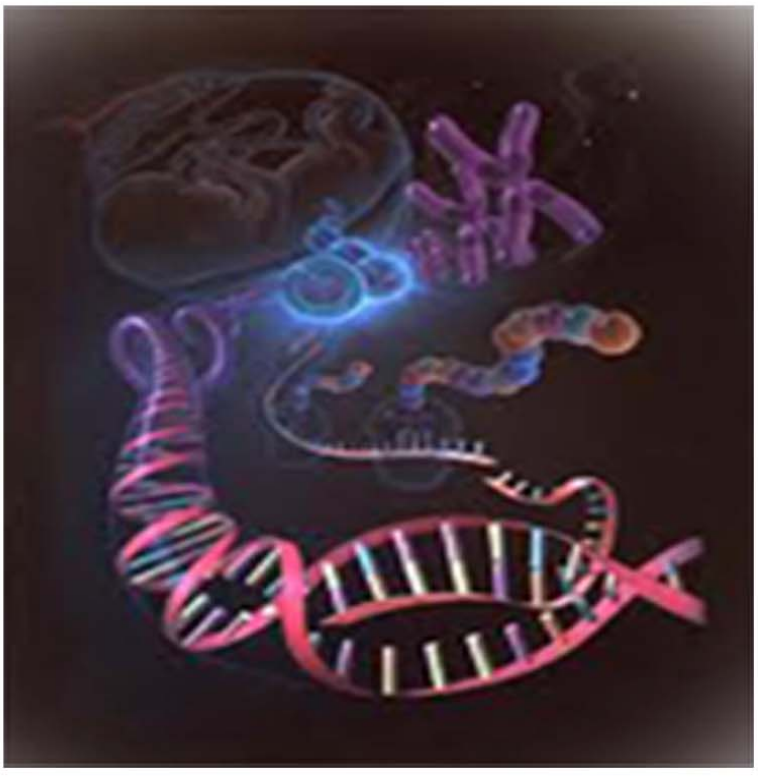
IJLBPR

ISSN 2250 - 3137
Vo1. 2, No. 4, October 2013

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 2, No. 4, October 2013

Research Paper

ASSESSMENT OF ANTIOXIDATIVE POTENTIAL OF *ACACIA NILOTICA* (L.) WILLD EX DEL. VIA *IN VITRO* MODELS

Jyoti Mahajan[1] and Saroj Arora[1]*

*Corresponding Author: **Saroj Arora,** ✉ sarojarora.gndu@gmail.com

The present study envisages the antioxidant activity of different fractions of leaves of *Acacia nilotica* Willd. Ex. Del. The leaves of *Acacia nilotica* after drying and grinding were serially extracted with solvents of increasing polarities to obtain the hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions. The total phenolic and flavonoid content of the fractions was determined. The antioxidant capacity was done by using Total Antioxidant Capacity (TAC), Cupric ion Reducing Antioxidant Capacity (CUPRAC) and lipid peroxidation assays. It was found that the fractions exhibited the radical scavenging activity in a dose dependent manner. The maximum total phenolic and total flavonoid content was found in ethyl acetate fraction 93.3 GAE mg/g and 61.19 RE mg/g. A maximum inhibition of 93.25 ascorbic acid equivalents (mg/g) of ethyl acetate fraction followed by chloroform was observed in total antioxidant capacity assay. In CUPRAC assay, the ethyl acetate fraction was found to have maximum reduction potential of 99.06% at 200 µg/ml of concentration whereas in lipid peroxidation assay the hexane fraction exhibited the maximum inhibition (86.85%).

Keywords: *Acacia nilotica*, Antioxidant assays, CUPRAC, Lipid peroxidation

INTRODUCTION

The role of free radicals and active oxygen in the pathogenesis of human diseases including cancer, aging and atherosclerosis has been recognized (Halliwell and Gutteridge, 1992). Electron acceptors such as molecular oxygen, react rapidly with free radicals to become radicals themselves also referred to as Reactive Oxygen Species (ROS). The ROS include superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) (Grisham and Mc Cord, 1986). Lipid peroxidation which involves a series of free radical mediated chain reaction process, is also associated with several types of biological damage. Therefore much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit peroxidation and to protect the biomolecules from damage due to free radicals.

[1] Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, Punjab, India.

Antioxidants are used to protect human beings from the ill-effect caused by oxidative stress that is exerted by enhanced production of ROS as a result of exposure to pollutants. The body has several mechanisms to counteract oxidative stress either by naturally generated endogenous antioxidants (in situ) or supplied externally (exog enous antioxidants (Halliwell and Gutteridge, 2007).

A great number of aromatic, spicy, medicinal and other plants contain chemical compounds, exhibiting antioxidant properties. Source of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson, 1990). Many of these antioxidant compounds possess antiinflammatory, antiatherosclerotic, antitumor, anticarcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Sala *et al.,* 2002). Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolic compounds are of great interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food (Kahkonen *et al.,* 1999; Rice *et al.,* 1995).

Acacia nilotica (L.) Willd Ex Del. (Fabaceae) is an vital multipurpose plant (Kaur *et al*., 2005). It is also popular ornamental avenue tree in India. The medicinal plants promote self healing and good health in ayurvedic medicine practices and have been acknowledged to provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases. It also serves as a source of polyphenols (Singh *et al.,* 2009a). Phenolic compounds exert their protective effect by acting as blocking agents or suppressing agents, in the prevention of formation of carcinogens from precursor substances (Manach *et al.,* 2004). Several studies have reported that extracts from *Acacia* species, rich in phenolics have strong antioxidant activities (Singh *et al.,* 2004; Arora *et al.,* 2005; Kaur *et al.,* 2005). *A. nilotica* offers a variety of bioactive components such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7-diglucoside, rutin, derivatives of (+)-catechin-5-gallate and m-catechol. They have spasmogenic, vasoconstrictor, antihypertensive, mutagenic, carcinogenic, spasmodic, inflammatory, oxidant and platelet aggregatory properties (Singh *et al.,* 2007).

MATERIALS AND METHODS

Chemicals/Reagents

Ammonium molybdate, Sodium phosphate, Sulphuric acid, Aluminium Chloride, Sodium Hydroxide, Folin-Ciocalteu reagent, Copper Chloride, Neocuproine, Ammonium Acetate, TBA (2-Thiobarbituric Acid), TCA (Trichloroacetic acid), Potassium Chloride, Tris-HCl, Ferrous Ammonium Sulphate, Ascorbic Acid, Hydrochloric acid, Sodium nitrite, Sodium carbonate.

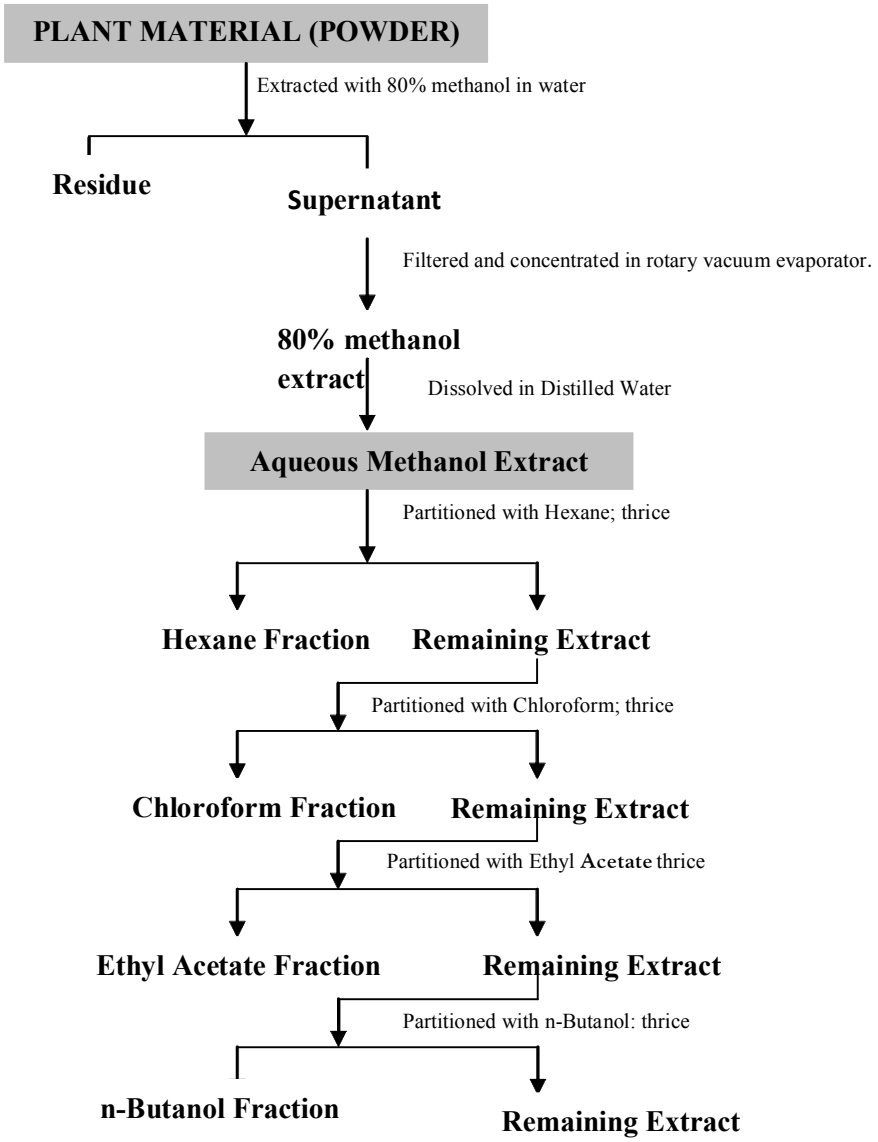
Plant Material

The leaves of *Acacia nilotica* (L.) Willd Ex Del. were collected from trees growing in the Guru Nanak Dev University Campus, Amritsar. Botanical identification was made from Herbarium of Department of Botanical and Environmental Sciences, GNDU, Amritsar.

Plant Extract

The leaves of plant were thoroughly washed with tap water, dried at room temperature and ground to fine powder. The powdered leaves were extracted as per the protocol shown in Flow Chart 1. The concentrated solutions were then lyophilized to get the dry powder of respective fractions.

Flow Chart 1: Extraction Procedure Using Maceration Method



Determination of Phytoconstituents

The total phenolic and flavonoid content of different fractions was estimated using different methods.

Total Phenolic Content

The total phenolic content in the different fractions of *A. nilotica* was determined by Folin-Ciocalteu method (Yu *et al*., 2002). In this method, 0.1 ml of extract solution was added to 0.9 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent (1:1) along with 1.5 ml of 20% sodium carbonate solution. The mixture was allowed to stand for 2 h at room temperature. After completion of reaction, the volume was raised to 10 ml by adding distilled water. The absorbance of mixture was measured at 765 nm. The amount of total phenolic content of different fractions was expressed as mg/g

Gallic acid equivalents from calibration curve of standard solution of gallic acid.

Total Flavonoid Content

The total flavonoid content of different fractions (TFC) was analyzed by using rutin as standard (Kim *et al.*, 2003). To 1 ml of extract solution (each of 100 μg/ml concentration), 4 ml of distilled water, 300 μl of sodium nitrite and aluminium chloride were added. The mixture was incubated at room temperature for 5 min. After the completion of incubation, 2 ml of sodium hydroxide was added and final volume of solution was raised to 10 ml by addition of distilled water. The absorbance of samples was measured at 510 nm. The total flavonoid content for all the fractions was expressed in terms of Rutin equivalents (mg/g). The curve absorbance versus concentration is described by the equation: y = 0.0016 x –0.0006 (R^2 = 0.9951) where x = concentration and y = absorbance

Determination of Antioxidant Activities

The antioxidant potential of different fractions of *Acacia nilotica* was measured by employing the following methods:

Total Antioxidant Capacity (Molybdate Ion Reduction Assay)

The total antioxidant capacity was measured by spectrophotometric method given by Prieto *et al.* (1999) with slight modifications. Briefly, 300 μl of extract solution was added to 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95°C for 1.5 h. After incubation, the mixture was cooled at room temperature and the absorbance was read at 695 nm against blank. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicates and values are expressed as mg/g AAE of extract.

Cupric Ion Reducing Antioxidant Assay – CUPRAC Assay

The cupric ion reducing potential of different fractions was determined by Apak *et al*. (2007) method. To the mixture [1 ml (10 mM) copper (II) chloride, 1 ml (7.5 mM) neocuproine and 1 ml (1.0 M, pH 7) ammonium acetate buffer solution], added 100 μl of extract solution. The reaction mixture was allowed to stand for 30 min at room temperature and absorbance was measured at 450 nm. An increase in absorbance indicates the increased reduction ability. The percentage reduction for the fractions at different concentrations was calculated by using the formula: [1-(Absorbance of control-Absorbance of sample)/Absorbance of control]*100.

Lipid Peroxidation Assay

The method of Ohkawa et al., 1979 was used with some minor modifications for determination of lipid peroxidation. In this procedure, 0.5 ml of extract solution, 0.5 ml of homogenate (egg yolk 10% in 0.15 M KCl) and 0.5 ml of reaction mixture (0.16 mM Ferrous ammonium sulphate, 30 mM Tris-HCl, 0.6 mM Ascorbic acid) were added. The above mixture was incubated for half hour at 37°C. After the completion of incubation, 2 ml of TBA (10% TCA, 0.5% TBA, 0.25 N HCl) was added and further incubated on water bath/oven at 80°C for 1 h or 100°C for 30 min. The mixture was cooled in ice bucket and centrifuged at 2000 rpm for 10 min. The optical density of the supernatant (pink color) was read at 532 nm against blank solution. The percentage inhibition was calculated by using the formula: [(Absorbance of control-Absorbance of sample)/Absorbance of control]*100.

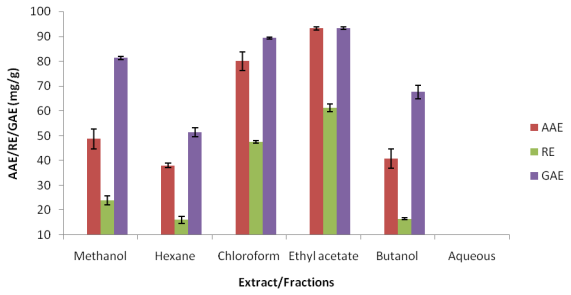
Statistical Analysis

All the measurements were taken in triplicates and presented as mean ± SE.

RESULTS

Figure 1 shows the total phenolic content of methanol fractions of *A. nilotica* in terms of mg/g GAE. The analysis of phenolic content showed comparative abundance of phenolic compounds in ethyl acetate fraction followed by chloroform and methanol fractions. Ethyl acetate fraction showed highest phenolic content of 93.3 mg/g GAE while chloroform and methanol fractions was having 89.3 and 81.3 mg/g GAE. The aqueous fraction showed the least phenolic amount.

Figure 1: Total Antioxidant Capacity, Flavonoid and Phenolic content of Different Fractions of *Acacia nilotica*, Total Antioxidant Capacity is expressed as Ascorbic Acid Equivalents (AAE); Flavonoid content is expressed in terms of Rutin Equivalents (RE) and Total Phenolic Content as Gallic Acid Equivalents (GAE)



The total flavonoids were measured spectrophotometrically. Figure 1 depicts the total flavonoid content of extract/fractions in terms of mg/g RE of extract. It was observed that fraction of ethyl acetate showed the highest TFC (61.19 mg/g RE), whereas in other extract/fractions the flavonoid content was found in the following order: chloroform (47.44) > methanol (23.88) > n-butanol (16.56) > hexane (16.12). The aqueous fraction showed the flavonoid content in negligible amount.

The antioxidant activity of fractions of *A. nilotica* (leaves) was determined by employing TAC, cupric ion reducing antioxidant capacity and lipid peroxidation assays. Figure 1 depicts the total antioxidant capacity of methanolic fraction of leaves of *Acacia nilotica*. Different extract/ fractions *of Acacia nilotica* showed very potent antioxidant capacity. Among all the fractions studied, it was observed that the ethyl acetate fraction showed the highest total antioxidant capacity viz. 93.25 mg/g AAE and the aqueous extract showed the least antioxidant capacity that is 1.75 mg/g AAE. The antioxidant capacity of different fractions was observed to be in the following order: chloroform (80 mg/g AAE)> methanol (48.75 mg/g AAE)> n-butanol (40.75 mg/g AAE) > hexane (38 mg/g AAE) > aqueous (1.75 mg/g AAE).

Figures 2 and 3 depict the inhibition percentages of different fractions of leaves of plant in comparison to standard compound which was used as natural antioxidant compound. In CUPRAC assay, the ethyl acetate fraction of *Acacia nilotica* was found to have effective potential in converting Cu^{2+} to Cu^{+} with reduction potential of 99% at 200 µg/ml and 91% at 180 µg/ml concentrations. The intensity of colored

Figure 2: Reduction Potential of Different Fractions of *Acacia nilotica* as Measured by Cupric Ion Reducing Antioxidant Capacity

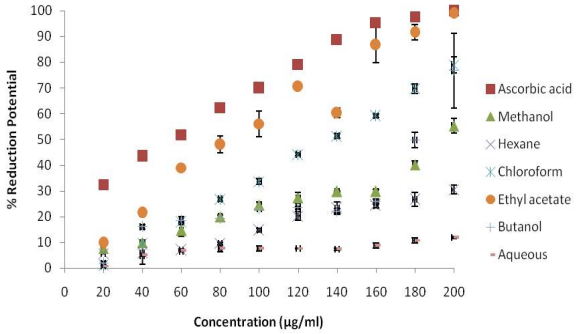
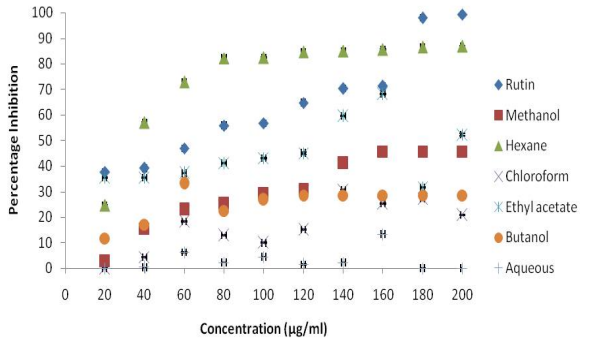


Figure 3: The Effect of Different Fractions of *Acacia nilotica* in Lipid Peroxidation Assay



complex, i.e., chromophore [Cu (I)-Nc] increased with the electron donating ability of the extract. Ascorbic acid was used as standard compound (Figure 2). The ability of different fractions in converting the Cu^{2+} to Cu^{+} was observed in the following order: chloroform (78.91%)> n-butanol (76.7%)> methanol (55.28%)> hexane (30.45%) and aqueous (11.84%) at 200 µg/ml of concentrations.

Lipid peroxidation process proceeds via radical chain reaction resulting in the formation of lipid hydroperoxides (LOOH). The results of lipid peroxidation assay showed that the hexane extract/fraction of *A. nilotica* showed maximum percentage inhibition as more peroxyl radical scavenging potential (86.85%) than ethyl acetate fraction (52.30%) at 200 µg/ml of concentration whereas the methanol, n-butanol and chloroform fractions also showed the detectable scavenging potential, viz., 45.75%, 28.57% and 20.81%, respectively (Figure 3).

DISCUSSION

Medicinal plants have a long history of use in therapy throughout the world and still make an important part of traditional medicine. The use of medicinal plants is perhaps the oldest method of coping with illness. *A. nilotica* is reported to have habitual medicinal uses such as appetite enhancer, strength and nutrient supplement for sore joints, stomach ache and clear out circumcision wounds. Antioxidants have been implicated in many diseases such as malaria, cardiovascular disease, gastric ulcer, diabetes, malignant tumors, rheumatic joint inflammation, cataracts disease (Chang *et al.,* 2001; Kahkonen *et al.,* 1999). Natural antioxidants such as flavonoids, phenolics, tannins, curcumin and terpenoids are found in this plant (Moskovitz *et al.,* 2002).

Extracts of medicinal herbs and spices are the most studied natural antioxidants (Yanishlieva *et al.,* 2006). Source of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and bark (Grisham *et al.,* 1998 ; Pratt *et al.,* 1990). A lot of these antioxidant compounds have antiinflammatory, atherosclerotic, antitumor, anticarcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Sala *et al.,* 2002). It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, flavor and also in providing health beneficial effects. Different phenolic compounds serve in plant defense mechanisms to counteract ROS in order to survive and prevent molecular damage and damage by microorganisms, insects and herbivores (Vaya *et al.,* 1997), whereas flavonoids are a group of polyphenolic compounds with known properties such as free radical scavenging activity, inhibition of hydrolytic and antioxidative enzyme and antiinflammatory action have been isolated from plants (Omale *et al.,* 2008; Frankel 1995; Pourmorad *et al.,* 2006).

A. nilotica belonging to family Fabaceae has highest total phenolic and flavonoid content that is 93.3 mg/g GAE and 61.19 mg/g RE respectively. As seen in Figure 1, the total antioxidant capacity of the ethyl acetate fraction of plant showed the highest antioxidant capacity as 93.25 mg/g AAE. The different antioxidant capacities of the plant extracts depend upon the composition of extracts. The antioxidant activities of different fractions were measured using CUPRAC and lipid peroxidation assays.

In general, the important feature of CUPRAC assay is its versatility, i.e., applicability to both hydrophilic and lipophilic antioxidants (Apak *et al.,* 2004; Apak *et al.,* 2005) by a simple change of solvent. The chromogenic redox reagent used for the CUPRAC assay was bis(neocuproine) copper(II) chelate and this reagent is useful at pH 7. The absorbance of the Cu(I)-chelate formed as a result of redox reaction with reducing polyphenols was measured at 450 nm. The chromogenic oxidizing reagent of the developed CUPRAC method, i.e., bis(neocuproine) copper(II) chloride (Cu(II)-Nc) reacts with n-electron reductant antioxidants (AO). In this process, the reactive Ar-OH groups of polyphenolic antioxidants are oxidized to the corresponding quinones (ascorbic acid is oxidized to dehydroascorbic acid) and Cu(II)-Nc is reduced to the highly coloured Cu(I)-Nc chelate showing maximum absorption at 450 nm. The color was due to the Cu(I)-Nc chelate formed. The bis(neocuproine)copper(I) cation chromophore is soluble both in water and organic media, therefore the CUPRAC method is capable to assay both hydrophilic and lipophilic antioxidants (Apak *et al*., 2007). The percentage inhibition of different fractions of plant was done and it has been observed that the results showed dose dependent manner. The ethyl acetate fraction of *Acacia nilotica* was found to have effective potential in converting Cu^{2+} to Cu^{+} with reduction potential of 99% at 200 µg/ml.

LPO has been broadly defined as the oxidative deterioration of polyunsaturated lipids (Kappus, 1991). Lipid peroxidation is a complex system where generation of the initiator molecule is followed by chain initiation, propagation, branching and termination reactions (Catala, 2006; Schnitzer *et al.,* 2007; Fukuzawa, 2008). LPO occurs mainly in biomembranes, where the content of unsaturated fatty acids is relatively high. The scavenging peroxyl radicals acts due to their hydrogen donating property. Fe^{2+} induced lipid peroxidation is also a good system for assessing antioxidant action of different extracts. One of the degradation products of lipid peroxidation is malondialdehyde which causes cell damages form a pink colour chromogen with thiobarbituric acid. The antioxidant compounds present in the extract scavenged the hydroxyl radicals generated in the Fenton reaction in the liver homogenate.

In lipid peroxidation assay, the potential of hexane fraction was found to be highest in scavenging the peroxyl radical as compared to ethyl acetate fraction in CUPRAC assay at 200 µg/ml of concentration. In this assays, as the concentrations of the extract was increased the inhibition of lipid peroxidation was also increased. The broad range of antioxidant activity of the extracts indicates the potential of the plant as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits.

ACKNOWLEDGMENT

The authors are thankful to University Grants Commission (UGC) New Delhi, UPE (University with Potential for Excellence) and CPEPA (Center with Potential for Excellence in particular Area) for providing financial assistance. We are also thankful to Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar for providing necessary laboratory facilities.

REFERENCES

1. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B, Berker KI and Ozyurt D (2007), "Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the cupric assay", *Molecules,* Vol .12, pp. 1496-1547.
2. Apak R, Guclu K, Ozyurek M and Karademir SE (2004), "Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method", *Journal of Agricultural and Food Chemistry,* Vol 52, pp. 7970-7981.
3. Apak R, Guclu K, Ozyurek M, Karademir SE and Altun M (2005), "Total antioxidant capacity assay of human serum using copper (II)-neocuproine as chromogenic oxidant: The CUPRAC method", *Free Radical Research*, Vol 39, pp. 949.
4. Arora S, Brits E and Kaur S (2005), "Evaluation of genotoxicity of medicinal plant extracts by the comet and VITOTOX_ tests," *Journal of Environmental Pathology, Toxicology and Oncology*, Vol. 24, pp. 193-200
5. Catala A (2006), "An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay: review", *International Journal of Biochemistry and Cell Biology,* Vol. 38, pp. 1482–1495.
6. Chang S T, Wu J H, Wang S Y, Kang P L, Yang N S and Yur L F (2001), "Antioxidant potential of extracts from different agro wastes: stabilization of corn oil", *Journal of Agricultural and Food Chemistry,* Vol. 49, pp. 3420–3424
7. Frankel E (1995), " Nutritional benefits of flavonoids", *International conference of food factors: chemistry and cancer prevention,* Hamamatsu, Japan, Abstract C6-2.
8. Fukuzawa K (2008), "Dynamics of lipid peroxidation and antioxidation of alphatocopherol in membranes: review", *Journal of Nutritional Science and Vitaminology (Tokyo),* Vol. 54, pp. 273–285.
9. Grisham M B and Mccord J Z (1998), "Chemistry and cytotoxicities of reactive oxygen metabolites", *American Physiological Society Bethesda*, Vol. 9, pp. 1-8.
10. Grisham M B and McCord J M (1986), "Chemistry and cytotoxicities of reactive oxygen metabolites", American Physiological Society, Bethesda, pp. 1-8.
11. Halliwell B and Gutteridge J M C (1992), "Free radicals, antioxidant and human disease", *Journal of Laboratory and Clinical Medicine*, Vol. 119, pp. 598-620
12. Halliwell B and Gutteridge J M C (2007), *Free radicals in biology and medicine*, Oxford, UK: Clarendon Press, 4th Edition.

13. Kahkonen M P, Hopia A I, Vuorela H J, Rauha J P, Pihlaja K, Kujala T S and Heinonen M (1999), "Antioxidant activity of plant extracts containing phenolic compounds", *Journal of Agriculture Food Chemistry*, Vol 47, pp. 3954-3962

14. Kappus H (1991), "Lipid peroxidation-Mechanism and biological relevance*",* in *Free radicals and Food Additives,* O I Aruoma and B Halliwell (Eds.)*,* Taylor and Francis, London.

15. Kaur A, Singh J, Kamboj SS, Saxena AK, Pandita RM and Shamnugavel M (2005), "Isolation of an *N*-acetyl-D-glucosamine specific lectin frm the rhizomes of *Arundo donax* with antiproliferative activity", *Phytochemistry*, Vol. 66, pp. 1933-1940.

16. Kaur K, Michael H, Arora S, Arkonen PH and Kumar S (2005), "In vitro bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from *Acacia nilotica* (L.) Willd. Ex Del". *Journal of Ethnopharmacology*, Vol. 99, pp. 353-360.

17. Kim D, Jeong S and Lee CY (2003), "Antioxidant capacity of phenolic phytochemicals from various cultivars of plums", *Journal of Food Chemistry*, Vol. 81, pp. 321-326.

18. Manach C, Scalbert A, Morand C, Remesy C and Jimenez L (2004), "Polyphenols: food sources and bioavailability", *American Journal of Clinical Nutrition*, Vol. 79, pp. 727-747.

19. Moskovitz J, Yim MB and Chock PB (2002), "Free radicals and disease", *Journal of Biochemistry,* Vol 397, pp. 354-359

20. Ohkawa H, Ohishi N and Yagi K (1979), "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction", *Journal of Analytical Biochemistry*, Vol 95, pp. 351-358, (with modifications).

21. Omale J and Okafor P N (2008), "Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*", *Journal of African Biotechnology*, Vol. 7, pp. 3129-3133.

22. Pourmorad F, Hosseinimehr S J and Shanabi Majd N (2006), "Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants", *Journal of African Biotechnology*, Vol. 5, pp. 1142-1145.

23. Pratt D E and Hudson B J F (1990), "Natural antioxidants not exploited commercially," *Journal of Food Antioxidants*, Elsevier, Amsterdam, pp. 171-192.

24. Prieto P, Pineda M and Aguilar M (1999), "Spectrophotometric quantization of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E", *Journal of Analytical Biochemistry*, Vol. 269, pp. 337-341.

25. Rice E C, Miller N J, Bolwell G P, Bramley P M and Pridham J B (1995), "The relative antioxidant activities of plant derived polyphenolic flavonoids", *Journal of Free Radicals Research*, Vol. 22, pp. 375-383.

26. Sala A, Recio M D, Ginner R M, Manez S, Tournier H, Schinella G and Rios J L (2002), "Anti-inflammatory and antioxidant properties of *Helichrysum italicum", Journal*

of Pharmacy and Pharmacology, Vol. 54, pp. 365-371.

27. Schnitzer E, Pinchuk I and Lichtenberg D (2007), "Peroxidation of liposomal lipids", *Journal of European Biophysics*, Vol. 36, pp. 499-515.

28. Singh B N, Singh B R, Singh R L, Prakash D, Sarma B K and Singh H B (2009a), "Antioxidant and anti-quorum sensing activities of green pod of *Acacia nilotica* L", *Journal of Food Chemistry Toxicology*, Vol. 47, pp. 778-786.

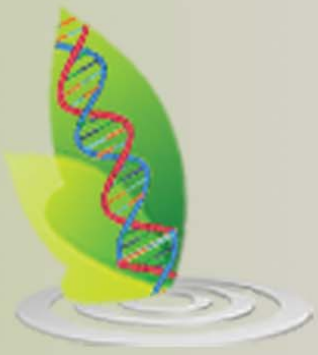
29. Singh R, Singh S, Kumar S and Arora S (2004), "Hydroxyl radical scavenging potential of acetone extract/fractions of *Acacia nilotica* (L.) Willd. Ex Del", *International Journal of Bioscience Reporter*, Vol. 2, pp. 440-446.

30. Singh R, Singh S, Kumar S and Arora (2007), "Evaluation of antioxidant potential of ethylacetate extract/fractions of *Acacia auriculiformis* A, Cunn", *Journal of Food Chemistry and Toxicology,* Vol. 45, pp. 216-223.

31. Vaya J, Belinky PA and Aviram M (1997), "Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity toward LDL oxidation", *Free Radical Biology and Medicine,* Vol. 23, pp. 302-313.

32. Yanishlieva NV, Marinova E and Pokorny J (2006), "Natural antioxidants from herbs and spices", *European Journal of Lipid Science and Technology*, Vol. 108, pp. 776-793.

33. Yu L, Haley S, Perret J, Harris M, Wilson J and Qian M (2002), "Free radical scavenging properties of wheat extracts", *Journal of Agricultural and Food Chemistry*, Vol. 50, pp. 1619-1624.



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijlbpr@gmail.com or editor@ijlbpr.com
Website: www.ijlbpr.com

ISSN 2250-3137
9 772250 313001 04