Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 1403–1410
Food Chemistry
www.elsevier.com/locate/foodchem

Free radical-scavenging activity of acetone extract/fractions of *Acacia auriculiformis* A. Cunn.

Rajbir Singh [a], Sukhpreet Singh [b], Subodh Kumar [b], Saroj Arora [a,*]

[a] *Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, Punjab, India*
[b] *Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, Punjab, India*

Received 10 April 2006; received in revised form 11 September 2006; accepted 24 October 2006

Abstract

The antioxidant potency of acetone extract/fractions of *Acacia auriculiformis* A. Cunn. was investigated by employing various *in vitro* systems, such as DPPH, deoxyribose (site- and non-site-specific), reducing power, chelating power and lipid peroxidation in rat liver homogenate. The bark powder of the plant was extracted with different solvents by a maceration method in order of increasing and decreasing polarities and then partitioned (Flow Charts 1 and 2). It was observed that the fractions were comparatively more effective than the crude acetone extract in all the assays. Maximum inhibitory activities noticed were 72.3%, 91.7%, 1.63, 83.3%, and 70.9% in DPPH, deoxyribose, reducing power, chelating power and lipid peroxidation assays, respectively. The inhibitory potential was compared with known antioxidants (ascorbic acid and BHT) and correlated with the total phenolic content in crude extract and fractions. Fractions rich in polyphenolic content were more effective than crude extract. Further studies are underway to isolate and elucidate the structures of the active principles.

Keywords: Free radical; *Acacia auriculiformis*; Antioxidant assays; Cardiovascular diseases; DPPH; Transition metals

1. Introduction

Reactive oxygen species (ROS) cause DNA strand breakage, sister chromatid exchanges and DNA–DNA and DNA–protein cross-links, in addition to base modifications, which have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age (Cadenas & Davies, 2000; Honda, Casadesus, Paterson, Perry, & Smith, 2004; Marnett, 2000; Miquel & Romano-Bosca, 2004; Uchida, 2000). Since free radicals play a key role in the pathology of diseases, the supply of antioxidants, via the food chain, is of great importance for a healthy life (Scalbert & Williamson, 2000). Foods of plant origin, such as fruits, vegetables and medicinal plants have been suggested as natural sources of antioxidants (Auddy et al., 2002; Choi et al., 2002; Mantle, Eddeb, & Pickering, 2000).

Bearing this in mind, the present work was designed to investigate the antioxidative activity of acetone extract/fractions of *Acacia auriculiformis* A. Cunn. by employing DPPH radical, deoxyribose (site-specific and non-site-specific), reducing power, chelating power and lipid peroxidation *in vitro* assays.

Acacia auriculiformis A. Cunn. is a vigorously growing, deciduous or evergreen tree, possibly attaining 30 m height. It belongs to the family Mimosaceae, and is found to be rich in methylglucuronic acid, glucuronic acid, galactose, arabinose and rhamnose (Anderson, 1978). It is reported to have central nervous system-depressant, spermicidal and filaricidal activities, due to the presence of tannins and triterpenoid saponins (Garai & Mahato, 1997; Ghosh, Sinha Babu, & Sukul, 1993; Parkashi, Ray, Pal, & Mahato, 1991).

* Corresponding author. Tel.: +91 183 451048; fax: +91 183 258820.
E-mail address: jrosh1@rediffmail.com (S. Arora).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.10.056

2. Materials and methods

2.1. Chemicals

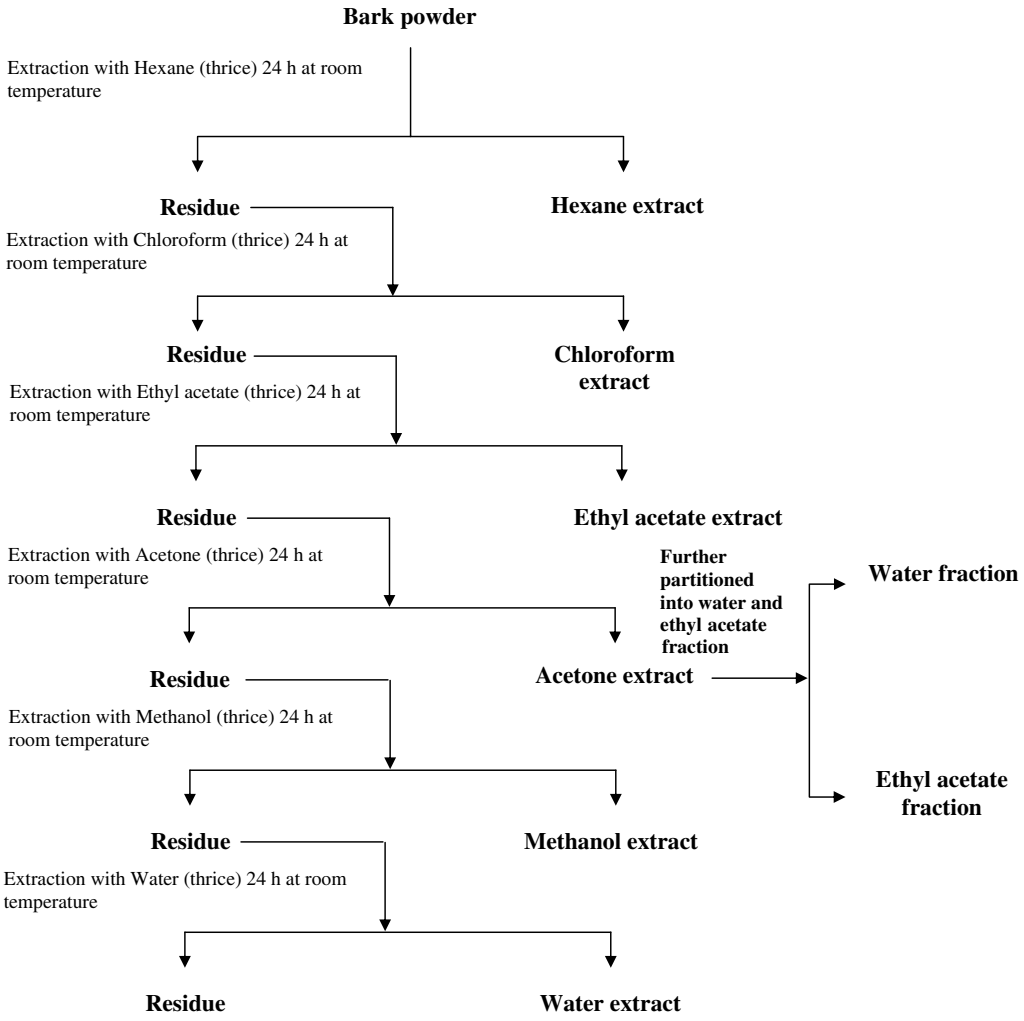
1′-1′Diphenylpicryl-hydrazyl (DPPH), and 2-thiobarbituric acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and deoxyribose was obtained from Lancaster Synthesis Inc. USA. All other chemicals, namely potassium ferricyanide, trichloroacetic acid, ferric chloride, EDTA, hydrogen peroxide, L-ascorbic acid, sodium hydroxide, BHA, Folin-Ciocalteu reagent, sodium carbonate and other solvents, were procured from CDH and were of analytical grade.

2.2. Preparation of extract

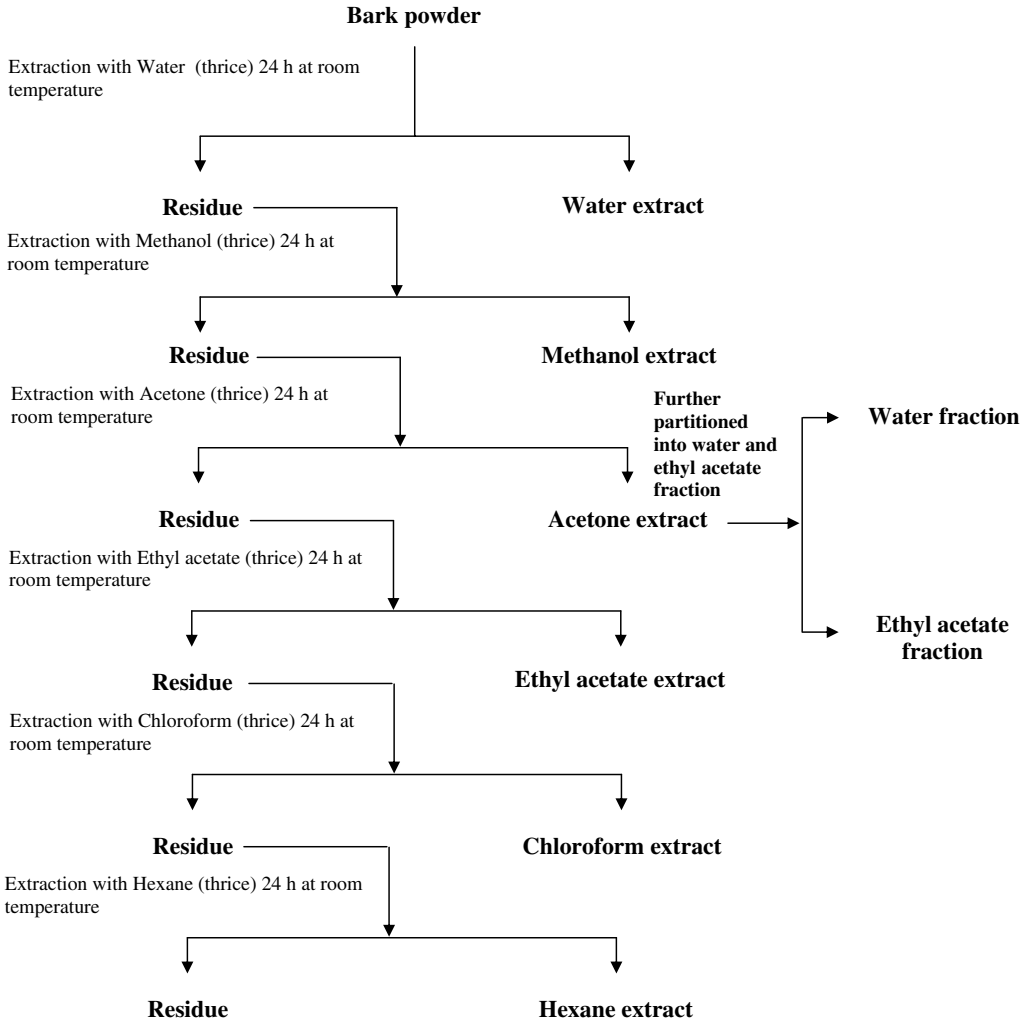
The dried and fine powdered bark material was extracted by adding solvents in increasing order of solvent polarity, namely hexane, chloroform, ethyl acetate, acetone, methanol and water and in reverse order (Flow Charts 1 and 2). After filtering through folded filter paper (Whatman No. 1), the supernatant in different solvents was recovered and this process was repeated thrice with each solvent. Then the respective solvents from the supernatant were evaporated in a vacuum rotary evaporator to obtain the crude extract (CE). From the different crude extracts, the crude acetone extract was partitioned in double-distilled water and ethyl acetate to obtain the water fraction (WF) and ethyl acetate fraction (EAF). For checking the antioxidant activity, each extract/fraction was dried and redissolved in methanol.

2.3. Determination of total phenolics

The total phenolic content (TPC) of the extract/fractions of *Acacia auriculiformis* was determined by the method of Folin-Ciocalteu (Kujala, Loponen, Klika, & Pihlaja, 2000), using gallic acid as standard. To 100 μl of extract/fractions (20 μg/ml) were added 500-μl of (50%) Folin-Ciocalteu reagent, followed by the addition of 1 ml of 20% Na_2CO_3 solution. After a 20 min incubation at room temperature, the absorbance was measured at 730 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per gram of samples.



Flow Chart 1. Maceration extraction of bark powder of *Acacia auriculiformis* by increasing order of solvent polarity.



Flow Chart 2. Maceration extraction of bark powder of *Acacia auriculiformis* by decreasing order of solvent polarity.

2.4. Antioxidant testing assays

2.4.1. General

The antioxidant activity of the acetone extract/fractions was addressed by employing standard methods.

2.4.2. DPPH scavenging assay

The extracts/fractions were measured in terms of hydrogen-donating or radical-scavenging ability using the stable radical, DPPH·, following the method given by Blois (1958) with modification. Briefly, the reaction mixture contained 300 μl of extract/fraction (concentrations 1–100 μg/ml) and 2 ml of DPPH· (0.1 mM in methanolic solution). The reaction mixture was then placed in the cuvette holder of the spectrophotometer (Shimadzu-1601) and read at 517 nm against the blank, which did not contain the extract/fraction. L-Ascorbic acid was used as the positive control. The percent DPPH· decolorization of the sample was calculated.

2.4.3. Reducing power assay

The reducing power of extract was determined by the method of Oyaizu (1986) with modifications. Different concentrations of extract (1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. Aliquots (2.5 ml) of trichloroacetic acid (10%) were added to the mixture, which was then centrifuged for 10 min at 1036*g*. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (2.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power.

2.4.4. Deoxyribose degradation assay

The non-site- and site-specific deoxyribose assays were performed, following the method of Halliwell, Gutteridge, and Aruoma (1987) and Arouma, Grootveld, and Halliwell (1987) with slight modifications. In non-site-specific deoxyribose assay Briefly, the extracts (from 1–100 μg/ml) were mixed with a Haber–Weiss reaction buffer [10 mM $FeCl_3$, 1 mM EDTA (pH 7.4), 10 mM H_2O_2, 10 mM deoxyribose, and 1 mM L-ascorbic acid] and the final volume of all mixtures was made to 1.0 ml. The mixture was then incubated at 37 °C for 1 h and heated at 80 °C for 30 min with 1 ml of

2-TBA (0.5% 2-TBA in 0.025 M NaOH, 0.02% BHA) and 1 ml of 10% trichloroacetic acid (TCA) in a water bath for 45 min. After cooling, absorbance of the mixture was measured at 532 nm. A site-specific assay was performed, following slight modifications in which the EDTA was replaced with the same volume of phosphate buffer, and the percentage inhibition was calculated.

2.4.5. Chelating effects on ferrous ions

The chelating effect on ferrous ions was determined according to the method of Dinis, Madeira, and Almeida (1994) with some modifications. The extracts/fractions (0.25 ml) were mixed with 1.75 ml of methanol and 0.25 ml of 250 mM $FeCl_2$. This was followed by the addition of 0.25 ml of 2 mM ferrozine, which was left to react at room temperature for 10 min before determining the absorbance of the mixture at 562 nm.

2.4.6. 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 (Halliwell & Guttridge, 1989). Normal male rats (250 g) were used for the preparation of liver homogenate. The perfused liver was isolated, and a 10% (w/v) homogenate was prepared with a homogenizer at 0–4 °C with 0.15 M KCl. The homogenate was centrifuged at 800*g* for 15 min and the clear cell-free supernatant was used for the study of *in vitro* lipid peroxidation. Different concentrations (50–400 μg/ml) of extract/fractions dissolved in methanol were taken in test tubes. One millilitre 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, centrifuged and the absorbance of the supernatants was measured at 532 nm.

2.5. Statistical analysis

All experiments were repeated at least three times. Results are reported as means ± SE.

3. Results and discussion

3.1. General

Owing to the complexity of the oxidation–antioxidation processes, it is obvious that no single testing method is capable of providing a comprehensive picture of the anti-

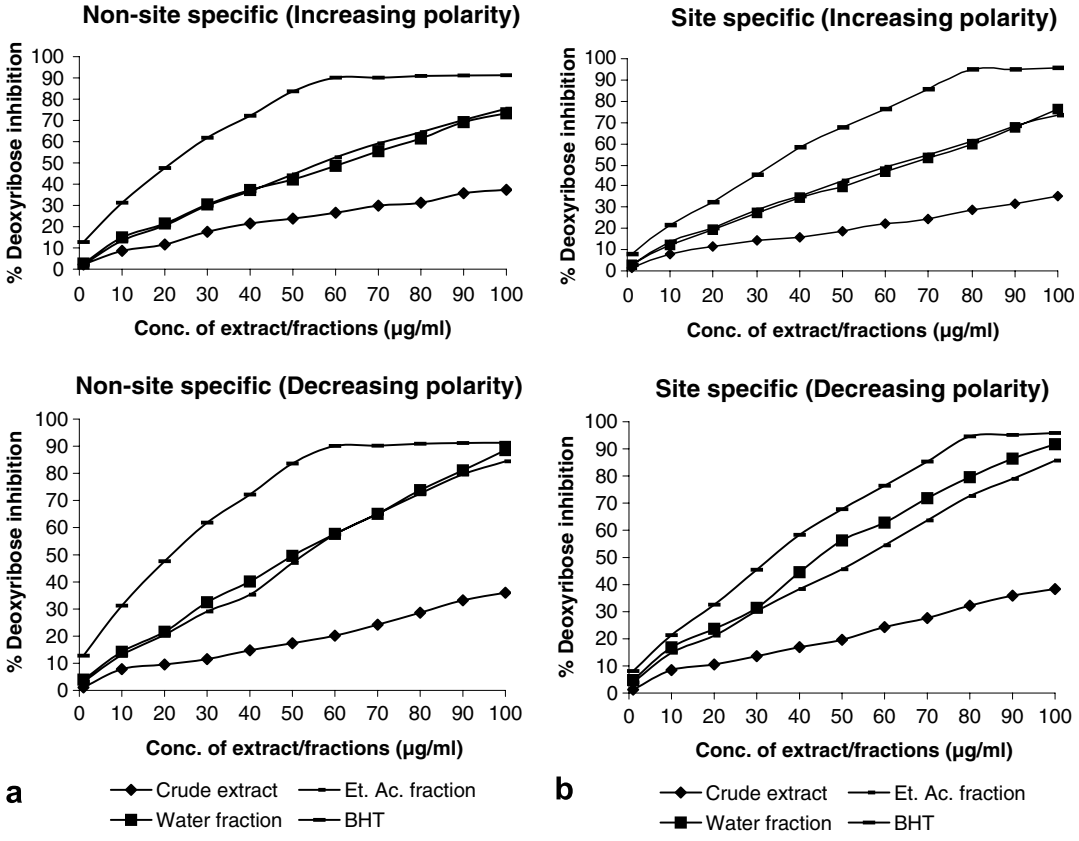


Fig. 1. Scavenging of hydroxyl radicals by acetone extract/fraction of *Acacia auriculiformis* in non-site-specific (a) and site-specific (b) deoxyribose degradation assay, respectively.

oxidant profile of a studied sample. Preliminary studies confirmed that a multimethod approach is necessary in the assessment of antioxidant activity. Independently of the chosen method, suitable reference antioxidants should be tested for comparison. A combination of rapid, sensitive, and reproducible methods, preferably requiring small sample amounts, should be used whenever an antioxidant activity screening is designed. It was observed that, in general, the extracts/fractions prepared by decreasing order of solvent polarity showed more inhibitory potency than did the extracts/fractions obtained in solvents in reverse order. So the results of the former scheme are described in detail in a further section.

3.2. DPPH scavenging assay

The acetone extract/fractions of *Acacia auriculiformis* quenched DPPH free radical in a dose-dependent manner because, as the concentration of extract/fractions increased, the DPPH$^{\cdot}$ quenching activity also increased (Fig. 2a). The order of effectiveness of the extract and fractions was: water fraction (72.3%) > ethyl acetate fraction (62.3%) > crude extract (31.0%) for increasing order of solvent polarity and water fraction (65.7%) > ethyl acetate fraction (62.3%) > crude extract (26.9%) for decreasing order of solvent polarity.

The observed antioxidant activity of the extract/fractions may be due to the neutralization of free radical character of DPPH$^{\cdot}$, either by transfer of an electron or hydrogen atom (Naik et al., 2003). The ability of the extract/fractions to scavenge the DPPH radical has also been related to the inhibition of lipid peroxidation (Rekka & Kourounakis, 1991).

3.3. Deoxyribose scavenging assay (site-specific and non-site-specific)

Fig. 1a and b shows the effects of acetone extract and its fractions in deoxyribose scavenging assays (non-site- and site-specific, respectively). It was observed that all the extract/fractions were effective in scavenging the hydroxyl radicals in site-specific assay as well as in non-site-specific assay but the change was comparatively greater in the site-specific than in the non-site-specific assay, which indicated their strong chelating power.

The order of effectiveness of the extracts/fractions as antioxidants in site-specific assay was: water fraction (91.7%) > ethyl acetate fraction (85.7%) > crude extract (38.3%) and in non-site-specific assay, the order of inhibition was: water fraction (88.6%) > ethyl acetate fraction (84.5%) > crude extract (36.0%).

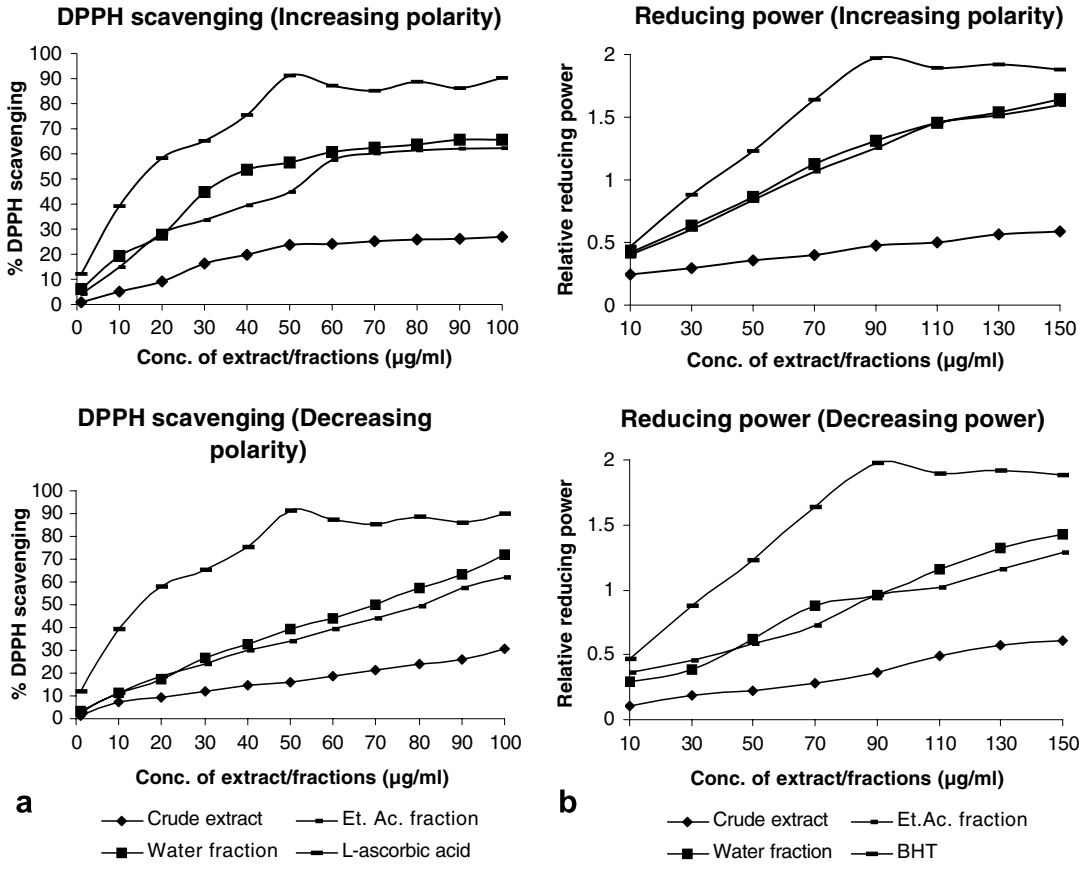


Fig. 2. Scavenging of the DPPH radical and reducing power potential of acetone extract/fraction of *Acacia auriculiformis* by DPPH (a) and reducing power assay (b), respectively.

The potencies of extract/fractions, in both the assays, indicate their efficacy as chelating agents, as well as their capacity to compete with deoxyribose for OH radicals which are produced free in solution from a Fe^{2+}–EDTA chelate (Asamari, Addis, Epley, & Krick, 1996).

3.4. Chelating power assay

Fig. 3a depicts the effect of extract/fractions in the chelating power assay. It was observed that the water fraction (73.5%) showed more metal ion-chelation activity than did the ethyl acetate fraction (70.2%) or the crude extract (36.9%) obtained by increasing order of solvent polarity. By contrast, with extract/fractions of decreasing order of solvent polarity, the maximum effect was exhibited by ethyl acetate fractions (83.3%), as compared to water fractions (68.2%) and crude extract (38.5%) at 400 μg/ml concentration.

The results obtained with this assay strengthen the observation made in the deoxyribose assay, wherein extract/fractions showed more effects in site-specific than in non-site-specific assays. A possible explanation of the chelating power of the extracts is the ability of the extracts to reduce iron and then form Fe^{2+}-extract/fraction complexes that are inert. This study is in conformity with the observation made in the literature that binding of iron to flavonoid antioxidants can suppress the accessibility of the iron to oxygen molecules by changing the redox potential, thus converting the ferrous ion to ferric and thereby inhibiting oxidative damage. Furthermore, it has been reported that non-flavonoid polyphenolics can reduce iron and then form Fe^{2+}–polyphenol complexes that are inert (Laughton, Halliwell, Evans, & Holult, 1987).

3.5. Reducing power assay

The results obtained in the reducing power assay are shown in Fig. 2b. The water fraction had more reducing potential (1.64, 1.42*) than did the ethyl acetate fraction (1.60, 1.29*) or crude extract (0.588, 0.608*) in increasing and decreasing* order of solvent polarities, respectively. As mentioned in the literature, the reducing power evaluation is an important parameter and it related to antioxidant activity because the extract/fractions acted as reductones, which inhibited LPO by donating a hydrogen atom, thereby terminating the free radical chain reaction (Duh, 1998; Duh, Tu, & Yen, 1999; Tanaka, Kuie, Nagashima, & Taguchi, 1988; Yen & Chen, 1995).

3.6. Lipid peroxidation assay

We measured the potential of acetone extract/fractions of *Acacia auriculiformis* to inhibit lipid peroxidation in rat liver homogenate, induced by the $FeCl_2$–H_2O_2 system

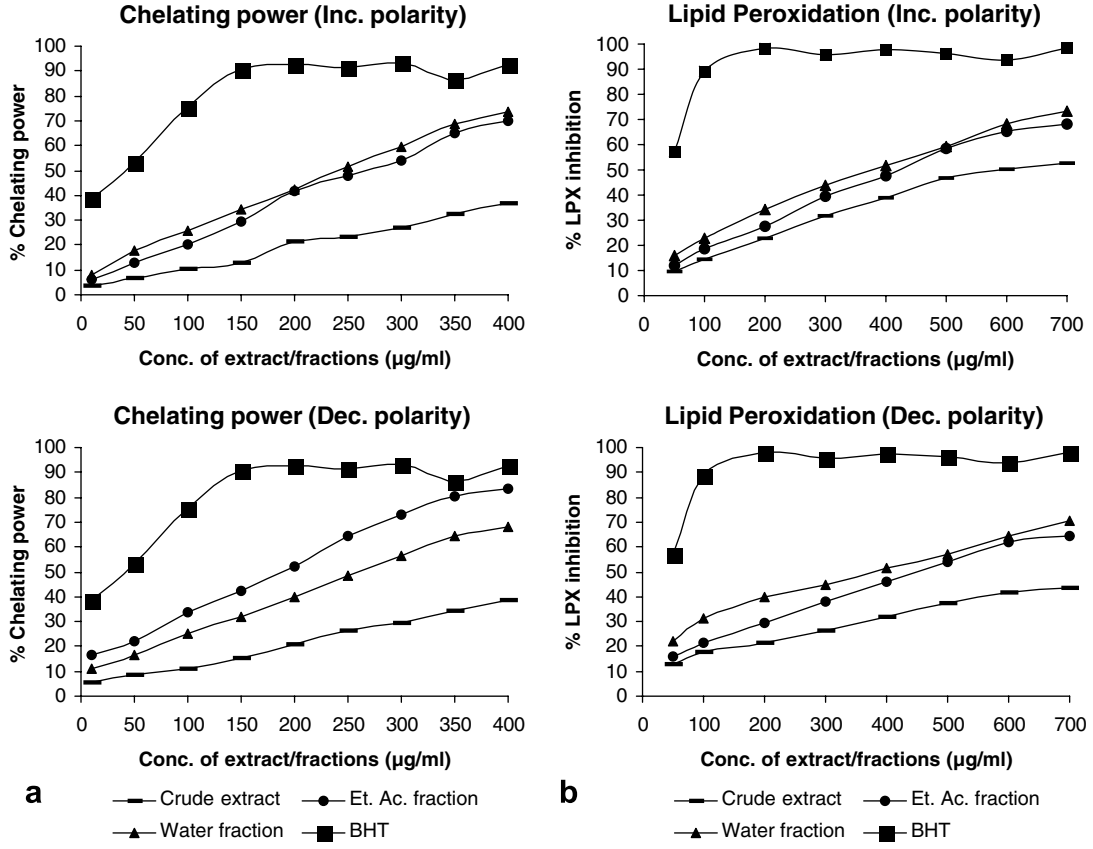


Fig. 3. Chelating power (a) and lipid peroxidation (LPX inhibition) potential of acetone extract/fractions of *Acacia auriculiformis*, respectively.

Table 1
Total phenolic content (TPC) of acetone extracts/fractions of *Acacia auriculiformis*, mg/g, as gallic acid equivalents (GAE)

Acetone extract	TPC
Crude extract	300
Ethyl acetate fraction	495
Water fraction	775

(Fig 3b). The hydroxyl radicals, generated via Fenton reaction, were observed to be scavenged significantly by co-incubation of rat liver homogenate with varying concentrations of extract/fractions. Many workers have employed this system to assess the biological activity of various natural plant-derived biomolecules (Halliwell et al., 1987; Pin-Der-Duh, 1998). The water fraction exhibited more LPX inhibition (73.3%, *70.9%) than did the ethyl acetate fraction (68.2%, *64.6%) or crude extract (52.7%, *43.6%) at 700 μg/ml concentration of extract/fractions, for both increasing and *decreasing orders of solvent polarities, respectively.

A critical analysis of the results obtained in different assays shows that the fractions were comparatively more effective than were the crude extracts. In an attempt to identify the antioxidant principle in the extract/fractions, the total phenolic content was determined (Table 1). The total phenolic content was 300, 495, 775 mg gallic acid equivalents (GE) in each gram of the plant extract for crude extract, ethyl acetate fraction and water fraction, respectively. The amount of phenolic compounds was observed to be greater in fractions than in crude extracts. The free radical-scavenging activity in different assays can be linked to the presence of phenolic compounds in the extract/fractions because these compounds exhibit important mechanisms of antioxidative activities (Yildirim et al., 2000). The greater TPC (775 mg GE/g), detected in the water fraction, suggests that this fraction may serve as a dietary source of phenolic substances, which may act as antioxidants for disease prevention and/or general health promotion through improved nutrition.

Though other antioxidants were also probably present in these extract/fractions, phenolic compounds could make a significant contribution to their bioactivity. It is pertinent to mention that the results obtained in the present study are in conformity with our previous results on antimutagenic activity against genotoxic injury by NPD, sodium azide and 2-aminofluoerene in the Ames *Salmonella* histidine reversion assay and antioxidant activity employing different in vitro methods (Arora et al., 2005; Kaur et al., 2002; Kaur, Micheal, Arora, Harkonen, & Kumar, 2005; Singh, Singh, Kumar, & Arora, 2004). The work further reveals that the *Acacia* species, could be an interesting source of antioxidants of potential use in different fields, namely food, cosmetics, and pharmaceuticals. A detailed chemical investigation of these extract/fractions is underway to identify the compounds responsible for the antioxidant activity.

References

Anderson, D. M. W. (1978). Chemotaxonomic aspects of the chemistry of Acacia gum exudates. *Kew Bulletin, 32*(3), 529–536.

Arora, S., Britis, E., Kaur, S., Kaur, K., Sohi, R. S., Kumar, S., et al. (2005). Evaluation of genotoxicity of medicinal plant extracts by the comet and VITOTOX test. *Journal of Environmental, Pathology, Toxicology and Oncology, 24*(3), 193–200.

Arouma, O. I., Grootveld, M., & Halliwell, B. (1987). The role of iron in ascorbate-dependent deoxyribose degradation. *Journal of Inorganic Biochemistry, 29*, 289–299.

Asamari, A. M., Addis, P. B., Epley, R. J., & Krick, T. P. (1996). Willd rice hull antioxidants. *Journal of Agricultural and Food Chemistry, 44*, 126–130.

Auddy, B., Ferreira, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F., et al. (2002). Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *Journal of Ethnopharmacology, 84*, 131–138.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 26*, 1199–1200.

Cadenas, E., & Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine, 29*, 222–230.

Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., et al. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science, 163*, 1161–1168.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivates (acetoaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archive of Biochemistry and Biophysics, 315*, 161–169.

Duh, P. D. (1998). Antioxidant activity of burdock (*Arctium lappa Linne*): its scavenging effect on free radical and active oxygen. *Journal of the American Oil Chemist's Society, 75*, 455–465.

Duh, P. D., Tu, Y. Y., & Yen, G. C. (1999). Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium Ramat*). *Lebensmittel Wissenschaft und Technologie, 32*, 269–277.

Garai, S., & Mahato, S. B. (1997). Isolation and structure elucidation of three triterpenoid saponins from *Acacia auriculiformis*. *Phytochemistry, 44*, 137–140.

Ghosh, M., Sinha Babu, S. P., & Sukul, N. C. (1993). Antifilarial effect of two triterpenoid saponins isolated from *Acacia auriculiformis*. Indian. *Journal of Experimental Biology, 31*, 604–606.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: a simple "test tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry, 165*, 215–219.

Halliwell, B., & Guttridge, J. M. C. (1989). *Free radicals in biology and medicine* (2nd ed.). Tokyo, Japan: Japan Scientific Societies Press.

Honda, K., Casadesus, G., Paterson, R. B., Perry, G., & Smith, M. A. (2004). Oxidative stress and redox iron in Alzheimer's disease. *Annals of New York Academy of Science, 1012*, 179–182.

Kaur, K., Arora, S., Hawthorne, M. E., Kaur, S., Kumar, S., & Mehta, R. G. (2002). Correlative study of antimutagenic and chemopreventive activity of *Acacia auriculiformis* A. Cunn. and *Acacia nilotica* (L) Willd. Ex Del. *Drug and Chemical Toxicology, 25*(1), 39–63.

Kaur, K., Micheal, M., Arora, S., Harkonen, P., & Kumar, S. (2005). In vitro bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from *Acacia nilotica* (L) Willd. Ex Del. *Journal of Ethnopharmacology, 99*, 353–360.

Kujala, T. S., Loponen, J. M., Klika, K. D., & Pihlaja, K. (2000). Phenolic and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effects of cold storage on the content of total phenolics and three individual compounds. *Journal of Agricultural and Food Chemistry, 48*, 5338–5342.

Laughton, M. J., Halliwell, B., Evans, P. J., & Holult, J. R. S. (1987). Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myricetin. *Biochemical Pharmacology, 36*, 717–720.

Mantle, D., Eddeb, F., & Pickering, A. T. (2000). Comparison of relative antioxidant activities of British medicinal plant species in vitro. *Journal of Ethnopharmacology, 72*, 47–51.

Marnett, L. (2000). Oxyradicals and DNA damage. *Carcinogenesis, 21*, 361–370.

Miquel, J., & Romano-Bosca, A. (2004). Oxidative stress and antioxidant diet supplementation in ageing, arterosclerotic and immune dysfunction processes. *ARS Pharmacy, 45*(2), 91–109.

Naik, G. H., Priyadarsini, K. I., Satav, J. G., Banavalikar, M. M., Sohoni, P. P., Biyani, M. K., et al. (2003). Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry, 63*, 97–104.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition, 44*, 307–315.

Parkashi, A., Ray, H., Pal, B. C., & Mahato, S. B. (1991). Sperm immobilizing effect of triterpene saponins from *Acacia auriculiformis*. *Contraception, 43*, 475–483.

Pin-Der-Duh, X. (1998). Antioxidant activity of burdock (*Arctium lappa Linne*): its scavenging effect on free radical and active oxygen. *Journal of the American Oil Chemists Society, 75*, 455–461.

Rekka, E., & Kourounakis, P. N. (1991). Effect of hydroxyethyl rutosides and related compounds on lipid peroxidation and free radical scavenging activity. Some structural aspects. *The Journal of Pharmacy and Pharmacology, 43*, 486–491.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition, 130*, 2073–2085.

Singh, R., Singh, S., Kumar, S., & Arora, S. (2004). Hydroxyl radical scavenging potential of acetone extract/fractions of *Acacia nilotica* (L.) Willd. Ex Del. *International Journal of Bioscience Reporter, 2*, 440–446.

Tanaka, M., Kuie, C. W., Nagashima, Y., & Taguchi, T. (1988). Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi, 54*, 1409–1414.

Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. *Free Radical Biology and Medicine, 28*, 1685–1696.

Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry, 43*, 27–32.

Yildirim, A., Mavi, A., Oktay, M., Kara, A. A., Algur, O. F., & Bilaloglu, V. (2000). Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argenteaDesf* ex DC), sage (*Salvia triloba* L.), and black tea (*Camellia sinensis*) extracts. *Journal of Agricultural and Food Chemistry, 48*, 5030–5034.