Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(1): 04-07

Received: 11-11-2015
Accepted: 13-12-2015

Vitthalrao B Khyade
Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati; Dist. Pune – 413115, India.

Correspondence
Vitthalrao B Khyade
Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati; Dist. Pune – 413115, India.

Antioxidant activity and phenolic compounds of mulberry, *Morus alba* (L) (Variety: Baramatiwali)

Vitthalrao B Khyade

Abstract
Mulberry, *Morus alba* (L) is the significant plant at the Malegaon Sheti Farm of Agricultural Development Trust, Baramati. The mulberry leaves are mainly utilized for feeding the larval instars of silkworm, *Bombyx mori* (L). They are also commonly used to prepare traditional dessert among peoples of Baramati for a long time. Water was used as an extraction agent by the peoples in this area. This extracted leaves were used by the peoples of Baramati because it has natural color, flavor and fragrance properties. In the present attempt, the leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) were extracted by using 80% methanol in the laboratory. The extracted content was analyzed and found to be high in antioxidant activity of phenolic compound. The result shows that the extracted content of the leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) had high amount of total phenolic compound (44.66µg of GAE/mg of fresh weight). The plants secondary metabolites normally play role in cancer treatment. Cytotoxicity activity of the extracted leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) was evaluated on MCF-7 (Breast cancer cell line), HT-29 (colorectal cancer cell line), and WRL-68 (normal liver cell line), and the results show that, cytotoxicity activity (IC50 values 78.87µg/ml, 77.50µg/ml, and 78.29µg/ml, respectively). Methanolic extract of the leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) mulberry leaf had antioxidant effects, which may suggest its use as food supplement for cancer patients.

Keywords: *Morus alba* (L) (Variety: Baramatiwali); antioxidant activity; total phenolic content; anticancer activity; cytotoxicity.

1. Introduction
The utilization of plant based product in food supplements and health industries were increased tremendously in the current decade. This was believed to be due to carcinogenic related problems with the usage of artificial or chemicals based products. Therefore, a lot of studies have been done by the researcher all over the world to determine the active biocomponent in plant, which could be replaced artificial products. For example, uses *Punica granatum* (Chaitra, *et al*, 2012) [1], uses coconut shell (Verma, *et al*, 2012) [2] and uses *Carica papaya* (Shaziy and Goyal, 2012) [3]. Many other researchers were also embarked on the study to identify the possibility of using plant component to solve human health problems (Kanife, *et al*, 2012; Bobade and Khyade, 2012; Khyade, *et al*, 2012 and Jitendta and Dilip Kumar, 2012) [4, 5, 6, 7]. Besides that, a lot of studies were also undertaken by the researcher to use plant material as a solution for the environmental pollution, for example, Muthusamy, *et al*, 2012 [8] have embarked on a study to utilize Maize Cob to remove heavy metals in industrial wastewater. Meanwhile, study by Aweng, *et al*, 2012 [9] and showed that, *Cassia alata* has a potential to be use as coagulant in water treatment. Similarly with the finding from Mangale Sapana, *et al*, 2012 [10], where they found that, *Moringa oleifera* could be use as natural absorbent and antimicrobial agent in water treatment.
Morus alba, known as white mulberry, is a short-lived, fast-growing, small to medium-sized mulberry tree, which grows to 10–20 m tall. The species is native to northern China, and is widely cultivated and naturalized elsewhere (United States, Mexico, Australia, Kyrgyzstan, Argentina, etc.). The mulberry is widely cultivated to feed the silkworms employed in the commercial production of silk. It is also notable for the rapid release of its pollen, which is launched at over half the speed of sound. The mulberry, *Morus alba* (L) is basically a shrub belongs to Family: Moraceae (Khyade, *et al*, 2012) [6]. Baramati and surrounding areas mostly depend on agriculture as the main source of income. The land in the region is very well irrigated because of left bank Canal irrigation from Veer Dam. Nira river also provides direct irrigation water to farms.

The special features of Malegaon Sheti farm of Agricultural Development Trust Baramati lies in having well established mulberry garden for commercial silk production. The variety of mulberry, *Morus alba* (L) at Malegaon Sheti farm of Agricultural Development Trust Baramati is herewith named as "Baramatiwali". The major nutritional components (moisture, ash, lipids, proteins, fibres, carbohydrates, and total sugar) were found to be in the suitable range along with good computed energy. Total dry weight, pH, and titratable acidity (percent citric acid) were (17.60±1.94)–(21.97±2.34) mg/100 g, (3.20±0.07)–(4.78±0.15), and (0.84±0.40)%–(2.00±0.08)%, respectively. Low riboflavin (vitamin B_2) and niacin (vitamin B_3) contents were recorded in all the fruits, while ascorbic acid (vitamin C) was in the range from (15.20±1.25) to (17.03±1.71) mg/100 g fresh weight (FW). The mulberry fruits were rich with regard to the total phenol and alkaloid contents, having values of (880±7.20)–(1650±12.25) mg/100 g FW and (390±.22)–(660±5.25) mg/100 g FW, respectively. Sufficient quantities of essential macro-(K, Ca, Mg, and Na) and micro-(Fe, Zn, and Ni) elements were found in all the fruits. K was the predominant element with concentration ranging from (1270±9.36) to (1731±11.50) mg/100 g, while Ca, Na, and Mg contents were (440±3.21)–(576±7.37), (260±3.86)–(280±3.50), and (24±3.51)–(360±4.20) mg/100 g, respectively. The decreasing order of micro-minerals was Fe>Zn>Ni. The radical scavenging activity of methanolic extract of fruits was concentration-dependent and showed a correlation with total phenolic constituents of the respective fruits. Based on the results obtained, mulberry fruits were found to serve as a potential source of food diet and natural antioxidants (Vitthalrao B. Khyade, 2004). The mulberry fruits were nutritionally rich and may be useful in a balanced diet. Higher phenolic contents with antioxidant activity further increase their nutritive as well as phytomedicine potentials. The current work is the first of its kind, providing new reference data and also public awareness regarding consuming these unconventional fruits. The present study is also a step towards the standardization of these fruits as potential healthy foods, which may also be used in food and pharmaceutical industries (Lin and Tang, 2006) [25]. Mulberry can grow in a wide range of climatic, topographical, and soil conditions, which can affect the chemical composition and nutritional status of plants. However, mulberry, *Morus alba* (L) (Variety: Baramatiwali) cultivated at the Malegaon Sheti Farm of Agricultural Development Trust, Baramati has not been subject to these kinds of studies. Therefore, the current study was undertaken to investigate it's antioxidant potentials and the contents of phenolic compounds.

2. Material and Methods

2.1 Sample Collection: The fresh leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) cultivated at the Malegaon Sheti Farm of Agricultural Development Trust, Baramati (India).

2.2 Sample Extraction: The mulberry leaves samples were blended in 80% methanol. Then, the solvent was removed from samples using rotary evaporator. Freeze dryer was used to transform the samples into powder form.

2.3 Total Phenolic Content (TPC) Assay: The total phenol content was assayed according to the Folin-Ciocalteu method (Singleton and Rossi, 1965) [18]. Test samples were diluted to a concentration of 1 mg/ml in methanol. Test solution (0.05 mL of 1 mg/mL solution) was added to 3 mL of distilled water and swirled before added with 0.25 mL of Folin-Ciocalteu phenol reagent. After 2 min, 0.75mL of 20% sodium carbonate was added and the volume made up to 5 mL with distilled water. The mixture was vortexed and left for 2 h, after which the absorbance was measured at 760 nm. A mixture without test solution was used as blank. 1 mg/mL solution of gallic acid was used as standard, and the total phenolic content of each extract was expressed as percentage of the phenolic content of gallic acid (taken as 100%). Spectrophotometer measurements were performed at least three times for each separate concentration of standard and samples, and in triplicate.

2.4 Cytotoxicity assays: The cytotoxicity activities of test samples were performed against MCF-7 (Breast cancer cell line), HT-29 (colorectal cancer cell line), and WRL-68 (normal liver cell line). Briefly, cells in 100 µl of medium per well were seeded in 96-well flat-bottomed micro titer plate. After 24 hours of incubation in a 5 % CO2 humidified incubator at 37 °C, 100 µl of media containing test samples were added into micro filter plate in quadruplicate at various concentrations. After 72 hours incubation, the cells were fixed by adding 50 µl of TCA for 30 min at RT, rinsed with tap water and stained with 0.4% SRB (in 1%acetic acid) to remove unbound dye, air-dried and solubilised in 100 µl of 10 mM unbuffered tris base solution. The plates were read in micro-plate reader at 490 nm. Results were expressed as the dose that inhibited 50% control growth after the incubation period (IC50). The values were estimated by plotting drug concentration (µg/ml) against the percentage of viable cells compared to control. The tests were repeated in at least three independent experiments.

3. Results and Discussion

The extracts of leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) cultivated at the Malegaon Sheti Farm of Agricultural Development Trust, Baramati (India) give rise to purple liquid. The use of the mulberry leaves as natural food products have more advantages compared to other product which contained synthetic/artificial coloring, fragrance and flavorant. Natural food product can provide beneficial values such as antioxidant and anticancer that synthetic product do not provide. Furthermore, FDA (food and drug administration) also has firmer rules on the use of synthetic coloring such as tartrazine and amaranth for food coloring agent.

Analysis of the mulberry leaf extract shows high antioxidant activities of phenol contents with 44.66µg of GAE/mg (44.66 mg GAE/g) of fresh weight. The high level activities of antioxidant in mulberry leaves give major advantage for medicinal purposes. NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NFkB plays a key role in regulating the immune response to infection. Conversely, incorrect regulation of NF-kB has been linked to cancer.

The nuclear factor (NF-kB) proposed to be a pivotal protein in the link between inflammation and cancer (Pikarsky, *et al*, 2004). NF-kB characterizes all inflammatory responses and is also a major hallmark of tumors (Lin and Karin, 2003). Antioxidant supplementation can block NF-kB activation and inhibit NF-kB activity through mechanisms distinct from redox regulation (Huang, *et al*, 2001). Meanwhile, cytotoxicity activity of the extracted mulberry leaves which was tested on breast cancer cell line, colorectal cancer cell line and normal liver cell line shows that, cytotoxicity activity, IC50 values 78.87µg/ml, 77.50µg/ml, and 78.29µg/ml, respectively. Cytotoxicity test of extract of leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) cultivated at the Malegaon Sheti Farm of Agricultural Development Trust, Baramati (India)

against cancer cell line have proved to inhibit cancer cell line which is one of the major causes of human death.

Table 1: Cytotoxicity of mulberry, *Morus alba* (L) (Variety: Baramatiwali) against cancer cell lines

Cell Lines	IC50 (µg/ml)
MCF-7 (Breast cancer cell line)	78.81
HT-29 (Colorectal cancer cell line)	77.50
WRL-68 (Normal liver cell line)	78.29

4. Conclusion

The significance findings of this study was that the leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) cultivated at the Malegaon Sheti Farm of Agricultural Development Trust, Baramati (India) demonstrated much stronger antioxidant activity and contained significantly more phenolics (44.66 mg GAE/g) than common vegetables and fruits which are considered as good natural sources of dietary antioxidants. Based on literature reviews, red apple contain 11.6 mg GAE/ g 21, sour prickly pears contain 2.07 mg GAE/g22, red pitaya contains 0.424 mg GAE/ g23 and white mulberry contain 19.24 mg GAE/g24. The mulberry, *Morus alba* (L) (Variety: Baramatiwali) cultivated at the Malegaon Sheti Farm of Agricultural Development Trust, Baramati (India) might be a potential source of excellent natural antioxidants and anticancer to be use also as natural color, flavour and fragrance for food preparation. Phenolic antioxidants in the mulberry, *Morus alba* (L) (Variety: Baramatiwali) cultivated at the Malegaon Sheti Farm of Agricultural Development Trust, Baramati (India) and it's antioxidative properties would play a role in preventing and treating cancer.

5. Acknowledgement

The author like to express the deepest appreciation to Agricultural Development Trust, Baramati (India) laboratory facilities.

6. References

1. Chaitra H, Madhuri M, Nitisha ST, Arijit D, Sourav B, Rohit KC. Evaluation of Antimicrobial Properties, Phytochemical Contents and Antioxidant Capacities of Leaf Extracts of Punica granatum L., ISCA Journal of Biological Sciences. 2012; 1(2):32-37.
2. Verma V, Bhardwaj A, Rathi S, Raja RB. A Potential Antimicrobial Agent from Cocos nucifera mesocarp extract; Development of a New Generation Antibiotic, ISCA Journal of Biological Sciences. 2012; 1(2):48-54.
3. Shaziy B, Goyal PK. Anthelmintic effect of Natural Plant (Carica papaya) extract against the Gastrointestinal nematode, Ancylostoma caninum in Mice, ISCA Journal of Biological Sciences. 2012; 1(1):2-6.
4. Kanife UC, Odesanmi OS, Adekumle AA, Doherty VF. Effects of Ethanol Extracts of Healthy and Infected Panicum maximum (Jacq.) Floret on liver and kidney function profile and histopathology in Sprague-dawley rats, Research Journal of Recent Sciences. 2012; 1(5):8-13.
5. Bobade SN, Khyade VB. Influence of Inorganic Nutrients on the activity of Enzyme, Nitrate reductase in the leaves of Mulberry, Morus alba (L) (M-5 variety), Research Journal of Recent Sciences. 2012; 1(5):14-21.
6. Khyade VB, Shukla KK, Sarawade JP. Juvenoid activity of some non mulberry plant extractives through inhibition of chitin deposition in the integument of fifth instar larvae of silkworm, Bombyx mori (L) (Race: PM x CSR2), Research Journal of Recent Sciences, 2012, 1(ISC-2011), 6-6.
7. Jeetendra S, Dilip Kumar A. Ethno Medicinal Plants used by Tribal Communities for the Treatment of Snakebite in West Nimar MP, India, ISCA Journal of Biological Sciences. 2012; 1(2):77-79.
8. Muthusamy P, Murugan S, Smitha M. Removal of Nickel ion from Industrial Waste Water Using Maize Cob, ISCA Journal of Biological Sciences. 2012; 1(2):7-11.
9. Aweng ER, Anwar AI, Siti Rafiqah MI, Suhaimi O. Cassia alata as a Potential Coagulant in Water Treatment, Research Journal of Recent Sciences. 2012; 1(2):28-33.
10. Mangale Sapana M, Chonde Sonal G, Raut PD. Use of Moringa oleifera (Drumstick) seed as Natural Absorbent and an Antimicrobial agent for Ground Water Treatment, Research Journal of Recent Sciences. 2012; 1(3):31-40.
11. Salah EL-Kousy, Mona Mohamed, Shimaa Mohamed. Phenolic and biological activities of Vitex trifolia aerials parts, Life Science Journal. 2012; 9(2):670-677.
12. Thompson L. What Substance Gives Plants Their Green Color? http:wwwwehow.com/how-does 5470613 substance-gives-plants-green-color.html [12 October 2010], 2009.
13. Blakesley K. Peak Fall Colors: Why Do Leaves Change Color? http://webchace.googleusercontent.com/ search?q=cache:yXAaAmRgvSQJ:www.associatedconten t. com/article/224519/peak fall colors why do leaveschange.html+factor+destroying+chlorophyll&cd=1 & cd=1&hl=en&ct=clnk&client=firefox-a [12 October 2010], 2009.
14. Holcorft DM, Kader AA. Carbon Dioxide- Induced Changes in color and Anthocyanin Synthesis of Stored Strawberry Fruit, Hort Science, 1999; 36(7):1244-1248.
15. Romero C, Bakker R. Interaction Between Grape Anthocyanins and Pyruvic Acid, with Effect of pH and Acid Concentration on Anthocyanin Composition and Color in Model Solutions Interaction, Journal of Agriculture Food Chemical. 1999; 47(8):3130-3139.
16. Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-kB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion and metastasis, Oncogene, 2001; 20:4188-4197.
17. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, Life Sciences, 2003; 74:2157-2184.
18. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, American Journal of Enology and Viticulture. 1965; 16:144-158.
19. Pikarsky E, Porat RM, Stein I, Bramovitch RA, Amit S, Kasem S *et al.* functions as a tumour promoter in inflammation-associated cancer, Nature, 2004; 431:461-466.
20. Lin A, Karin M. NF-kB in Cancer: a Market Target, Semin, Cancer Biology, 2003; 13:107-114.
21. Henriquez C, Almonacid S, Chiffelle I, Valenzuela T, Araya M, Cebezas L *et al.* Determination of Antioxidant Capacity, Total Phenolic Content and Mineral Composition of Different Fruit Tissue of Five Apple Cultivars Grown in Chile, Chilean Journal of Agricultural Resources. 2010; 70(4):523-536.
22. Osorio-Esquivel O, Ortiz-Moreno A, Alvarez AB, Dorora-Alvarez L, Giuti MM. Phenolics betacyanins and antioxidant activity in Opuntia joconostle fruits, Food Research International. 2011; 44(7):2160-2168.
23. Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and anti proliferative activities of red pitaya,

Food Chemistry 2006; 95(2):319-327.
24. Gungor N, Sengul M. Antioxidant Activity, Total Phenolic Content and Selected Physicochemical Properties of White Mulberry (Morus Alba L.) Fruits, International Journal of Food Properties. 2008; 11(1):44-52
25. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation, 2006, Food Chem. 2007;101(1):140-147. doi: 10.1016/j.foodchem.2006.01.014.