Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Suppl 1, 2012 ISSN - 0974-2441

Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF SESAMUM INDICUM SEEDS

H.S. VISHWANATH, K.R. ANILAKUMAR*, S.N.HARSHA, FARHATH KHANUM AND A.S. BAWA

[1]**Biochemistry and Nutrition Discipline, Defence Food Research Laboratory Mysore-570011, INDIA,Email:**anilakumarkr@gmail.com

Received: 30 August 2011, Revised and Accepted: 20 October2011

ABSTRACT

The white and black varieties of *Sesamum indicum* were extracted in ethanol and the extracts were assayed for their antioxidant activities. The study revealed that both the extracts showed antioxidant activity. Respect to its abilility in inhibiting the lipid peroxidation. The hydroxyl radical scavenging by the white sesame extract was found to be more than that of black sesame. The white sesame seed extract was markedly a more potent scavenger of superoxide anion than the black one. The reducing power of the seed extracts was in substantiation with the antioxidant property. Fe^{++} chelation by the extracts was found to be high. It is concluded that the sesame seed extracts possess high antioxidant activity and that the white variety elicit better antioxidant activity than the black one.

Keywords: *Sesamum indicum*; plyphenols; lipid peroxidation; superoxides; reducing power.

INTRODUCTION

Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals and hydroxyl radicals, as well as non-free radical like hydrogen peroxide species and the singlet oxygen (Halliwel 1995; Squadriato et al 1998; Yildrim et al 2001). Also, excessive generation of ROS, induced by various stimuli and which exceed the antioxidant capacity of the organism, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity, and cancer (Kourounakis et al 1999; Gulcin et al 2002 and Gulcin et al 2003). The World Health Organization (WHO) estimated that approximately 80 percent of the world populations rely primarily on traditional medicine as source for their primary health care (Farnsworth et al 1985). Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions, or from exogenous factors (Cerutti 1991). Polyphenols present in plants, fruits and vegetables are an important source of natural antioxidants as they act as reducing agents, hydrogen donors, singlet oxygen quenchers and potential metal chelators (Kahkonen et al 1999; Rice-Evans et al 1995). *In vivo*, some of these ROS play positive roles such as energy production, phagocytosis, regulation of cell growth and intercellular signalling, or synthesis of biologically important compounds (Halliwell 1997).However, ROS may also be very damaging as they can induce oxidation of lipids, causing membrane damage, decreasing membrane fluidity, and leading to cancer via DNA mutation (Cerutti 1994). A potent scavenger of these ROS may serve as a possible preventative against free radical mediated diseases (Ames et al 1995).

Sesamum indicum (Sesame) is an ancient spice, one of the first recorded plants used for its seeds. It has been used for thousands of years and is still an oil seed of worldwide significance. Sesame oil is very rich in protein and is used in margarine production and as cooking oils. Non-culinary uses include its application as an ingredient in soap, cosmetics, lubricants and medicines. In southern India it is used to anoint the body and hair. Sesame meal is excellent feed for poultry and livestock. Sesamolin has insecticidal properties and is used as a synergist for pyrethrum insecticides (Morris 2002.) Sesame oil is used as a solvent, oleaginous vehicle for drugs, and skin softener. Chlorosesamone obtained from roots of sesame has antifungal activity (Begum et al. 2000). Sesame oil is a pharmaceutical aid used as a solvent for intramuscular injections and has nutritive, demulcent, and emollient properties (Tyler et al. 1976) and has been used as a laxative.

Sesamin, sesamolin and myristic acid found in sesame have been found to possess antioxidant and health promoting activities (Kato et al.1998; Sirato- Yasumotosumoto et al. 2001). The seed consumption appears to increase plasma gamma-tocopherol andenhances vitamin E activity, which is believed to prevent cancer and heart disease (Cooney et al. 2001). Indians have used sesame oil as an antibacterial mouthwash and for relieving anxiety and insomnia (Annussek 2001). A clinical trial proved the effectiveness of sesame oil for treating nasal mucosa dryness rather than isotonic sodium chloride solution (Johnson *et al.* 2001). In addition, sesame oil contains large amounts of linoleate in triglyceride form which selectively inhibited malignant melanoma growth (Smith and Salerno 2001). The medicinal, industrial and pharmaceutical characteristics of the seeds are described in a recent review (Anilakumar et al. 2010).

Several studies indicated that antioxidant activity of many plant foods are highly correlated to their total phenolics (Parr and Bolwel 2000, Dewanto *et al* 2002). The purpose of this study was to assess the antioxidant activities of the extracts of sesame, both black and white varieties as well as with regard to the lipid peroxidation and pro-oxidant activity.

MATERIALS AND METHODS

Chemicals and reagents: Ethanol and distilled water were used as solvent for extraction of antioxidant compounds. H_2SO_4, NaOH, HCl, DPPH, BHA, gallic acid, Folin-Ciocalteu reagent, $FeCl_2$, ferrozine, potassium ferricyanide, EDTA, ascorbic acid, TCA, $FeCl_3$, Na_2CO_3, catachin were of analytical grade and were stored at prescribed conditions in the laboratory.

Preparation of seed extract

Sesame (*Sesamum indicum* L.), black and white varieties were purchased from local market. The seeds were ground in mixer separately. 10g of the powder was weighed and suspended in 100ml of 90% ethanol and kept for shaking for 2hrs.After filtration the samples were subjected for vacuum evaporation. The extract was redissolved in a known volume of 90% ethanol and assayed for its antioxidant activity.

DPPH radical scavenging activity

For determination of the antioxidant activity of the black sesame and white sesame extracts, the stable , 1 diphenyl-2-picryl hydrazyl (DPPH) radical was used (Sun and Ho 2005). An aliquot 0.5ml of DPPH solution was diluted in 4.5 ml of methanol, and 30µl of ethanolic solution of the black sesame and white sesame extract and standard BHA and TBHQ was added. A control without extract/standard was also maintained. The mixture was shaken vigorously and allowed to stand for 45 minutes in the dark and the absorbance was measured at 515nm. The antioxidant activity of the extract was calculated using the formula,

$$\% \text{ scavenging activity} = \frac{\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Total phenolic content

The amount of total polyphenolic compounds was measured by the method described by Taga *et al.* (1984). 15mg of extract was dissolved in 1ml of 90% ethanol. A 10µl aliquot of the resulting solution was added to 2ml of 2% Na_2CO_3 and after 2 minutes 100µl of Folin-ciocalteu reagent (diluted with water 1:1) was added. After a further 30 minutes, the absorbance was measured at 750nm. The concentration was calculated using gallic acid as standard, and the results were expressed as mg gallic acid equivalents per gm extract.

Total flavonoid content

The determination of flavonoids was performed according to the colorimetric assay of Delcour and de Varebeke (1985). About 50mg of the extract was diluted in 10ml of 90% ethanol and 40µl of this solution was pipetted into a test tube. A total of 5ml of the chromogen reagent (1.0 g of 4-dimethylaminocinnamaldehyde dissolved in a cooled mixture of 250 ml of HCl and 750 ml of methanol) was added to the extract solution, and after 10 min the absorbance was read at 640 nm against a blank. A calibration curve was prepared with (+)-catechin and the results were expressed as (+)-catechin equivalents.

Reducing power assay

Reducing power of the sample was determined according to the method of Oyaizu (1986). 1.0 ml of different concentrations of sample (to produce final concentration 5-25mg/ml) was mixed with 2.5ml of potassium ferricyanide (1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50^0C for 20 minutes, 2.5ml of TCA (10%) was added to it and centrifuged at 800xg rpm for 10 minutes. 2.5 ml of supernatant was added to 2.5 ml of water and 0.5 ml of ferric chloride (0.1%). Absorbance was measured at 700nm.

Superoxide anion ($O_2^{\cdot-}$) radical scavenging activity

Measurement of superoxide anion scavenging activity of ethanol and water extracts was based on the method described by Liu et al (1997). Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the radiation of NBT. In this experiment, the superoxide radicals were generated in 3ml of Tris-HCl buffer (16Mm, pH 8.0) containing 1ml of NBT (50µM) solution, 1ml NADH (78µM) solution and (100-300µg/ml) of sample extract. The reaction was started by adding 1ml of PMS solution (10µM) to the mixture. The reaction mixture was incubated at 25^0C for 5min and the absorbance was measured at 560nm against blank sample. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The inhibition percentage of superoxide anion generation was calculated by using the following formula:

$$\% \text{ scavenging activity} = \frac{\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Hydroxyl ($OH^{\cdot}$) radical scavenging activity

Deoxyribose degradation assay was performed as per the method of Halliwell *et al*. (1997), with slight modification. Briefly, different concentrations of extracts were mixed with 200 mM $FeCl_3$ and 1.04 mM EDTA (0.2 ml, 1:1), 1 mM H_2O_2 (0.1 ml), 28 mM deoxyribose (0.1 ml) and 1 mM ascorbic acid (0.1 ml) and the final volume was made to 1.1 ml with phosphate buffer (0.2 mM, pH 7.2). The mixture was incubated at 37° C for 1 hr. Then, 1 ml of thiobarbituric acid (1% in 50 mMNaOH) and 1 ml of 5% TCA was added followed by boiling in a boiling water-bath for 20 min. After cooling, absorbance of the mixture was measured at 532 nm and the percentage inhibition was calculated

$$\% \text{ scavenging activity} = \frac{\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Metal chelating activity

The chelating of ferrous ions of the extracts was evaluated by the method of Dinis et al (1994). Briefly the extracts samples (200-1000µg/ml) were added to a solution of 2mmol/l $FeCl_2$ (0.05ml). The reaction was initiated by the addition of 5mmol/l Ferrozine (0.2ml) and the mixture was shaken vigorously and left for standing at room temperature for 10min. Absorbance of the solution was than measured spectrophotometrically at 562nm. The percentage of inhibition of Ferrozine-Fe^{2+} complex formation was calculated from

$$\% \text{ scavenging activity} = \frac{\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}}}{\text{Absorbance}_{\text{control}}} \times 100$$

All the values were expressed as mean of 3 determinations.

RESULTS AND DISCUSSION

Spices have been recognized to possess several medicinal properties and have been effectively used in India and other countries. Apart from the traditional use, many beneficial physiological effects have been brought to the fore by extensive studies (Anilakumar et al. 2001, Khanum and Anilakumar 2004, Srinivasan 2005). Hence this study was taken up to evaluate the comparative effects of, black and white sesame seed extracts for their antioxidant activities. The yield of ethanolic extract of black sesame and white sesame in powder form were found to be 19.8mg/g and 78.4mg/g respectively.

The free radical scavenging ability of black and white sesame extracts were analysed by DPPH method (Fig 1). This assay is one of the tests used to prove the ability of the components of spice extracts to act as donors of hydrogen atoms. The black sesame and white sesame extracts showed a significant effect in inhibiting DPPH, reaching up to 56.73% and 61.16% respectively at a concentration of 1mg/ml compared with that of standard antioxidants butylatedhydroxyanisole (BHA) (72.62%) and *tert*-butylhydroquinone (TBHQ) (48.97%). This indicates that the radical scavenging potential of the extracts studied was less than that of BHA and slightly higher than that of TBHQ.

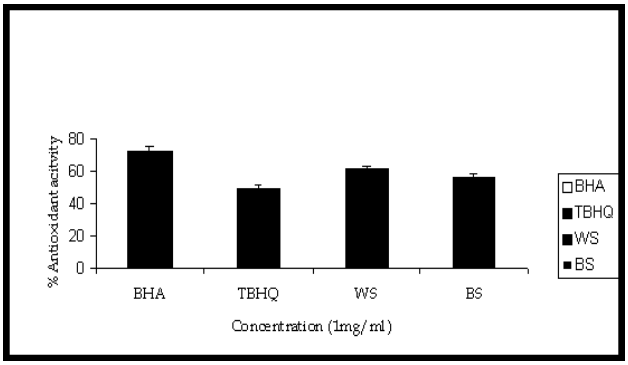

Fig. 1: Percentage of antioxidant activity of black sesame and white sesame by DPPH radical scavenging method.

Both black sesame and white sesame seed extracts displaced hydroxyl radical scavenging activity (Fig 2). The hydroxyl radical scavenging activity of white sesame seed extract was found to be more than their of black sesame seed extract. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species capable of damaging biomolecules of the living cells (Gorden 1990; Halliwell 1991).Hydroxyl radical has the capacity to cause DNA strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, this radical species is considered as one of the quick initiators of the lipid oxidation process (Kappus 1991; 9. Robak et al 1998).

Superoxide radical scavenging activity was shown by both black sesame and white sesame seed extracts (Fig 3). White sesame seed extract was a more potent scavenger of superoxide anion than the black sesame seed extract. Superoxide radicals are generated during the normal physiological process mainly in mitochondria. Although superoxide anion by itself is a weak oxidant, it generates dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to the oxidative stress (Mayer and Isaksen 1995; Babu et al 2001). Hence superoxide radical scavenging by antioxidants has physiological implications.

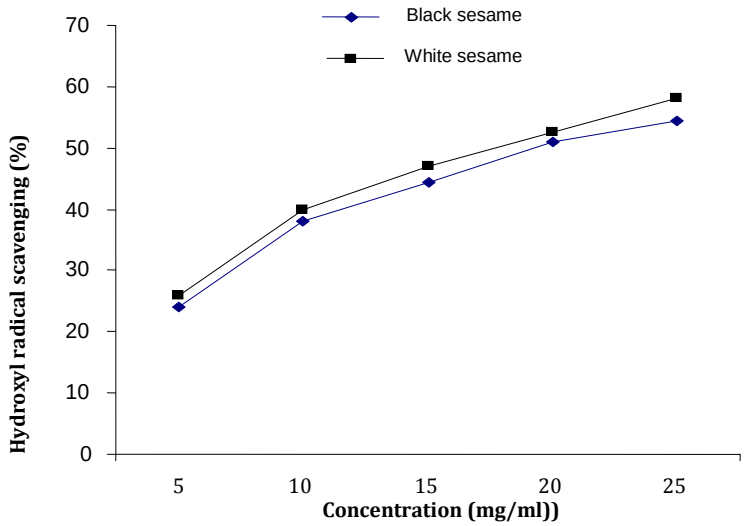

Fig 2: Effect of ethanol extracts from black and white sesame seeds on hydroxyl radical scavenging activity.

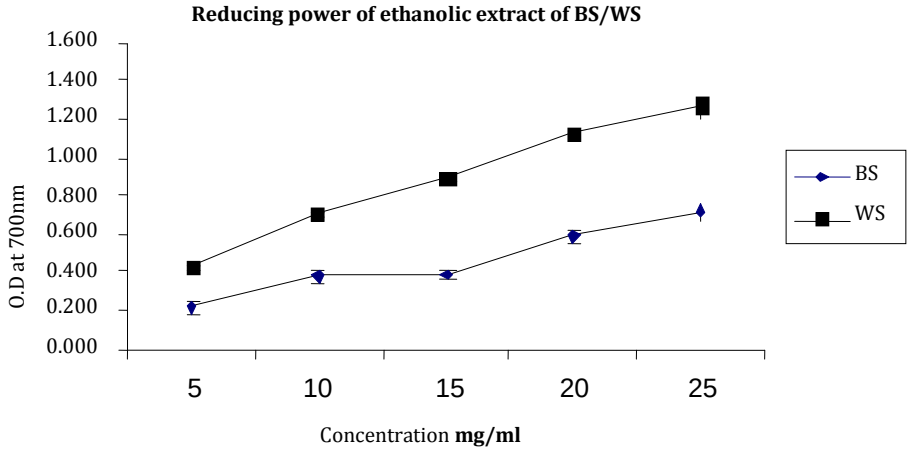

Fig 3: Effect of ethanol extracts from black and white sesame seeds on superoxide radical scavenging activity.

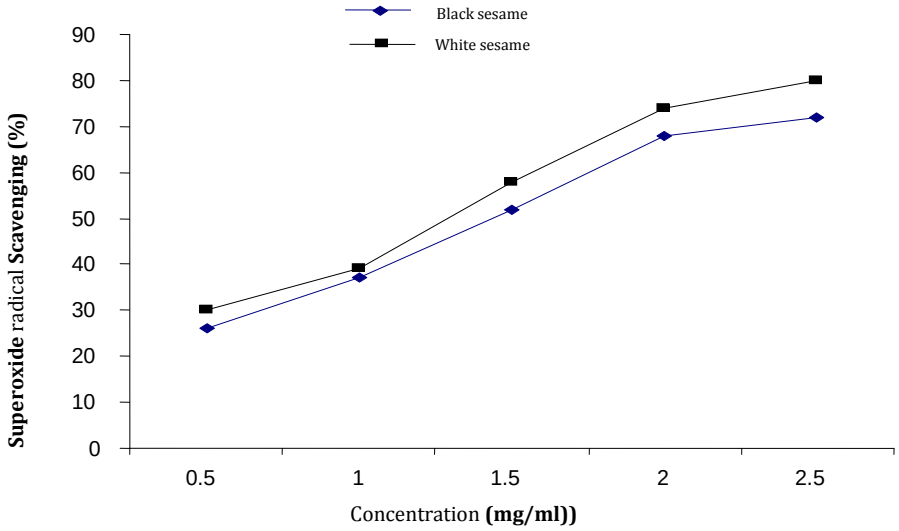

Fig 4: Reducing power of ethanol extracts from black and white sesame seed.

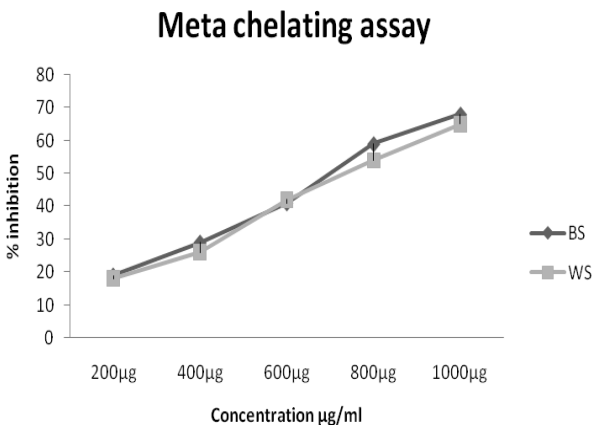

Fig. 5 Metal chelating activity of ethanol extracts from black and white sesame seeds.

The reducing power of the extract, which may serve as a significant reflection of antioxidant activity, was determined using a modified Fe3+ to Fe2+ reduction assay, whereby the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of the samples (Ebrahimzadeh et al 2008).The effect of reducing power of the extract on potassium ferricyanide is given in Fig 4. The reducing power of both the extracts increased as concentration increased. At the concentration of 25mg/ml, the black sesame and white sesame extracts showed significant reducing power. Our results are in accordance with an earlier work (Tanaka et al. 1988).

Transition metals have been proposed as the catalysts for the initial formation of radicals. Chelating agents may stabilize transition metals in living systems and inhibit generation of radicals, consequently reducing free radical-induced damage. Black sesame and white sesame seed extracts were evaluated for Fe^{++} chelating activity (Fig 5). It is known that the binding of the known pro-oxidant viz. Fe^{++} may prove beneficial in preventing oxidation of lipids. At the concentration of 1mg/ml, both the black sesame and white sesame extracts showed inhibition of metal chelation. This indicates that the chelating activity of black sesame and white sesame extract does contribute to its antioxidant activity.

This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng et al 2001). In fact, many medicinal plants contain large amounts of antioxidants such as polyphenols. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson et al 2001; Djeridane et al 2006). The result strongly suggests that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents. Table 1 shows that the white sesame seed extract contain better polyphenols and flavonoids contents than that of black sesame. This could be the reason for the better antioxidant activities of the white sesame seed than that of the black variety. In this study, the sesame seed extracts as compared to the synthetic antioxidant was found to possess potential antioxidant activity. More work is however needed, to define the optimum dietary concentrations for obtaining the greatest stability in the food products. From the foregoing it is concluded that white variety of sesame seed possesses better antioxidant property than black sesame seed *in vitro*.

Table 1: Antioxidant activity of polyphenols and flavonoid contents of sesame seed extracts.

Spice extracts	AOA%	Polyphenols mg/g	Flavonoids mg/g
Black sesame	56.73±1.39	1.38± 0.08	0.05±0.008
White sesame	61.16±2.09	2.88±0.15	0.12±0.009

CONCLUSION

The properties of the sesame extracts under study compared with the synthetic antioxidant, determine its potential as a natural preservative, applicable in the food and pharmaceutical industries. More work is needed to define the optimum dietary combinations for obtaining the greatest stability in the resultant product. This will eventually require sophisticated feed formulations and better understanding of the nutrient impact of the byproducts that are traditionally used as food. Sesame is an indispensable component of Indian culinary. With regard to antioxidant properties of sesame extracts established in this work, it can be successfully used as a key ingredient in halva, tahini, and bread dip and in other colourful rice and noodle dishes for its aroma and flavour. From the foregoing it is concluded white sesame seed posesses better antioxidant property than black sesame seed *in-vitro.*

REFERENCES

1. Ames BN, Gold LS, Willet WC, (1995) The causes and prevention of cancer. *Proceedings of the National Academy of Sciences* USA. 92, 5258–5265.
2. Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, Steinberg FM, (2001) Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. *Biochemical and molecular action of nutrients*. *J. Nutr.* 131, 2837-2842.
3. AnilakumarKR, Ajay Pal, FarhathKhanum, Amarinder Singh Bawa (2010) Nutritional, medicinal and industrial uses of sesame(*Sesamumindicum L*) seeds-An overview, Agri Cons Scien (acs),75,4(159-168).
4. AnilakumarKR, NagarajNS and Santhanam, K (2001) Effect of coriander seeds on hexachlorocyclohexane- induced lipid peroxidation in rat liver. Nutr. Res. *21*, 1455–1462.
5. Annusek G (2001) Sesame oil. In *Gale encyclopedia of alternative medicine.* Gale and Group and Lookshmart.
6. Babu BH, Shylesh, B.S. and Padikkala J, (2001) Antioxidant and hepatoprotective
effect of Alanthusicicifocus, Fitoterapia, 72, 272-277.
7. BegumS, FurumotoT and FukuiH (2000) A new chlorinated red naphthoquinone from roots of Sesamumindicum. Biosci. Biotech.Biochem. *64*, 873-874.
8. Cerutti P, (1994) Oxy-radicals and cancer. *Lancet.* 344, 862-863.
9. Cerutti PA, Oxidant stress and carcinogenesis. *Eur J Clin Invest.* 21, 1991, 1–11.
10. CooneyRV, CusterLJ, OkinakaL and FrnkeAA (2001) Effects of dietary seeds on plasma tocopherol levels. Nutr. Cancer. *39*, 66-71.
11. DelcourJA, and deVarebekeDJ (1985) A new colourimetric Assay for Flavonoids in pilsner Beers. J.Inst. Brew. *91*, 37-40.
12. DewantoV, WuX, AdomKK and LiuRh (2002) Thermal processing enhances the nutritional values of tomatoes by increasing total antioxidant activity. J Agric Food Chem. *50*, 3010-3014.
13. Dinis, Madeira, & Almeida (1994) T.C.P. Dinis, V.C.M. Madeira and L.M. Almeida, Action of phenolic derivates as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Archives of Biochemistry and Biophysics315, pp. 161–169
14. Djeridane A, Yousfi M, Nadjemi B, Boutassouma D, Stocker P, Vidal N, (2006) Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* 97, 654-660.
15. Ebrahimzadeh M.A., Hosseinimehr S.J., Hamidinia A. and Jafari M. (2008): Antioxidant and free radical scavenging activity of *Feijoasallowiana*fruits peel and seeds. *Pharmacologyonline*1, 7-14.
16. FarhathKhanum and Anilakumar KR (2004) Anticarcinogenic properties of garlic: A review. Crit Rev Food SciNutr. *44*, 449-479.
17. Farnsworth NR, Akerela O, Bingel AS, Soejorto DD, Guo Z, (1985) Medicinal plants in therapy. *Bull WHO* 63, 965-981.
18. HalliwellB and Gutteridge JMC (2000) In *Free radicals in Biology and Medicine,* Third edition. Clarendon, Oxford, England.

19. Halliwell B, (1997) Antioxidants and human diseases: a general introduction. *Nutr Rev.* 55, S44–S52.
20. HussainMSandChandrasekaraN (1994). Effect of curcumin and capsaicin on the regression of pre-established cholesterol gallstones in mice. Nutr Res.*14,* 1561-1574.
21. JohnsonJ, Bratt BM, Michel-BarronO, GlennowC and PetrusonB (2001) Pure sesame oil vs. isotonic sodium chloride solution as treatment for dry nasal mucosa. Arch. Otolaryngol Head Neck Surg.*127,* 1353-1356.
22. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kulaja TS, (1999) Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.,*47, 3954-3962.
23. KatoMJ, ChuA, DavinLB and LewisNG (1998) Biosynthesis of antioxidant lignans in Sesamumindicum seeds. Phytochemistry. *47*, 583-591.
24. Liu, F., Ooi,V.E.C., & Chang, S.T. (1997). Free radical scavenging activity of mushroom polysaccharide extracts. Life science, 60, 763-771
25. Morris JB (2002) Food, industrial, nutraceutical, and pharmaceutical uses of sesame genetic resources. In: Trends in new crops and new uses. *(* J. Janick and A. Whipkey eds.) . 153–156 ASHS Press, Alexandria.
26. Oyaizu M (1986) Studies on product of browning reaction prepared from glucose amine. Jap. J. Nutr.*44*, 307-315.
27. Parr AJ and Boiwell GP (2000) Phenols in plant and in man. The potential for possible nutritional enhancement of the diet by modifying the potential content or profile. J. Sci. Food Agri. *80*, 985-1012.
28. PhangJM, PooreCM, LopaczynaskaJCMand YehGC (1993) Flavanol stimulated efflux of 7, 1,2-dimethyl benz (a) anthracine in multi-drug resistant breast cancer cells. Cancer Res. *53,* 5977-5988.
29. ReddyACP, and LokeshBR (1994) Studies on the anti-inflammatory activity of spice principles and dietary n-3 fatty acids on carrageenan-induced inflammation in rat. Ann. Nutr. Metab. *38,* 349-358.
30. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM. Pridham JB, (1995) The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Radic.Res*., 22, 375-383.
31. Robak, J. and Gryglewski, R.J, (1998) Flavonoids are scavengers of superoxide anions,Biochem . Pharmcol., 37, 837-841.
32. Sirato-YasumotoS,Katsuta M, Okuyama Y, Takahashi Y andIde T (2001) Effect of sesame seeds rich in sesamin and sesamolin on fatty acid oxidation in rat liver. J. Agr.Food Chem. *49*, 2647-2651.
33. SmithDE and SalernoJW (2001) Selective growth inhibition of a human malignant melanoma cell line by sesame oil *in vitro*. Prostaglandins Leukot. Essent. Fatty Acids. *46*, 145-150.
34. SrinivasanK (2005) Role of spices beyond food flavoring: Nutraceauticals with multiple health effects. Food Rev. Inter.*21,* 167-188.
35. Sun T and Ho C (2005) Antioxidant activities of buckwheat extracts. Food Chem. *90*, 743-749.
36. Taga MS, Miller EE, Pratt DE and Chia (1984) Seeds as a source of natural lipid antioxidants. J.Am. Oil Chem. Soc. *61*, 928-931.
37. Tanaka M, Kuie CW, Nagashima Y and Taguchi T (1988) Applications of antioxidativeMaillard reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi.*54,* 1409-1414.
38. Tyler VE, Brady LR and Robbers JE (1976) Lipids. In: Pharmacognosy. Lea and Febiger, Philadelphia. P.A. P121-122
39. Zainol M, Abd-Hamid A, Yusof S and Muse R. (2003) Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of Centellaasiatica(L.) urban. Food Chem. *81,* 575-581.
40. Zheng W, Wang SY, (2001) Antioxidant activity and phenolic compounds in selected herbs. *J. Agric.Food Chem.* 49, 5165–5170.
41. Halliwell B (1995). How to characterize an antioxidant: an update. Bioch. Soc. Sym. 61, 85-91.
42. Squadriato G L, Peyor W A, (1998). Oxidative chemistry of nitric oxide: the role of superoxide, peroxynitrite, and carbon dioxide. Free Radical Biol Med. 25, 392-403.
43. Yildrim A, Oktay M, Bilaloglu V, (2001). The antioxidant activity of the leaves of Cydonia vulgaris. Turkish J. Med. Sc. 31, 23-27.
44. Kourounakis AP, Galanakis D, Tsiakitzis K, (1999). Synthesis and pharmacological evaluation of novel derivatives of anti-inflammatory drugs with increased antioxidant and anti-inflammatory activities. Drug Dev. Res. 47, 9-16.
45. Gulcin I, Buyukokuroglu M E, Oktay M, Kufrevioglu I O (2002). On the in vitro antioxidant properties of melatonin. J. Pineal Res. 33, 167-171.
46. Gulcin I, Buyukokuroglu M E, Oktay M, Kufrevioglu I O, (2003). Antioxidant and analgesic activities of turpentine of PinusnigraArn. Subsp. Pallsiana (Lamb.) Holmboe. J Ethnopharmacol. 86, 51-58

.