Asian Journal of Chemistry Vol. 21, No. 7 (2009), 5073-5084

Radical Scavenging and Antioxidant Potential of Aqueous and Organic Extracts of Aerial Parts of *Litchi chinensis* Sonn.

ISLAM U. KHAN, MUHAMMAD N. ASGHAR*, SADAF IQBAL,
TANVEER H. BOKHARI and ZAHEER-UD-DIN KHAN†
Materials Chemistry Laboratory, Department of Chemistry,
GC University, Lahore-54000, Pakistan
Tel: (92)(332)4522509; E-mail: mnasghar2003@yahoo.com

Extracts of medicinal plants have been reported to be very much effective in the control of chronic diseases due to their high antioxidant contents. Focus of the present study is the exploitation of safer and cheaper sources of the antioxidants based on the natural origin. *Litichi chinensis* Sonn. is used for its hypoglycemic activity in the traditional medicinal system of Indian subcontinent. The antioxidant activity of the aqueous and organic extracts of leaves, stem and fruit pulp of *L. chinensis* was investigated using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulpohonic acid) (ABTS) decolourization assay, the ferric reducing anti-oxidant power (FRAP) assay, 2.2'-diphenyl-1-picrylhydrazil (DPPH) assay, the total phenolic content (TPC) assay and the total antioxidant activity assay. Using the ABTS decolourization assay, *L. chinensis* extracts showed a wide range of antioxidant activity in terms of trolox equivalent antioxidant capacity (TEAC); from 5.64-0.126 mM for leaves of *L. chinensis* (LCL), from 4.12-0.23 mM for stem (LCS) and from 0.66-0.24 mM for fruit pulp of *L. chinensis* (LCPL). The total phenolic content assay exhibited 1.72, 1.46 and 1.41 mM gallic acid equivalents for ethyl acetate, aqueous (before partitioning) and aqueous (after partitioning) fractions of the stem respectively. The ferric reducing antioxidant power assay indicated high reducing power of leaves and stem of *Litchi chinensis*. Methanol, 1-butanol, aqueous and ethyl acetate fractions of all the parts of *Litchi chinensis* showed strong DPPH and peroxyl radicals scavenging activity. The results obtained in the present study demonstrated that all the pats studied are potential sources of natural antioxidants. Although the radical scavenging and inhibition of lipid peroxidation studies show usefulness of *Litchi chinensis* extracts *in vitro* conditions, yet verification of their *in vivo* activity and especially during disease conditions needs further experimentation.

Key Words: ABTS, FRAP, TPC, TEAC, *Litchi chinensis* Sonn.

INTRODUCTION

Oxygen based free radicals and other reactive species, collectively named as reactive oxygen species (ROS) are produced *in vivo* during oxidation[1]. Reactive oxygen species are not only associated with lipid peroxidation leading to food

†Department of Botany, GC University, Lahore-54000, Pakistan.

deterioration, but are also involved in development of a variety of diseases, including cellular ageing, mutagenesis, carcinogenesis, coronary heart disease, diabetes and neurodegeneration[2,3]. Foods rich in antioxiants may be used to help the human body in reducing the oxidative damage by ROS[4,5]. Various phytochemicals from teas, spices and herbs have been shown to have their role in the suppression of ROS *in vivo*[6]. Keeping in view the positive role of phytochemicals on human health, different antioxidant compounds including butylated hydroxytoluene (BHT), propyl gallate (PG), *tert*-butylhydroquinone (TBHQ), *etc.* are synthesized in laboratory[7]. However, these compounds have been charged with damaging liver and causing cancer to experimental animals[8,9]. Therefore, exploitation of safer and cheaper antioxidants of natural origin is highly desired[10].

Litchi chinensis Sonn., a member of Spindaceae family, has its origin in China and is now widely spread in the tropical and sub-tropical regions of the world[11]. Litchi fruit is high in vitamin C and the essential mineral potassium[12]. Different anthocyanins, phenolics, tannins have been identified and quantified in pericarp and peel of litchi[13-15].

Antioxidant activity of the aerial parts including stem, leaves and fruit pulp has not been as much studied as that of peel and pericarp of this plant. Focus of the present study is to investigate the antioxidant potential of various aqueous and organic extracts of aerial parts of *L. chinensis* by using antioxidant assays including the ABTS radical cation decolourization, the FRAP assay, the TPC assay, the DPPH decolourization assay and the total antioxidant power using linoleic acid emulsion system.

EXPERIMENTAL

All the chemicals used were of analytical reagent grade. Methanol, 1-butanol, chloroform, *n*-hexane, ethyl acetate, glacial acetic acid, potassium persulphate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, iron(III) chloride and iron(II) chloride were purchased from E. Merck while trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), ABTS (2,2'-azino*bis*-(3-ethylbenzothiazoline-6-sulphonic) diammonium salt), gallic acid, Tween 20 (polyoxyethylenesorbitan monolaurate), iron(II) sulphate, potassium thiocyanate, 1,1-diphenyl-2-picrylhydrazil (DPPH), 2,4,6-tripyridyl-*s*-triazine (TPTZ), Folin-Ciocalteau's reagent (FC Reagent) were purchased from Fluka (Switzerland).

Fresh *Litichi chinensis* fruit was purchased from local fruit market and its leaves and stem were collected from Botanic Garden of GC University, Lahore, Pakistan. Pulp of the fruit was separated manually. Leaves, stem and pulp were allowed to dry at room temperature under shade. A finely ground amount equal to 10 g each of leaves (LCL), stem (LCS) and pulp (LCPL) was soaked in methanol in 1:20 w/v separately in cork-fitted flasks for 24 h at 30 ºC and 240 rpm. The extract obtained the next day was filtered and stored at 4 ºC while the residue was reextracted in another 200 mL methanol under the same conditions. Both the filtrates

obtained were mixed and a small volume of methanolic extract equal to about 5 mL was stored in a capped bottle at 4 ºC, while rest of the extract was concentrated through rotary evaporator at 30 ºC. The residue obtained was suspended in appropriate volume of distilled water. Subsequently different fractions of aqueous extract were obtained using organic solvents (25 × 3 mL) of varying polarity. The aqueous and organic extracts were stored in refrigerator at 4 ºC.

$ABTS^{\bullet+}$ Assay protocol: $ABTS^{\bullet+}$ Assay protocol as developed by Re *et al.*[16]. was followed. ABTS was dissolved in doubly distilled water to a 7 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. For the study of antioxidant activity of standard antioxidant and plant samples, the ABTS stock solution was diluted with PBS buffer (pH 7.4) to an absorbance of 0.700 (±0.020) at 734 nm and equilibrated at 30 ºC. For plant extracts, the dilution was made in the respective solvents. After addition of 10 µL of neat or diluted stock solution to 2.99 mL of diluted $ABTS^{\bullet+}$ solution (A = 0.700 ± 0.020), the absorbance reading was taken at 30 °C exactly 1 min after initial mixing up to 8 min. Appropriate solvent blanks were run in each assay. The percentage inhibition of absorbance was calculated by the following formula and was plotted as a function of concentration of antioxidants and of Trolox for the standard reference data:

$$\% \text{ Inhibition}_{(\text{at } 734 \text{ nm})} = (1 - A_f/A_o) \times 100$$

where A_o is the absorbance of radical cation solution before addition of sample/standard antioxidants while A_f is the absorbance after addition of the sample/standard antioxidants. Each measurement was made in triplicate and at least three times at each concentration level of standards and sample.

Total phenolic content assay: Total soluble phenolics in *L. chinensis* plant extracts were determined with Folin-Ciocalteu reagent according to the method of Singleton *et al.*[17] using gallic acid as a standard phenolic compound. A volume of 40 µL of each sample and standard was transferred into separate test tubes and to each added 3.16 mL water and 200 µL of Folin-Ciocalteu's reagent. The mixture was mixed well, waited for 8 min and then added 600 µL of sodium carbonate solution with continuous stirring. The solution was left at 40 ºC for 0.5 h and absorbance of each solution at 765 nm against the blank (without phenolic solution) was determined. A curve was plotted between absorbance and concentration. The concentration of total phenolic compounds of all fractions of litchi plants was determined as milligrams of gallic acid equivalent (GAE) by using the following equation that was obtained from the standard gallic acid graph.

$$\text{Absorbance} = 0.0555 \times \text{Gallic acid (mg/L)}$$

Ferric reducing antioxidant power assay (FRAP): FRAP assay was performed as described by Benzie and Strain method[19]. Experiment was conducted at 37 ºC at pH 3.6 with a blank sample. FRAP reagent was prepared by mixing 25 mL acetate buffer (pH 3.6), 2.5 mL (10 mmol/L) TPTZ solution and 2.5 mL (20 mmol/L)

$FeCl_3.6H_2O$ solution. 300 µL freshly prepared FRAP reagent was warmed to 37 ºC and a reagent blank reading was taken at 593 nm; 10 µL of sample/standard antioxidant was then added along with 30 µL H_2O. Absorbance readings were taken after every 1 min for 4 min. The change in absorbance (ΔA_{593}) between final reading and reagent blank was noted. For reference data ΔA_{593} values for a range of trolox concentrations were noted and a standard curve was drawn. Final results were expressed as micromole trolox equivalents (TE) per gram on dried basis (µmol TE\g, db).

Diphenyl-2-picrylhydrazyl radical scavenging capacity assay (DPPH assay): Free radical scavenging activity of water and organic solvent extracts was measured by 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\bullet+}$) using the method of Shimada *et al.*[19]. DPPH solution (3 mL, 25 mg/L) in methanol was mixed with appropriate volumes of neat or diluted sample solutions. The reaction progress of the mixture was monitored at 515 nm over a time period. Upon reduction, the colour of the solution faded. The percentage of the DPPH remaining was calculated as:

$$\% \ DPPH_{rem} = [DPPH]_{rem}/[DPPH]_{t=0} \times 100$$

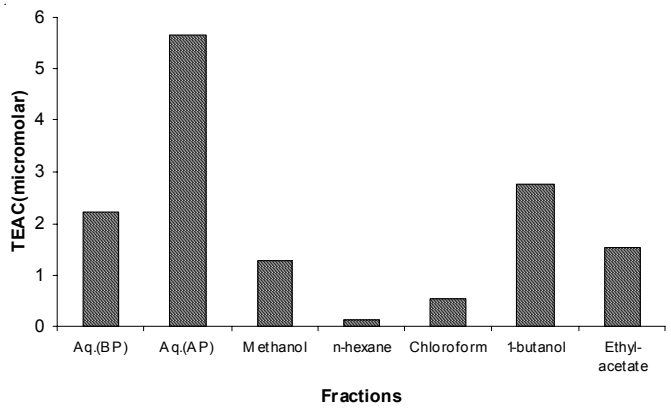
where $[DPPH]_{rem}$ corresplnds to absorbance of DPPH solution at time t while $[DPPH]_{t=0}$ is the absorbance before the addition of sample or standard antioxidant.

A kinetic curve showing the scavenging of DPPH radical in terms of decrease in absorbance at 593 nm as a function of time (min) was plotted for each fraction of the samples.

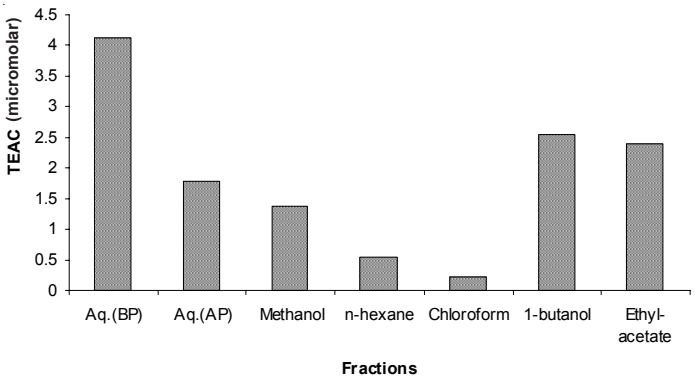
Total antioxidant activity determination: Total antioxidant activity of aqueous and organic extracts of litchi plant was determined according to the method employed by Mitsuda *et al.*[20]. The solution, which contain 100 mL each of neat or diluted plant extract of litchi plant in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). Each solution was then incubated at 37 ºC in sealed bottles in dark. The solution without added extract was used as blank, while the solutions containing 100 mL (50 µg/20 µL) of Trolox was used as positive control. At intervals of 24 h during incubation, 0.1 mL of each solution was transferred to a beaker containing 3.7 mL of ethanol. After addition of 0.1 mL each of $FeCl_2$ (20 mM in 3.5 % HCl) and thiocyanate solution (30 %) to the ethanolic sample, the solution was stirred for 1 min. The absorption values of the solutions measured at 500 nm were taken as lipid peroxidation values.

RESULTS AND DISCUSSION

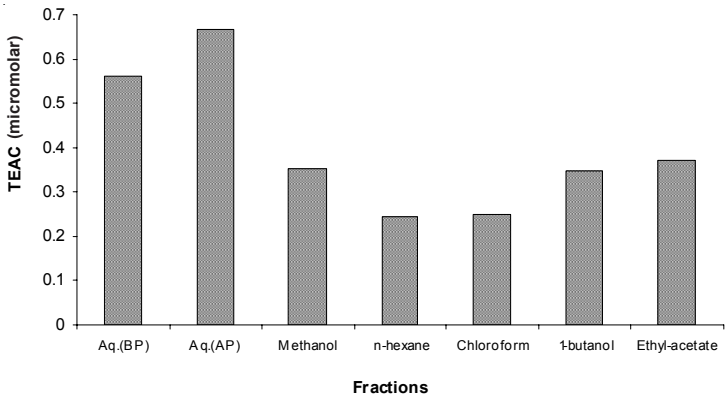
ABTS Radical cation decolourization assay: ABTS decolourization assay which is electron-transfer-based assay, was applied to evaluate the antioxidant potential of various extracts of litchi plant. The antioxidant components having a redox potential lower than that of ABTS radical cation have the ability to scavenge the colour of the radical proportionate to their amount. TEAC values were obtained by calculating the % inhibition on addition of the sample and subsequently comparing with a calibration curve formed against Trolox as standard antioxidant. Bar graphs were plotted between TEAC values and their corresponding fraction of the sample (Fig. 1).



(A)



(B)



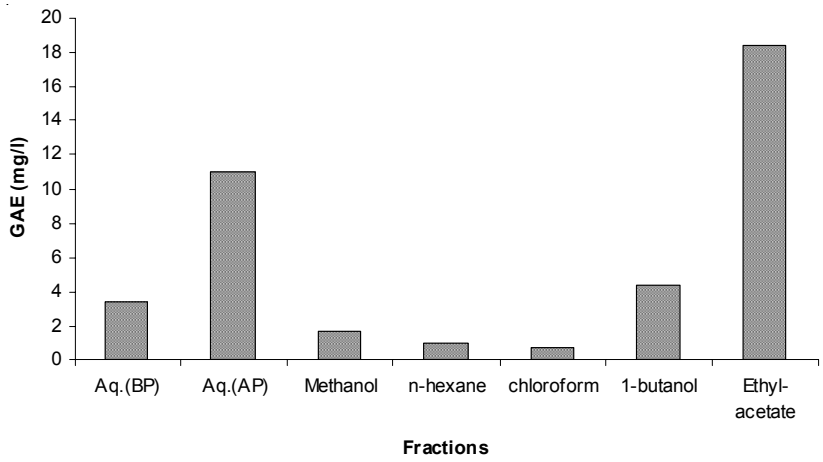
(C)

Fig. 1. TEAC (micromolar) values of the extracts of (A) leaves, ethyl acetate is 10 times diluted (B) Stem, all fractions are 10 times diluted (C) pulp of *L. chinensis* using the ABTS radical cation decolourization assay

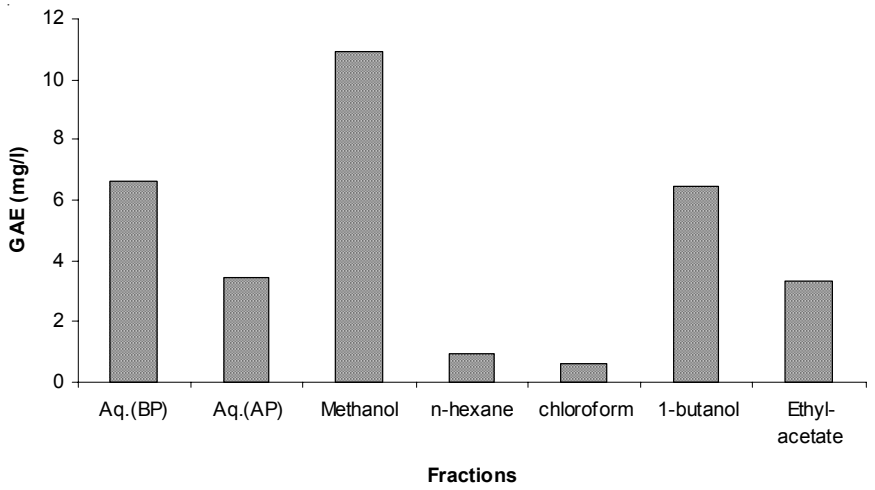
LCS and LCL showed relatively higher antioxidant activity as compared to the extracts of LCPL. TEAC values ranged from 5.64-0.126 µM for aq. (AP) and *n*-hexane of LCL, respectively, from 4.12-0.230 µM for aq. (BP) and chloroform fractions of LCS, respectively and for LCPL from 0.66-0.24 µM for aq. (AP) and *n*-hexane, respectively. Overall aq. (BP) showed highest while *n*-hexane of LCS showed the least TEAC values.

Total phenolic content assay: The FCR actually measures reducing capacity of phenolic compounds. Phenolic compounds have been reported to be powerful antioxidants due to presence of hydroxyl groups in their structures. Phenolic compounds react with FCR only under basic conditions. Dissociation of a phenolic proton leads to phenolate anion, which is capable of reducing FCR. The blue compound formed between phenolate and FCR is independent of the structure of phenolic compounds, therefore ruling out the possibility of coordination complex formed between the metal centre and phenolic compounds. Despite the undefined chemical nature of FCR, the total phenol assay by FCR is a convenient, simple and reproducible assay.

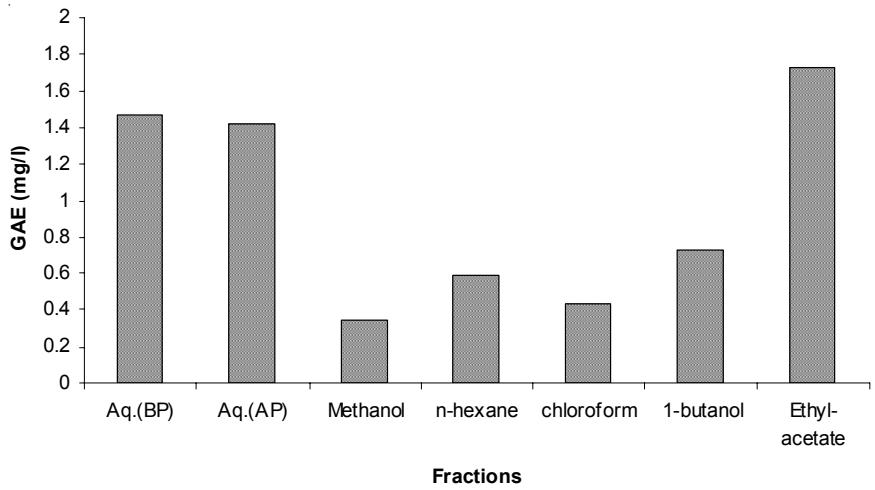
Different parts of LCPL were found to be containing less phenolic contents as compared with those of LCS and LCL. Of the leaves, the total phenoloic contents of aq. (AP) and ethyl acetate fractions were highest at 10.9 µM of gallic acid equivalent/ 10 µL of leaves fraction, respectively, followed by 4.05 and 3.75 µM of GAE/40 µL of 1-butanol and aq. (B.P) fractions respectively (Fig. 2A-C). Of the stem, the total phenolic contents of methanol, aq. (B.P), 1-butanol, ethyl acetate fractions were found highest at 11.45m 6.77, 6.59, 3.34 µM of gallic acid equivalents/40 µL of corresponding fraction. Ethyl acetate, aq. (B.P), aq. (A.P) and 1-butanol were found to the the highest in TPC as compared with other fractions of the pulp. Although extracts of fruit pullp showed low values of TPC, but the total phenolic contents tended to be highest in leaves followed by stem and fruit pulp.



(A)



(B)

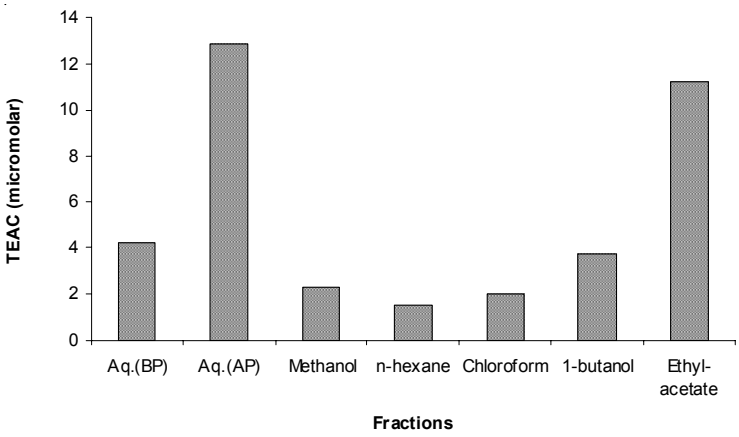


(C)

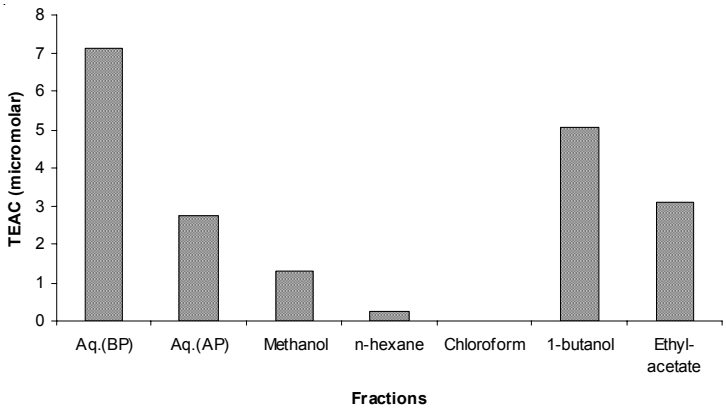
Fig. 2. GAE values of the extracts of A) leaves B) stem,methanolic fraction 10 times diluted C) pulp *L. chinensis* using TPC assay

Ferric reducing antioxidant power assay: FRAP assay was used to assess "antioxidant or reducing power" of biological and abiological samples through the reduction of ferric to ferrous ions at low pH. The conversion of ferric to ferrous causes formation of a coloured ferrous-tripyridyltriazine (TPTZ) complex. TEAC values of different aqueous and organic fractions of the medicinal plants were evaluated using Trolox as standard reductant.

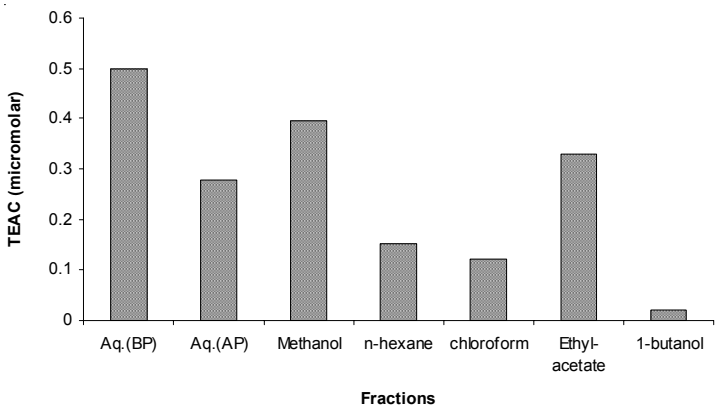
LCL and LCS showed good reducing power while LCPL showed low TEAC values. In all the three parts aq. (AP), aq. (BP), methanol and 1-butanol fractions showed high TEAC values (Fig. 3A-C).



(A)



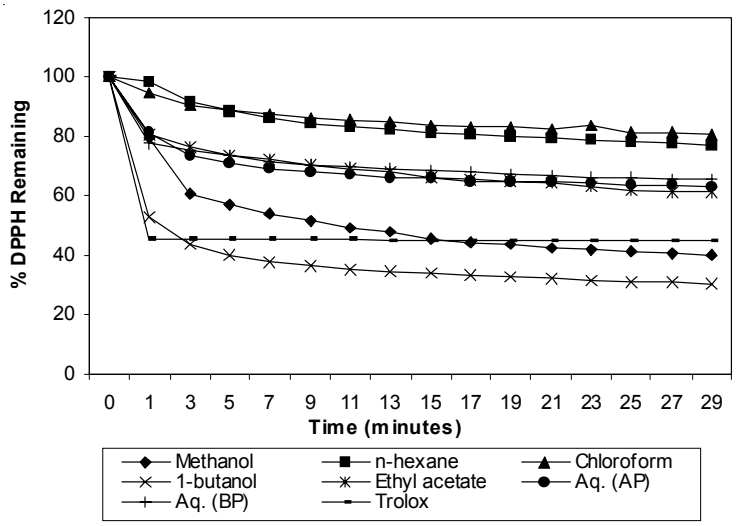
(B)



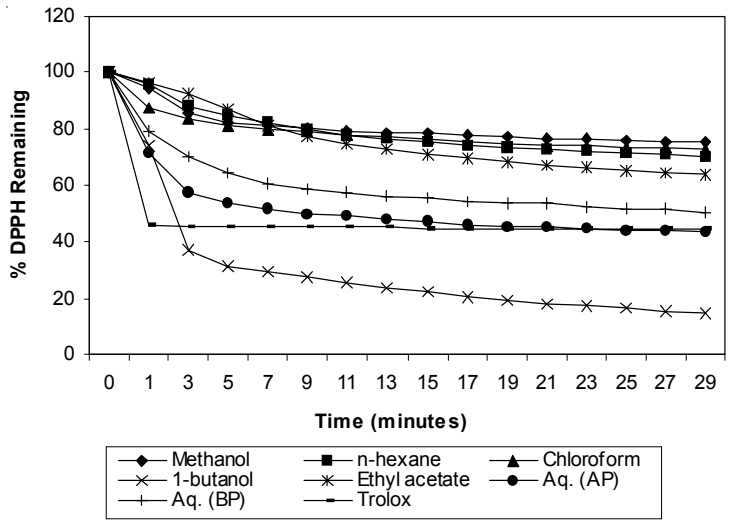
(C)

Fig. 3. TEAC values of the extracts of (A) leaves (methanol, *n*-butanol and aq. BP are 10 times diluted) (B) stem (except chloroform all are 10 times diluted) (C) pulp of *L. chinensis* using the FRAP assay

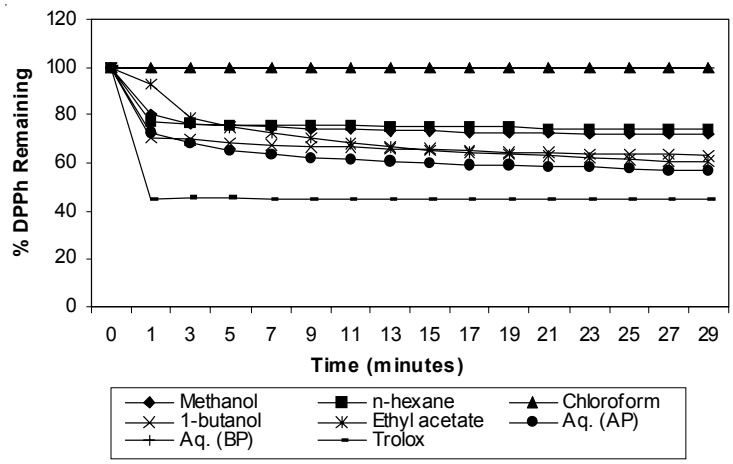
DPPH Radical scavenging capacity assay: DPPH Radical scavenging capacity assay was employed to investigate the reaction kinetics of sample antioxidants with DPPH radical. Upon reaction with sample antioxidants, the colour of DPPH solution fades. The reaction progress can be monitored by noting the absorbance readings at 515 nm as a function of time. % $DPPH_{rem.}$ was plotted against the time (min). Methanol, 1-butanol, aq. (AP) and aq. (BP) and ethyl acetate fractions of all the plant extracts showed maximum scavenging of DPPH free radical. Scavenging of DPPH free radical started at a greater pace for the first two minutes, which continued at relatively slower pace for the rest of the time (Fig. 4A-C).



(A)



(B)



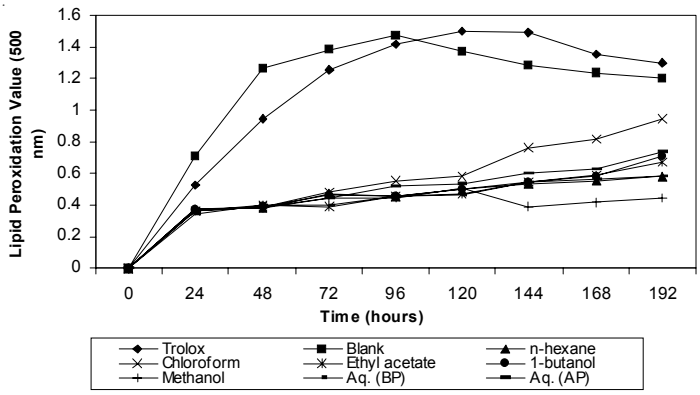
(C)

Fig. 4. % DPPH remaining values of the extract of (A) leaves (aq. BP, 1-butanol and methanol 5 times diluted, ethyl acetate 15 times diluted) (B) stem (chloroform 3 times concentrated. aq. (AP) and ethyl acetate 10 times diluted, respectively) (C) pulp (ethyl acetate and aq. (AP) 10 times conc. 1-butanol and *n*-hexane 15 times conc, methanol 13 times conc.) of *L. chinensis* as a function of time

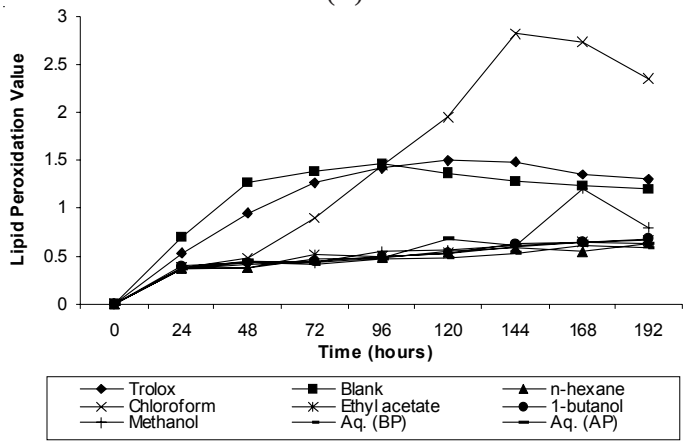
The scavenging effect of different fractions of leaves, stem and fruit pulp on the DPPH radical decreased in the order of ethyl acetate > 1-butanol > methanol > aq. (BP) > trolox > aq. (AP) > *n*-hexane > chloroform; aq. (AP) > ethyl acetate > 1-butanol > trolox > aq. (BP) > *n*-hexane > methanol > chloroform and trolox > aq.(BP) > chloroform > aq.(AP) > ethyl acetate > methanol > 1-butanol > *n*-hexane, respectively.

The kinetics of the reaction between the sample and the DPPH radical showed that the extracts contained a mixture of different types of antioxidant components, of which some reacted instantaneously with DPPH radical and discharged its colour while some took longer period of time to react completely.

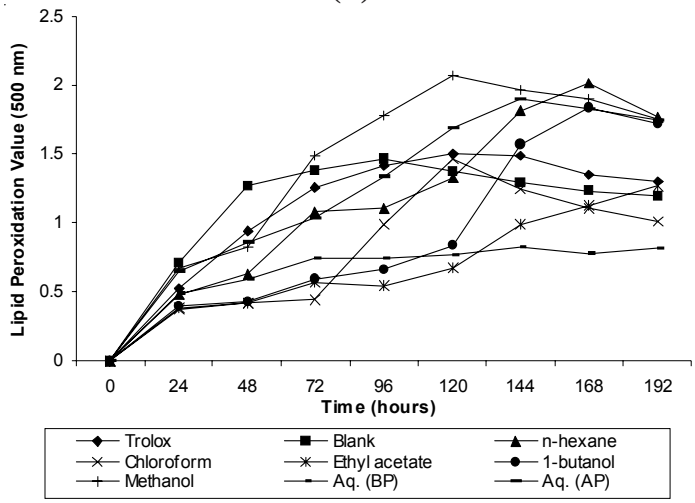
Total antioxidant activity assay: Thiocyanate method was employed to determine total antioxidant activity of the extracts of the traditional medicinal plants. During linoleic acid oxidation, peroxides are formed. These peroxides have ability to oxidize Fe^{2+} to Fe^{3+}. Fe^{3+} ion on reaction with thiocyanate ions (SCN^{-}) forms complex which can be measured spectrophotometrically at 500 nm. The added standard or sample solutions try to inhibit or slow down oxidation of linoleic acid and thus formation of peroxide values. Thus low peroxidation values indicate high antioxidant power of the sample and *vice versa*. Antioxidant activity of the aqueous and organic fractions of the plants was determined as a function of time. Standard antioxidant Trolox was used as positive control. The results showed that *n*-butanol, Aq. (AP), Aq. (BP) and methanol fractions of all the parts of *L. chinensis* had potent peroxyl radical scavenging activity (Fig. 5 A-C). The antioxidant components of the samples were so potent that even after incubation of 192 h; there was a slight increase in the peroxidation value.



(A)



(B)



(C)

Fig. 5. Peroxidation values of extracts of (A) leaves (B) stem (C) pulp of *L. chinensis* using thiocyanate method

The results presented here show a useful data about the total antioxidant activity and the ability of *L. chinensis* extracts to metal chelation activity and to slow down lipid oxidation in *in vitro* systems. They would certainly prove helpful in correlating the antioxidant activity of *L. chinensis* extracts with their antidisease activity. Furthermore, the extracts may be used as good source of natural antioxidants for maintaining health conditions. As *L. chinensis* is abundantly available and potent antioxidant, so extracts may be used in phytochemical drugs and food industry.

REFERENCES

1. O. Bloknina, E. Virolainen and K.V. Fagerstedt, *Ann. Bot.*, **91**, 179 (2003).
2. B. Halliwell and J.M.C. Gutteridge, Free Radicals in Biology and Medicines, Oxford University Press, Oxford, edn. 3 (1999).
3. J. Moskovitz, K.A. Yim and P.B. Choke, *Arch. Biochem. Biophys.*, **397**, 354 (2002).
4. I. Gulcin, M. Oktay, O.I. Kufrevioglu and A. Aslan, *J. Ethnopharmacol.*, **79**, 325 (2002).
5. J.L. Mau, G.R. Chao and K.T. Wu, *J. Agric. Food Chem.*, **49**, 5461 (2001).
6. C.T. Ho, T. Ferraro, Q. Chen and R.T. Rosen, in eds.: C.T. Ho, T. Osawa, M.T. Huang and R.T. Rosen, Phytochemicals in Teas and Rosemary and Their Cancer-Preventive Properties, Food Phytochemicals for Cancer-Prevention, II. Tea, Spices and Herbs, ACS Symposium Series 547, American Chemical Society, Washington, D.C., pp. 2-9 (1994).
7. F.R. Sherwin, in ed.: R. Branen, Antioxidants, Food Additives, Marcel Dekker, New York, pp. 139-193 (1990).
8. H.C. Grice, *Food Chem. Toxicol.*, **24**, 1127 (1986).
9. H.P. Wichi, *Food Chem. Toxicol.*, **26**, 717 (1988).
10. M. Oktay, I. Gulcin and O.I. Kufrevioglu, *Lebensmittel-Wissenchaft und Technologie*, **36**, 263 (2003).
11. C.M. Menzal, *Sci. Horticult.*, **21**, 201 (1983).
12. H.S. Lee and L. Wicker, *Food Chem.*, **40**, 263 (1991).
13. D.L. Zhang, P.C. Quantick and J.M. Grigor, Postharvest Bio and Tec, pp. 165-172 (2000).
14. P. Sarni-Manchado, E. Le Roux, C. Le Guerneve, Y. Lozano and V. Cheynier, *J. Agric. Food Chem.*, **48**, 5995 (2000).
15. X. Zhang, X. Pang, C. Yang, Z. Ji and Y. Jiang, *Food Chem.*, **84**, 601 (2004).
16. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C.A. Rice Evans, *Free Radical Biol. Med.*, **26**, 1231 (1999).
17. V.L. Singleton and J.A. Rossi, *Am. J. Enol. Viticult.*, **16**, 144 (1965).
18. I.E.F. Benzie and J.J. Strain, *Anal. Biochem.*, **239**, 70 (1996).
19. K. Shimada, K. Fujikawa, K. Yahara and T. Nakamura, *J. Agric. Food. Chem.*, **40**, 945 (1992).
20. H. Mitsuda, K. Yuasumoto and K. Iwami, *Eiyo to Shokuryo*, **19**, 210 (1996).
21. C. Rice-Evans, N.J. Miller and G. Paganga, *Trends Plant Sci.*, **2**, 152 (1997).

(*Received*: 31 March 2008; *Accepted*: 27 April 2009) AJC-7438