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Full Length Research Paper

Antioxidant potential and content of phenolic compounds in ethanolic extracts of selected parts of *Andrographis paniculata*

Arash Rafat[1], Koshy Philip[1]* and Sekaran Muniandy[2]

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

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The present study investigated the antioxidant capacity and total content of phenolic compounds in an ethanolic extract of leaves, stems and fruits of *Andrographis paniculata*, a medicinal plant. The antioxidant activity of the samples was evaluated by the red cell hemolysis assay, free radical scavenging and superoxide dismutase activities. The Folin–Ciocalteu reagent assay was used to estimate the phenolic content of extracts. Leaf extracts showed the highest antioxidant potential followed by stem and fruit extracts with the rabbit erythrocytes hemolysis and superoxide dismutase activity assays. However, *A. paniculata* fruit extracts exhibited the highest DPPH free radical scavenging activity compared to the other extracts. A positive correlation between free radical scavenging capacity and the content of phenolic compounds was found in the fruit, leaf and stem extracts of this plant.

Key words: *Andrographis paniculata*, erythrocytes hemolysis, superoxide dismutase, radical scavenging, phenolic content.

INTRODUCTION

Free radicals generated in the human body may increase the risk of chronic diseases such as cancer and cardio-vascular diseases. These free radicals are usually produced through aerobic respiration. Although the human body produces antioxidant enzymes to neutralize free radicals (Rimbach et al., 2005), a diet rich in edible antioxidants is recommended to assist the human body to protect itself from food borne free radicals.

A variety of plant secondary metabolites have been reported to act as antioxidants and amongst them phenolic compounds form a major group. There are several reports on the contribution of phenolic compounds to the antioxidant potential of different plant species. Cai et al. (2004), for example, reported a positive linear correlation between the total content of phenolic compounds and the antioxidant activities for aqueous and methanolic extracts of Chinese medicinal plants. Similarly, a positive correlation was reported for aqueous and methanolic extracts of different Jordanian plant species (Tawaha et al., 2007).

In view of dissimilar compounds or different amounts of same compounds being present in different parts of the plant body, the antioxidant potential of these parts of the plant could also be different. For example, different levels of antioxidant activities for Siamese neem tree leaves, fruits, flowers and stems were reported by Sithisarn et al. (2005).

The extremely bitter and characteristic taste of *Andrographis paniculata*, of the Acanthaceae family, gives it the term “King of bitters”. *A. paniculata* is a well known medicinal plant in South and Southeast Asia and is traditionally used for treating a variety of ailments. Several recent studies have validated some of the medicinal properties of this plant and its use in traditional medicine. Such properties include its antimicrobial activity (Singha et al., 2003), hepatoprotective capacity (Trivedi and Rawal, 2001), antimalarial activity (Rahman et al., 1999) and antidiarrhoeal potential (Gupta et al., 1993). *A. paniculata* has also been reported to have antioxidant potential by many authors. More recently, Sheeja et al.

*Corresponding author. E-mail: kphil@um.edu.my. Tel: +60123951839

(2006) reported on the antioxidant activity of *A. paniculata* using both in vitro and in vivo systems of whole plant extracts. The effects of the aqueous extract of *A. paniculata* on the antioxidant defence system in liver-*lymphoma* bearing AKP mice has been reported by Verma and Vinayak (2008). Meanwhile, Neogy et al. (2008) reported that Andrographis significantly reduced lipid peroxidation and protein oxidation in liver, kidney, heart, lung and spleen during nicotine induced oxidative stress.

All of the studies on the antioxidant potential of *A. paniculata* have relied on extracts of the whole plant. For the reasons elaborated above, we were keen to study the parts of the plant separately for their antioxidant activity and to correlate this with their content of phenolic compounds. Hence, in the present study, the antioxidant activities of ethanolic extracts of three different parts of *A. paniculata* (leaf, stem and fruit) were evaluated using three *in vitro* methods. Total phenolic content of the ethanolic extracts of specific plant parts was also measured to study the total content of phenolic compounds.

MATERIALS AND METHODS

Chemicals

Hydrogen peroxide (H_2O_2) was obtained from UNI-CHEM® (South Kearny, N.J.). 2,2–diphenyl-1-picrylhydrazil (DPPH*), Tert-butylated hydroxytoluene (BHT) and ascorbic acid (vitamin C) were purchased from Sigma-Aldrich (St. Louis, Mo) while 95% ethanol was obtained from Systerm ChemAR®. Tween 80 (Polyoxyethylene sorbitan mono-oleate) was purchased from Hopkin and Williams (London).

Sample preparation and extraction

Leaves, stems and fruits of one of the local variety of *A. paniculata* were collected. The explants were cleaned with tap water and air-dried at room temperature in the dark separately. Dried parts of the plant (3 g) were ground to produce fine homogenous powders using an electrical blender (Super Blender National®, Japan). The fine plant powder was soaked in 40 ml of 95% ethanol at room temperature for 72 h in the dark. The solution was then filtered through Whatman® 5 filter paper (Whatman International, Maidstone) and evaporated to dryness using a rotary evaporator (Heidolph WB2000, Germany) at a temperature below 40ºC. A stock solution (100 mg/ml) of the plant extracts was prepared in 5% Tween 80 dissolved in isotonic phosphate buffer (IPB), pH 7.4 and kept at 4ºC until required for experiments. The working solution (10 mg/ml) for assays was made by diluting the stock solution with IPB.

Antioxidant activity evaluation methods

i. Rabbit erythrocytes haemolysis assay

Preparation of erythrocyte suspension: Blood sample was withdrawn from the marginal vein of healthy normal New Zealand white rabbits using a 26Gx1/2" needle (TERUMO®, Belgium) and aspirated into Silicone Coated Blood Collection Tube (Vacutainer®, BECTON DICKINSON, USA). The cells were collected by centrifugation of rabbit blood at 1000 Xg at 4°C for 20 min. The buffy coat and plasma were removed using a pipette. The final erythrocyte suspension was produced by adding an equal volume of IPB (pH 7.4) after washing the cells thrice with IPB.

Hemolysis assay: Pretreatment stage was carried out by adding 1 ml of *A. paniculata* ethanolic extract working solution (concentration of 10 mg/ml) to 500 µl of erythrocyte suspension and incubating at 37°C for 40 min. A positive control for this experiment was prepared by pretreating the erythrocyte suspension with 1 ml of 10 mg/ml concentration of vitamin C dissolved in IPB. The non-pretreated erythrocyte suspension was used as the negative control. The volume of all pretreated and non-pretreated erythrocyte suspensions were adjusted to 9 ml by adding IPB. Oxidative stress was then induced by adding 1 ml of 10 mM hydrogen peroxide (H_2O_2) and incubated at 37°C for 150 min. After incubation, the released hemoglobins into the supernatant of the mixtures were measured using Spectronic $20D^+$ spectrophotometer (USA) at 540 nm. Erythrocyte hemolysis in pure water was based on complete erythrocytes hemolysis (100%) while hemolysis of the pretreated and non-pretreated erythrocytes was expressed as a percentage of this value (method adapted from Reddy et al., 2007).

ii. Free radical scavenging activity

This assay was based on the method described by Bozin et al. (2006) with some modifications. Briefly, 950 µl of 90 µM 2,2–diphenyl-1-picrylhydrazil (DPPH*) solution was added to 50 µl of the working samples (10 mg / ml concentration) and made up to a final volume of 4 ml with 95% ethanol. After the mixtures were vigorously shaken, they were incubated at room temperature in the dark for 2 hours. The reduction of solution colour caused by scavenging of the free radicals (DPPH*) was measured at 515 nm using a spectrophotometer. The capability of samples to scavenge DPPH* was obtained by comparison of sample colour reduction effect with the control (mixture without working solution) using the following equation and expressed as percentage values.

$$\text{DPPH radical scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

The positive control was prepared using Tert-butylated hydroxytoluene (BHT) at a concentration of 10 mg/ml.

iii. Superoxide dismutase (SOD) activity assay

SOD Assay Kit-WST (Dojindo Molecular Technologies, Gaithersburg) was applied to determine the Superoxide Dismutase (SOD) activity. Reaction mixtures of the kit were mixed with 20 µl of working solution samples and after a gentle shaking they were inoculated at 37°C for 20 min. The inhibition by SOD activity on the reaction of xanthine oxide generated superoxide with a tetrazolim salt was determined by measuring the absorbance of the mixtures at 450 nm using a microplate reader. The positive control in this study was made by adding 20 µl of ascorbic acid at a concentration of 10 mg/ml instead of working solution sample. All inhibition rates were expressed as percentage of the negative control (without sample). The method used in this kit is a protocol modified from Sakudo et al. (2008).

Total phenolic content determination

A Folin-Ciocalteau method based on Slinkard and Singleton (1977) report was applied to determine the total amount of phenolic compounds in different parts of *A. paniculata.* Working solution

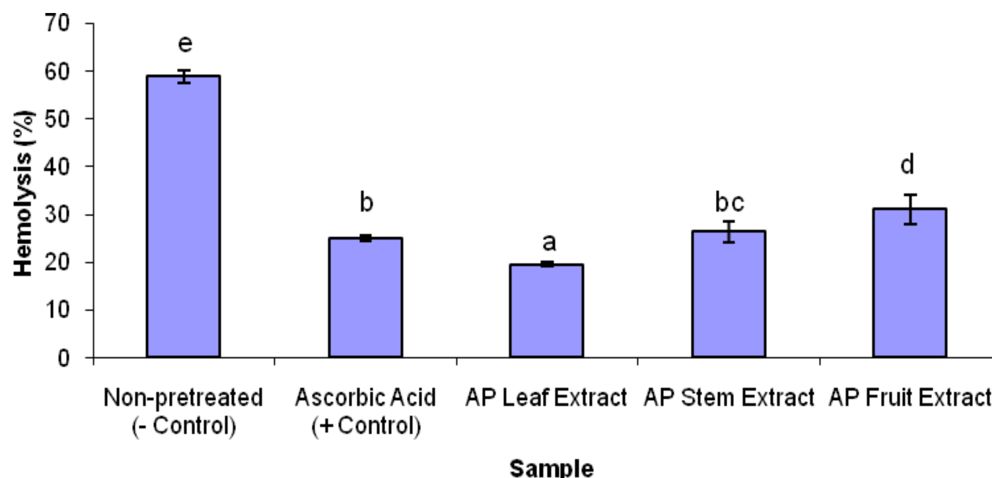

Figure 1. Antioxidant activity of selected parts (leaf, stem, and fruit) of *Andrographis paniculata*: hemolysis of rabbit erythrocytes expressed as percentage values. Ascorbic acid was used as the positive control. The mean changes between the samples were analyzed by one-way ANOVA followed by Duncan's Multiple Comparison Test. Samples represented with different letters (a, b, d and e) are significantly different ($p < 0.05$) from each other including + control (b). bc represents statistically insignificant difference between bc and b.

samples (20 µl) of each plant part was added to 100 µl of 2N Folin-Ciocalteu reagent as recommended by the above protocol. The mixture was made up to a final volume of 1600 µl using distilled water. Lastly 300 µl of sodium carbonate solution (0.2 mg/ml) was added and incubated at 37°C for 45 min. Absorbance values of the solutions were measured at 760 nm. Total phenolic contents were determined as a gallic acid equivalent (GAE) based on Folin-Ciocalteau calibration curve using gallic acid (ranging from 50 to 1000 mg/ml) as the standard and expressed as mg gallic acid per gram of dry sample.

Statistical analysis

All experiments were carried out in triplicate and data were subjected to one-way analysis of variance (ANOVA) using SPPS version 15. Duncan's Multiple Range Test (DMRT) was used to compare the means.

RESULTS

Hemolysis assay

The maximum erythrocyte hemolysis (100%) was achieved using ultra pure water and other hemolytic values from free radical damage were presented as percentage based on the former. The results of the rabbit erythrocytes hemolysis assay are shown in Figure 1. A mean of 58.9% hemolysis of erythrocytes was obtained with 1 mM hydrogen peroxide (negative control). Percentage of hemolysis in samples pre-treated with leaf extract (19.45%), stem extract (26.36%), and fruit extract (30.97%) were significantly lower than the negative control. Among the samples used, the *A. paniculata* leaf extract significantly ($p < 0.05$) reduced the percentage of hemolysis when compared to the positive control (24.92%). However, there was no significant difference between haemolytic reduction using *A. paniculata* stem extract and ascorbic acid (positive control) while the fruit extract showed significant difference with the positive control in terms of hemolysis reduction. Hence, in terms of haemolysis, the best result in minimizing the haemolytic effect of hydrogen peroxide was obtained with leaf extract.

DPPH free radical scavenging activity

Decolouration due to reaction of antioxidants in samples with the stable free DPPH radical was measured spectrophotometerically. Results (Figure 2) show the free radical scavenging potential of *A. paniculata* fruit extract (88.13%) is significantly higher than both *A. paniculata* leaf extract (86.87%) and *A. paniculata* stem extract (80.48%). All samples had significantly lower DPPH free radical scavenging activity compared to the positive control (94.86%).

SOD activity

The result of this enzyme based antioxidant capacity assay (Figure 3) showed *A. paniculata* leaf extract had the highest inhibition rate (80.41%) among the three different plant part extracts that was significantly higher than the *A. paniculata* stem extract SOD activity (77.53%) and *A. paniculata* fruit extract SOD capacity (73.20%). None of the examined parts of *A. paniculata* extracts used in this study could achieve a higher inhibition rate

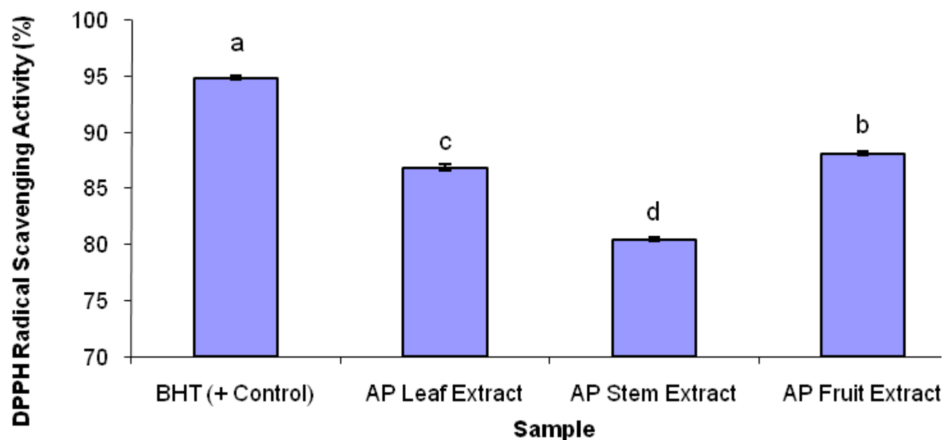

Figure 2. Antioxidant activity of selected parts (leaf, stem, and fruit) of *Andrographis paniculata*: neutralization of DPPH radical of samples in the free radical scavenging activity assay. Tert-butylated hydroxytoluene was used as the positive control. The mean changes between the samples were analyzed by one-way ANOVA followed by Duncan's Multiple Comparison Test. Samples represented with different letters (b, c and d) are significantly different ($p < 0.05$) from each other including the + control (a).

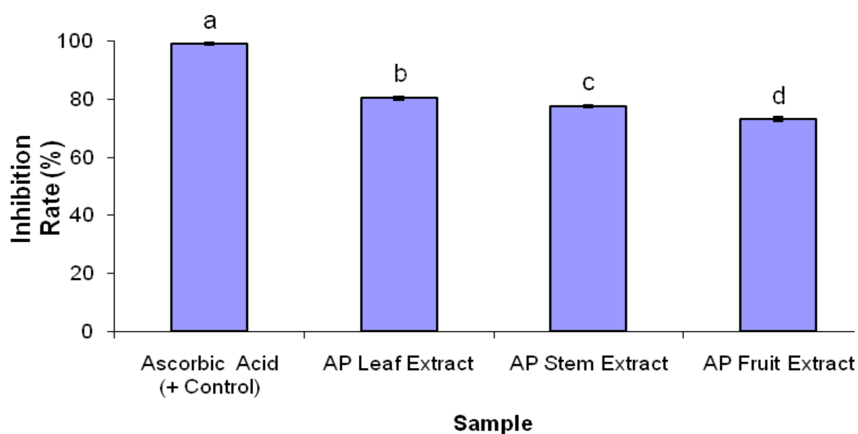

Figure 3. Antioxidant activity of selected parts (leaf, stem, and fruit) of *Andrographis paniculata*: SOD activities of samples are presented as inhibition rate. Ascorbic acid was used as the positive control. The mean changes between the samples were analyzed by one-way ANOVA followed by Duncan's Multiple Comparison Test. Samples represented with different letters (b, c and d) are significantly different ($p < 0.05$) from each other including + control (a).

than the positive control (99.20%).

Total phenolic content

The calculation of total phenolic content of *A. paniculata* extracts was carried out using the standard curve of gallic acid and presented as gallic acid equivalents (GAE) per gram (Table 1). *A. paniculata* fruit extract contained the highest amount of phenolic compounds while the lowest amount is present in *A. paniculata* stem extract. All three examined parts of the *A. paniculata* plant had significantly different contents of phenolics.

The sample means were compared by one-way ANOVA followed by Duncan's Multiple Comparison Test. Samples represented with different letters are significantly different ($p < 0.05$) between the different plant parts.

DISCUSSION

In this study antioxidant activities of three important plant parts (leaf, stem and fruit) of *A. paniculata* were evaluated.

Table 1. Total phenolic contents of *Andrographis paniculata* leaf, stem and fruit extracts.

Andrographis paniculata **morphological part**	**Total phenolic contents (mg of GAE/g)**
Leaf	75.86 ± 0.82 [b]
Stem	55.02 ± 0.35 [c]
Fruit	181.00 ± 1.48 [a]

Due to the important physiological functions of phenolic compounds especially polyphenols, the total phenolic content of ethanolic extracts of these parts was measured. The results of both rabbit erythrocytes hemolysis and SOD activity assays showed similar trends with respect to antioxidant activities with leaf extracts having the highest activity followed by stem and fruit extracts. However, the antioxidant capacity of the extracts determined by DPPH free radical scavenging assay showed that fruit extract antioxidant potential was higher than leaf extract antioxidant potential followed by stem extract antioxidant potential. Although the result of radical scavenging assay of fruit extract was not in agreement with both hemolysis and SOD activity assays, all three methods showed *A. paniculata* leaf extract had a higher antioxidant potential than *A. paniculata* stem extract. Based on these results, it is also suggested that different methods must be applied to determine the antioxidant activity of samples as a single method alone may not be reliable.

Kruawan and Kangsadalampai (2006) reported the DPPH scavenging effect (58.02 %) and total phenolic contents (38.65 ± 4.26 mg of GAE/g) of the aqueous extract of *A. paniculata* leaves. Compared to our results above, it could be stated that the ethanolic extract of *A. paniculata* leaves had a higher stable DPPH free radical scavenging activity (86.87%) and higher content of total phenolic compounds (75.86 ± 0.82 mg of GAE/g) than aqueous extract. This difference between *A. paniculata* antioxidant activity of ethanolic extract and aqueous extract might be attributed to the higher temperature used in the preparation of the latter causing damage to some compounds with antioxidant activity and/or different solubility of elements in water and ethanol. Different parts of the plant produce different compounds or different amounts of a compound due to their differential gene expression. This particularly affects the antioxidant potential of the different parts of a given plant. Based on our review of the current literature, the present study is the first to compare the antioxidant capacity of different parts of the *A. paniculata* plant. In our study, three different methods were used to evaluate the antioxidant activity of three different parts of *A. paniculata* namely the leaves, stems and fruits. Surveswaran et al. (2007) as well as Sheeja et al. (2006) evaluated the antioxidant potential of the whole *A. paniculata* plant. The current results in our paper contribute to a better understanding of the antioxidant values of each tested morphological part of *A. paniculata.*

Several studies showed there is a difference between antioxidant capacity of different varieties and cultivars of the same plant. For example, Kedage et al. (2007) showed the different antioxidant properties of eleven grape (*Vitis vinifera*) varieties while Henríquez et al. (2009) reported the different antioxidant activities of five Chile apple cultivars. Most of the *A. paniculata* antioxidant activity studies used the Indian varieties of the plant (for example, Surveswaran et al., 2007 and Sheeja et al., 2006). To our knowledge this the first study of antioxidant activity in an indigenous Malaysian variety of *A. paniculata.* Moreover Surveswaran et al. (2007) used methanolic extracts of the Indian varieties while our study employed ethanolic extraction.

Only a combination of different methods of antioxidant activity evaluation can give more reliable results. Although a wide range of antioxidant potential evaluation methods have been used in different *A. paniculata* antioxidant capacity studies, our study is the first in which the *in vitro* erythrocytes hemolysis model is utilised to determine the antioxidant capacity of *A. paniculata.*

Several studies (Cai et al., 2004; Beta et al., 2005; Tawaha et al., 2007; Othman et al., 2007) found a good correlation between total content of phenolic compounds and the antioxidant activity in different plants. Other studies however contradicted the above statement. For example, Nsimba et al. (2008) showed that the antioxidant activity of *Chenopodium quinoa* and *Amaranthus spp.* seeds, determined using three different assays (carotene bleaching, FRAP and DPPH), poorly correlated with total content of phenolic compunds. There were also no significant relationships between the antioxidant activities (determined using three different methods namely FRAP, DPPH and carotene bleaching assays) and total contents of phenolic compounds for fifteen genotypes of selected Turkey Zizyphus jujube Mill. fruits (Kamiloğlu et al., 2009). Based on the results obtained in the present study, there is a positive linear correlation between total contents of phenolics and DPPH free radical scavenging activities of all three examined parts of *A. paniculata.* This correlation confirms that phenolic compounds play an important role as free radical scavengers. On the other hand, although the highest amount of total phenolics was obtained from *A. paniculata* fruit extract, the lowest inhibition rate in SOD activity assay and the lowest hemolysis protection potential in rabbit erythrocytes hemolysis assay were also obtained from *A. paniculata* fruit extract. It might show that *A. paniculata* leaves and stems may contain other

non-phenolic compounds which have a higher inhibition activity and protection against erythrocytes hemolysis. There are not many studies on antioxidant activities of components extracted from *A. paniculata* like neoandrographolide (Liu et al., 2007), andrographolide (Jada et al., 2007) and andrograpanin (Ji et al., 2005) that are reported to have medicinal usages.

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REFERENCES

Beta T, Nam S, Dexter JE, Sapirstein HD (2005). Phenolic Content and Antioxidant Activity of Pearled Wheat and Roller-Milled Fractions. Cereal Chem. 82(4): 390-393.

Bozin B, Mimica-Dukic N, Simin N, Anackov G (2006). Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. J. Agric. Food. Chem. 54: 1822–1828.

Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74: 2157-2184.

Gupta S, Yadava JNS, Tandon JS (1993). Antisecretory (antidiarrhoeal) activity of Indian medicinal plants against Escherichia coli enterotoxin-induced secretion in rabbit and guinea pig ileal loop models. Pharmaceut. Biol. 31(3): 198-204.

Henríquez C, Almonacid S, Escobar B, Chiffelle I, Gómez M, Speisky H (2009). Antioxidant content and activity in different structures of five apple cultivars grown in Chile. Acta Hort. 841: 275-280.

Jada SR, Subur GS, Matthews C, Hamzah AS, Lajis NH, Saad MS (2007). Semisynthesis and *in vitro* anticancer activities of andrographolide analogues. Phytochemistry 68(6): 904-12.

Ji LL, Wang Z, Dong F, Zhang WB, Wang ZT (2005). Andrograpanin, a compound isolated from anti-inflammatory traditional Chinese medicine *Andrographis paniculata*, enhances chemokine SDF-1alpha-induced leukocytes chemotaxis. J. Cell Biochem. 95(5): 970-978.

Kamiloğlu Ö, Ercisli S, Sengül M, Toplu C, Serçe S (2009). Total phenolics and antioxidant activity of jujube (Zizyphus jujube Mill.) genotypes selected from Turkey. Afr. J. Biotechnol. 8(2): 303-307.

Kedage W, Tilak JC, Dixit GB, Devasagayam TP, Mhatre M (2007). A study of antioxidant properties of some varieties of grapes (*Vitis vinifera* L.). Crit. Rev. Food Sci. Nutr. 47(2): 175-185.

Kruawan K, Kangsadalampai K (2006). Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extracts of herbs. Thai J. Pharmaceut. Sci. 30: 28-35.

Liu J, Wang ZT, Ji LL (2007). I*n vivo* and In *vitro* Anti-inflammatory Activities of Neoandrographolide. Am. J. Chin. Med. 35(2): 317-328.

Neogy S, Das S, Mahapatra SK, Mandal N, Roy S (2008). Amelioratory effect of *Andrographis paniculata* Nees on liver, kidney, heart, lung and spleen during nicotine induced oxidative stress. Environ. Toxicol. Pharmacol. 25(3): 321-328.

Nsimba R, Kikuzaki H, Konishi Y (2008). Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds. Food Chem. 106: 760-766.

Othman A, Ismail A, Ghani NA, Adenan I (2007). Antioxidant capacity and phenolic content of cocoa beans. Food Chem. 100: 1523-1530.

Rahman NNNA, Furuta T, Kojima S, Tabane K, Ali-Mohd M (1999). In vitro and in vivo study revealed that malarias medicinal plants, *Piper sarmentosum*, *Andrographis paniculata* and *Tinospora* crispa produce considerable antimalarial effect. J. Ethnopharmacol. 64: 249–254.

Reddy CSSS, Subramanyam MVV, Vani R, Devi SA (2007). In vitro models of oxidative stress in rat erythrocytes: Effect of antioxidant supplements. Toxicol *In vitro* 21(8): 1355-1364.

Rimbach G, Fuchs J, Packer L (2005). Application of nutrigenomics tools to analyze the role of oxidants and antioxidants in gene expression. In: Rimbach G, Fuchs J, Packer L (eds.), Nutrigenomics, Taylor and Francis Boca Raton Publishers, FL, USA, pp. 1-12.

Sakudo A, Nakamura I, Tsuji S, Ikuata K (2008). GPI-anchorless human prion protein is secreted and glycosylated but lacks superoxide dismutase activity. Int. J. Mol. Med. 21: 217-222.

Sheeja K, Shihab PK, Kuttan G (2006). Antioxidant and anti-inflammatory activities of the plant *Andrographis paniculata* Nees. Immunopharm. and Immunotoxicol. 28: 129–140.

Singha PK, Roy S, Dey S (2003). Antimicrobial activity of *Andrographis paniculatA.* Fitoterapia 74: 692-694.

Sithisarn P, Supabphol R, Gritsanapan W (2005). Antioxidant activity of Siamese neem tree (VP1209). J Ethnopharmacol. 99: 109-112.

Slinkard K, Singleton VL (1977). Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Viticul. 28: 49-55.

Surveswaran S, Cai YZ, Corke H, Sun M (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem. 102: 938 – 953.

Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem. 104: 1372-1378.

Trivedi NP, Rawal UM (2001). Hepatoprotective and antioxidant property of *Andrographis paniculata* (Nees) in BHC induced liver damage in mice. Indian J. Exp. Biol. 39(1): 41-6.

Verma N, Vinayak M (2008). Antioxidant action of *Andrographis paniculata* on lymphom*A.* Mol. Biol. Rep. 35: 535-540.