antioxidants such as vitamin A, C, E, polyphenolic compounds and flavonoids which prevent free radical damage and reducing risk of chronic diseases such as diabetes, cirrhosis, nephrotoxicity etc.,[5,6] Hence, the study of antioxidant status during the presence of free radical can be used as an index of protection against the development of degenerative processes under experimental condition for therapeutic measures.[7]

Trichosanthes dioica Roxb (family: Cucurbitaceae) is a dioecious perennial plant, grown throughout India and it is known as the pointed gourd. It is mainly cultivated as a vegetable crop. It has been used for overcoming problems like constipation, fever, skin infection and wounds. The fruits are used as a remedy for spermatorrhoea and also used for reduce body temperature and as a laxative.[8] The leaves are easily digestable and used for the preparation of syrup for convalescents and good for maintaining healthy heart and brain.

Clitoria ternatea Linn (family: Fabaceae) is a perennial twining herb found in India, China, Philippines and Madagascar. It is commonly called Shankpushpi.[9] In traditional Ayurvedic medicine, it has been used as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent.[10] The root extracts was used traditionally to cure whooping cough, infertility, gonorrhea, to control menstrual discharge and also as an aphrodisiac.[11]

The present study was done to investigate the flavonoids, total phenolics and antioxidant (free radical scavenging) activities in ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea*.

MATERIALS AND METHODS

Collection and authentication of plant materials

Fresh unripe fruit and leaf of *Trichosanthes dioica* Roxb. (*T.dioica*) and the leaf of *Clitoria ternatea* L. (*C.ternatea*) were collected from SKM Herbal Research Centre, Erode, Tamil Nadu, India. The plants were identified and authenticated by Dr. V.R.Mohan, Associate Professor, Department of Botany, V.O.Chidambaram College, Tuticorin. A voucher specimen (No. VOCB 2307 and VOCB 2453) was deposited in Ethno pharmacology Unit, Research Department of Botany, V.O.C College, Tuticorin, Tamil Nadu, India.

Preparation of the plant extracts

Freshly collected leaf and fruit of *T.dioica* and leaf of *C.ternatea* were washed with distilled water and the fruits were cut into small pieces. Both fruits and leaves

were dried under shade for several days. The shade dried leaves and fruits were coarsely powdered separately. The powdered materials were kept in airtight containers until use.

About 500 gm of dried coarse powdered samples were weighed and subjected to 1250 ml of ethanol in a Soxhlet extractor for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper separately and the extracts were concentrated in vacuum at 60ºC using a rotary evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50ºC for 8 hrs. Later the extracts were used for quantitative phytochemical analysis and *in vitro* antioxidant studies.

Quantitative phytochemical analysis

Flavonoids and total phenolics are considered to be the most important phytochemicals that are responsible for the pharmacological activities. Flavonoids and total phenolic content were estimated[12,13] respectively.

In vitro antioxidant activity

Antioxidant studies were performed by DPPH radical scavenging method[14], hydroxyl radical scavenging method[15], superoxide radical scavenging method[16], reducing power assay[17] and total antioxidant capacity by Phosphomolybdenum method.[18]

Statistical analysis

In vitro antioxidant assays and measurement values are expressed as means of triplicate analysis of the samples (n=3) ± SD.

Calculation of 50% inhibitory concentration (IC_{50})

The concentration (mg/dl) of the plant extracts required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition was calculated using the formula

Percentage inhibition =

$$\frac{\text{Absorbance of the control - Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

RESULTS

Quantification of phytochemicals

The quantitative analyses of phytochemicals such as flavonoids and total phenolics in the ethanolic extracts of the investigated plants were given in Table 1.

DPPH radical scavenging activity

The radical scavenging activity of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* were tested using the 'stable' free radical, DPPH was shown in Table 2. Among the three different ethanolic extracts, the highest percentage of scavenging activity on DPPH was seen in *C.ternatea* leaf followed by *T.dioica* fruit and least activity was seen in *T.dioica* leaf at 300 µg/ml concentration. The IC_{50} value of the ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* was 256.41, 205.76 and 147.92 respectively (lower IC_{50} value indicates higher antioxidant activity).When compared to the standard tannic acid, IC_{50} value was higher in the test samples.

Table 1: Quantitative analysis of phytochemicals in ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea*

Phytochemicals	*T.dioica*		*C.ternatea* leaf
	Leaf	Fruit	
Flavonoids (mg RE/ gm extract)	36.1±2.02	48.2±1.06	74.5±3.03
Total phenolics (mg GAE/ gm extract)	26.0±0.03	38.1±0.03	98.2±1.02

Hydroxyl radical scavenging activity

Hydroxyl radicals are major active oxygen species causing lipid peroxidation and enormous biological damage. Hydroxyl radical scavenging activity of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* were shown in Table 3.

Among the studied plant samples, ethanolic extract of *C.ternatea* leaf exhibited highest hydroxyl radical scavenging activity followed by fruit and leaf of *T.dioica*. When compared to the standard mannitol, IC_{50} value was higher in the test samples.

Superoxide radical scavenging activity

Superoxide radical plays an important role in plant tissues and it is involved in the formation of other cell damaging free radicals. The leaf and fruit of *T.dioica* and leaf of *C.ternatea* extracts were subjected to superoxide radical scavenging assay and the results were shown in Table 4.

The results showed that ethanolic extract of *C.ternatea* leaf (300 µg/ml) exhibited the maximum superoxide radical scavenging activity of 39.75±0.54% than the other two extracts. The IC_{50} value of the ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* and standard (quercetin) in this assay was 555.55, 495.04, 387.59 and 52.1 respectively.

Reducing power assay

Table 5 showed the reducing ability of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea*. Absorbance of the solution increased with an increased concentration of the sample. At 300 µg concentrates, the *C.ternatea* leaf extract showed the highest reducing activity followed by *T.dioica* fruit extract and least activity was seen in *T.dioica* leaf extract. The increased absorbance at higher concentration indicated the strong reducing power potential of the extracts.

Table 2: Effect of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* on DPPH radical scavenging activity

Sample Concentration (µg/ml)	DPPH radical scavenging activity (%)				
	Sample			Standard Concentration (µg/ml)	Standard - Tannic acid
	T.dioica		*C.ternatea* leaf		
	Leaf	Fruit			
60	13.34±1.23	15.51±1.85	28.71±1.01	2	16.09±2.6
120	22.32±0.76	33.15±1.21	42.94±0.56	4	28.84±0.7
180	34.28±1.72	42.82±2.00	54.03±0.56	6	33.26±1.2
240	48.76±1.62	58.63±0.91	69.74±1.01	8	36.87±0.7
300	57.30±1.43	71.77±1.28	72.02±0.76	10	41.61±1.6
IC_{50} (µg/ml)	256.41	205.76	147.92	IC_{50} (µg/ml)	13.1

Values are mean of three independent analyses of the extract ± standard deviation (n = 3)

Table 3: Effect of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* on hydroxyl radical scavenging activity

Sample Concentration (µg/ml)	Hydroxyl radical scavenging activity (%)				
	Sample			Standard Concentration (µg/ml)	Standard - Mannitol
	T.dioica		*C.ternatea* leaf		
	Leaf	Fruit			
60	12.30±0.51	13.10±1.82	15.95±0.34	10	16.03±0.1
120	25.04±1.54	25.97±1.43	31.91±1.26	20	18.11±1.1
180	36.58±0.53	39.67±0.34	45.65±0.92	30	33.11±1.2
240	48.97±0.51	50.80±1.43	61.81±1.21	40	55.12±1.1
300	58.29±0.90	64.13±1.37	76.16±0.34	50	67.55±1.2
IC_{50} (µg/ml)	251.25	233.64	195.31	IC_{50} (µg/ml)	38.3

Values are mean of three independent analyses of the extract ± standard deviation (n = 3).

Table 4: Effect of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* on superoxide radical scavenging activity

Sample Concentration (µg/ml)	Superoxide radical scavenging activity (%)				
	Sample			Standard Concentration (µg/ml)	Standard - Quercetin
	T.dioica		*C.ternatea* leaf		
	Leaf	Fruit			
60	5.45±0.54	6.59±0.69	7.43±0.53	10	9.1±0.1
120	10.24±0.59	12.88±0.59	15.08±0.62	20	18.2±0.11
180	15.65±0.67	17.85±0.89	22.38±0.53	30	22.1±0.21
240	21.37±0.52	23.79±0.87	30.56±0.46	40	35.2±0.13
300	27.79±0.66	30.43±0.66	39.75±0.54	50	48.3±0.14
IC_{50} (µg/ml)	555.55	495.04	387.59	IC_{50} (µg/ml)	52.1

Values are mean of three independent analyses of the extract ± standard deviation (n = 3).

Total antioxidant capacity by Phosphomolybdenum method

The result of Phosphomolybdenum assay was presented in Table 6. The ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* have recorded 179.22±6.04, 185.01±4.00 and 302.51±5.23 mg ascorbic acid eq/gm extract respectively.

DISCUSSION

Several methods have been developed to estimate the antioxidant capacity of different plant materials.[19] A single assay is not sufficient to evaluate the total antioxidant activity.[20]

DPPH radical scavenging is considered to be good *in vitro* model widely used to assess antioxidant efficacy of single compound as well as for different plant extracts within a very short period of time.[21] Unlike laboratory generated

free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelating and enzyme inhibition.[22,23] In radical form, DPPH disappears on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting in the colour change from purple to yellow, due to the formation of diphenyl picryl hydrazine. It could be taken as an indication of the hydrogen donating ability of the extracts.[24,25]

Table 5: Reducing power assay of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea*

Sample Concentration (µg/ml)	Absorbance (700 nm)		
	***T.dioica* leaf**	***T.dioica* fruit**	***C.ternatea* leaf**
60	0.009±0.004	0.004±0.002	0.099±0.006
120	0.014±0.003	0.050±0.007	0.352±0.005
180	0.048±0.002	0.109±0.004	0.506±0.004
240	0.073±0.004	0.134±0.004	0.733±0.002
300	0.101±0.005	0.169±0.002	0.926±0.006

Values are mean of three independent analyses of the extract ± standard deviation (n = 3).

Table 6: Phosphomolybdenum assay of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea*

Sample	Phosphomolybdenum (mg ascorbic acid eq/g extract)
T.dioica leaf	179.22 ± 6.04
T.dioica fruit	185.01 ± 4. 00
C.ternatea leaf	302.51 ± 5.23

Values are mean of three independent analyses of the extract ± standard deviation (n = 3).

In the present study, the percentage of DPPH scavenging effect increases with the concentration of samples in 60 µg to 300 µg in all the samples. As compared to 60 µg concentrations, 300 µg concentrations of leaf (76.71%) and fruit (78.38%) of *T.dioica* and leaf of *C.ternatea* (60.13%) increased DPPH radical scavenging activity. Among the extracts, the leaf extract of *C.ternatea* appeared to have the highest potential for DPPH radical scavenging activity indicated by the lowest IC_{50} value. The results indicated that the extracts with their proton donating ability, could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.[26]

Hydroxyl radical scavenging activity

Hydroxyl radicals are one of the quick indicators of the lipid peroxidation process by abstracting hydrogen atom from unsaturated fatty acids or simply auto-oxidation of polyunsaturated fatty acids found primarily in membranes.[27] Scavenging of hydroxyl radicals is an important antioxidant activity, because of its very high reactivity, which can easily cross the cell membranes at specific sites and reacts with most of the biomolecules and furthermore cause tissue damage and cell death. Thus, removing hydroxyl radical is very important task for the protection of living systems.[28]

In the present study, hydroxyl radical scavenging activity of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* showed a dose dependent hydroxyl radical scavenging activity. Out of the five concentrations tested for hydroxyl radical scavenging activity, higher concentrations (240 and 300 µg/ml) have demonstrated good hydroxyl radical scavenging activity. 120 and 180 µg/ml concentrations showed slightly better inhibition than 60 µg concentration. As compared to the values of *T.dioica* leaf and fruit, *C.ternatea* leaf has recorded 23.46% and 15.79% respectively increased hydroxyl radical scavenging activity at 300 µg/ml concentration along with increased inhibition capacity. IC_{50} values of the extracts were more than the standard mannitol. The values of DPPH radical scavenging activity was more or less equal to the values of hydroxyl radical scavenging activity.

Superoxide radical scavenging activity

Superoxide anions are the most common free radicals in *in vivo*. It is produced from molecular oxygen due to oxidative enzymes of body as well as *via* non-enzymatic reaction such as auto-oxidation by catecholamines.[29]

Superoxide anion is a reduced form of molecular oxygen and is generated in a variety of biological systems. Mitochondria generate energy by using an electron transport chain reaction, reducing molecular oxygen to water. Some of the electrons escapes from the chain reaction of mitochondria and directly react with oxygen and form superoxide anion. It plays an important role in the formation of more dangerous reactive oxygen species, including hydrogen peroxide, hydroxyl radical and singlet oxygen that have potential for reacting with biological macromolecules including lipids, proteins and DNA and thereby, inducing tissue damage.[30,31] The concentration of superoxide anions increase under conditions of oxidative stress.[32] Overproduction of superoxide anion radical contributes to redox imbalance and associated with harmful physiological consequences.[33]

In the current study, the ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* were shown to have significant superoxide radical scavenging activity and it was dose dependent. As compared to the values of leaf and fruit of *T.dioica*, *C.ternatea* leaf recorded 30.08% and 23.44% respectively increased superoxide radical scavenging activity at 300 µg/ml concentration. The plant extracts scavenge superoxide radicals by combining with superoxide radical ions to form stable radicals, thus terminating the radical chain reaction.[34]

The highest antioxidant activity was noted in the extract of *C.ternatea* leaf followed by *T.dioica* fruit and leaf respectively. It might be due to the presence of good amount of phenolics and flavonoids in *C.ternatea* leaf

when compared to other two samples. Many researchers have reported close relationship between phenolic content and antioxidant activity of the plant extracts.[35,36] Superoxide radical scavenging activity is increased with increasing flavonoid content.[37] This study showed correlation between antioxidant activity and flavonoids and phenolic content of the plant extracts. But there are some reports that showed no correlation between antioxidant activity and phenolic content of certain medicinal plants.[38]

Reducing power assay

In reducing power assay, the presence of antioxidants in the sample reduced Fe^{3+}/ ferricyanide complex to the ferrous form. This assay measures the electron-donating capacity of an antioxidant.[39,40] This reducing capacity of compounds could serve as an indicator of potential antioxidant properties and increase in absorbance could indicate an increase in reducing power.[41] In the present investigation, a higher absorbance was seen in the samples which indicated a higher reducing power. All the studied plant samples exhibited good reducing activity. But, the ethanolic extract of leaf of *C.ternatea* was shown to have significant reducing power. It may be suggested that the ethanolic extracts of test plants have high redox potentials and can act as reducing agents.

Higher amounts of reductone, could react with free radicals to stabilise and block radical chain reactions.[42] Reductants may serve as a significant indicator of the antioxidant capacity.[43]

Total antioxidant capacity

Phosphomolybdenum assay is based on the reduction of Mo (VI) to Mo (V) in the presence of antioxidant compounds and the subsequent formation of a green phosphate Mo (V) complex at acidic pH. In the present analysis, when compared to leaf and fruit of *T.dioica,* leaf of *C.ternatea* has 40.75% and 38.84% respectively higher ascorbic acid equivalents. The good antioxidant activity might be attributed to the presence of phytochemicals present in the ethanolic extracts of leaf of *C.ternatea* followed by fruit and leaf of of *T.dioica*.

Phytochemicals have long been recognized to possess many properties including antioxidant, anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic effects.[44] They can have complementary and overlapping mechanisms of action in the body including, modulation of detoxification enzymes, delay of the oxidation process, inhibition of the polymerization chain initiated by free radicals and other subsequent oxidizing reactions, stimulation of the immune system and modulation of hormone mechanism.[45] As plants produce significant amount of antioxidants to prevent the oxidative stress caused by protons and oxygen, they represent a potential source of new compounds with antioxidant activity.

CONCLUSION

Based on the results obtained in the present investigation, it can be concluded that ethanolic extract of leaf of *C.ternatea* appeared to have the highest potential for free radical scavenging activity than other two test samples. This may be attributed to the presence of good amounts of bioactive compounds (flavonoids and total phenolics) that may serve as effective antioxidants.

REFERENCES

1. Halliwell B, Free radicals, antioxidants and human disease: Curiosity, cause or consequence, The Lancet, 344, 1994, 721-724.
2. Baynes JW, Role of oxidative stress in development of complications in diabetes, Diabetes, 40, 1991, 405-412.
3. Scartezzini P, Speroni E, Review on some plants of Indian traditional medicine with antioxidant activity, Journal of Ethnopharmacology, 71(1-2), 2000, 23-43.
4. Osawa T, Mechanism of ageing and development, Elseiver, 3, 1999, 133-139.
5. Marx JL, Oxygen free radicals linked to many diseases, Science, 235, 1987, 529-531.
6. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Viña-Ribes J, Functional food science and defence against reactive oxidative species, British Journal of Nutrition, 80(1), 1998, S77-112.
7. Fathi H, Ebrahimzadeh MA, Antioxidant and free radical scavenging activities of *Hypericum perforatum* L. (St. John's wort), International Journal of Forest soil and Erosion, 3(2), 2013, 68-72.
8. Kirtikar KR, Basu BD, Indian Medicinal Plants, Edition II, Vol II, Jayyed Press, Allahabad, 1975, 1110-1111.
9. Kulkarni C, Pattanshetty JR, Amruthraj G, Effects of alcoholic extract of *Clitoria ternatea* Linn. on central nervous system in rodents, Indian Journal of Experimental Biology, 26, 1988, 957-960.
10. Mukherjee PK, Kumar V, Kumar NS, Heinrich M, The Ayurvedic medicine *Clitoria ternatea* from traditional use to scientific assessment, Journal of Ethanopharmacology, 120, 2008, 291–301.
11. Fantz, Paul R, Ethnobotany of *Clitoria* (*Leguminosae*), *Economic Botany* (New York Botanical Garden Press), 45(4), 1991, 511-520.
12. Zhishen J, Mengcheng T, Jianming W, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chemistry, 64, 1999, 555-559.
13. Lachman J, Hamouz K, Orsak M, Pivec V, Potato tubers as a significant source of antioxidant human nutrition, Rostl Vyr, 46, 2000, 231-236.
14. Blois MS, Antioxidant determination by the use of a stable free radical, Nature, 181, 1958, 1199-1200.
15. Halliwell B, Gutteridge JMC, Aruoma OI, The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals, Analytical Biochemistry, 165, 1987, 215-219.
16. Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B, Antioxidant activity of *Caesalpinia digyna* root, Journal *of* Ethnopharmacology, 113, 2007, 284-291.
17. Kumar RS, Hemalatha S, *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites, Journal of Chemical and Pharmaceutical Research, 3, 2011, 259-267.
18. Prieto P, Pineda M, Aguilar M, Spectrophotometric quantification of antioxidant capacity through the formation of a

phosphomolybdenum complex: specific application of vitamin E, Analytical Biochemistry, 269, 1999, 337-341.

19. Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y, Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay, Nutrition Research, 23, 2003, 1719–1726.

20. Frankel EN, Meyer AS, The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants, Journal of Agricultural Food Science, 80, 2000, 1925–1941.

21. Katalinic V, Milos M, Kusilic T, Jukic M, Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols, Food Chemistry, 94, 2006, 550-557.

22. Yen GC, Hung CY, Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian-tsao (*Mesona procumbens* Hemsl.), Food Research International Journal, 33, 2000, 487–492.

23. Mau JL, Lin HC, Song SF, Antioxidant properties of several speciality mushrooms, Food Research International Journal, 35, 2002, 519–526.

24. Oktay M, Guloin I, Kufrevioglu OI, Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts, *LWT* – Food Science and Technology, 36, 2003, 263–271.

25. Shon MY, Kim TH, Sung NJ, Antioxidants and free radical scavenging activity of *Phellinus baumii* (Phellinus of *Hymenochaetaceae*) extracts, Food Chemistry, 82, 2003, 593-597.

26. Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen UP, Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements, Sensors, 7, 2007, 2080–2095.

27. Kappus H, Lipid peroxidations mechanism and biological relevance, In free radicals and food additives, Aruoma O.I., Halliwell B., Eds.; Taylor and Francis: London, U.K, 1991, 59-75.

28. Yang CH, Li RX, Chuang LY, Antioxidant activity of various parts of *Cinnamomum cassia* extracted with different methods, Molecules, 17, 2012, 7294-7304.

29. Naskar S, Islam A, Mazuumder UK, Saha P, Haldar PK, Gupta M, *In vitro* antioxidant potential of hydromethanolic extract of *Phoenix dactylifera* fruits, Journal of Scientific Research, 2, 2010, 144-157.

30. Halliwell B, Gutteridge JMC, Oxygen toxicology, oxygen radicals, transition metals and disease, Biochemistry Journal, 219, 1984, 1-4.

31. Lee J, Koo N, Min DB, Reactive oxygen species, aging and antioxidative neutraceuticals, Comprehensive Reviews *in* Food Science and Food Safety, 3, 2004, 21-33.

32. Lee JC, Kim HR, Kim J, Jang YS, Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. *saboten,* Journal of Agricultural and Food Chemistry, 50(22), 2002, 6490-6496.

33. Pervaiz S, Clement M, Superoxide anion: oncogenic reactive oxygen species, International Journal of Biochemistry and Cell Biology, 39, 2007, 1297-1304.

34. Wang T, Jonsdottir R, Olafsdottir G, Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds, Food Chemistry, 116, 2009, 240-248.

35. Velioglu YS, Mazza G, Gao L, Oomah BD, Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, Journal of Agricultural and Food Chemistry, 46, 1998, 4113-4117.

36. Deighton N, Brennan R, Finn C, Davies HV, Antioxidant properties of domesticated and wild Rubus species, Journal of Science Food Agriculture, 80, 2000, 1307-1313.

37. Choudhary RK, Swarnkar PL, Antioxidant activity of phenolic and flavonoid compounds in some medicinal plants of India, Natural Product Research, 25(11), 2011, 1101-1109.

38. Saikia LR, Sristisri Upadhyaya, Antioxidant activity, phenol and flavonoid content of some less known medicinal plants of Assam, International Journal of Pharma and Biosciences, 2(2), 2011, 383-388.

39. Yen GC, Chen HY, Antioxidant activity of various tea extracts in relation to their antimutagenicity, Journal of Agricultural and Food Chemistry, 43, 1995, 27–32.

40. Hinneburg I, Dorman HJD, Hiltunen R, Antioxidant activities of extracts from selected culinary herbs and spices, Food Chemistry, 97, 2006, 122-129.

41. Umamaheswari M, Chatterjee TK, *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract, African Journal of Traditional, Complementary and Alternative Medicine, 5, 2008, 61-73.

42. Isabel Ferreira CFR, Paula Baptista, Miguel Vilas-Boas and Lillian Barros, Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity, Food Chemistry, 100, 2007, 1511–1516.

43. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V, Comparison of antioxidant and antimicrobial activities of *Tilia* (*Tilia argentea* Desf ex DC), sage (*Salvia triloba* L.), and Black tea (*Camellia sinensis*) extracts, Journal of Agricultural and Food Chemistry, 48, 2000, 5030-5034.

44. Eastwood MA, Interaction of dietary antioxidant *in vivo*: how fruit and vegetables prevent disease, Quarterly Journal of Medicine, 92(9), 1999, 527-530.

45. Halliwell B, Aruoma OI, DNA damage by oxygen derived species, Its mechanism, measurement and significance, American Journal of Clinical Nutrition, 57, 1991, 715-725.

Source of Support: Nil, **Conflict of Interest:** None.