Research in Pharmacy 3(4): 01-08, 2013 ISSN : 2231-539X
www.researchinpharmacy.com

Regular Article

Exploration of Antioxidant Properties in Various Extracts of *Bryophyllum pinnatum* (Lank.)

Sindhu S and S Manorama

PG and Research Dept. of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-29, Tamil Nadu, India
Corresponding author email: ssindhu389@gmail.com

A lot of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) named Rasayana proposed for their interesting antioxidant activities. The purpose of this study was to evaluate antioxidant activities of various solvent extract of *Bryophyllum pinnatum* (Lank.). Total phenols and flavonoids were analyzed according to the Folin–Ciocalteu method and total antioxidant activity of various solvent extracts of *Bryophyllum pinnatum* (Lank.) leaves was assessed by metal chelating assay, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as well as 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Ethanol extract exhibited excellent antioxidant activity, as measured using 1,1-diphenyl-2-picryhydrazyl (DPPH) and total antioxidant assays.

Keywords: *Bryophyllum pinnatum*, Total phenol content, Total flavonoid content, ABTS, DPPH, Metal chelating assay.

Medicinal plants have been used to treat human diseases in the East for centuries. People are becoming increasingly interested in medicinal plants because of their good therapeutic performance and low toxicity. In recent years, studies on antioxidant activity of Chinese medicinal plants have increased remarkably due to increased interest in their potential of being used as a rich and natural source of antioxidant compounds (Liu, 2000; Ou, 2003). Free radicals have been claimed to play a key role, affecting human health by causing severe diseases, such as cancer and cardiovascular diseases by cell degeneration. These free radicals can be generated during normal body function, and can be acquired from the environment.

The oxidative damage might be prevented or limited by dietary antioxidants (Dasgupta, 2006). Phytochemicals, such as phenolic compounds, are considered beneficial for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (Larson, 1988; Pereira *et al.*, 2007; Pulido *et al.*, 2000; Velioglu *et al.*, 1998). These compounds have been reported to be well correlated with antioxidant potential (Katalinic *et al.*, 1998). The current focus is toward natural antioxidants, especially plant polyphenolics. It is of interest to investigate the antioxidant properties of herbal infusions especially those traditionally used in folk medicine.

Received: 4.6.13; Revised: 17.7.2013; Accepted: 19.7.2013

Bryophyllum pinnatum (Lank.) Oken, syn. *B.calucinum* or *Kalanchoe pinnata* (Crassulaceae) is a perennial succulent herb. Extracts of *Bryophyllum pinnatum*, have been used by modern physicians mainly as a psychiatric sedative. Identified active ingredients include bufadienolides, flavonoids, glycosides, steroids and organic acids (Marriage, 1971; Gaind, 1972; Gaind, 1974; Do'ben, 1987; Costa *et al*., 1995). In traditional medicine, the leaves of this plant have been reported to possess antimicrobial (Metha Bhat, 1952; Akinpelu, 2000; Oliver-Bever, 1983), antifungal (Misra, 1979), anti ulcer (Pal, 1991), anti-inflammatory & analgesic (Pal, 1989; Pal, 1992) and antihypertensive (Ojewole, 2002) activities. It is astringent, sour in taste, sweet in the post digestive effect and has hot potency. It is well known for its haemostatic and wound healing properties. The plant has considerable attention for their medicinal properties and finds application in folk medicine, as well as in the contemporary medicine. The aim of the present study was to examine the total phenolic content, total flavonoid content and antioxidant properties by various models.

Materials and Methods

Plant material

Bryophyllum pinnatum (Lank.) leaves were collected from Perundurai, Erode district. They were authenticated by Botanical Survey of India, Southern Circle, Coimbatore.

Preparation of the extract

Plant materials (leaves) were washed with distilled water and shade dried. The dried samples were manually ground to a fine powder. The coarsely powdered parts were exhaustively extracted with hexane, Chloroform, Ethyl acetate, Acetone and ethanol for 48 h using Shaker. The filtrate was then evaporated to dryness under reduced pressure using rotary vacuum evaporator. The extracts were lyophilized until further use.

Chemicals

Sodium carbonate, Folin-Ciocalteu reagent, Potassium acetate, Aluminium chloride, Gallic acid, Rutin, Dimethyl Sulphoxide (DMSO), DPPH (1,1-diphenyl-2-picryl hydrazine), $ABTS^{\bullet+}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), Ascorbic acid, Potassium persulfate, Phosphate buffer, Potassium Ferricyanide, Trichloroacetic acid (TCA), Ferric chloride ($FeCl_3$), Ferrous sulphate (FeSO4), ferrozine, BHT (Butylated hydroxytoluene), EDTA (Ethylene diamine tetra acetic acid), Trolex and all other chemicals were of analytical grade.

Total Phenolic Content

The total phenolic content of the extract was determined using the method of McDonald *et al.* (2001) with slight modifications. Absorbance values were measured at 765 nm and the standard curve was drawn after an incubation of 40 minutes in dark to determine the total phenolic content. All determinations were carried out in triplicate. The total phenolic content in the extract were presented as mg Gallic Acid Equivalents (GAE)/ g extract.

Determination of Total Flavonoid Content

Total Flavonoids of extracts were estimated as mg Rutin Equivalents (RE)/g extract, from the Rutin calibration curve. The reaction mixture was prepared by mixing 0.5 ml of extract solutions with 1.5ml of 95% ethanol followed by 0.1 ml (10 g/l) Aluminium chloride and 0.1 ml (98.5 g/l) of Potassium acetate. Each reaction flask was then immediately diluted with 2.8 ml of

distilled water and mixed. The absorbance of reaction mixture was read at 415 nm (Miliauskas *et al.*, 2004).

DPPH scavenging activity

DPPH (1, 1-diphenyl-2-picryl hydrazine) free radical-scavenging capabilities of methanolic extracts were evaluated by the method of Blois (1958). Briefly, different concentrations (50, 100,150,200 and 250 mg/ml) of the extracts were pipetted out to the test tubes. 100µL of 0.2 mM alcoholic DPPH solution was added to the samples. These samples were vortexed, and incubated in dark at room temperature for 30 min. The absorbance was measured at 517 nm against blank samples. Decreased absorbance of the sample indicates DPPH free radical scavenging capability (Gulcin, 2004a; Gulcin, 2004b).

ABTS radical scavenging assay

2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity assay was carried out using procedures described by Re *et al.* (1999). ABTS radical cations are produced by reacting ABTS 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (7 mM) and potassium persulfate (2.45 mM) and incubating the mixture at room temperature in the dark for 16 hour. The solution thus obtained was further diluted with 89% ethanol to give an absorbance of 0.700 at 734 nm. 20-100µL of the test sample were added to 2 ml of ABTS and the absorbance was recorded at 734 nm after 30 minutes of incubation (Auddy *et al.*, 2003). Trolox was used as reference standard. The percent inhibition was calculated from the following equation:

$$\text{Percentage of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Ferrous ion-chelating ability

The ferrous ion-chelating (FIC) assay reported by Singh and Rajini (2004) was adopted. 2 mM FeSO4 (100µl) was mixed with different concentrations of extracts (1000, 2000, 3000, 4000 and 5000 µl), followed by 5mM ferrozine (500 µl). Absorbance was measured at 562 nm after 10 min. The ability of extracts to chelate ferrous ions was calculated as follows:

$$\text{Percentage of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Results and Discussion

Total phenolic content and Total Flavanoid content

Total phenolic content of all extracts in BP leaves were found to be 1.15 ± 0.11 (Hexane), 1.78 ± 0.08 (Chloroform), 3.03 ± 0.10(Ethyl acetate), 1.88 ± 0.04 (Acetone) , 2.56 ± 0.07 (Ethanol) and 3.98 ± 0.06 (Standard Gallic acid equalents), where as Total Flavanoid content was found to be 3.83 ± 0.19 (Hexane), 6.71 ± 0.38 (Chloroform), 6.10 ± 0.65(Ethyl acetate)(Fig 1), 4.70 ± 0.05(Acetone) , 7.85 ± 0.06 (Ethanol) and 4.03 ± 0.07 (Standard Rutin equalents). Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds (Djeridane *et al.*, 2006). Flavonoids play an important role in antioxidant system in plants. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron

and copper and inhibition of enzymes responsible for free radical generation (Benavente-Garcia *et al.*, 1997).

DPPH

The DPPH test intends to measure the hydrogen atom or electron donor capacity of the extracts to the stable radical DPPH formed in solution (Tepe *et al.*, 2005). The activity was expressed as the concentration of sample necessary to give a 50% reduction in the sample absorbance (IC_{50}). Five extracts exhibited considerable DPPH free radical scavenging activity as indicated by their IC_{50} values are shown in Figure 2. IC_{50} indicates the potency of scavenging activity. Standard BHT was found to have an IC_{50} of 125.45µg/ml. In comparison to standard BHT, hexane, chloroform, ethyl acetate and ethanol extract of *BP leaves* showed IC_{50} of 110.61, 180.50, 143.26, 153.84 and 144.09 respectively. Isolation of antioxidant compounds from plant is possible through extraction with different solvents and it depends on the nature of extracting solvents (Tatiya *et al.*, 2011).

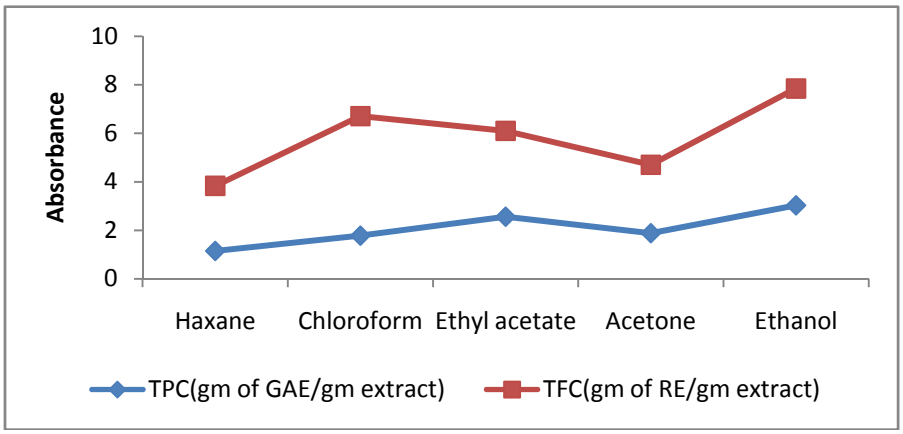

Fig.1. Total phenolic content and total flavonoid content in various extracts of BP leaf

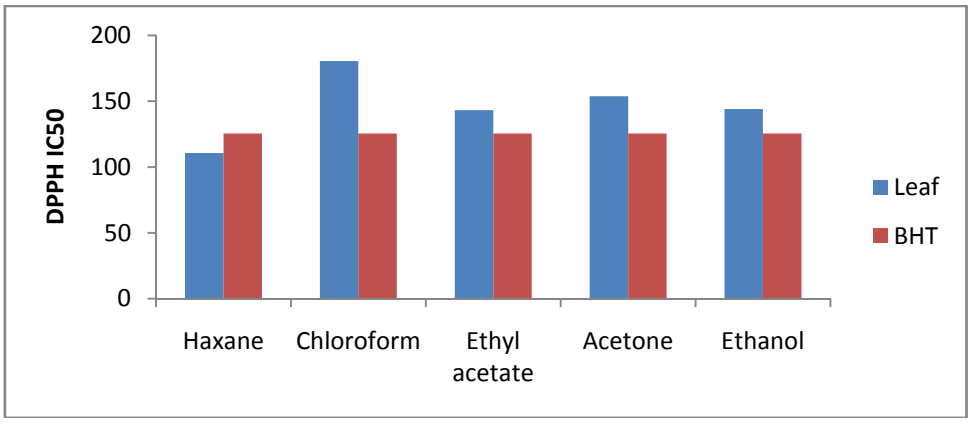

Fig.2. DPPH (IC_{50} value) of different extracts of BP

ABTS

Rice-Evans *et al.* (1996) [34] have reported that phenolic compounds may play a major role in scavenging the free radicals. In the present study, all the BP extracts could readily scavenge ABTS radical cation indicating the presence of phytochemical components such as flavonoid and phenolics, which substantiate their antioxidant action. In the present investigation, ethanol extract of leaf (5420.2µmol of TE/g DW) and Acetone leaf (4934.2µmol of TE/g DW) samples registered the highest amount of ABTS radical quenching ability than the other three extracts (Fig 3). The reaction of ABTS radical cation has been widely used to measure the antioxidant capacity of natural extracts (Cai *et a.*, 2006).

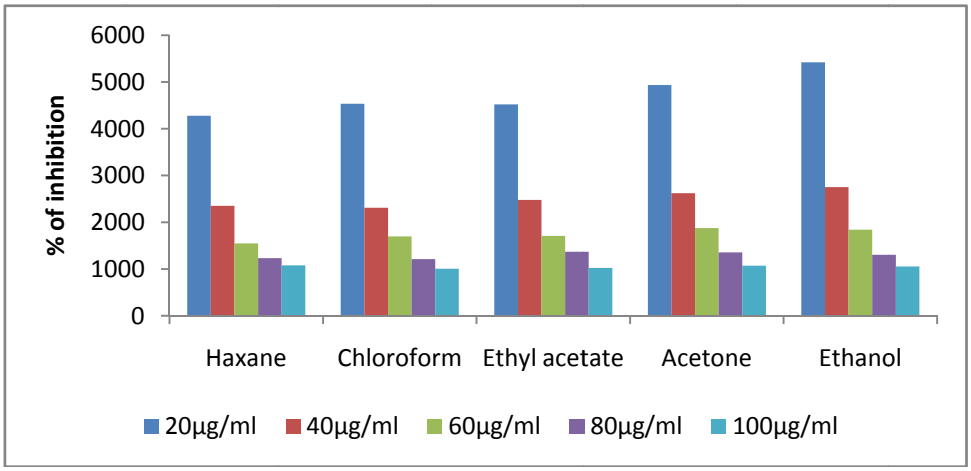

Fig.3. Antioxidant activity (ABTS) of various extract of BP

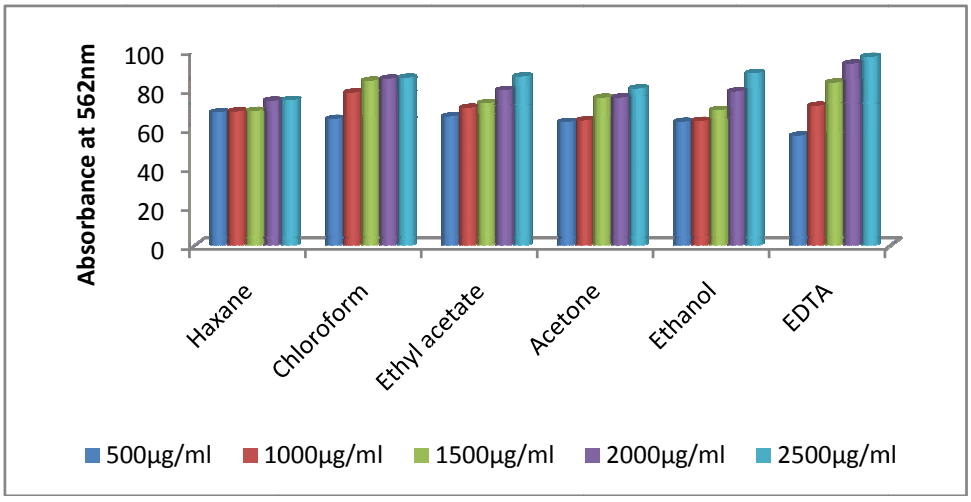

Fig.4. Free radical-scavenging activity of sample extracts in metal chelating assay

Metal chelating

The metal chelating capacity may significantly influence the course of oxidative reactions, thus metal binding compounds are included to the class of oxidation inhibitors. Iron

is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components (Smith *et al.*, 1992). Chelating agents may inhibit radical generations by stabilizing transition metals consequently reducing free radical damage. In addition, some phenolic compounds exhibit antioxidant activity through the chelating of metal ions (Zhao *et al.*, 2008). Iron binding capacity in terms of percent inhibition of the ethyl acetate extract of BP at 2500µg/ml was higher (86.67%) than the chloroform extract leaf at 2500µg/ml (85.95%) (Fig.4). However, it was comparable to that of the reference standard, EDTA.

Conclusions

The many pharmacological effects of phenolic compounds and flavonoids are linked to their ability to act as strong antioxidants and free radical scavengers, to chelate metals. Various solvent extracts from *Bryophyllum pinnatum* leaves showed varying degrees of antioxidant activity in different test systems in a dose-dependent manner. These results indicated that the plant *Bryophyllum pinnatum* has many chemical compounds able to scavenge free radicals. Therefore, the plant has promising compounds to be tested as potential antioxidant drugs for the treatment of diseases resulting from oxidative stress Therefore, it is suggested that further work could be performed on the isolation and identification of the antioxidant components in *Bryophyllum pinnatum*.

References

Akinpelu, D.A. (2000): Anti-microbial activity of *Bryophyllum pinnatum* leaves. *Fitoterapia*., 71(2):193-194.

Auddy, B., Ferreira, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F., Tripathi, P.C., Seal, T., Singh, N. and Rajini, P.S. (2004) Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, 85: 611–616.

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

Cai, Y.Z., Sun, M., Xing, J., Luo, Q., Corke, H. (2006): Structure radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sciences*, 78: 2872-2888.

Costa, S., Jossang, A., Bodo, B. (1995): Proprie´te´s biologiques et phytochimie des kalanchoe. In: Boiteau P, Allorge-Boiteau L, editors. Kalanchoe de Madagascar. Syste´matique, e´cophysiologie et phytochimie. Paris: Karthala; p. 219–35.

Dasgupta, N. and De, B. (2006): Antioxidant activity of some leafy vegetables of India: A comparative study. *Food Chemistry*, 101: 471–474.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouma, D., Stocker, P., Vidal N. (2006): Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97, 654–660.

Do'ben, H.,(1987): Phytochemische. Untersuchungen an zwei Arten der Gattung Bryophyllum.. Bonn: Rheinische Friedrich-Wilhelms University.

Gaind, K. and Gupta, R.(1972): Alkanols, triterpens and sterols of *Kalanchoe pinnata*. *Phytochemistry,* 11:1500–2.

Gaind, K., Gupta, R. (1974): Identification of waxes from the leaves of *Kalanchoe pinnata*. *Planta Medica*, 23:193–7.

Gulcin , Mshvildadze, V., Gepdiremen, A., Elias, R. (2004b): Antioxidant activity of saponins isolated from ivy: a-Hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside, *F. Planta Medica.*, 70: 561- 563.

Gulcin, I., Beydemir, Alici, H.A., Elmasta_ M., Buyukokurolu, M.E. (2004a): *In vitro* antioxidant properties of morphine. *Pharmacological Research*, 49: 59- 66.

Katalinic, V., Milos, M., Modun, D., Music, I. & Boban, M.(1998): Antioxidant effectiveness of selected wines in comparison with (+) catechin. *Food Chemistry*, 86: 593–600.

Larson, R.A. (1988): The antioxidants of higher plants. *Phytochemistry*, 27(4): 969–978.

Liu, F. and Ng, T.B. (2000): Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sciences*, **66**: 725-735.

Marriage, P.B., Wilson, D.G. (1971): Analysis of the organic acids of *Bryophyllum calycinum*. *Canadian Journal of Biochemistry*, 49: 282–95.

McDonald, S., Prenzler, P.D., Autolovich, M., Robards, K. (2001): Phenolic content and antioxidant activity of olive oil extracts. *Food Chemistry*, 73:73-84.

Mehta Bhat, J.U. (1952): Studies on Indian medical plant II; Bryophyllin, a new antibacterial substance from the leaves of *Bryophyllum*, *Calyciunum* Salsib. *J. University Bombay*. 21:21-25.

Miliauskas, G., Venskutonis, P.R., Van Beek, T.A. (2004): Screening of radical scavenging activity of some medicinal plants and aromatic plant extract. *Food Chemistry*, 85:231-237.

Misra, S. and Dixit, S.N. (1979): Anitfungal activity of leaf extract of some higher plants. *Acta Botanica Indica.* , 7:147-150.

Ojewole, J.A.O. (2002): Antihypertensive properties of *Bryophyllum pinnatum* (Clam) Oken leaf extracts. *American Journal of Hypertension*, 15(4): A34-A39.

Oliver-Bever, B. (1983): Medicinal plants in tropical west Africa. III Anti-infection therapy with higher plants. Journal of *Ethnopharmacology,* 1983; 9:1–83.

Ou, B.X., Huang, D.J. and Hampsch-Woodill, M. (2003): When east meets west: The relationship between yin-yang and antioxidation oxidation. *The journal of the Federation of Americans Societies for Experimental Biology*, 17: 127–129.

Pal, S. and Nag Chaudhuri, A.K. (1989): Preliminary studies on the anti-inflammatory and analgesic activities of *Bryophyllum pinnatum* (Lam). *Medical Science Research*, 17:561-562.

Pal, S. and Nag Chaudhuri, A.K. (1991): Studies on the antiulcer activity of a *Bryophyllum pinnatum* leaf extract in experimental animals. Journal of *Ethnopharmacology*, 33:97-102.

Pal, S. and Nag Chaudhuri, A.K. (1992): Further studies on the anti-inflammatory profile of the methanolic fraction of the fresh leaf extract of *Bryophyllum pinnatum*. *Fitoterapia*, 63:451-459.

Pereira, J.A., Oliveira, I., Sousa, A., Valenta~o, P., Andrade, B.P., Ferreira, C.F.R.I., *et al.*. (2007): Walnut (*Juglans regia* L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food and Chemical Toxicology*, 45: 2287–2295.

Pulido, R., Bravo, L. and Saura-Calixto, F. (2000): Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, 48: 3396–3402.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice- Evans, C. (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine,* 26: 1231 – 1237.

Rice-Evans, C.A., Miller, N.M. and Paganda, G. (1996): Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20, 933–956.

Smith, C., Mitchinson, M.J., Arouma, O.I., Halliwell, B. (1992): Stimulation of lipid peroxidation and hydroxyl radical generation by the contents of human atherosclerosis lesions. *Biochemistry Journal*, 15: 905-910.

Tatiya, A.U., Tapadiya, G.G., Kotecha, S. and Surana, S.J. (2011): Effect of solvents on total phenolics, antioxidant and antimicrobial properties of *Bridelia retusa* Spreng. Stem bark, *Indian Journal* of Natural *Products* and *Resources*, 2(4):442-447.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M.(2005): Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chemistry*, 90, 333–340.

Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. (1998): Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, 46: 4113–4117.

Zhao, H., Fan, W., Dong, J., Lu, J., Chen, J. and Shan, L. (2008): Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chemistry*, 107: 296.