Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(5): 18-23

Received: 05-07-2016
Accepted: 06-08-2016

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Evaluation of antioxidants level in *Triticum aestivum*. L

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Abstract
Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular and anti-inflammatory diseases. The results indicate that the plant extract has better DPPH scavenging action and reducing power compared to the individual plant extract indicating synergistic and supra additive effect of phytochemicals present in the extract. This present study proves that the plant extracts have considerable antioxidant property and further detailed studies and characterization of these plant extracts can contribute to their medicinal and pharmaceutical applications. The assessment of *in vitro* antioxidant status of *T. aestivum* against a broad spectrum of free radical scavengers provide strongly a valid tool that *T. aestivum* could serve as a natural source of antioxidant defense. Almost all the antioxidants were noted to be significant in counteracting the free radical scavenging activity. The *in vitro* antioxidant status provides a new insight to assess the hepatoprotective effect of *T. aestivum*.

Keywords: Antioxidant activity, DPPH, free radicals, ethanol extract

Introduction
Plants are the richest resource of drugs of traditional system of medicine, modern medicine, pharmaceutical intermediates and chemical entities for the synthetic drugs. The use of plants and plants product as a medicine could be traced as far back as he begining of human civilization [1]. Various types of plants have been used not only for dietary supplements but also as traditional folk treatments for many health problems [2]. The use of folk medicine is widespread and herbaceous plants comprise an important source of bioactive compounds possessing potent biological properties. They have played a significant role in traditional medicine since ancient times and still represent an important source of natural antioxidants that might lead to the development of novel drugs [3]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [4]. Antioxidants act as a defence mechanism that protects against oxidative damage and include compounds to remove or repair damaged molecules. It can prevent/ retard the oxidation caused by free radicals and sufficient intake of antioxidants is supposed to protect against diseases [5].
The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer [6], cardiovascular disease [7], neural disorders [8], Alzheimer's disease [9], mild cognitive impairment [10], Parkinson's disease [11], alcohol induced liver disease [12], ulcerative colitis [13], aging [14] and atherosclerosis [16]. Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery [17].
Wheat, (*Triticum* species) a cereal grass of the Gramineae (Poaceae) family, is the world's largest edible grain cereal-grass crop. The wheat plant is an annual grass. In early growth stages the wheat plant consists of a much-compressed stem or crown and numerous narrowly linear or linear-lanceolate leaves. For over fifty years, researchers have known that the cereal plant, at this young green stage, is many times richer in levels of vitamins, minerals and

proteins as compared to seed kernel, or grain products of the mature cereal plant [18].

Today, wheatgrass is quickly becoming one of the most widely used supplemental health foods and is available in many health food stores as fresh produce, tablets, frozen juice, and powder. Wheatgrass contains vitamins, minerals, enzymes, amino acids, polysaccharides, and large amounts (70%) of chlorophyll. In line with folk sayings, the health benefits of wheatgrass may include improved digestion, blood pressure reduction, heavy metal detoxification from the bloodstream, immune system modulation, and gout alleviation. Several papers have indicated that wheatgrass has anti-tumor activities, anti-oxidant properties (2), and a therapeutic effect on distal ulcerative colitis. In addition, wheatgrass may help prevent some disorders, including diabetes and heart disease [19].

The objectives of the present study, we assessed the antioxidant level in leaves of *Triticum aestivum*. It was also aimed to observe the antioxidant properties in terms of DPPH scavenging activity and reducing power of this plant extract.

Materials and Methods

Plant Materials and preparation of extract

The leaves of *T. aestivum* were collected from tray. The samples were shade dried at room temperature and then ground to a fine powder in a mechanic grinder. The powdered material was then extracted using solvent ethanol in the ratio 1:10 using soxhlet apparatus. After extracting all colouring material the solvent was removed by evaporating in water bath which give rise to a solid mass of the extract.

DPPH assay: Various concentrations of ethanolic extract of the sample (4.0 ml) were mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture were shaken vigorously and left to stand for 30 mins, and the absorbance was measured at 517 nm. Ascorbic acid was used as control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

$$\% \text{ decolorization} = [1-(ABS_{sample}/ABS_{control})] \times 100$$

IC_{50}value (mg extract/ml) was the inhibitory concentration at which DPPH radicals were scavenged by 50%. Ascorbic acid was used for comparison [20].

ABTS assay: Samples were diluted to produce 0.2 to 1.0 µg/ml. The reaction was initiated by the addition of 1.0 ml of diluted ABTS to 10 µl of different concentration of ethanolic extract of the sample or 10 µl of methanol as control. The absorbance was read at 734 nm and the percentage inhibition was calculated. The inhibition was calculated according to the equation $I = A_1/A_0 \times 100$, where A_0 is the absorbance of control reaction, A_1 is the absorbance of test compound [21].

Hydroxyl scavenging assay: The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM$FeSo_4$, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and varying concentrations of the extract [18]. After incubation for 1 hour at 37 °C, the absence of the hydroxylated salicylate ecomplex was measured at 562 nm. The percentage scavenging effect was calculated as Scavenging activity = $[1-(A_1-A_2)/A_0] \times 100\%$.

Where A_0 was absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without sodium salicylate [22].

Super oxide radical assay: The 3 ml reaction mixture contained50µl of lMNBT, 150µl of lM NADH with or without sample and trisbuffer (0.02 M, pH 8.0). The reaction was started by adding 15µl of lM PMS to the mixture and the absorbance change was recorded to 560 nm after 2 mins. Percent inhibition was calculated against a control without the extract [23].

Reducing power: The reaction mixture contained 2.5 ml of various concentrations of ethanolic extract of the sample, 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The control contained all the reagents except the sample. The mixture was incubated at 50 °C for 20 min and were terminated by the addition of 2.5 ml of 10% (W/V) of trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 min. 5.0 ml of the supernatant upper layer was mixed with 5.0 ml measured at 700 nm against blanks that contained distilled water and phosphate buffer. Increased absorbance indicates increased reducing power of the sample. EC_{50} value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and were obtained by interpolation from linear regression analysis. Ascorbic acid was used for comparison [24].

NO scavenging assay: The interaction of ethanolic extract of the sample with nitric oxide was assessed by the nitrite detection method. Nitric oxide was generated from sodium nitroprusside and measured by Griess illosvory reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generated nitric oxide, which interacts with oxygen to produce nitrite, which can be estimated by the use of Griess illosvory reagent. In the present experiment, nitrite ion was measured by using Griess illosvory reagent, which is modified by using naphthyl ethylene diamine dihydro chloride instead of 1-naphthyl amine. Nitric oxide radical generated from sodium nitroprusside (SNP) was measured. Briefly, the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphate-buffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25 °C for 180 min. The $NO^{\cdot}$ radical thus generated interacted with oxygen to produce the nitrite ion ($NO^{\cdot}{}_2$) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent. The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride cas measured at 546 nm [25].

FRAP assay: The stock solution of 10 mM 2, 4, 6- tripyridyl-s- triazine (TPTZ) in 40 mMHCL, 20 mM$FeCl_3$. $6H_2O$ and 0.3 M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml acetate buffer. It were freshly prepared and warmed to 37 °C. 900 µl FRAP reagent were mixed with 90 µl water and 30 µl test ethanolic extract of the sample and standard antioxidant solution. The reaction mixture was then incubated at 37 °C for 30 mins and the absorbance was recorded at 595 nm. An intense blue colour complex was formed when ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex was reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded [26].

Statistical analysis

All values are expressed as Mean ± SD. The significance of differences between the means of the tests and controls were calculated by one way ANOVA.

Results

DPPH

The reaction capability of DPPH radical was determined by the decrease in its absorbance at 515 nm induced by antioxidants. At 200-1000 µg, the antioxidant activities of ethanolic extract of *T. aestivum* and the standard ascorbic acid were 24.11-38.12% and 26.11-40.11%, respectively. The extract exhibited concentration-dependent radical scavenging activity, that is, the higher the concentration, the scavenging potential. The DPPH radical scavenging activity of ethanolic extract of *T. aestivum* is shown in the Fig 1, which clearly indicates the dose-dependent DPPH scavenging activity of *T. aestivum*, with an IC50 value of 820 mg, and ascorbic acid with an IC50 value of 670 mg.

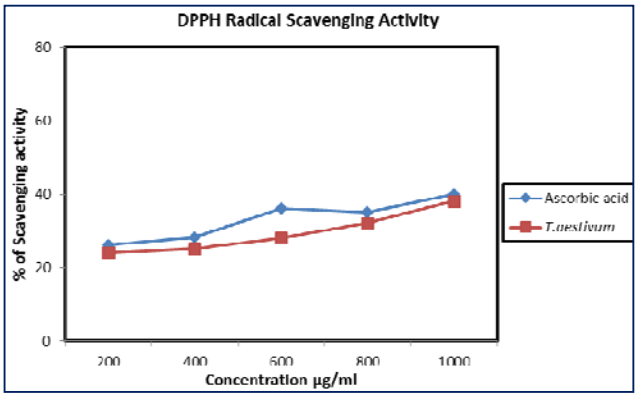

Fig 1

Superoxide scavenging

Superoxide radical reduced NBT to a blue-colored formation that was measured at 560 nm. At 200-1000 µg, the superoxide scavenging activity of ethanolic extract of *T. aestivum* was 17.40-59.50% and that of the standard ascorbic acid was 19.5-60.30%. The superoxide scavenging activity of ethanolic extract of *T. aestivum* and standard ascorbic acid is shown in Figure 2. The ethanolic extract of *T. aestivum* exhibited concentration-dependent radical scavenging activity, that is, percentage inhibition increased with sample concentration.

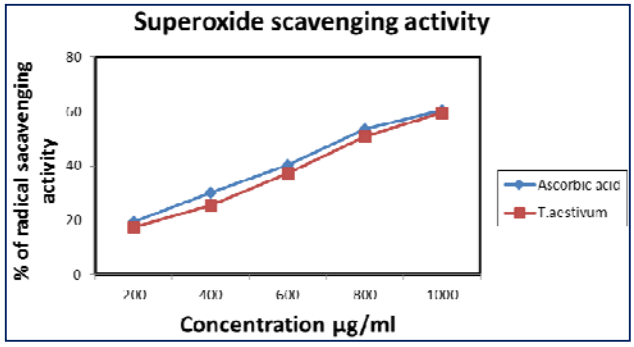

Fig 2

Reducing power

Ethanolic extract of *T. aestivum* displayed considerable reducing power, primarily due to its effect as an electron donor, and thereby hatting radical chain reactions by converting free radicals to more stable products. Increasing absorbance at 700 nm indicated an increase in reductive ability. Fig-3 shows dose response curves for the reducing power of the extract. The extracts showed good reducing power that was comparable with that of ascorbic acid. The antioxidant activity confirmed the medicinal importance of plants as naturally occurring antioxidants.

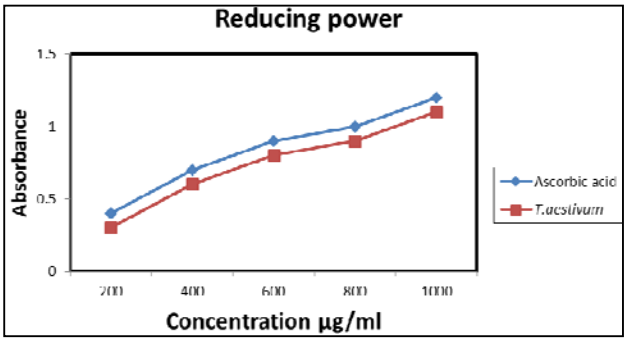

Fig 3

Nitric oxide scavenging activity

The minimum *in vitro* nitric oxide scavenging activity of the plant extract was 27.8% at 200 µg concentration, whereas the maximum *in vitro* activity was 62.5% at 1000 µg concentration. The percentage inhibition was increased with increasing concentration of the extract. However, activity of ascorbic acid was more pronounced than that of our extract.

Fig. 4 clearly indicates the dose-dependent nitric oxide scavenging activity of *T. aestivum*.

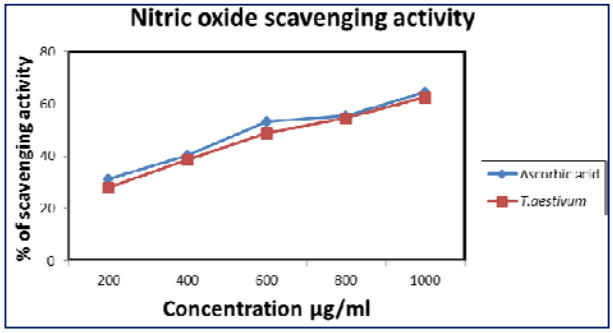

Fig 4

FRAP

The ferric reducing ability of the extract at 200-1000 µg was in the range of 0.06-0.15 and that of the standard ascorbic acid was 0.07-0.16. The FRAP values for the extract were significantly lower than those of ascorbic acid. Fig-5 shows that there was an increase in absorbance from 0.073 to 0.138.

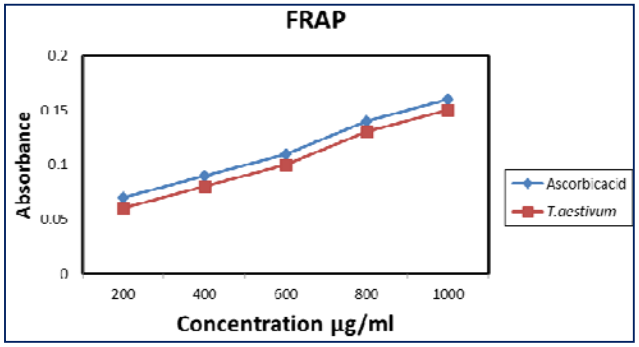

Fig 5

ABTS

Proton radical scavenging is an important attribute of antioxidants. ABTS is a protonated radical that has a characteristic maximum at 734 nm, which decreases with the scavenging of proton radicals. Fig-6 shows the ABTS

scavenging ability of *T. aestivum*. The scavenging effect of ABTS radical increased with concentration. The ethanolic extract of the leaves of *T. aestivum* were fast and effective scavengers of the ABTS radical. The plant extract showed antioxidant activities, proving their capacity to scavenge ABTS.

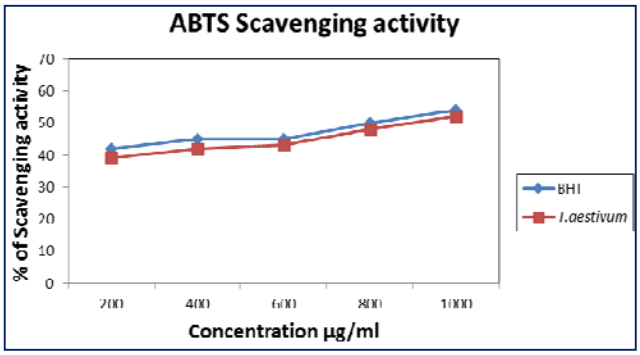

Fig 6

Hydroxyl radical scavenging

The potential of an ethanolic extract of *T. aestivum* to inhibit hydroxyl-radical-mediated deoxyribose damage was assessed at a concentration of 200-1000 µg/mL. The sample exhibited minimum activity of 17.0% at 200 µg and maximum activity of 58.0% at 1000 µg, showing that the hydroxyl radical scavenging activity occurred in a dose-dependent manner (Fig. 7). The results indicate the scavenging potential of *T. aestivum* against hydroxyl radicals.

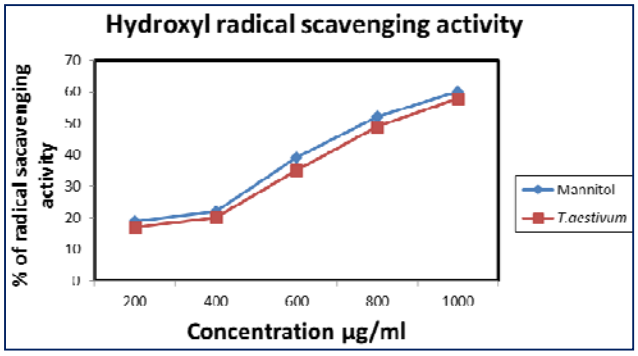

Fig 7

Discussion

DPPH

The free radical scavenging activity of ethanolic extract of *T. aestivum* and also that of ascorbic acid was evaluated through its ability to quench the synthetic DPPH radical. There are many methods for evaluating the antioxidant activity of both natural and artificial compounds. The DPPH assay constitutes a rapid and low cost method that has frequently been used for evaluation of the antioxidative potential of various natural products [27]. Therefore, in the present study, *T. aestivum* was screened for its possible antioxidant and radical scavenging activity by DPPH. The radical scavenging reaction of ascorbic acid with DPPH was essentially instantaneous; the reaction of DPPH with *T. aestivum* was also fast but slower compared to that with ascorbic acid. It is usually noticeable as discoloration of ethanolic extract of plant samples from purple to yellow; hence, DPPH is widely used to evaluate the free radical scavenging capacity of antioxidants [28].

Superoxide scavenging

Superoxides are produced from molecular oxygen by oxidative enzymes as well as via nonenzymatic reactions such as auto-oxidation by catecholamines [29]. Superoxide anions play an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, protein and DNA [30, 31]. The superoxide scavenging activity of *T. aestivum* was investigated, because the extract has the potential to scavenge superoxide anions. Fig. 2 clearly indicates that *T. aestivum* is a potent scavenger of superoxide radicals.

Reducing power

The reducing power of a compound acts as an indicator of its potential antioxidant activity. The reducing properties are generally associated with the presence of reductones, which exhibit antioxidant activity by breaking the chain reactions by donating hydrogen atoms [32]. Reductones also react with certain precursors of peroxide, thus preventing formation of the latter [33]. We showed that *T. aestivum* had comparable reducing power towards ascorbic acid.

Nitric oxide scavenging activity

Nitric oxide is a free radical produced in mammalian cells involved in regulation of various physiological processes. However, excess production of nitric oxide is associated with several diseases. The development of substances to prevent the overproduction of nitric oxide has become a new research target for treating chronic inflammatory diseases [34]. In the Fig. 4. ABTS scavenging activity of ethanolic extract of *T. aestivum* and Butylated hydroxytoluene (BHT). Values are expressed as mean _ SD (n ¼ 3) indicate triplicate measurement. Fig. 3. Ferric reducing antioxidant power of ethanolic extract of *T. aestivum* and Butylated hydroxytoluene. Values are expressed as mean standard deviation (n ¼ 3) indicate triplicate measurement. Fig. 5. Hydroxyl radical scavenging activity of ethanolic extract of *T. aestivum* and mannitol. Values are expressed as mean standard deviation (n ¼ 3) indicate triplicate measurement [35, 36]. Fig. 6. Present study, the ethanolic extract of *T. aestivum* was checked for its inhibitory effect on nitric oxide production. Nitric oxide radical generation from sodium nitroprusside at physiological pH was inhibited by *T. aestivum*.

FRAP

In FRAP, ferric-ferric cyanide complex is reduced to the ferrous form depending on the presence of antioxidants. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity; higher absorbance indicates a higher ferric reducing power [37]. We showed that *T. aestivum* had comparable ferric reducing power to that of synthetic antioxidants.

ABTS

The ABTS radical cation decolorization assay can measure the relative antioxidant ability to scavenge the radical ABTS as compared with BHT, and is an excellent tool for determining the antioxidant capacity of hydrogen-donating antioxidants. The blue and green ABTS radical cation was generated prior to adding antioxidant containing samples prevents interference, which stable absorbance was achieved, by adding the ethanolic extract of *T. aestivum* and the scavenging ability measured in terms of discolorization at 734 nm [39].

Hydroxyl radical scavenging

Hydroxyl radicals are highly potent oxidants, which can react with biomolecules in living cells and cause severe damage. In the present study, administration of leaf extract to the reaction mixture significantly inhibited the hydroxyl radical activity, with a maximum inhibition of 58.06% and 56.12% being observed with the standard and *T. aestivum*, respectively [40].

Conclusion

As our knowledge of the mechanisms of human diseases has increased, particularly metabolic diseases such as diabetes, liver disease and hypertension, the role played by highly reactive oxygen species such as free radicals has become increasingly relevant. Research on medicinal plants for natural antioxidants is also increasing. The present study indicates that the leaves of *T. aestivum* possess antioxidant properties and could serve as free radical inhibitors or scavengers, or act as primary antioxidants. With this kind of investigation it would be easier to treat and prevent the human damages occurring due to the free radical. Therefore, further research is needed for the isolation and identification of the active components in the extracts.

Acknowledgement

The author gratefully thank to the Department of Biochemistry, school of biosciences, Periyar university, Salem-11 for providing infrastructure facility to carry out the research.

References

1. Aparna A Bhairappa, Vrushali V Dhoble, Nikita V Potabatti. A comparative study on the antioxidant activity of selected medicinal plant extracts. European Journal of Biotechnology and Bioscience. 2016; 4(1):11-13.
2. Kaewpiboon C, Lirdprapamongko l K, Srisomsap C, Winayanuwattikun P, Yongvanich T, Puwaprisirisan P *et al*. Studies of the *in vitro* cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected Thai medicinal plants. BMC Compl Altern Med. 2012; 12: 217.
3. Subhashini N, Thangathirupathi A, Lavanya N. Antioxidant activity of *trigonella foenum-graecum* using various *in vitro* and *Ex vivo* models. Internat J Pharm Sci. 2011; 96-102.
4. Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. Am J Cardiol. 2003; 91:12-16.
5. Celiktar OY, Girgin G, Orhan H, Nichers HG, Bedir E, Sukan FV. Screening of free radicals scavengingcapacity and antioxidant activities of *Rosmarinus Officinale* extracts with focus on location and harvesting times. Eur Food Res Tech. 2007; 24:443-451.
6. Kinnula VL, Crapo JD. Superoxide dismutases in malignant cells and human tumors. Free Radic. Biol. Med. 2004; 36:718-744.
7. Singh U, Jialal I. Oxidative stress and atherosclerosis. Pathophysiology. 2006; 13:129-142.
8. Sas K, Robotka H, Toldi J, Vecsei L. Mitochondrial, metabolic disturbances, oxidative stress and kynurenine system, with focus on neurodegenerative disorders. J. Neurol. Sci. 2007; 257:221-239.
9. Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochim. Biophys. Acta. 2000; 1502:139-144.
10. Guidi I, Galimberti D, Lonati S, Novembrino C, Bamonti F, Tiriticco M, Fenoglio C, Venturelli E, Baron P, Bresolin N. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol. Aging. 2006; 27:262-269.
11. Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. Chem. Res. Toxicol. 2000; 13:135-160.
12. Arteel GE. Oxidants and antioxidants in alcohol induced liver disease. Gastroenterol. 2003; 124:778-790.
13. Ramakrishna BS, Varghese R, Jayakumar S, Mathan M, Balasubramanian KA. Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. J Gastroenterol Hepatol. 1997; 12:490-494.
14. Hyun DH, Hernandez JO, Mattson MP, de Cabo R. The plasma membrane redox system in aging. Aging Res. Rev. 2006; 5:209-220.
15. Upston JM, Kritharides L, Stocker R. The role of vitamin E in atherosclerosis. Prog. Lipid Res. 2003; 42:405-422.
16. Md. Nur Alam, Nusrat Jahan Bristi, Md. Rafiquzzaman. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal. 2013; 21:143-152
17. Tirgar1 PR, Thumber BL, Desai TR. Isolation, Characterization and Biological Evaluation of Iron Chelator from *Triticum Aestivum* (Wheat Grass). International Journal of Pharma and Bio Sciences. 2011; 2:288-296.
18. Chia-Che Tsai, Chih-Ru Lin, Hsien-Yu Tsai, Chia-Jung Chen, Wen-Tai Li, Hui-Ming Yu *et al*. The journal of biological chemistry. 2013; 288:17689-17697.
19. Singh RP, Chidambara Murthy KN, Jayaprakash GK. Studies on antiox- idant activity of pomegranate peel and seed extracts using *in vitro* models. J Agric Food Chem. 2002; 50:86-89.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice- Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999; 26:1231.
21. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry. 1989; 28:1057-1060.
22. Chou HJ, Kuo JT, En-Shyhlin. Comparative Antioxidant Properties of Water Extracts from Different Parts of Beefsteak Plant (Perilla frutescens). Journal of Food and Drug Analysis. 2009; 17(6):489-496
23. Gulcin I, Tel AZ, Kirecci E. Antioxidant, antimicrobial, antifungal, and antiradical activities of Cyclotrichium niveum (Boiss.) Manden and Scheng. Int J Food Prop. 2009; 11:450-471.
24. Garret DC. The quantitative analysis of drugs, champman and Hall, Japan. 1964; 3:456-458.
25. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996; 239:70-6.
26. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2003; 26:211-219.
27. Sanchez-Moreno C. Review: method used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci Technol Int. 2002; 8:121-137.
28. Hemmami T, Parihar MS. Reactive oxygen damage. Ind J Physiol Pharmacol. 1998; 42:440-452.
29. Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000; 63:1035-1042.
30. Wickens AP. Aging and the free radical theory. Respir Physiol. 2001; 128:379-391.

31. Meir S, Kaneer J, Akiri B. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves. J Agric Food Chem. 1995; 43:1813-1815.
32. Matsushige K, Basnel P, Kadola S, Namba T. Potent free radicals scavenging of dicaffeoylquinic acid derivatives from propolis. J Trad Med. 1996; 13:217-228.
33. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95:351-358.
34. Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J. 1992; 6:3051-3064.
35. Shen SC, Lee WR, Lin HY, Huang HC, Ch Ko, Yang LL. *In vitro* and *in vivo* inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin E (2) production. Eur J Pharmacol. 2002; 446:187-194.
36. Amarowicz R, Pegg RB, Rahimi-Maghadddam P, Barl B. Free radical scavenging capacity and antioxidant selected plant species from the Canadian prairies. Food Chem. 2004; 84:551-562.
37. Yim HS, Chye FY, Tan CT, Ng YC, Ho CW. Antioxidant activities and total phenolic content of aqueous extract of Pleurotus ostreatus (cultivated oyster mushroom). Malaysian J Nutr. 2010; 16:281-291.
38. Mathew S, Abraham TE. *In vitro* antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food Chem Toxicol. 2004; 44:198-206.
39. Gulcin I. Antioxidant and antiradical activities of L-carnitine. Life Sci. 2006; 78:803-811.