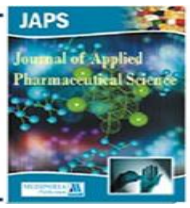
Journal of Applied Pharmaceutical Science
Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 12-07-2012
Revised on: 18-07-2012
Accepted on: 23-07-2012
DOI: 10.7324/JAPS.2012.2722

Screening of *in vitro* antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae)

Jamuna, S., S. Paulsamy and K. Karthika

Jamuna, S., S. Paulsamy and K. Karthika
Department of Botany, Kongunadu Arts and Science College, Coimbatore, India

For Correspondence
S. Paulsamy
Email: paulsami@yahoo.com

ABSTRACT

The aim of present study was to estimate the total phenolic and flavonoid contents and to investigate *in vitro* antioxidant potential of methanolic leaf and root extracts of the herb, *Hypochaeris radicata* L. (Asteraceae). Antioxidant activity was assessed by using 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$) assay, reducing power activity, [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] $ABTS^{\bullet+}$ assay and ferrous ion chelating activity. Here, butylated hydroxytoluene (BHT), ascorbic acid (ASA), trolox and EDTA were used as standard antioxidants. The total phenolic and flavonoid contents were also determined and expressed in gallic acid and quercetin equivalent respectively. The results of the study indicate that the methanolic extracts of the leaf and root of *H. radicata* posses significant scavenging activity against $DPPH^{\bullet}$ (97.99% for leaf and 96.44% for root at 250µg/ml each) and ferrous ions chelating activity (38.69% for leaf and 40.52% for root at 5000µg/ml each), reducing power activity (1.38 absorbance at 600µg/ml for leaf, 0.45 absorbance at 700 µg/ml for root) and free radical scavenging activity ($ABTS^{\bullet+}$) (2706.73 for leaf and 2028.37µmol for root TE/g). The free radical scavenging and antioxidant activities may be attributed to the presence of adequate phenolic (gallic acid content is 125.5µg/10mg in leaf and 133.06µg/10mg in root) and flavonoid compounds (105.76µg/2mg in leaf and 55.16µg/2mg in root). This study revealed that the methanolic extracts of both leaf and root of *H. radicata* has demonstrated significant antioxidant activity.

Keywords: *Hypochaeris radicata* L., total phenolic, flavonoids, $DPPH^{\bullet}$ assay, reducing power activity, $ABTS^{\bullet+}$ assay, ferrous ion chelating activity.

INTRODUCTION

Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species can initiate degenerative diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids are commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Brown and Rice-Evans, 1998). Currently, the possible toxicity of synthetic antioxidants has been criticized. Thus interest in natural antioxidant, especially of plant origin has greatly increased in recent years (Jayaprakash and Rao, 2000).

Free radicals arising from metabolism or environmental sources interact continuously in biological systems and their uncontrolled generation correlates directly with molecular level of many diseases (Huang *et al.,* 2005). Lots of research has clearly showed that free radicals would damage nearby structures including DNA, proteins or lipids. Radical scavenging antioxidants are particularly important in antioxidative-defence in protecting cells from the injury of free-radical (Youwei *et al.,* 2008). Plants are the good source of biologically active compounds known as phytochemicals. The phytochemicals have been found to act as antioxidants by scavenging free radicals and may have therapeutic potential for free radical associated disorders (Hausladen and Stamer, 1999). It is well known that free radicals are the major cause of various chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Scalbert *et al.,* 2005). Therefore, it is important to assess antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these herbal plants (Abdul *et al.,* 2012).

Hypochaeris radicata L. of Asteraceae family an one such plant species distributed in high hills of Nilgiris, the Western Ghats, India at temperate climate is expected to have antioxidant property as it is being used by the local communities for adding freshness and activeness (Paulsamy *et al.,* 2008). Therefore to have the scientific validation on antioxidant properties, the leaves and roots of this species were taken for the present study. The methanolic extracts of both parts of *H. radicata* were used to investigate the antioxidant activity in terms of free radical scavenging activity ($DPPH^{\bullet}$), reducing power activity, $ABTS^{\bullet+}$ assay and ferrous ion chelating activity.

MATERIALS AND METHODS

Collection and identification of plant material

The plant material was collected from Kattabettu, (2100ms above msl), the Nilgiris, Western Ghats, India. The plant was authenticated by Dr. P. Sathyanarayana, Botanical Survey of India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2010-11/Tech/153.

Preparation of plant extracts

Fresh plant material was washed under running tap water, air dried and powdered. About 50g of coarsely powdered plant materials (50g/250ml) were extracted in a soxhlet extractor for 8 to 10 hours, sequentially with petroleum ether, chloroform, ethyl acetate, methanol and water. The extracts obtained were then concentrated and finally dried to a constant weight. Dried extracts were kept at 20^0C until further test were carried out. For stock solutions, 10mg/ml of methanolic leaf and root extracts were dissolved in dimethyl sulffoxide (DMSO).

Determination of total phenolics content

The total soluble phenols present in the methanolic leaf and root extracts were determined by using the Folin-Ciocalteu reagent according to the procedure reported by Singletion *et al.*, (1999). About 500µl (20mg/ml) of plant sample was added to 25ml of distilled water and 1ml of Folin-Ciocalteu reagent (1:10). Then this mixture was kept at room temperature for 3 minutes, after then 1.5ml of 2% sodium bicarbonate was added, soon after vortexting the reaction mixture for 1 hour at room temperature, the absorbance was measured at 760nm. All the tests were performed in triplicates and the results were averaged. The concentration of total phenolic compounds in methanolic leaf and root extracts was determined as microgram of gallic acid equivalent by using an equation that was obtained from the standard gallic acid graph (10-300 µg/ml).

Determination of total flavonoids content

The aluminium chloride colorimetric assay was used for total flavonoids determination, as described by Zhishen *et al.* (1999). 100µl (20mg/ml) of the extract was mixed with 2.5 ml of distilled water and 300µl of 5% sodium nitrate. Then, it was incubated at room temperature for 5 minutes and 300µl of 10% aluminium chloride, 2ml of 1M sodium hydroxide and 1ml of distilled water were added. Then, absorbance of the reaction mixture was measured at 512nm, along with the standard, quercetin and blank. The total flavonoids content was determined as microgram, quercetin equivalent by using the standard, quercetin graph, obtained by comparing the calibration curve prepared from a reference solution containing quercetin (10-300µg/ml).

In vitro antioxidant activity

The free radical scavenging activity of the methanolic leaf and root extracts of the study species, *H. radicata* was determined by using various *in vitro* assays such as $DPPH^{\bullet}$ assay, reducing power assay and $ABTS^{\bullet+}$ assay and ferrous ion chelating activity.

Free radical scavenging activity ($DPPH^{\bullet}$)

The free radical scavenging activity of methanolic extract of *H. radicata* was measured by using 2, 2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$) method of Blois (1958). 0.2mM solution of $DPPH^{\bullet}$ in methanol was prepared and 100µl of this solution was added to various concentrations of methanolic leaf and root extracts at the concentrations of 50, 100, 150, 200 and 250µg/ml. After 30 minutes, absorbance was measured at 517nm. Butylated hydroxytoluene (BHT) was used as the reference material. All the tests were performed in triplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

$$\text{Percentage of inhibition} = \frac{Abs_{cntrl} - Abs_{test} \times 100}{Abs_{cntrl}}$$

The scavenging reaction between $DPPH^{\bullet}$ and an antioxidant, H-A can be written as

$$(DPPH^{\bullet}) + (H\text{-}A) \longrightarrow DPPH\text{-}H + (A^{\bullet})$$

(Purple) → (Yellow)

Reducing power activity

Reducing power assay was determined according to the method of Yildirim *et al.*, (2001). Different concentrations of methanolic extracts of leaf (200, 300, 400, 500 and 600µg/ml) and root (300, 400, 500, 600 and 700µg/ml) of the study species were mixed with 1ml of 200mM sodium phosphate buffer (pH 6.6) and 1ml of 1% potassium ferriccyanide followed by incubation at 50^0C for 20 minutes. After adding 1ml of 10% trichloro acetic acid, the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant was taken out and mixed with 2ml of distilled water and 0.5ml of 1% ferric chloride. After incubation for 10 minutes, the absorbance was measured at 700nm. Higher absorbance of the reaction mixture indicates reductive potential of the extracts (Yang *et al.*, 2002, Rajeshwar *et al.*, 2005, Koksal *et al.*, 2011). All the tests were performed in triplicates and ascorbic acid was used as reference standard.

Free radical scavenging activity ($ABTS^{\bullet+}$)

The total antioxidant activity of the samples was measured by [2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] $ABTS^{\bullet+}$ radical cation decolorization assay according to the method of Re *et al.* (1999). $ABTS^{\bullet+}$ was produced by reacting 7mM $ABTS^+$ aqueous solution with 2.4mM potassium persulfate in the dark for 12-16 hours at room temperature. The radical was stable in this form for more than two days when stored in the dark at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30^0C to give an absorbance of 0.7000±0.02 at 734 nm. Then, 2ml of diluted $ABTS^{\bullet+}$ solution was added to the sample concentration at 20µl (1mg/ml). After 30 minutes of incubation at room temperature, the absorbance was recorded at 734nm and percentage of inhibition was calculated. Trolox was used as a reference standard. Triplicates were performed.

Ferrous ion chelating activity

The chelating of ferrous ions by leaf and root methanolic extracts of *H. radicata* was estimated by the method of Singh and Rajini (2004). The different concentrations of methanolic extracts (1000, 2000, 3000, 4000 and 5000µg/ml separately for leaf and root) were mixed with 100µl of 2mM ferrous sulphate solution and 300µl of 5mM ferrozine. The mixture was incubated at room temperature for 10 minutes. The absorbance of the solution was measured at 562nm. Ethylene diammine tetra acetate (EDTA) was used as standard. All the tests were performed in triplicate and percentage of inhibition was calculated by using this formula,

$$\text{Percentage of inhibition} = \frac{Abs_{cntrl} - Abs_{test} \times 100}{Abs_{cntrl}}$$

Statistical analysis

The statistical comparison among the groups were performed with one way analysis of variance (ANOVA) test using a statistical package program SPSS 10 and the significance of the difference between means was determined by Duncan's Multiple Range Test (DMRT) at ($p<0.05$) significant level. (Statsoft Inc, Tulsa, USA).

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases (Roy *et al.*, 1994). They are also involved in autoimmune disorder like rheumatoid arthritis etc. (Rao *et al.*, 2004). Our results demonstrated that the methanolic extracts of leaf and root of *H. radicata* possess free radical scavenging activity *in vitro* models like $DPPH^{\bullet}$, $ABTS^{\bullet+}$, reducing power activity and ferrous ion chelating assays.

Total phenolics content

Phenolic compounds are known as powerful chain breaking antioxidant (Shahidi and Wansundeara, 1992) and they are very important plant consistuents because of their scavenging ability, which is due to their hydroxyl groups (Hatano *et al.*, 1989). In methanolic leaf and root extracts of *H. radicata*, the total phenolic content was found to be 125.5µg/10mg and 133.06µg/10mg respectively in terms of gallic acid equivalent (Table 1). In addition it has been determined that the highest extraction yield was found in root extract.

Table. 1: Extraction yield, total phenolics and flavonoids content of methanolic leaf and root extracts of *Hypochaeris radicata.*

Sample	Extraction yield (MeOH)	Total phenolic content	Total flavonoid content
	% yield (W/W)	[Gallic acid equivalent (µg/10mg)]	[Quercetin equivalent (µg/10mg)]
Leaf	18.4	125.5 ± 0.5	105.76 ± 0.75
Root	25.6	133.06 ± 0.6	55.16 ± 0.76

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Total flavonoids content

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activities (Umamaheswari *et al.*, 2008). They are capable of effectively scavenging the reactive O_2 species because of their phenolic hydroxyl groups and so they are potent antioxidants also (Cao *et al.*, 1997). The total flavonoids content of methanolic leaf and root extracts of *H. radicata* was determined to be 105.76µg/2mg and 55.165µg/2mg respectively inturms of quercetin equivalent (Table 1).

Free radical scavenging activity ($DPPH^{\bullet}$)

$DPPH^{\bullet}$ is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract (Bhuiyan *et al.*, 2009). Scavenging of $DPPH^{\bullet}$ radical is related to the inhibition of lipid peroxidation (Rekka and Kourounakis 1991). $DPPH^{\bullet}$ is usually used as a substance to evaluate the antioxidant activity (Tara Chand *et al.*, 2012). Antioxidants either transfer an

electron or a hydrogen atom to DPPH$^{\bullet}$, thus neutralizing its free radical character (Pan *et al.*, 2008). DPPH$^{\bullet}$ test, which is based on the ability of DPPH$^{\bullet}$, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action (Raquibul Hasan *et al.*, 2009). The DPPH$^{\bullet}$ assay has been largely used as a quick, reliable and reproducible parameter to search the *in vitro* general antioxidant acitivity of pure compounds as well as plant extracts (Koleva *et al.*, 2002). The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir *et al.*, 1995). In the present study, the percentage of scavenging effect on the DPPH$^{\bullet}$ radical was concomitantly increased with the increase in the concentration of both leaf and root methanolic extracts from 50 to 250 µg/ml. The percentage of inhibition was existing from 54.91 at 50µg/ml to 98.36 at 300 µg/ml for leaf extract and for root extracts, they were 23.27 at 50µg/ml and 96.72 at 250 µg/ml (Table 2). From the results it is known that the species, *H. radicata* possess hydrogen donating capabilities for methanolic leaf extract and does scavenging free radicals. Furthermore, it was noticed that the leaf extract has more pronounced scavenging activity than that of the standard, BHT (Table 2).

Table. 2: Free radical scavenging activity (DPPH) of methanolic leaf and root extracts of *Hypochaeris radicata.*

S. No.	Sample concentration (µg/ml)	Leaf extract % of inhibition	Root extract % of inhibition	BHT % of inhibition
1.	50	$54.21^{a} \pm 0.66$	$23.4^{a} \pm 0.33$	$36.24^{a} \pm 0.31$
2.	100	$90.86^{b} \pm 0.37$	$56.52^{b} \pm 0.38$	$42.21^{b} \pm 0.38$
3.	150	$93.25^{c} \pm 0.62$	$75.49^{c} \pm 0.45$	$49.39^{c} \pm 0.34$
4.	200	$96.3^{d} \pm 0.51$	$95.36^{d} \pm 0.47$	$42.16^{d} \pm 0.40$
5.	250	$97.99^{e} \pm 0.36$	$96.44^{e} \pm 0.33$	$57.15^{e} \pm 0.24$

BHT was used as reference standard.
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Reducing power activity

Reducing power activity is often used to evaluate the ability of natural antioxidant to donate electron (Yildirim *et al.*, 2000, Dorman *et al.*, 2003). Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Duh, 1998; Duh *et al.*, 1999; Yildirim *et al.*, 2000). The reducing power activity of methanolic leaf and root extracts of *H. radicata* increased consistently with the increase in the volume of extract from 200µg to 600µg for leaf and 300µg to 700µg for root. When compared with the root extract (0.433 at 700µg/ml), leaf extract showed higher absorbance (1.092 at 600µg/ml). It is known further that the reducing power activity of leaf extract was far higher than the standard, ascorbic acid (Table 3).

Free radical scavenging activity (ABTS$^{\bullet+}$)

The decolorization of the ABTS$^{\bullet+}$, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734nm (Re *et al.*, 1999). ABTS$^{\bullet+}$ was generated by incubating ABTS$^{\bullet+}$ chromophore through the reaction (Wolfenden *et al.*, 1982). The presence of specific chemical compounds in the extracts of *H. radicata* may inhibit the potassium persulfate activity and hence reduced the production of ABTS$^{\bullet+}$. This study reports that the methanolic leaf extract of *H. radicata* has highest antioxidant activity (2706.73 µmol/g) than that of its root counter part (2028.37µmol/g) (Table 4).

Ferrous ion chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, it is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components (Smith *et al.*, 1992).

The metal chelating ability of the methanolic leaf and root extracts was measured by the formation of ferrous ion-ferrozine complex. Ferrozine combines with ferrous ions forming a red coloured complex which absorbs at 562n m (Yamaguchi *et al.*, 2000). It was reported that the chelating agents which forms σ bond with a metal, are effective as secondary antioxidants, because they reduce the redox potential there by stabilizing the oxidized form of the metal ion (Duh *et al.*, 1999). Iron binding capacity in terms of percent inhibition of the methanolic extract of *H. radicata* at 5000µg/ml was higher for root (40.52%) than the leaf (38.96%) (Table 5). However, it was not comparable to that of the reference standard, EDTA.

CONCLUSION

Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food production. An *in vitro* antioxidant study provides scientific evidence to prove the traditional claims to the Asteraceae member, *H. radicata.* On the basis of the results obtained in the present study, it was concluded that the methanolic leaf and root extracts of this species possess significant antioxidant activity.

Presence of adequate amount of phenol and flavonoid compounds may account for this fact. So these findings of present study suggest that this plant have a potential source of natural antioxidant. Further studies are warranted for the isolation and characterization of antioxidant compounds, and also *in vivo* studies are needed for understanding their mechanism of action as antioxidants.

Table. 3: Reducing power activity of methanolic leaf and root extracts of *Hypochaeris radicata.*

S. No.	Sample concentration (µg/ml)	Leaf extract (Absorbance at 700nm)	Ascorbic acid (Absorbance at 700nm)	Sample concentration (µg/ml)	Root extract (Absorbance at 700nm)	Ascorbic acid (Absorbance at 700nm)
1.	200	$0.67^{a} \pm 0.44$	$0.39^{a} \pm 0.04$	300	$0.16^{a} \pm 0.01$	$0.55^{b} \pm 0.03$
2.	300	$0.71^{b} \pm 0.02$	$0.55^{b} \pm 0.03$	400	$0.18^{a} \pm 0.01$	$0.64^{c} \pm 0.04$
3.	400	$0.78^{c} \pm 0.01$	$0.64^{c} \pm 0.04$	500	$0.22^{b} \pm 0.02$	$0.86^{d} \pm 0.03$
4.	500	$1.05^{de} \pm 0.01$	$0.86^{d} \pm 0.03$	600	$0.36^{c} \pm 0.02$	$0.98^{e} \pm 0.02$
5.	600	$1.38^{e} \pm 0.54$	$0.98^{e} \pm 0.02$	700	$0.45^{d} \pm 0.02$	$1.09^{f} \pm 0.04$

Ascorbic acid was used as reference standard.
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Table. 4: ABTS activity of methanolic leaf and root extracts of *Hypochaeris radicata*.

Sample	Total antioxidant activity (µmol TE/g extract)
Leaf	2706.79 ± 2.21
Root	2028.37 ± 3.15

Total antioxidant activity (µmol equivalent trolox).
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Table. 5: Ferrous ion chelating activity of methanolic leaf and root extracts of *Hypochaeris radicata.*

S. No.	Sample concentration (µg/ml)	Leaf extract % of inhibition	Root extract % of inhibition	EDTA % of inhibition
1.	1000	34.68^{a} ± 0.48	30.49^{a} ± 0.37	56.28^{a} ± 0.19
2.	2000	36.34^{b} ± 0.28	31.57^{b} ± 0.09^{b}	71.55^{b} ± 0.33
3.	3000	37.02^{bc} ± 0.46	32.60^{c} ± 0.26	83.46^{c} ± 0.20
4.	4000	37.59^{d} ± 0.35	34.68^{d} ± 0.45	93.19^{d} ± 0.21
5.	5000	38.69^{e} ± 0.23	40.52^{e} ± 0.34	96.69^{e} ± 0.15

EDTA, reference standard.
Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

REFERENCES

Abdul-Lateef-Molan, Abbas Mohammed Faraj, Abdulkhaliq saleh Mahdy. Antioxidant activity and phenolic content of some medicinal plants traditionally used in Northern Iraq. J of Phytopharmacology. 2012; 2(2): 224-233.

Bhuiyan MAR., Hoque MZ., Hossain SJ. Free Radical Scavenging Activities of *Zizyphus mauritiana*. World J. Agr. Sci.2009; 5: 318-322.

Blois MS. Antioxidant determination by the use of a stable free radical nature. Nature.1958; 26: 1199-1200.

Bohm BA., Stuessy TF. "Flavonoids of the sun frower family (Asteraceae)". Springer. New York (2001).

Brown JE., Rice-Evans CA. Luteolin-Rich artichoke extract protect low density lipoprotein from oxidation *in vitro*. Free Radical Research. 1998; 29: 247-255.

Cao G., Sofic E., Prior RL. Antioxidant and pro-oxidative behavior of flavonoids: Structure activity relationships. Free Radical.Biol. Med. 1997; 22: 749-760.

Dorman HJD., Peltoketo A., Hiltunen R., Tikkanen MJ. Characterisation of the antioxidant properties of deodourisation aqueous extracts from selected Lamiaceae Herbs. Food Chem. 2003; 83: 255-256.

Duh PD. Antioxidant activity of burdock (*Arctium lappa* Linne.): Its scavenging effect on free-radical and active oxygen. J. Am. Oil Chem. Soc. 1998; 75: 455-461.

Duh PD., Du PC., Yen GC. Action of methanolic extract of mung beans hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. Food Chem. Toxicol. 1999; 37: 1055-1061.

Hatano T., Edamatsu R., Mori A. Effect of interaction of tannins and related poyphenols on superoxide anion radical and on DPPH radical. Chem Pharm Bull. 1989; 37: 2016-2021.

Hausladen A., Stamer JS. Nirosative stress. Method in Enzymology. 1999; 300: 389-395.

Huang DJ., Chen HJ., Lin CD., Lin YH. Antioxidant and antiproliferative activities of water spinach (*Ipomea aquatica* Frosk.) constituents. Bot Bull Acad Sin. 2005; 406: 99-106.

Jayaprakash GK., Rao LJ. Phenolic constituents from lichen *Parmontrema stuppeum*. Food control. 2000; 56: 1018-1022.

Koksal E., Bursal E., Dikici E., Tozoglu F., Gulcin I. Antioxidant activity of *Melissa officinalis* leaves. J. Med. Plant. Res. 2011; 5: 217-222.

Koleva II., Van Beek TA., Linssen JPH., de G root A., Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 2002; 13: 8-17.

Meir S., Kanner J., Akiri B., Hada SP. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaf. J. Agri. Food chem. 1995; 43: 1813-1819.

Pan Y., Wang K., Huang S., Wang H., Mu X., He C. et al. Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longum* Lour.) peel. Food Chemistry. 2008; 106: 1264-1270.

Paulsamy S, Vijaya kumar KK, Murugesan M, Sivashanmugam M, Senthilkumar P, Suresh D. Medicinal and other Economic Plants of Shola understories, the Nilgiris, Western Ghats. International Book Distributors, India (2008).p.112.

Rajeshwar Y., Senthil Kumar GP., Gupta M., Mazumder UK. Studies on *in vitro* antioxidant activities of methanol extract of *Muccuna pruriens* (Fabaceae) seeds. Eur J Drug Res. 2005; 13: 31-39.

Rao MS., Raman MV. Biochemical systematic and Ecology. 2004; 32:447-448

Raquibul Hasan SM., Mokarram Hossain MD., Raushanara A., Mariam J., Ehsanul Hoque Mazumder MD., Shafiqur Rahman. DPPH free radical scavenging activity of some Bangladesh medicinal plants. Full length Research paper. 2009; 3 (11): 875-879.

Rekka E., Kourounakis PN. Effect of hydroxyethyl rutenosides and related compounds on lipid peroxidation and free radical scavenging activity-some structural aspects. J. Pharm Pharmacol. 1991; 43: 486-491.

Roberta Re, Nicoletta Pellegrini, Anna Proteggente, Ananth Pannala, Min Yang Catherine Rice-Evans. Antioxidant activity applying an improved ABTS Radical cation decolorization assay. Free radical Biology and Medicine. 1999; 26: 1231-1237.

Roy H., Burdon. Free Radical Damage and its control, Elsevier Science B.V. Netherlands.1994, pp 125.

Scalbert A., Manach C., Remesy C., Morand C. Dietary polyphenols and the prevention of diseases critical. Reviews in Food Sciences and Nutrition. 2005; 45: 287-306.

Shahidi F., Wanasundara PKJPD. Phenolic antioxidants. Food Sci. Nut. 1992; 32: 67-103.

Singh N., Rajini RS. Free radical scavenging activity of an aqueous extract of potato peel. Food Chem. 2004; 85: 611-616.

Singletion VL., Orthofer R., Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. Methods Enzymol. 1999; 299: 152-178.

Smith C., Halliwell B., Aruoma OI. Protection by albumin against the pro-oxidation action of phenolic dietary components. Food Chem Toxicol. 1992; 30: 483-489.

Tara Chand, Anil Bhandari, Bhupendra K. Kumawat, Pawank Basniwal, Sanjay Sharma, Rajesh Verma. *In vitro* antioxidant activity of alcoholic extract of seed of *Cucumis callosus* (Rottl.) cogn. American Journal of Pharmtech Research. 2012; 2(3): 2249-3387.

Umamaheswari, Chatterjee TK. *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. Afr. J. Traditional, complementary and Alternative Medicines. 2008; 5(1): 61-73.

Wolfenden BS.., Willson RL. Radical-cations as reference chromogens in kinetic studies of one-electron transfer reactions: pulse radiolysis studies of [2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonate)]

J. Chem. Soc. Perkin Trans. 1982; 2: 805-812.

Yamaguchi F., Ariga T., Yoshimara Y., Naxazawa H. Antioxidant and antiglycation of carcinol from *Garcinia indica* fruit rind. J. Agri. Food Chem. 2000; 48: 180-185.

Yang JH., Lin HC., Macl JL. Antioxidant properties of several commercial mushrooms. Food Chem. 2002; 77:229-235

Yildirim A., Mavi A., Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. Journal of Agricultural and Food Chemistry.2001; 9: 4083-4089.

Yildirim A., Mavi A., Oktay M., Kara AA., Algur OF., Bilaloglu V. Comparison of antioxidant and antimicrobial activities of tilia (*Tilia arentea* Desf. Ex. D.C.) sage (*Salvia triloba* L.) and black tea (*Camellia sinensis* L.) extracts. J. Agr. Food Chem. 2000; 48(10): 5030-5034.

Youwei Z., Jinlian Z., Yonghong P. A comparative study on the free radical scavenging activities of some fresh flowers in Southern China. LWT-Food Sci. Technol. 2008; 41: 1586-1591.

Zhishen J., Mengcheng T., Jainming W. Research on antioxidant activity of flavonoids from natural materials. Food Chem. 1999; 64: 555-559.