Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jopr

Original Article

Efficacy of *Helicteres isora* L. against free radicals, lipid peroxidation, protein oxidation and DNA damage

Vinay Kumar [a,*], *Mansi Sharma* [a], *Melissa Lemos* [a], *Varsha Shriram* [b]

[a] *Department of Biotechnology, Modern College, Ganeshkhind, Pune 411053, Maharashtra, India*
[b] *Department of Botany, Prof. Ramkrishna More College, Akurdi, Pune 411044, Maharashtra, India*

ARTICLE INFO

Article history:
Received 24 April 2013
Accepted 21 May 2013
Available online 27 June 2013

Keywords:
DPPH
Fenton's reaction
Free radicals
Lipid peroxidation

ABSTRACT

Background: Free radicals are implicated with cellular disorders through their detrimental actions on proteins, lipids and DNA and are causative factors for large number of degenerative diseases and aging process. Antioxidants of plant origin hold great significance and have therefore gained utmost importance in recent past. Present investigation was a step in this direction, with an objective to comprehensively evaluate the protective effects of *Helicteres isora* fruits against free radicals, protein oxidation and DNA damage.

Methods: Aqueous (AqE), aqueous-methanol (AqME), methanol (ME) and acetone (AE) extracts of mature pods (fruits) were obtained and concentrated *in vacuo*. Total phenols, flavonoids, ascorbic acid and carotenoids were estimated from extracts using standard methods. Antioxidant activities of extracts with varying concentrations (200–1000 μg/ml) were determined by total antioxidant activity (TAA), ferric reducing antioxidant power, DPPH, and OH radical scavenging assays besides lipid peroxidation inhibition. Extracts were assessed for protection against AAPH (2,2′-Azobis(2-methylpropionamidine) dihydrochloride) induced-protein oxidation using SDS-PAGE and Fenton's reagent induced-DNA damage using DNA nicking assay.

Results: The results postulated that the plant is a rich source of total phenols, flavonoids and ascorbic acid. Extracts showed concentration-dependent free radical scavenging activities and lipid peroxidation inhibition. Amongst all four extracts, AqME showed highest antioxidant potential in terms of reducing power (360 ± 5.9 gallic acid equivalent: GAE), TAA (150 ± 5.6 GAE), scavenging of free radicals including DPPH (75.6%) and OH (100%) besides maximal (97.4%) lipid peroxidation inhibition at 1000 μg/ml concentration. All the extracts barring ME effectively protected the DNA from hydroxyl radical-induced damage. Similarly, fruit extracts effectively protected the AAPH-induced-protein oxidation.

Conclusions: H. isora fruits exhibited broad-spectrum antioxidant potencies against free radicals and significantly ameliorated various impairments associated with free radical formation including lipid peroxidation, protein oxidation and DNA damage.

* *Corresponding author*. Tel.: +91 9767839708; fax: +91 20 25650931.
E-mail address: vinaymalik123@gmail.com (V. Kumar).

0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2013.05.017

1. Introduction

Free radicals, the molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital are generated naturally in living organisms as byproducts of endogenous metabolism and are even known to play significant roles in cell signaling. However, when generated in excess, they are known to be associated with cellular disorders through their actions on proteins, lipids and DNA.[1] Free radicals cause DNA damage-induced mutation and chromosomal damage, causes biomolecular oxidation besides oxidizing the cellular thiols, which eventually affects key enzymes and lipid peroxidation[2,3] and as a result, are thought to underline the process of ageing and causes over 100 diseases including cataractogenesis, cardiovascular problems, inflammatory disorders, neurodegenerative diseases, immune system decline and carcinogenesis.[1–4] Antioxidants play an imperative role in scavenging free radicals and providing protection against oxidative stress and associated diseases, and hence received a great deal of attention in recent past.

In contemporary times, a noticeable upsurge of interest has been evidenced in evaluating the antioxidant potentials of medicinal plants for scavenging free radicals and therefore reducing the oxidative stress-induced tissue injuries. The possible detrimental effects of synthetic antioxidants have further enhanced the interest in searching for potential antioxidants of plant origin.[5,6] Consequently, the antioxidants of phyto-origin have seen an unprecedented demand in biopharmaceuticals, nutraceuticals besides their use as food additives.

Helicteres isora L. (*Sterculiaceae*) commonly known as East Indian screw tree, is medicinally important sub-deciduous small tree. Various parts of the plant have traditional usage against colic, cough, asthma and diabetes.[7–9] The fruits are astringent, stomachic, vermifugal, and useful in flatulence[10] besides antispasmodic.[11] Roots and barks possess hypolipidemic, hypoglycemic and antinociceptive activities,[9,12–14] Our group has reported plasmid-curing activities from fruits.[15] The present study was aimed to evaluate *H. isora* fruit extracts for total phenols and flavonoid contents, efficacy in free radical scavenging, lipid peroxidation inhibition, and protein oxidation and DNA damage protection activities using *in vitro* models.

2. Materials and methods

Mature pods of *H. isora* were collected from Satara region of Western Ghats, India. Samples were authenticated by Dr. Rani Bhagat, at Anantrao Pawar College, Pune (Ref. No. APCP/21/2012-13).

2.1. Plant extracts

One Kilogram powder of shade dried pods was soaked in 3 L acetone/methanol/aqueous-methanol (1:1) or distilled water. The extract was prepared by cold percolation for 24 h at room temperature (RT: 26±2 °C). The filtrate was concentrated *in vacuo* at 40, 40, 56 and 60 °C to get acetone (AE), methanol (ME), aqueous-methanol (AqME), and aqueous extracts (AqE), with 2.74%, 3.10%, 4.20% and 4.9% yield, respectively.

2.2. Phytochemical investigation

Total phenols were estimated using Folin–Ciocalteu method[16] and expressed as mg gallic acid equivalents (GAE) g^{-1} extract. Total flavonoids were estimated using modified Marinova et al[17] and expressed as mg quercetin equivalents/g extract. Total ascorbic acid was estimated by 2,4-dinitrophenylhydrazine method.[18] Carotenoids were estimated by following Jensen[19] and concentration was expressed as mg β-carotene equivalents/g extract.

2.3. Determination of antioxidant activities

2.3.1. Total antioxidant activity (TAA)

The assay is based on the reduction of Mo(VI) to Mo(V) by sample compound and formation of green colored phosphate/Mo(V) complex at acidic pH (4.0).[20] 0.1 ml of extract from varying concentrations (200–1000 μg/ml) was added to 1 ml reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95 °C for 90 min and the absorbance was measured at 695 nm after cooling the samples and TAA was expressed as GAE.

2.3.2. FRAP (ferric reducing antioxidant power)

The spectrophotometric method is based on reduction of Fe^{3+}-tetra(2-pyridyl)pyrazine (TPTZ) complex to Fe^{2+}-tripyridyltriazine at low pH.[21] FRAP reagent contained 300 mM acetate buffer, 10 ml TPTZ dissolved in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in 10:1:1 ratio. Five hundred μl standard was added to 1 ml reaction mixture and incubated at 37 °C for 30 min. Absorbance was taken at 593 nm against blank and FRAP values were expressed as GAE.

2.3.3. DPPH radical scavenging activity

The antioxidant activity of the plant extract was examined on the basis of the scavenging effect on the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity as described by Braca et al.[22] Ethanolic solution of DPPH 0.05 mM (300 μl) was added to 40 μl extract with 200–1000 μg/ml concentrations. After 5 min, absorbance was measured at 517 nm. The radical scavenging activity of the plant extract was expressed as % inhibition against control.

2.3.4. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test extract for hydroxyl radical generated by Fenton's reaction.[23] The reaction mixture contained deoxyribose (2.8 mM in KH_2PO_4–KOH buffer, pH 7.4), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), with 200–1000 μg/ml concentrations of extracts in a final volume of 1.0 ml. The reaction mixture was incubated for 1 h at 37 °C. Deoxyribose degradation was measured as thiobarbituric acid (TBA) assay. One ml of TBA (1%) and 1 ml of TCA (2.8%) were added to above mixture and incubated at 100 °C for 20 min. The development

Table 1 – Total phenols, flavonoids, ascorbic acid and carotenoid contents of *H. isora* extracts.

Extract	Total phenols* (mean ± SE)	Flavonoids^ (mean ± SE)	Ascorbic acid# (mean ± SE)	Carotenoids$ (mean ± SE)
AE	17.3 ± 0.9^{a}	24.0 ± 0.8^{c}	25.6 ± 1.3^{a}	1.3 ± 0.2^{c}
ME	29.5 ± 1.1^{b}	31.5 ± 1.1^{d}	35.1 ± 1.9^{b}	$1.6 \pm 0.2^{b,c}$
AqME	40.1 ± 1.7^{d}	29.9 ± 0.7^{b}	105.3 ± 2.9^{d}	$1.8 \pm 0.3^{a,b}$
AqE	34.2 ± 1.2^{c}	26.2 ± 0.8^{a}	40.0 ± 1.7^{c}	1.4 ± 0.1^{a}

*Gallic acid; ^quercetin; #ascorbic acid and; $β-carotene equivalents mg g^{-1} extract.
Means within a column followed by different letters are significantly different at $P \leq 0.05$ according to DMRT.

of pink color was measured at 532 nm and % inhibition was calculated.

2.3.5. *Determination of inhibition of lipid peroxidation*

Lipid peroxidation inhibition was evaluated using modified Halliwell and Gutteridge[24] method. Freshly excised goat liver was minced using glass Teflon homogenizer in cold phosphate buffered saline (pH 7.4). 10% homogenate was prepared and filtered to obtain a clear homogenate and this process was carried on ice. Varying concentrations (200–1000 μg/ml) of the extracts were added to the liver homogenate and lipid peroxidation was initiated by adding 100 μl ferrous sulfate (15 mM) to 3 ml of the tissue homogenate. After 30 min, 100 μl aliquot was taken in a tube containing 1.5 ml of 10% TCA. After 10 min, tubes were centrifuged and supernatant was mixed with 1.5 ml of 0.67% TBA in 50% acetic acid. The mixture was heated for 30 min in a boiling water bath. The intensity of the pink colored complex was measured at 535 nm. The degree of lipid peroxidation was assayed by estimating the TBARS (TBA-reactive species) content and results were expressed as percentage inhibition.

2.4. *DNA damage protection activity*

The ability of different extracts to protect DNA (pBR322, Merck, India) from damaging effects of hydroxyl radicals generated by Fenton's reagent (FR) was assessed by modified DNA nicking assay.[25] The reaction mixture contained 2.5 μl of DNA (0.25 μg) and 10 μl FR (30 mM H_2O_2, 500 μM ascorbic acid and 800 μM $FeCl_3$) followed by the addition of 5 μl of extracts and the final volume was made 20 μl with DW. The reaction mixture was then incubated for 45 min at 37 °C and followed by addition of 2.5 μl loading buffer (0.25% bromophenol blue, 50% glycerol). The results were analyzed on 0.8% agarose gel electrophoresis using EtBr-staining.

2.5. *Protein oxidation inhibition activity*

Oxidation of BSA (5 μg) in phosphate buffer was initiated by 25 mM AAPH[26] and inhibited by different *H. isora* extracts (50 μg/ml). After incubation of 2 h at 37 °C, 0.02% BHT was added to prevent the formation of further peroxyl radical. The samples were then electrophoresed using 12% SDS-PAGE using the Protean® II System (Bio-Rad, USA) and the gel was stained with 0.25% CBB R-250.

2.6. *Statistical analyses*

The results are presented as means of 3 replicates ± standard error (SE). Means were compared through Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$, using MSTAT-C software. The graphs were plotted using Microcal Origin 6.0.

3. Results and discussion

Results depicted in Table 1 revealed that the plant is a rich source of phenols, flavonoids and ascorbic acid; and their quantities showed solvent-type-dependent variations. Several reports have shown a correlation between higher amounts of polyphenols in plants and correspondingly their higher antioxidant potential[16,25–27] as they inhibit free radical formation and/or interrupt propagation of autoxidation.[28] Our results supported these hypotheses. Phenolic contents were found in the range of 17.3–40.1 GAE mg/g extract. Overall, AqME showed maximum amounts of polyphenols followed by

Table 2 – FRAP and total antioxidant activities (TAA) of *H. isora* extracts.

Conc. of extracts (μg/ml)	TAA of various extracts (GAE) (mean ± SE)				FRAP of various extracts (GAE) (mean ± SE)			
	AE	ME	AqME	AqE	AE	ME	AqME	AqE
200	12 ± 1.2^{a}	29 ± 2.0^{a}	42 ± 2.3^{a}	21 ± 1.5^{a}	50 ± 1.7^{a}	198 ± 3.1^{a}	260 ± 3.7^{a}	200 ± 4.1^{a}
400	27 ± 2.4^{b}	40 ± 3.2^{b}	54 ± 2.9^{b}	32 ± 2.4^{b}	140 ± 3.9^{b}	229 ± 3.4^{b}	310 ± 4.9^{b}	220 ± 5.5^{b}
600	38 ± 2.9^{c}	57 ± 4.5^{c}	88 ± 3.3^{c}	36 ± 2.1^{b}	170 ± 3.7^{c}	240 ± 4.0^{c}	330 ± 5.2^{c}	222 ± 5.9^{b}
800	51 ± 2.8^{d}	78 ± 4.8^{d}	99 ± 3.8^{d}	42 ± 2.5^{c}	190 ± 2.8^{d}	250 ± 4.1^{d}	350 ± 4.4^{d}	230 ± 5.6^{c}
1000	62 ± 3.7^{e}	80 ± 4.9^{d}	150 ± 5.6^{e}	45 ± 2.8^{c}	200 ± 3.1^{e}	270 ± 3.9^{e}	360 ± 5.9^{e}	239 ± 4.9^{d}

GAE: Gallic acid equivalents.
Means within a column followed by different letters are significantly different at $P \leq 0.05$ according to DMRT.

ME, AqE and AE, respectively. Likewise, AqME showed significantly higher amount of ascorbic acid.

Antioxidant potential of plants is generally attributed to phytochemicals present and the synergies between them and therefore, should not to be evaluated by a single method. Hence, in order to explore and understand possible mechanisms, array of antioxidant assays including TAA, FRAP, DPPH and OH radical scavenging assays were performed for evaluating antioxidant activities of *H. isora*. These results validated the traditional usage of this plant against aging and diabetes and shown a broad-range of antioxidant properties.

The results of TAA and FRAP scavenging activity are summarized in Table 2. Extracts showed concentration-dependent TAA. AqME showed highest TAA whereas AE showed lowest TAA among all the extracts. The results presented in Table 2 showed notable antioxidant potential of extracts of *H. isora* in terms of FRAP in a dose-dependent manner. AqME showed highest ferric reducing power with 360 ± 5.9 GAE followed by ME (270 ± 3.9 GAE), AqE (239 ± 4.9 GAE) and AE (200 ± 3.1 GAE) at 1000 µg/ml extract concentration. Since antioxidant capacity is directly correlated with the reducing capacity of plants and their products, the FRAP assay is considered as a reliable method for evaluation of antioxidant potentials of plant extracts and compounds[21] and our results are in conformity of these hypotheses.

The reduction capacity of stable DPPH radicals was determined by decrease in its absorbance at 517 nm induced by the antioxidants present in extracts and the results are illustrated in Fig. 1. All extracts showed tendency to quench the DPPH radicals in a concentration-dependent fashion. AqME proved a potent free radical scavenger and showed DPPH inhibition followed by AqE, ME and AE, respectively, at 1 mg/ml concentration. Many authors have attributed higher free radical scavenging ability of plants to their phenol contents and their ability to donate hydrogen atom.[2,6,16] Likewise,

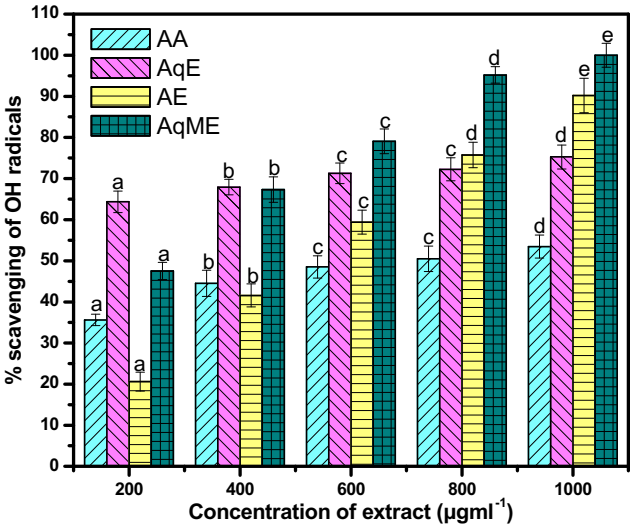

Fig. 2 – OH scavenging activities of *H. isora*. Each value represents mean ± SE. The bars with different letters are significantly different at $P \leq 0.05$ according to DMRT.

OH radical scavenging activity was also observed maximum in AqME (Fig. 2).

One of the major consequences of free radical formation is the oxidative damage to cellular components including lipid membranes, and is believed to be associated with pathology of many diseases and conditions.[16] Therefore, inhibition of lipid peroxidation is considered as most important index of antioxidant potential. Fig. 3 illustrates that this plant has tremendous potential in terms of lipid peroxidation inhibition. AqME offered a good degree of protection against the

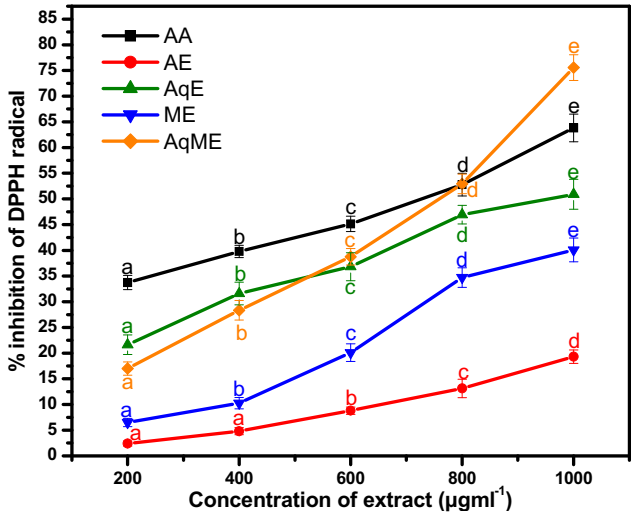

Fig. 1 – DPPH radical scavenging activities of *H. isora*. Each value represents mean ± SE. The lines with the same color and symbol with different letters are significantly different at $P \leq 0.05$ according to DMRT.

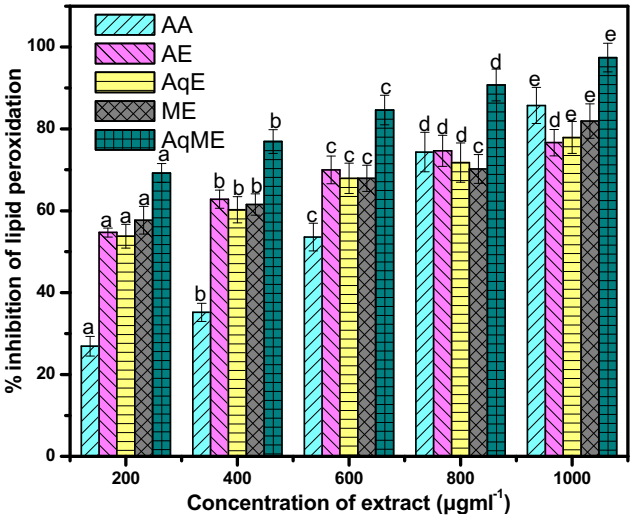

Fig. 3 – Inhibition of lipid peroxidation in goat liver by *H. isora*. Each value represents mean ± SE. The bars with different letters are significantly different at $P \leq 0.05$ according to DMRT.

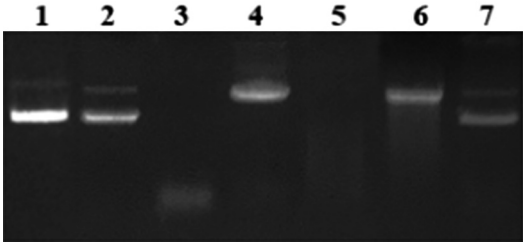

Fig. 4 – DNA damage protecting activities of *H. isora*. Lane 1: pBR322 DNA; Lane 2: DNA + FR + quercetin (50 μg/ml); Lane 3: DNA + Fenton's reagent (FR); Lane 4: DNA + FR + AqME; Lane 5: DNA + FR + ME; Lane 6: DNA + FR + AE; Lane 7: DNA + FR + AqE.

biological end-point of oxidative damage and showed 97% lipid peroxidation inhibition at 1 mg/ml concentration.

Extracts were evaluated for their oxidative damage protective activity against a model DNA pBR322 and the results are illustrated in Fig. 4. Hydroxyl radicals generated by Fenton's reaction are known to cause DNA damage as in the present investigation, Fig. 4 (lane 3) showed absence of DNA band and only a smear of degraded DNA was observed. All the extracts except methanol showed observable protection of DNA intactness. Free radicals are known for DNA strand breaking and damage which eventually contributes to carcinogenesis, mutagenesis and cytotoxicity.[16] Various researchers have reported the similar results and used plant extracts and fractions for DNA protection against oxidative damage.[16,28] One of the interesting finding of present study was that ME did not show significant DNA protection activity which can be attributed to its inability to scavenge OH radicals (Fig. 2).

It can be postulated from the results depicted in Fig. 5 that AAPH degraded BSA protein (lane 3). However, pre-treatment of *H. isora* fruit extracts effectively protected the protein from AAPH-induced oxidation, which can be seen in terms of restoration of band intensity in the gel. These results hold

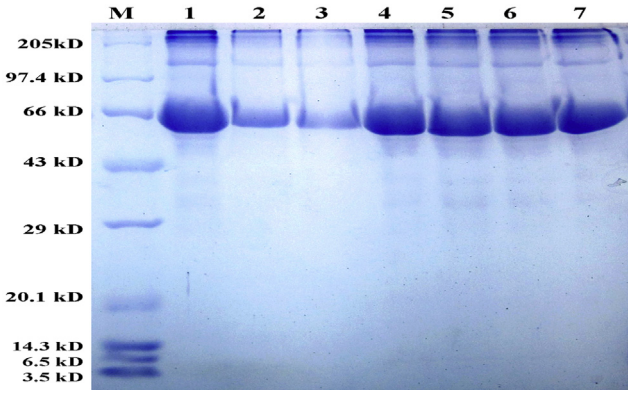

Fig. 5 – The inhibitory activities of *H. isora* against protein oxidation. Lane M: Marker; Lane 1: BSA; Lane 2: BSA + AAPH + ascorbic acid; Lane 3: BSA + AAPH; Lane 4: BSA + AAPH + AqE; Lane 5: BSA + AAPH + AqME; Lane 6: BSA + AAPH + ME; Lane 7: BSA + AAPH + AE.

significance and may have a positive role in inhibiting several stress or toxicity induced-protein oxidation.[26]

Conflict of interest

All authors have none to declare.

Acknowledgment

Authors thank the Principals of Modern College and Prof. Ramkrishna More College, Pune for encouragement and support to carry out this work.

REFERENCES

1. Barros L, Cabrita L, Boas MV, Carvalho AM, Ferreira ICFR. Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chem*. 2011;127:1600–1608.
2. Elzaawely AA, Tawata S. Antioxidant capacity and phenolic content of *Rumex dentatus* L. grown in Egypt. *J Crop Sci Biotech*. 2012;15:59–64.
3. Murdifin M, Wahyudin E, Lawrence GS, Subehan Manggau MA, Alam G. Phytochemical analysis and antioxidant activity of *Mezzetia parviflora* Becc. woodbark extract. *Pharmacognos J*. 2012;4:18–21.
4. Chua M, Tung Y, Chang S. Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. *Bioresour Technol*. 2008;99:1918–1925.
5. Wang H, Liu F, Yang L, et al. Oxidative stability of fish oil supplemented with carnosic acid compared with synthetic antioxidants during long-term storage. *Food Chem*. 2011;128:93–99.
6. Muruhan S, Selvaraj S, Viswanathan PK. *In vitro* antioxidant activities of *Solanum surattense* leaf extract. *Asian Pac J Trop Biomed*. 2013;3:28–34.
7. Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Allahabad: Lalit Mohan Basu; 1981.
8. Kumar G, Banu GS, Murugesan AG, Pandian MR. Hypoglycemic effect of *Helicteres isora* bark extract in rats. *J Ethnopharmacol*. 2006;107:304–307.
9. Venkatesh S, Laxmi KS, Reddy BM, Ramesh M. Antinociceptive activity of *Helicteres isora*. *Fitoterapia*. 2007;78:146–148.
10. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian Medicinal Plants*. New Delhi, India: CSIR; 1956:131.
11. Pohocha N, Grampurohit ND. Antispasmodic activity of the fruits of *Helicteres isora* Linn. *Phytother Res*. 2001;15:49–52.
12. Chakrabarti R, Vikramadithyan RK, Mullangi R, et al. Antidiabetic and hypolipidemic activity of *Helicteres isora* in animal models. *J Ethnopharmacol*. 2002;81:343–349.
13. Venkatesh S, Reddy GD, Reddy YS, Sathyavathy D, Reddy BM. Effect of *Helicteres isora* root extracts on glucose tolerance in glucose-induced hyperglycemic rats. *Fitoterapia*. 2004;75:364–367.
14. Kumar G, Murugesan AG. Hypolipidemic activity of *Helicteres isora* L. bark extracts in streptozotocin induced diabetic rats. *J Ethnopharmacol*. 2008;116:161–166.
15. Shriram V, Jahagirdar S, Latha C, et al. Antibacterial and antiplasmid activities of *Helicteres isora* L. *Indian J Med Res*. 2010;132:94–99.

16. Gul M, Bhakshu L, Ahmed F, Anand K, Qureshi I, Ghazi I. Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using *in vitro* assays. *BMC Complement Altern Med*. 2011;11:64.
17. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J Univ Chem Technol Metall*. 2005;40:255–260.
18. Kapur A, Haskovic A, Copra-Janicijevic A, et al. Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables. *Bull Chem Technol Bosnia Herzegovina*. 2012;38:40–43.
19. Jensen A. Chlorophyll and carotenoids. In: Hallebust JA, Craigie JS, eds. *Handbook of Physiochemical and Biochemical Methods*. Cambridge: Cambridge University Press; 1978:5–70.
20. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to determination of vitamin. *Anal Biochem*. 1999;269:337–341.
21. Benzie IF, Strain JJ. Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of total biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Meth Enzymol*. 1999;299:15–27.
22. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniaeflora*. *J Ethnopharmacol*. 2002;79:379–381.
23. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcuminoid. *Int J Pharm*. 1990;58:235–242.
24. Halliwell B, Gutteridge JMC. Protection against lipid peroxidation. In: *Free Radicals in Biology and Medicine*. Tokyo: Japan Scientific Societies Press; 1989:2.
25. Lee JC, Kim HR, Kim J, Jang YS. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. *Saboten J Agric Food Chem*. 2002;50:6490–6496.
26. Kumar PG, Navya K, Ramya EM, Venkataramana M, Anand T, Anilakumar CR. DNA damage protecting and free radical scavenging properties of *Terminalia arjuna* bark in PC-12 cells and plasmid DNA. *Free Rad Antiox*. 2013. http://dx.doi.org/10.1016/j.fra.2013.04.001.
27. Senthilkumar R, Parimelazhagan T, Chaurasia OP, Srivastava RB. Free radical scavenging property and antiproliferative activity of *Rhodiola imbricata* Edgew extracts in HT-29 human colon cancer cells. *Asian Pac J Trop Med*; 2013:11–19.
28. Chaudhary A, Rampal G, Sharma U, et al. Anticancer, antioxidant activities and GC-MS analysis of glucosinolates in two cultivars of broccoli. *Med Chem Drug Dis*. 2012;2:30–37.