INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

# Antioxidant Activity of *Senna surattensis* Leaves

**Ellappan Thilagam[1]*, Chidambaram Kumarappan[1], Balasubramanian Parimaladevi[2] and Subhash Chandra Mandal[1]**

[1]Pharmacognosy and Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Faculty of Engineering and Technology, Jadavpur University, Kolkata,West Bengal, India.

[2]Centre for research and development, PRIST university, Thanjavur, Tamilnadu, India.

**ABSTRACT**

**Objectives:** The present study was aimed to investigate the *Invitro* free radical scavenging activity of ethanolic extract of *Senna surattensis* Burm.f. **Methods:** This study assess the antioxidant potency of ethanol extract of *Senna surattensis* (EESS) leaves by using established *In vitro* models which includes 1, 1- diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, superoxide radical scavenging, inhibition of lipid peroxidation, reducing powers, and estimation of the amount of total phenolic content using Ascorbic acid and α– tocopherol as standards. **Results:** The ethanol extract of *S.surattensis* leaves showed potent inhibition of lipid peroxidation compared with Ascorbic acid ($IC_{50}$ 212.16 µg/ml, 256.15µg/ml respectively). The ethanol extract exhibited a good scavenging effect of superoxide radical, hydroxyl radical, DPPH and reducing powering with dose dependently. **Conclusions:** The ethanolic extract of *S.surattensis* showed potent *invitro* inhibition of free radicals and acts as an effective antioxidant.

**Keywords:** Antioxidant, phenolic content, radical scavenging, *Senna surattensis*.

## 1. INTRODUCTION

Free radicals and other reactive oxygen species are generated by exogenous chemicals or endogenous metabolic processes in food systems or the human body. The radicals may cause oxidative damage by oxidizing biomolecules and results in cell death and tissue damage[1]. They have been implicated in cardiovascular diseases and diabetes mellitus as well as in the process of aging[2-3]. In a normal healthy state, endogenous antioxidants act as the body's effective defense system against free radicals. However, in the diseased state, additional antioxidants from the diet and other sources such as medicinal plants are required for effective recovery. Because lots of synthetic antioxidants such as Butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) used in foods are suspected of being responsible for liver damage and carcinogenesis[4-5].

Numerous plant extract and plant constituents have proven to show free radical scavenging and /or anti-oxidant activities. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation[6]. *Senna surattensis* Burm.f./*Cassia surattensis* Burm.f.syn.*C.glauca* Lam. (Caesalpinaceae) is commonly known as *Glaucos cassia*. It is a small tree or large shrub, distributed throughout India. Bark and leaves are useful in diabetes and gonorrhoea[7], aerial parts is used to treat diabetes[8].The plant is known for its use in diabetes, gonorrhoea and blennorrhoea[9]. The beads made from wood are hanged in neck to cure jaundice[10]. The plant found to contain anthraquinone, flavonol glycosides, chrysophanol, physcion, kaemferide and quercetin[11-13]. As there is no scientific proof and data available about the antioxidant properties of this plant, we explored the

antioxidant potency of the extract using various models *in vitro.*

## 2. METHODS AND MATERIALS

### 2.1. Plant material and extract preparation

The fresh leaves of *Senna surattensis* were collected from Thiruchirappalli, Tamil Nadu, India, in December 2006 and authenticated by the Botanical survey of India (BSI), Coimbatore, India (REF NO:BSI/SC/5/23/06-07/Tech-1638). An authentic voucher specimen was deposited in the Herbarium of Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India for further reference. . The leaves were air dried at room temperature without exposure to sunlight and coarsely powdered (300g) and extracted with ethanol in a soxhlet apparatus. Solvent was evaporated completely under reduced pressure in rotary evaporator (SUPERFIT, India) at 40° C to obtain dry extract (yield 24.25 %w/w) and stored at -20° C in refrigerator until used.

### 2.2. Preliminary phytochemical analysis

The ethanol extract of *Senna surattensis* (EESS) was subjected to preliminary phytochemical analysis for the detection of phytoconstituent using standard chemical tests[14-15].

### 2.3. Estimation of total phenol content

The total phenolic content of EESS was determined using Folin-Ciocalteu reagent according to previously described method[16]. 1.0 mL of extract containing 1.0 mg of extract was transferred in to 100 mL Erlenmeyer flask then final volume was adjusted to 46 mL by addition of distilled water. Afterwards, 1 mL of Folin-Ciocalteu reagent was added to this mixture and after 3 min 3 mL of $Na_2CO_3$ (2 %) were added. Subsequently, mixture was shaken on a shaker for 2 h at room temperature and then absorbance was measured at 760 nm. The concentration of total phenolic content was determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph. The equation is given below

Absorbance = 0.001× Pyrocatechol (µg) + 0.0033.

### 2.4. In vitro antioxidant activity

#### 2.4.1. DPPH radical Scavenging activity

DPPH radical scavenging activity of EESS was carried out by previously described method[17]. 0.1 Mm solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3ml of extract solution at different concentrations (20, 40, 80, 160, 320, 640 µg/mL). After 30 min incubation in dark, the absorbance was measured at 517 nm. Ascorbic acid was used as a reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All the tests were performed in triplicate. The percentage DPPH radical scavenging that is calculated in the following equation

$$\% \text{ DPPH radical scavenging} = [(A_0-A_1)] /A_0\times 100$$

Where $A_0$ is the absorbance of the control, $A_1$ is the absorbance of the extract/standard.

#### 2.4.2. Superoxide anion radical scavenging activity

The superoxide anion scavenging activity of EESS was determined by previously described method, this was slightly modified [18]. The reaction mixture consisting of 1 mL NBT solution containing 156 µM NBT dissolved in 1.0 mL 100 mM phosphate buffer, pH 7.4, 1 ml NADH solution (468 µM NADH dissolved in 1 ml 100 mM phosphate buffer, pH 7.4), and 0.1 ml of various concentration of EESS and reference compound, (20, 40, 80, 160, 320 and 640 µg/mL) were mixed. The reaction was started by adding 100 µl phenazine methosulfate solution containing 60 µM phenazine methosulfate in 100 mM phosphate buffer, pH 7.4. The reaction mixture was incubated at 25ºC for 5 min and absorbance at 560 nm was measured against control samples. All tests were performed in triplicate. Percentage inhibition was calculated by comparing the results of test samples with those of the control not treated with the extract. Ascorbic acid was used as reference compound. The percentage inhibition activity was calculated as

$$\% \text{ Inhibition} = [(A0-A1)] /A0\times 100$$

Where A0 is the absorbance of the control, A1 is the absorbance of the extract/standard.

#### 2.4.3. Hydroxyl radical scavenging activity

The ability of ethanol extract of *Senna surattensis* to scavenge the hydroxyl radical generated by the Fenton reaction was measured according to previously described modified method [19]. The Fenton reaction mixture containing 200 µL of 10mM $FeSO_4 \cdot 7H_2O$, 200 µL of 10 mM EDTA and 200 µL of 10 mM 2-deoxyribose was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) containing 200 µL of various concentrations of EESS (20 to 640 µg/mL). Thereafter, 200 µL of 10 mM $H_2O_2$ was added to the mixture before incubation for 4 h at 37° C. Later, 1 mL of 2.8 % TCA and 1mL of 1 % TBA were added and placed in a boiling water bath for 10 min. Then, the resultant mixture was allowed to cool up to room temperature and centrifuged at 395 × *g* for 5min. Absorbance was recorded at 532 nm.

#### 2.4.5. Lipid peroxidation by thiobarbituric Acid (TBA) Assay

TBA reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532 nm [20]. Normal albino rats of the Wister strain were used for the preparation of liver homogenate. The perfused liver was isolated, and 10% (w/v) homogenate was prepared using a Potter Elvehjem homogenizer at 0-4 °C with 0.15 M KCl. The homogenate was centrifuged at 800 × *g* for 15 min, and clear cell-free supernatant was used for the study of *in vitro* lipid peroxidation. Different concentrations (20 to 640µg/ml) of EESS (dissolved in Double distilled water) were taken in various test tubes. One milliliter of 0.15 M KCl and 0.5 mL of rat liver homogenates were added to the test tubes. Peroxidation was initiated by adding 100 µl of 0.2 mM ferric chloride. After incubation at 37 °C for 30 min, the reaction was stopped by adding 2 mL of ice-cold HCl (0.25 N) containing 15% trichloroacetic acid (TCA), 0.38% TBA, and 0.5% BHT. The reaction mixtures were heated at 80 °C for 60 min. The samples were cooled and centrifuged, and the absorbance of the supernatant was measured at 532nm. An identical experiment was performed to determine the amount of lipid peroxidation obtained in the presence of inducing agents without extract.

**2.4.5. Measurement of reducing Power**

Reducing power was investigated by previously described method with slight modification[21]. The extract (0.75 mL) at various concentrations (20-640 µg/mL) was mixed with 0.75 mL of phosphate buffer (0.2M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1% w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (TCA) solution (10% w/v), and then centrifuged at 800 × g for 10 min. 1.5 mL of the supernant was mixed with 1.5 ml of distilled water and 0.1mL of ferric chloride solution (0.1% w/v). Absorbance of the resultant mixture was measured at 700 nm after 10 min. Increased absorbance of the reaction mixture indicated enhanced reducing power.

**2.5. Statistical analysis**

The experimental data were expressed as mean ± SEM. The concentration of the extract (µg/mL) that was required to scavenge 50% ($IC_{50}$) of radicals was calculated by using linear regression method of plots of the percent of antiradical activity against the concentration of the tested compounds using Microsoft Excel Software Programme.

**1. RESULTS AND DISCUSSION**

Preliminary phytochemical screening of EESS showed the presence of triterpenoid, steroids, phenolic, tannins and flavonoids. The total polyphenol content of EESS was detected 98 µg equivalent to pyrocatechol/mg of extract. Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups[22]. The polyphenolic compounds may contribute directly to the antioxidative action[23]. In addition, it was reported to play an important role in stabilizing lipid peroxidation[24]. The extract contain polyphenol is used for the prevention and cure of various diseases which is mainly associated with free radicals[25].

DPPH is a stable free radical in aqueous or ethanol solution and accepts an electron or hydrogen radical becomes a stable diamagnetic molecule. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants[23]. In the presence of an electron-donating anti-oxidant, the purple colour typical of the free DPPH radical diminishes in intensity, a change that can be followed spectrophotometrically at 517 nm. The radical scavenging activities of the extract measured as decolorizing activity following the trapping of the unpaired electron of DPPH .The inhibitory effect of these extracts may be attributed to the presence of phenolic compound. It is well known that antioxidants can seize the free radical chain of oxidation and form stable free radicals. This would not initiate or propagate further oxidation DPPH has been used extensively as a fee radical to evaluate reducing substances[26-27]. The scavenging activities of DPPH exerted by EESS and ascorbic acid were summarized in Figure 1, it indicated the free radical scavenging activity of EESS concentration dependent. The $IC_{50}$ value of EESS was found to be 284.96 µg/mL with respect to the standard drug ascorbic acid 117.70 µg/mL (Table 1).

Superoxide anion is derived from dissolved oxygen in the PMS/NADH-NBT system by phenazine methosulphate (PMS)-NADH coupling reaction reduces nitroblue tetrazolium (NBT) to a blue coloured formazan that is measured at 560 nm[28]. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive species, such as single oxygen and hydroxyl radicals[29]. Furthermore, superoxide radical is considered to play an important role in the peroxidation of lipids[30] .The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide radical scavenging activity of EESS was increased markedly with increase of concentration (20-640 µg/mL) shown in Figure 2. $IC_{50}$ value of EESS found to be 428.88 µg/mL and for ascorbic acid 237.34 µg/mL (Table 1).

Among the oxygen radicals specifically, the hydroxyl radical is the most reactive and severely damages adjacent biomolecules such as all proteins, DNA, PUFA, nucleic acid, and almost any biological molecule it touches. This damage

causes aging, cancer and several diseases[31]. The deoxyribose method is a simple assay to determine the rate constants for reactions of hydroxyl radicals[32]. When the mixture of $Feso_4$-EDTA, $H_20_2$ and ascorbate were incubated with deoxyribose in phosphate buffer (pH 7.4), the hydroxyl radicals generated attack the deoxyribose and result in a series of reactions that cause the formation of MDA. Any hydroxyl radical scavenger added to the reaction would compete with deoxyribose for the availability of hydroxyl radicals, thus reducing the amount of MDA formation. We herein tested the scavenging activity of EESS along with positive control α-tocopherol shown in Figure 3. The $IC_{50}$ value of EESS and α –tocopherol was found to be 144.81 µg/mL and 84.76 µg/mL respectively (Table 1)

The peroxy radical which is generated during lipid peroxidation is very reactive and it propagates the chain reaction thereby resulting in extensive destruction of fatty acids, ultimately leading to cell death[33]. Oxidation of unsaturated fatty acids in biological membranes leads to the formation and propagation of lipid radicals, uptake of oxygen, rearrangement of the double bonds in unsaturated lipids, and eventual destruction of membrane lipids, with production of breakdown products such as malondialdehydes[34]. Malondialdehyde serves as a convenient index for determining the extent of lipid peroxidation[35]. In particular $O_2^-$ and OH induce various injuries to the surrounding organs and play a vital role in some clinical disorders. Therefore removal of $O_2^-$ and OH is the effective defense of the living body against disease[36-37]. In the present study EESS effectively inhibited the lipid peroxidation induced by Iron/TBA/BHT complex in rat liver homogenate shown in Figure 4. $IC_{50}$ value for the inhibition of lipid peroxidation of EESS and standard ascorbic acid was found to be 212.16 µg/mL and 256.15 µg/mL respectively.

The antioxidant activities of natural components may have a reciprocal correlation with their reducing powers[38]. The reducing power increased with the increasing concentration of extract, and the activity may be due to presence of phenolic compounds in EESS. The activity may it act as electron donors and could react with free radical to convert them into more stable products and to terminate radical chain reaction. The reducing powers of EESS and ascorbic acid shown in Figure 5. EESS showed reducing power comparable with standard at higher concentration at 640 µg/mL.

In conclusion, the antioxidant and free radical scavenging activities of *Senna surattensis* leaves might be due to the presence of phenolic compounds in this extract which is confirmed by Folin-ciocalteu reagent test and phytochemical tests. The polyphenolic antioxidants such as flavonoids, tannins, coumarins, xanthons and more recently procyanidins scavenge radicals dose-dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies[39]. Further studies are in progress in our laboratory to evaluate the *in vivo* antioxidant potential of this extract in various animal models as well as to isolate its active bioactive constituents to determine the value of the ethno botanical approach for the treatment of oxidative stress related disease.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**ACKNOWLEDGEMENTS**

Authors are grateful to thank All India Council of Technical Education (AICTE), New Delhi, India, for providing financial support through its Quality Improvement Programme.

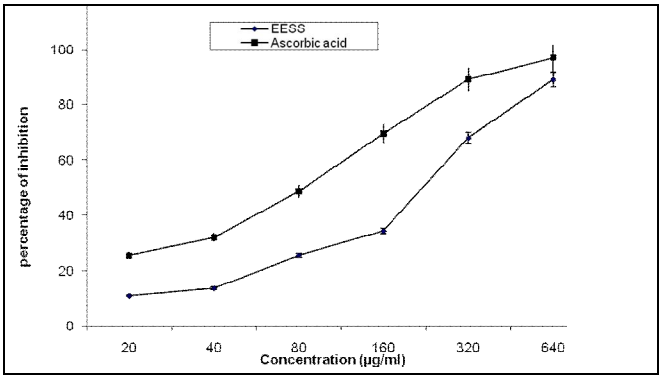


**Fig. 1: Effect of Ethanol extract of *Senna surattensis* on DPPH radical scavenging activity**

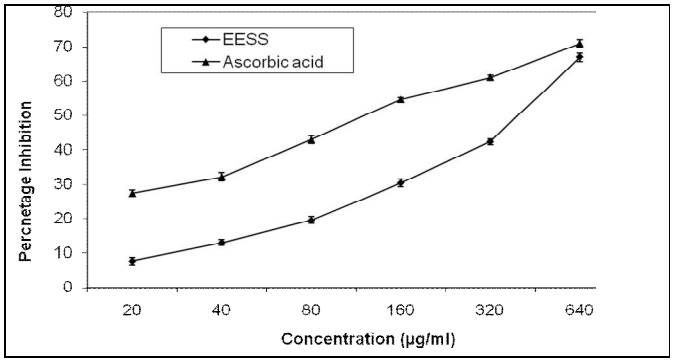


**Fig. 2: Superoxide Radical scavenging effect of Ethanol extract of *S.surattensis***

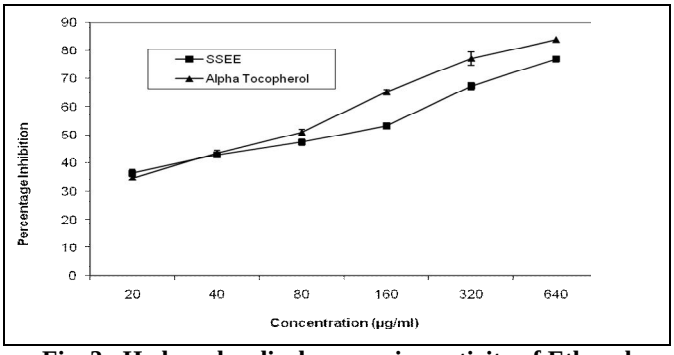


**Fig. 3: Hydroxyl radical scavenging activity of Ethanol extract of *S.surattensis***

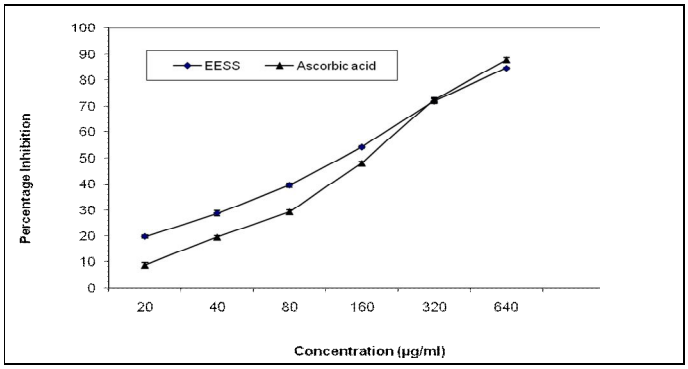


**Fig. 4: Effect of Ethanol Extract of *S. surattensis* on lipid peroxidation**

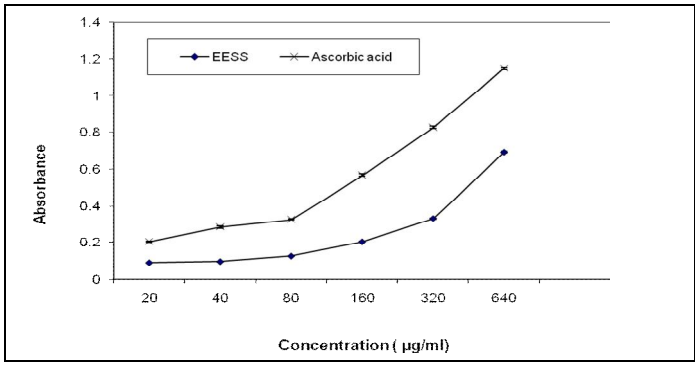


**Fig. 5: Effect of Ethanol extract of *S. surattensis* on reducing power**

**Table 1: Antioxidant activity of ethanol extract of *senna surattensis* (EESS)**

| Method | Inhibitors (µg / mL) | Inhibitory concentration ($IC_{50}$) (µg / mL) |
|---|---|---|
| DPPH radical | EESS (20 -640)<br>Ascorbic acid | 284.96<br>117.70 |
| Superoxide radical | EESS (20 -640)<br>Ascorbic acid | 428.88<br>237.34 |
| Hydroxyl radical | EESS (20 -640)<br>α-Tocopherol | 144.81<br>84.76 |
| Lipid peroxidation | EESS (20 -640)<br>Ascorbic acid | 212.16<br>256.15 |
| Reducing power | EESS (20 -640)<br>Ascorbic acid | - |

**REFERENCES**


1. Kehrer JP. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol. 1993;23:21-48.
2. Harman D. The aging process. Proc Natl Acad Sci USA 1981;78:7124-7128.
3. Droge W. Free radicals in physiological control of cell functions. Physiol Rev. 2002;82:47-95.
4. Wichi HC. Enhanced tumour development by butylated hydroxytoluene (BHT) in the liver, lung and gastro-intestinal tract. Food Chem Toxicology. 1986;24:1127-1130.
5. Grice HP. Enhanced tumour development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. Food Chem Toxicol. 1988;26:717-723.
6. Formica JV and Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol. 1995;33:1061-1080.
7. Singh V. Monograph on Indian Subtribe Cassiinae (Caesalpinaceae). India: Pawankumar Scientific Publisher. 2001;215-219.
8. Seetharami Reddi TVV, Ramarao Naidu BVA. Ethnomedicine and Human Welfare. India: Lambert Academic Publications. 2005;53.
9. Kirtikar KR, Basu BD. Indian Medicinal Plants. India: Lalit Mohan Basu Publishers. 1984;870-873.
10. Maheswari JK, Kalakoti BS, Lal B. Ethnomedicine of Bhil tribe of Jhabua District, M.P. Ancient Sci Life. 1986;5:255-256.
11. Tiwari HI, MisraI M. Phytochemical investigation of Cassia glauca bark. J Indian Chem Soc.1993;70:659.
12. Rai KN, Kaushalendra K and Singh J. Chemical constituents from the pods of Cassia glauca, Lam. Asian J Chem. 1997;9:558-560.
13. Hus KK and Hong WH. Isolation of quercetin from the flower of Cassia glauca. Chemical Abstracts 1965;62:3433.
14. Kokate CK. Practical Pharmacognosy. New Delhi: Vallabh Prakashan. 1994;107-113.
15. Harbone JB. Phytochemical Methods. London: Chapman and Hall.1998;60- 68.
16. Singleton VL, Orthofer RM and Ramuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin

Ciocalteu Reagent. Methods Enzymol. 1999;299:152-178.
17. Blois MS. Antioxidant determination by the use of stable free radical.Nature. 1958;181:1199-1200.
18. Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun.1972;46:849-853.
19. Chung SK, Osawa T and Kawakishi S. Hydroxyl radical scavenging effect of spices and scavengers from Brown mustard (Brassica nigra). Biosci Biotech Biochem. 1997;61:118-123.
20. Halliwell B, Gutteridge JMC and Aruoma OI. The deoxyribose method: a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. Annals Biochem.1987;165:215-19.
21. Oyaizu M. Studies on products of browning reaction: antioxidant activities of products of browning reaction prepared from glucose amine. Jap J Nutr. 1986;44:307-315.
22. Hatano T, Edmatsu R, Mori A, Fujita Y and Yasuhara E. Effect of interaction of tannins with co-existing substances. VI. Effect of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem Pharm Bull.1989;37:2016-2021.
23. Duh PD, Tu YY and Yen GC. Antioxidant activity of water extract of harng jyur (Chrysanthemum morifoliun Ramat). Lebensm-Wiss.u-Technol. 1999;32:269-315.
24. Yen GC, Duh PD and Tsai C. Relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem. 1993;41:67-70.
25. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol. 1983;30:1141-1148.
26. Schimada K, Jujikawa K, Yahara K and Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem. 1992;40:945-948.
27. Duh DU and Yen GC. Antioxidative activity of three herbal water extracts. Food Chem. 1997;60:639-645.
28. Khanam S, Schicprasad HN and Kashama D. In vitro antioxidant screening models: a review. Ind J Pharm Educ. 2004;38:180-94.
29. Aurand LW, Boonme NH and Gidding GG. Superoxide and singlet oxygen in milk lipid peroxidation. J Dairy Sci. 1977;6:363-9.
30. Dahl MK and Richardson T. Photo generation of superoxide anion in serum of bovine milk and in model system containing riboflavin and amino acids. J Dairy Sci.1978;61:400-7.
31. Aruoma OI. Free radicals stress, and antioxidants in human health and disease. J Am Oil Chem Soc. 1998;75:199-211.
32. Halliwell B and Guttridge JMC. Free Radicals in Biology and Medicine. Tokyo: Japan Scientific Societies Press;1989:18.
33. Ursini F, Maiorino M and Sevanian A. Oxidative stress: oxidants and Antioxidants. London: Academic Press;1991:279-386.
34. Slater TF, Cheeseman KH, Davies MJ and Xin W. Free radical mechanism in relation to tissue injury. Proc Nutr Soc.1987;46:1-12.
35. Esterbauer H. Free radical in liver injury, IRL Press, Oxford. 1985; 22-48.
36. Prasad K, Laxdal VA, Yu M and Raney BL. Evaluation of hydroxyl radical-scavenging property of garlic. Mol Cell Biochem. 1996;154:55-63.
37. Lin JM, Lin CC and Chen MF. Scavenging effects of Mallotus repandus on active oxygen species. J Ethno pharmacol. 1995;46:175-181.
38. Yen GC and Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. J Agric Food Chem. 1995;42:629-632.
39. Lee S, Suh S and Kim S. Protective effects of the green tea polyphenol (-) – epigallocatechin gallate against hippocampal neuronal damage after transient global ischemia in gerbils. Neurosci Lett. 2000; 287;191-94.