Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

# PHYTOCHEMICAL STUDIES AND ANTIOXIDANT ACTIVITIES OF *BRASSICA OLERACEA L.VAR.CAPITATA*

**MOHAMMED FAZIL AHMED*[1], A. SRINIVASA RAO[2], SHAIK RASHEED AHEMAD[1] AND MOHAMMED IBRAHIM[1]**

[1]Nizam Institute of Pharmacy & Research Center, Deshmukhi, Pochampally (M), Nalgonda, (AP), India 508284, [2]Bhaskar Pharmacy College, Yeknapally, Moinabad (Mandal), Hyderabad 500075. *[1]Email: mohdfazil_pharma@yahoo.co.in

*Received: 17 Jan 2012, Revised and Accepted: 05 Mar 2012*

**ABSTRACT**

The aim of this study was to investigate the antioxidant activities and phytochemical analysis of *Brassica oleracea L. var. capitata***.** The phytochemical screening was carried on the leaves extracts of *Brassica oleracea L. var. capitata* revealed the presence of some active ingredients such as Alkaloids, Tannins, Saponins, Phenols, Glycosides, Steroids, Terpenoids and Flavonoids. The ethanol leaves extract were also evaluated for their total phenolic contents and antioxidant activity using DPPH radical scavenging assay and reducing power assay**.** The result of the present study showed that the ethanolic leaves extract of *Brassica oleracea L. var. capitata* which contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity than Petroleum Ether, ethylacetate, chloroform and aqueous extracts. The high scavenging property of may be due to hydroxyl groups existing in the phenolic compounds.

**Keywords:** *Brassica oleracea L. var. capitata,* DPPH, Phytochemical screening, Phenolic compounds, Reducing power assay and Antioxidant activity.

## INTRODUCTION

Free radicals are chemical species possessing one or more unpaired electrons and usually make a molecule more reactive than the corresponding non-radical. The molecule acts as an electron acceptor and essentially 'steals' electrons from other molecules. Free radicals are referred to as oxidizing agents since they cause other molecules to donate their electrons.[1] They are produced continuously in cells, either as accidental byproducts of metabolism or deliberately. The most common cellular oxygen free radicals are superoxide radical ($O_2^-$), hydroxyl radical (OH) and nitric oxide (NO).[2] Other molecules, such as hydrogen peroxide ($H_2O_2$) and peroxynitrate (ONOO) are not free radicals themselves but can lead to their generation through various chemical reactions. An average cell utilizes 1013 molecules of $O_2$ per day. It is estimated that 1% of respired molecular oxygen will form reactive oxygen spices (ROS), thus approximately 1011 ROS are produced by each cell in a day. Cells normally employ a number of defence mechanisms against damage induced by free radicals.[2,3] Hence Antioxidants are extensively studied for their capacity to protect organism and cell from damage that are induced by oxidative stress.[4]

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidant defenses fall in to two main categories, those whose role is to prevent the generation of free radicals and those that intercept any radicals that are generated. Various animal studies have shown that antioxidants delay or protect against the oxidative damage produced by the free-radical reaction and a protective role against ailments mediated by free radicals is now well established.[5] There are increasing evidences that increased consumption of fruits and vegetables and intake of certain non-nutrients that are present in foods reduce the risk of various pathological events such as cancer [6,7] and cardio- and cerebro-vascular diseases.[8] Plant foods with apparent anticancer [9,10] and cardioprotective properties include varieties of Brassica oleracea[11], which have exhibited genotoxic properties[12]and high antioxidant and antimicrobial activities [13,14] in earlier studies. The vegetables are rich sources of many nutrients and antioxidant vitamins. *Brassica oleracea var. capitata (Cabbage)* (Family Brassicaceae) is an excellent source of vitamin C. It also contains significant amounts of glutamine, an amino acid that has anti-inflammatory properties. Cabbage can also be included in dieting programs, as it is a low calorie food.The present study was directed to investigate the antioxidant activity and phytochemical analysis of leaves extract of *Brassica oleracea var. capitata***.**

## MATERIALS AND METHOD

### Chemicals and Reagents

All chemicals and solvents used in the study were of analytical grade. Folin-Ciocalteu reagent (Merck Pvt. Ltd, India), Sodium chloride (S.D. Fine Chem, India), Sodium carbonet (Merck Pvt. Ltd, India),methanol and Catechol (Himedia Lab., India), 2, 2-Diphenyle-2-picryl hydrazyl (DPPH) and Vitamin C are obtained from (Himedia Lab., India).All solutions, including freshly prepared doubled distilled water. Stock solutions of the test extracts were prepared in ethanol. Appropriate blanks were used for individual assays.

### Plant collection and identification

The vegetable *Brassica oleracea L. var capitata* (cabbage) obtain from local market. The plant can be identified authenticated by Department of Botany, Research office (Botanist), Anwar-ul- loom, College of Pharmacy, Hyderabad.

### Extraction

The leaves of *Brassica oleracea L. var capitata* were dried under shade and powdered in a mechanical grinder. The powdered material (200gms) was extracted successively in Petroleum Ether, ethylacetate, chloroform, Ethanol and distilled water by cold percolation method using Soxhlet apparatus at 55°C for 18 h. The extracts was concentrated in vacuo and kept in vacuum desiccators for complete removal of solvent and weighed.

### Phytochemical investigation

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in Petroleum Ether, ethylacetate, chloroform, Ethanol and distilled water.[15, 16 &17]

### Phenolic Estimation

The total phenolic content of plant extracts were determined by using Folin-Ciocalteu Spectrophotometric method according to the method described by( Kim et al (2007).[18] Reading samples on a UV-vis spectrophotometer at 650 nm.The results of total phenolic content of the extract were expressed as Gallic acid equivalents (GAE) in mg/100g of fresh weight.

### DPPH free radical scavenging activity

DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al.* 2006.[19] The different concentrations of each of the extracts were prepared in ethanol and were added to 3ml of 0.1mM methanolic solution of

DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard.

Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

% Inhibition = (A0 - A1/ A0) X100

Where, A0 is the absorbance of the control (without test samples)

A1 is the absorbance of test samples.

All the tests were performed in triplicates and the results were reported as IC50, which is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%.

**Reducing power assay**

The reducing power of the extracts was evaluated according to Oyaizu, 1986. [20] Different amounts of ethanol extract were perched in aqueous solvent and diverse with 2.5 ml of 0.2M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. This mixture was incubated at 50oC for 20 min, 2.5 ml of 10% TCA was added to the blend and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was assorted with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicates increased reducing power.

**Statistical analysis**

All the tests were performed in triplicates and the results were pooled and expressed as mean ± standard error (SE).

**RESULTS AND DISCUSSION**

**The effect of different solvents on the yields of** *Brassica oleracea L. var capitata* **extracts.**

The significant variation in the yields of *Brassica oleracea L. var capitata* leaves extracts was shown using various fraction solvents. The yields of extracts using Petroleum Ether, ethylacetate, chloroform, Ethanol and distilled water in *Brassica oleracea L. var capitata* were 21.61gm,23.35gm, 24.84gm, and 27.78gm and 25.54 respectively. The variation in yield may be due to the polarity of the solvents used in the extraction process (Table-2). The results on the quantitative analysis of phytochemical constituents of different extracts of *Brassica oleracea L. var capitata* are shown in Table-1

**Free radical and antioxidative activity**

**Table-3** shows the results of the free radical (DPPH) scavenging activity in % inhibition. The result revealed that the ethanol fraction of *Brassica oleracea L. var capitata* exhibited the highest radical scavenging activity with 75.65±0.06 followed by its aqueous extract with 71.23±0.02, chloroform with 68.54±0.04, Ethylacetate with 61.34±0.02 and Petroleum Ether extract with 59.18±0.07. In overall comparison of different extracts, the ethanolic extract of *Brassica oleracea L. var capitata* show the highest scavenging activity. Methanol and ethanol has been proven as effective solvent to extract phenolic compounds.[21] In the present study, the values of ethanolic were higher than other extracts. Among solvents used in this study ethanol has showed the best effectiveness extracting phenolic components. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity.[22] **Fig 1**. Shows the antioxidant activities of *Brassica oleracea L. var capitata* in different solvents.

**Phenol content & antioxidant activity**

Therefore, we assume that DPPH free radical scavenging activity is related to the presence of bioactive compounds such as phenolic compounds in extract. The total phenolic content varied significantly between the different extracts of *Brassica oleracea L. var capitata*. The contents of total phenolic compounds in crude extract are shown in Table-1. The highest concentration of total phenol was (556mg/g) present in the ethanolic extract, followed by Chloroform extract (493mg/g), Ethylacetate extract (473mg/g), Petroleum Ether extract (452mg/g) and aqueous extract (402mg/g) of *Brassica oleracea L. var capitata*.

**$IC_{50}$ value**

Lower IC 50 value indicated a greater antioxidant activity[23]

$IC_{50}$ value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds. Results showed in table-2 reports $IC_{50}$ all extracts of *Brassica oleracea L. var capitata* showed lower $IC_{50}$ value, however ethanolic extract of *Brassica oleracea L. var capitata* being the lowest. **Fig. 2** Shows $IC_{50}$ values of *Brassica oleracea L. var capitata* leaf extracted in ethanol in comparison to Vitamin C**.** The ethanolic extract of *Brassica oleracea L. var capitata* exhibited significant activity with low $IC_{50}$ value. The antioxidant activity of *Brassica oleracea L. var capitata* extracts rise with the rising of polyphenol content of the extract. A linear relationship between the reciprocal of $IC_{50}$ value and the total polyphenol content was observed in this study, indicating that increasing the polyphenol content strengths the antioxidant activity. This finding is similar to that reported by Katsube et al. (2004). [24]

**Reducing power assay**

The reducing capacity of the extracts $Fe^{3+}$/ ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity.[25,26] The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom. The reduction of the $Fe^{3+}$/ ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution.[27] Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. Among different extracts, ethanolic extract of *Brassica oleracea* var. *capitata* showed highest reducing power activity **(Fig 3)** at concentrations of 2 to 10mg/ml. The reducing power of the ethanolic extract increased with increasing concentrations of the extract.

**Table 1: Results on the quantitative analysis of phytochemical constituents of different leaves extracts of *Brassica oleracea L. var capitata*.**

| S. No. | Constituents | Aqueous Extract | Petroleum Ether Extract | Ethanol Extract | chloroform | Ethyl acetate |
|---|---|---|---|---|---|---|
| 1. | Carbohydrates | + | + | + | + | + |
| 2. | Proteins | + | + | + | + | + |
| 3. | Amino acids | + | + | + | + | + |
| 4. | Alkaloids | + | + | + | - | + |
| 5. | Steroids | - | - | + | - | - |
| 6. | Tannins | + | + | + | + | + |
| 7. | Phenols | + | + | + | + | + |
| 8. | Flavonoids | + | + | + | + | + |
| 9. | Glycosides | + | + | + | + | + |
| 10. | Saponins | - | + | - | + | + |
| 11. | Terpenes | + | - | + | - | + |

**+** = Presence of phytochemical constituents; **-** = Absence of phytochemical constituents.

In present times, it is believed that the Vegetables contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals. This is often attributed to the antioxidants such as vitamin C, E, carotenoids, lycopenes and flavonoids that prevent free radical damages.[28,29] [30] These phytochemicals impart health benefits beyond basic nutrition.[31] The consumption of these vegetables may play a role in preventing human disease in which free radicals are involved, such as cancer, cardiovascular diseases and aging. Further, to elucidate a full profile of antioxidant activity against various ROS, comprehensive assays are needed.

**Table 2: Crude extracts, phenol contents & $IC_{50}$ Value in *Brassica oleracea L. var capitata*.**

| Solvent used | *Brassica oleracea L. var capitata.* | | |
|---|---|---|---|
| | Crude Extracts (gm) | Phenol content(mg/100g) | IC 50Value (µg/ml) |
| Petroleum Ether | 21.61 | 452 | 0.0087 |
| Ethylacetate | 23.35 | 473 | 0.0075 |
| Chloroform | 24.84 | 493 | 0.0069 |
| Ethanol | 27.78 | 556 | 0.0042 |
| water | 25.54 | 402 | 0.0065 |

**Table 3: Antioxidant activities of *Brassica oleracea L. var capitata*.in different solvents**

| Concentration of extracts (µg/ml) | Antioxidant activity (%) | | | | |
|---|---|---|---|---|---|
| | Petroleum Ether | Ethylacetate | Chloroform | Ethanol | Water |
| 20 | 52.23±0.03 | 52.65±0.04 | 57.11±0.02 | 64.31±0.01 | 58.65±0.03 |
| 40 | 53.25±0.02 | 53.87±0.04 | 59.88±0.08 | 67.13±0.02 | 54.17±0.06 |
| 60 | 54.61±0.09 | 55.35±0.09 | 61.43±0.05 | 69.99±0.05 | 66.17±0.08 |
| 80 | 56.27±0.08 | 58.12±0.01 | 65.44±0.01 | 72.76±0.06 | 68.26±0.09 |
| 100 | 59.18±0.07 | 61.34±0.02 | 68.54±0.04 | 75.65±0.06 | 71.23±0.02 |

Each value is expressed as the mean ± SD (n = 3).

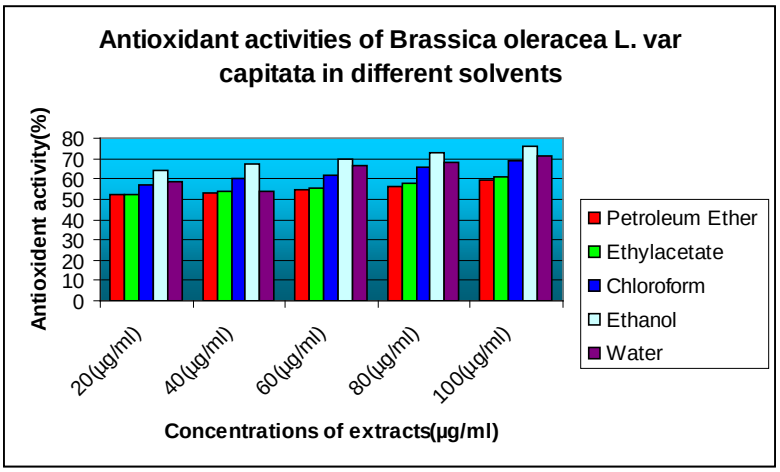


**Fig. 1: Antioxidant activities of *Brassica oleracea L. var capitata* in different solvents.**

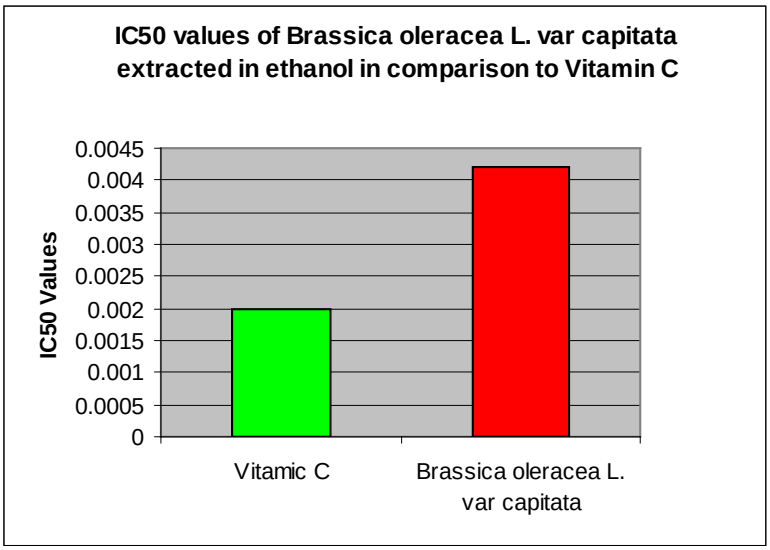


**Fig. 2: $IC_{50}$ values of *Brassica oleracea L. var capitata* extracted in ethanol in comparison to Vitamin C**

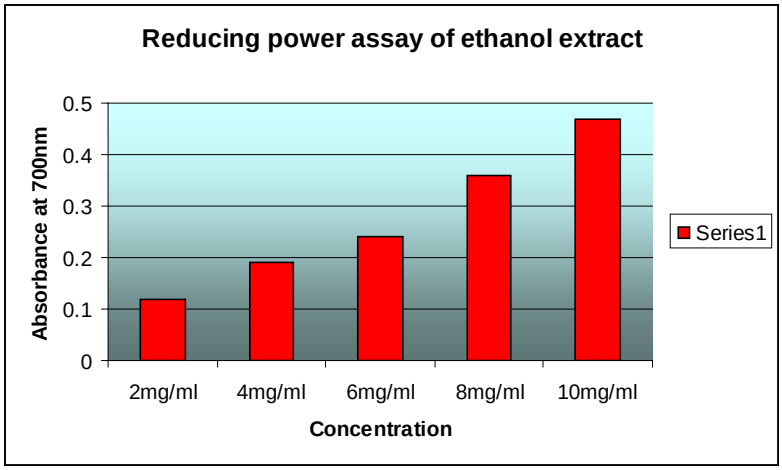


**Fig. 3: Reducing power assay of ethanol extract of *Brassica oleracea L. var capitata***

## CONCLUSION

The result of the present study showed that the ethanolic leaf extract of *Brassica oleracea L. var capitata* contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity. The high scavenging property of *Brassica oleracea L. var capitata* may be due to hydroxyl groups existing in the phenolic compounds. It is reported that phenols are responsible for the variation in the antioxidant activity of the plant.[32] They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals.[33, 34] Polyphenols are one of the major plant compounds with antioxidant activity. The –OH groups in phenolic compounds are thought have a significant role in antioxidant activity.[35] The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties.[36] In this study it is assumed that the high content of ascorbic acid in ethanol extract of *Brassica oleracea L. var capitata* might have accounted for their higher radical scavenging activities.

## ACKNOWLEDGMENT

My sincere thanks to ***Dr. A Srinavasa Rao***, *Principal, Bhaskar Pharmacy College* and ***Dr. Mohammed Ibrahim***, *Principal, Nizam Institute of Pharmacy*, for rendering their suggestions and helping me in each and every step of completing this research work successfully.

## REFERANCE

1. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet*, 1994, 344: 721-724.
2. Simonian NA, Coyle JT. Oxidative stress in neurodegenerative diseases. *Annu Rev Pharmacol Toxicol.* 1996; 36:83–106.
3. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 2002; 23:599–622.
4. Nithya S, Karthik N, Jayabharathi J. In vitro antioxidant activity of hindered piperidone derivatives. *Int J Pharm Pharm Sci* 2011; 3: 254 -256.
5. Cheeseman KH, Slater TF. Free radicals in medicine. *Br Med Bull* 1993; 49:479–724.
6. Goodwin JS, Brodwick M. Diet, aging, and cancer. *Clin Geriatr Med* 1995; 11:577–89.
7. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. Vegetable, Fruit, and Cereal Fiber Intake and Risk of Coronary Heart Disease among Men. *JAMA* 1996; 275:447-451.
8. Trease GE, Evans WC, A Text book of Pharmacognosy, 11th edition, Bailliere Tidall, London, 1978, 530.
9. Fowke, J.H., Longeope, C., Hebert, J., 2000. Brassica vegetable consumption shifts estrpgen metabolism in healthy postmenopausal women. *Cancer Epidem. Biomar. Prevent.* 9, 773–779.
10. Fowke, J.H., Chung, F.-L., Jin, F., Qi, D., Cai, Q., Conaway, C., Cheng, J.-R., Shu, X.-O., Gao, Y.-T., Zheng, W., 2003. Urinary isothiocyanate levels, Brassica, and human breast cancer. *Cancer Res*. 63, 3980–3986.
11. Beecher, C.W., 1994. Cancer preventive properties of varieties of Brassica oleracea. *Am. J. Clin. Nutr.* 59, 1166–1170.
12. Kassie, F., Parzefall, W., Musk, S., Johnson, I., Lamprecht, G., Sontag, G., Knasmüller, S., 1996. Genotoxic effects of crude juice from Brassica vegetables and juices extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. *Chem.-Biol. Interact*. 10, 1–16.
13. Roy, M.K., Takenaka, M., Isobe, S., Tsushida, T., 2007. Antioxidant potential, antiproliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: effects of thermal treatment. *Food Chem*. 103, 106–114.
14. Ayaz, F.A., Hayırlıoglu-Ayaz, S., Alpay-Karaoglu, S., Grúz, J., Valentová, K., Ulrichova, J., Strnad, M., 2008. Phenolic acid contents of kale (Brassica oleraceae L. var. acephala DC.) extracts and their antioxidant and antibacterial activities. *Food Chem*. 107, 19–25.
15. Trease GE and Evans WC. A text book of Pharmacognosy, 15th edition, 2002, Elsevier Company, Philadelphia, USA. pp.191-418.
16. Kokate, C.K., Purohith,A.P.&Gokhale, S.B. (1990). Pharmacognosy, Nirali Prakashan, Pune, 120.
17. Khandelwal, K.R. (2006). Practical Pharmacognosy techniques and experiments, 16 Edition, Nirali Prakashan, 149-156.
18. Kim KT, Yoo KM, Lee JW, Eom SH,Hwang IK, Lee CY. Protective effect of steamed American ginseng (Panax quinquefolius L.) on V79-4 cells induced by oxidative stress. *J. Ethnopharm* 2007; 111:443-445.
19. Wong SP, Lai PL, Jen HWK. Antioxidant activities of aqueous extracts of selected plants. *Food Chem* 2006; 99:775-783.
20. Oyaizu M. Studies on product of browning reaction prepared from glucose amine.*Jap J Nutr* 1986; 44:307–315.
21. Siddhuraju P, Becker K. Antioxidant properties of various extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *Journal of Agriculture and Food Chemistry* 2003; 51:2144-55.
22. Karadeniz F, Burdurulu HS, Koca N, Soyer Y. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Journal of Agriculture and Food Chemistry* 2005; 29, 297-303.
23. Torane RC, Kamble GS, Khatiwora E, Ghayal NA, Deshpande NR.Antioxidant capacity of leaves and stem of ehretia laevis . *Int J Pharm Pharm Sci* 2011; 3: 149 -151.
24. Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anuurad E, Shiwaku K, YamaneY. Screening for antioxidant activity in edible plant products: Comparision of low density lipoprotein oxidation

assay. *Journal of Agriculture and Food Chemistry* 2004; 52(8):2391-6.

25. Meir S, Kanner J. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves. *J Agric Food Chem* 1995; 43:1813-1819.
26. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF and Bilaloglu V. Comparison of antioxidant and antimicrobial activities of tilia (Tilia Agric argentea Desf ex DC), sage (Salvia triloba l.), and black tea (Camellia sinensis) extracts. *J Agric Food Chem*. 2000;48:5030-5034.
27. Zou, Y., Lu, Y., Wei, D. Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. in vitro. *J Agric Food Chem*.2004;52, 5032-5039.
28. Stahelin HB, Gey KF, Eichholzer M, Ludin E, Bernasconi F, Thurneysen J, Brubacher G. Plasma antioxidant vitamins and subsequent cancer mortality in the 12-year follow-up of the prospective Basel Study. *Am J Epidemiol* 1991; 133:766-775.
29. Steinberg D**.** Antioxidants and atherosclerosis. A current assessment. *Circulation* 1991; 84:1420-1425.
30. Willett WC. Micronutrients and cancer risk. *Am J Clin Nutr* 1994; 59: S265-S269.
31. Oomah BD, Mazza G, Functional foods, In: Francis FJ (ed) The Wiley encyclopedia of science and technology, 2nd edition, Wiley, New York, 2000, 1176–1182.
32. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life science* 2004;74:2157-84.
33. Pokorny J, Yanishlieva N, Gordon M. Antioxidants in food, Practical Applications,Cambridge Woodhead publishing limited 2001;72(5):145-71.
34. Pitchaon M, Suttajit M, Pongsawatmani R. Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. *Food Chem* 2007,100:1409-18.
35. Arumugam P , Ramamurthy P, Santhiya ST and Ramesh A (2006). Antioxidant activity measured in different solvent fractions obtained from Mentha spicata Linn.: An analysis by ABTS.+ decolorization assay. *Asia Pac. J. Clin. Nutr*. 119-124.
36. Rahman K (2007). Studies on free radicals, antioxidants, and cofactors. *Clin Interv Aging*. 2(2): 219–236.