PRAJÑĀ - **Journal of Pure and Applied Sciences Vol. 24 – 25 : 182 – 189 (2017)**
ISSN 0975 - 2595

IN VITRO ANTI-INFLAMMATORY, ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC CONTENTS IN METHANOLIC EXTRACT FROM VEGETABLES

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ABSTRACT

Anti-inflammatory and antioxidant activities are extensively used to screen chemopreventive foods. Four well-known vegetables bitter gourd, bottle gourd, drumstick leaves and ginger were assessed in this study. Total phenolics and flavonoid were estimated. The total antioxidant capacity was evaluated using FRAP; based on the scavenging ability of the cation radical DPPH RSA ABTS RSA and ORAC. Moreover, anti-inflammatory activity was determined based on the protein denaturation inhibition assay. The highest total phenolics and flavonoid content was observed in methanolic extract of drumstick leaves among four selected vegetables. Antioxidant capacity by FRAP was found to be highest in ginger, whereas scavenging activity by DPPH RSA and ABTS RSA was found to be high in drumstick leaves. The anti-inflammatory activity could be ranked based on the IC50 of the vegetables, as bitter gourd > ginger > bottle gourd > drumstick leaves. Additionally, a significant correlation existed between total phenolic content and antioxidant activity. This profound protective effect of vegetables may explain its extensive use in daily life and possible health benefits.

Key Words: In vitro anti-inflammation, protein denaturation, ORAC, Vegetables

INTRODUCTION

According to the World Health Organization, 46% of the global disease burden and 59% of global mortality are due to chronic diseases [1]. Inflammation has recently emerged as an important aspect of the pathophysiology of major chronic diseases of industrialized societies. Elevated plasma levels of inflammatory markers are risk factors, including obesity [2,3], insulin resistance, type 2 diabetes mellitus [4-6], metabolic syndrome [7] and many types of cardiovascular diseases [8,9].

Extensive research during the past two decades has revealed the mechanism by which continued oxidative stress can lead to chronic inflammation [10]. Inflammation is one of the manifestations of oxidative stress and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress. Inflammation and oxidative stress are postulated to impair beta-cell function and exacerbate insulin resistance in type 2 diabetes [11] and often coexist in atherosclerosis and cardiovascular disease [12].

Anti-inflammatory agents, including nonsteroidal anti-inflammatory drugs (NSAID) are widely used in treating these disorders. However, many of them have dose-dependent side effects and none of them are suitable for primary prevention, which significantly limit their use. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs and most of this therapy involves the use of plant extracts and their active components [13]. Epidemiological and experimental studies reveal a negative correlation between the consumption of diets rich in fruits and vegetables and the risks for chronic diseases [14-18]. These physiological functions of fruits and vegetables may be partly attributed by their abundance of phenolics including flavonoids and anthocyanins, and carotenoids [19-22]. Traditionally, deep colored fruits, vegetables or foods are recognized as more healthy to human body, especially in the oriental countries. There has been a growing interest in pigment components of fruits and vegetables, which may promote human health or lower the risk for disease [23]. Fruits and vegetables have had conferred the status of functional foods [24], they seem to be capable of delivering health benefits besides fulfilling physiological needs. Habitual consumption of fruits and vegetables confers significant benefits to human health [25]. *In vitro* studies as well as epidemiological data strongly suggest that foods containing phytochemicals with antioxidation and anti-inflammatory potential have strong protective effects against major disease risks including cancer and cardiovascular diseases [26-33]. In the present

study, the anti-inflammatory and anti-oxidant activity of different vegetables was evaluated by using a widely accepted model system.

MATERIALS AND METHODS

Vegetables and Sample Extraction: Plant parts of the four vegetables namely bitter gourd (*Momordica charantia*), bottle gourd (*Lagenaria siceraria*), ginger (*Zingiber officinale*) and drumstick leaves (*Moringa oleifera*) were purchased from the local market of Anand. About 2.0 g of plant material was extracted in 50 ml of acidified methanol at room temperature. The whole extracts were filtered, and used for further assays.

Determination of total phenolic and flavonoid content: Total phenolic content of extract was determined using Folin-Ciocalteu method [34]. Gallic acid was taken as standard and results were expressed as mg of GA equivalent per 100 g of sample. Flavonoid content was estimated using aluminium chloride colorimetric method using rutin as a standard [35] and the absorbance was read at 510 nm. Results were expressed as mg of rutin equivalent per 100 g of sample.

Ferric reducing antioxidant power assay (FRAP): The measure of FRAP of the methanolic extract was carried out by the method given by Benzie and Strain [36]. Results were expressed as mg Trolox Equivalent Antioxidant Capacity per 100 g of sample.

Scavenging effect on DPPH Radicals: The scavenging effects of methanolic extract from vegetables on DPPH were measured according to the method of Brand-Williams [37]. The extract was added to the methanolic solution of 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) and absorbance was recorded at 517 nm. Results were expressed in terms of mg of Trolox Equivalent Antioxidant Capacity per 100 g of sample.

Scavenging effect on ABTS Radicals: The scavenging effects of methanolic extracts on ABTS were estimated using the modified method of Re et al [38]. Percentage inhibition was calculated and the results were expressed in terms of TEAC per 100 g of sample.

Oxygen radical absorbance capacity (ORAC): The ORAC assay is based upon the inhibition of the peroxyl-radical-induced oxidation initiated by thermal decomposition of azo compounds. Modified method of Ou et al [39] was used for the estimation. The final values were calculated using the area under the curve and results were expressed as trolox equivalents in micromole per gm.

***In vitro* anti-inflammatory effects on protein denaturation inhibition assay:** Protein denaturation inhibition assay was done by the reported protocol with minor modifications [40]. The methanolic extracts with different concentration were treated with bovine albumin; turbidity was measured spectophotometrically at 660 nm. Diclofenac sodium was taken as standard and percent inhibition of protein denaturation was calculated.

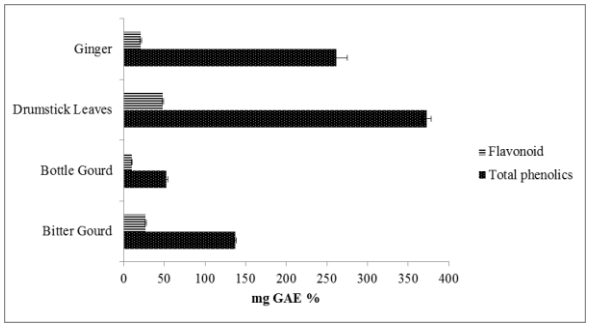
Statistical Analysis: Each experiment was performed in duplicate and expressed as mean±SD. Statistical analyses were performed using SPSS 20. Analyses of variance were performed using ANOVA. Significant difference (p<0.05) between the means were determined using Duncan's test. Pearson's correlation was calculated to study the relationship. Results were also subjected to multivariate data analysis by means of principal component analysis using SPSS 20.

RESULTS AND DISCUSSION

Fruits and vegetables are rich sources of antioxidants such as vitamin C, tocopherol, phenolics and β-carotene which contribute to their antioxidant or free radical or scavenging effects. Amongst these, phenolics serve as powerful antioxidants by virtue of the hydrogen-donating properties of their phenolic hydroxyl groups, as well as by donating electrons to stop free radical chain reactions emerging from oxidative stress [41].

Phenolic compounds are important contributors to functional quality and have an important role to play in counteracting reactive oxygen species (ROS) and thereby minimizing molecular damage [42]. Total phenolic content was found to vary significantly (p<0.05)

among vegetables and ranged from 52.41 to 372.82 mg GAE/100g depicting almost seven-fold variations (Figure 1). The hierarchy was bottle gourd<bitter gourd<ginger<drumstick leaves. The role of phenolics as natural antioxidants has attracted considerable interest due to their pharmacological functions. Increased consumption of phenolic compounds has been associated with the reduced risk of cardiovascular diseases and certain cancers [43-45]. Budrat [46] found 4.99 mg GAE/ gm of total phenolics content in bitter gourd by soxhlet extraction method. Le et al [47] found that aquous extract of drumstick leaves contains higher total phenolics content (105.04 mg gallic acid equivalents (GAE)/g), total flavonoids content (31.28 mg quercetin equivalents (QE)/g) and showed better antioxidant activity (85.77%).



Values are mean ± SD; n=4. Total phenolics content in mg gallic acid equivalent per 100 g; Flavonoid content in mg rutin equivalent per 100 g.

Figure 1: Total phenolics and Flavonoid content of the methanolic extract of vegetables.

The content of flavonoids also varied significantly (p<0.05) and ranged between 10.02 to 48.56 mg RT/ 100g (Figure 1). The content in increasing order was: bottle gourd<ginger<bitter gourd< drumstick leaves. Flavonoids have been reported to have beneficial health effects including anti-inflammatory, inhibition of platelet aggregation and inhibition of mast cell histamine release [47].

Table 1 shows the antioxidant capacity of the vegetables. Results showed that bottle gourd had the lowest antioxidant capacity measured by four different methods compared to other vegetables. Ginger had the highest ferric reducing antioxidant capacity compared to other vegetables. Drumstick leaves showed the highest scavenging activity on DPPH and ABTS among the studied vegetables. The hierarchy for ORAC was: bottle gourd<ginger<bitter gourd<drumstick leaves. Data obtained by Sreelatha [48] suggested that the extracts of drumstick leaves both mature and tender have potent antioxidant activity against free radicals. Water, aqueous methanol, and aqueous ethanol extracts of freeze-dried leaves of *Moringa oleifera* were examined for radical scavenging capacities and antioxidant activities by Siddhuraju [49]. They concluded that both methanol (80%) and ethanol (70%) were found to be the best solvents for the extraction of antioxidant compounds from drumstick leaves.

A correlation was observed between total phenolic content and flavonoid (r = 0.855), FRAP (r = 0.764), DPPH RSA (r = 0.997), ABTS RSA (r = 0.991) and ORAC (r = 0.593) in the selected vegetables. The results revealed that flavonoid content in the selected vegetables significantly (p<0.05) correlated with DPPH RSA (r = 0.879), ABTS RSA (r = 0.828) and ORAC (r = 0.807) (Table 2).

Table 1: Total Antioxidant Capacity by FRAP, DPPH RSA, ABTS RSA and ORAC of selected vegetables

	FRAP	DPPH RSA	ABTS RSA	ORAC
	(mg TE/100gm)			(micromole/gm)
Bitter Gourd	173.66±10.47^{b}	454.17±37.80^{b}	249.26±26.09^{b}	54.45±7.23^{c}
Bottle Gourd	65.89±10.76^{a}	173.33±7.22^{a}	39.20±3.22^{a}	28.54±5.86^{a}
Drumstick Leaves	764.31±33.39^{c}	1236.00±25.99^{d}	896.19±79.31^{d}	55.76±1.84^{c}
Ginger	1216.12±41.47^{d}	838.68±32.37^{c}	624.92±15.55^{c}	40.49±0.25^{b}
F-Value	1508.36*	1068.48*	323.90*	29.39*

Values are mean ± SD; n=4. Different alphabets within column are significantly different (p≤0.05) from each other. ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing antioxidant power assay; DPPH RSA, DPPH radical scavenging activity; ABTS RSA, ABTS radical scavenging activity; TE, trolox equivalent.

Table 2: Correlation between total phenolics, flavonoid and total antioxidant capacity of methanolic extracts of selected vegetables

	TP	Flavonoid	FRAP	DPPH RSA	ABTS RSA
Flavonoid	0.855**				
FRAP	0.764**	0.336			
DPPH RSA	0.997**	0.869**	0.743**		
ABTS RSA	0.991**	0.828**	0.787**	0.986**	
ORAC	0.593*	0.807**	0.166	0.604*	0.558*

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). TP, total phenolics; FRAP, ferric reducing antioxidant power assay; DPPH RSA, DPPH radical scavenging activity; ABTS RSA, ABTS radical scavenging activity.

However, FRAP content in the selected vegetables did not significantly correlate with flavonoid (r = 0.336) and ORAC (r =0.166) respectively. FRAP, ORAC and ABTS do not measure the same antioxidant phenomenon [50]. For example, FRAP is sensitive to single electron transfers while ORAC, to hydrogen atom transfer. The ABTS radical is thought to be sensitive to both single electron and hydrogen atom transfer. In effect, these three methods operate on different reaction mechanisms, accounting for the observation that they are not correlated with each other.

The percent inhibition and IC_{50} value of protein denaturation for vegetables were analyzed graphically and presented in figure 2 and table 3. The ability to inhibit protein denaturation by vegetables was displayed in the order of bitter gourd>ginger>bottle gourd>drumstick leaves. The lowest IC 50 value was observed for bitter gourd suggested the highest anti-inflammatory activity. Studies on different models revealed the same results as found in this study [51-53]. Juice of bottle gourd showed anti-inflamamtory activity against acute inflammatory models of paw edema [54,55]. Sulaiman [56] had evaluated *Moringa oleifera* aqueous extract for anti-inflammatory activity and they concluded that the aqueous extract of drumstick leaves exhibited significant anti-inflammatory activity in a dose dependent manner.

Principal component analysis is a variable reduction procedure and to develop a smaller number of artificial variables that will account for most of the variance in the observed variables. PCA was performed on the significant factor loading values, higher than or equal to 0.7 were used to identify the most important variables and attributes in each dimension, or principal components (PCs). First two PCs explained approximately 95.82% of total data variability, called PC1 (51.84%) and PC2 (43.98%) (Figure 3). In general, antioxidant capacity measured by FRAP, DPPH RSA and ABTS RSA located in the right hand of the component plot between attribute vectors indicate higher predictable capacity.

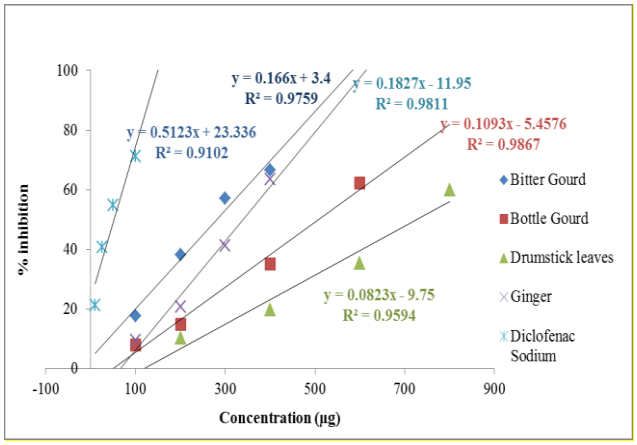


Figure 2: Anti-inflammatory activity of vegetables by protein denaturation inhibition assay

Table 3: Anti-inflammatory activity (% inhibition and IC_{50} value) of selected vegetables

Extracts	Concentration (µg)	% inhibition	IC_{50} (µg/ml)
Bitter Gourd	400	66.6	280.72
Bottle Gourd	400	35.1	507.31
Drumstick Leaves	400	19.8	726.00
Ginger	400	63.5	340.38
Diclofenac sodium	50	54.91	50.06

Values are mean ± SD; n=4.

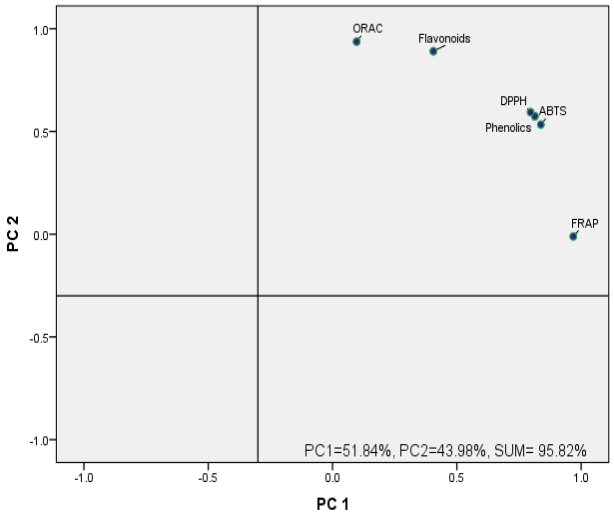


Figure 3: Principal component analysis (PCA) scatterplot obtained from the total phenolics, flavonoid and total antioxidant capacity by FRAP DPPH RSA, ABTS RSA and ORAC

CONCLUSION

In conclusion, this study found wide diversity in the phenolic, flavonoid content and antioxidant capacities in the selected vegetables. The methanolic extracts of vegetables exhibited anti-inflammatory activity which may possibly be due to the presence of phenolic compounds. The findings of the present study suggested that the extracts from several vegetables were found to possess strong antioxidant activity and scavenging effects on free radicals, that would have great importance as therapeutic agents in preventing or slowing the process of reactive oxygen species and associated oxidative stress related degenerative diseases. Hence it is recommended that the consumption of vegetables should be increased in the diet of human beings.

ACKNOWLEDGEMENT

This research work was supported by the Department of Science and Technology (DST)-INSPIRE fellowship (IF130975), New Delhi awarded to the first author.

REFERENCE

[1] Bengmark, S. (2006) : Impact of nutrition on ageing and disease. Current Opinion in Clinical *Nutrition & Metabolic Care*, **9(1):** 2-7.

[2] Ramos, E. J., Xu, Y., Romanova, I., Middleton, F., Chen, C., Quinn, R., ... & Meguid, M. M. (2003): Is obesity an inflammatory disease?. *Surgery*, **134(2):** 329-335.

[3] Williams, K.J., & Tabas,I. (2002): Atherosclerosis and inflammation. Science, **297(5581):** 521-522.

[4] Paresh, D., Ahmad, A., Ajay,C., Priya, M., & Rajesh, G. (2005): Metabolic syndrome:A comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation*, **111:** 1448-1454.

[5] Hanada, T., & Yoshimura, A. (2002): Regulation of cytokine signaling and inflammation. *Cytokine & growth factor reviews*, **13(4):** 413-421.

[6] Makarov, S.S. (2000): NF- κB as a therapeutic target in chronic inflammation:recent advances. *Molecular medicine today*, **6(11):** 441-448.

[7] Panagiotakos, D.B., Pitsavos, C., Yannakoulia, M., Chrysohoou, C., & Stefanadis, C. (2005): The implication of obesity and central fat on markers of chronic inflammation: the ATTICA study. *Atherosclerosis*, **183(2):** 308–315.

[8] Theuma, P., Fonseca, VA. (2003): Inflammation and emerging risk factors in diabetes mellitus and atherosclerosis. *Current diabetes reports*, **3(3):** 248–254.

[9] Strzelecka, M., Bzowska, M., Koziel, J., Szuba, B., Dubiel, O., Heinrich, M., & Bereta, J. (2005): Anti-inflammatory effects of extracts from some traditional Mediterranean diet plants. *Journal of Physiology and Pharmacology.* Supplement, **56(1):** 139-156.

[10] Evans, J.L., Goldfine, I.D., Maddux, B.A., & Grodsky, G.M. (2002): Oxidative stress amd stress activated signalling pathways: a unigying hypothesis of type-2 diabetes, *Endocrine Reviews*, **23(5):** 599-622.

[11] Wellen, K.E. & Hotamisligil, G.S. (2005): Inflammation, stress, and diabetes. *Journal of Clinical Investigation*, **115(5)**: 1111-1119.

[12] Strocker, R. & Keaney, J.F. (2004): Role of oxidative modifications in atherosclerosis. *Physiological Reviews*, **84(4):** 1381-1478.

[13] Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011): A review of the antioxidant potential of medicinal plant

species. *Food and bioproducts processing*, ***89*(3):** 217-233.

[14] Chen, C. C., Liu, L. K., Hsu, J. D., Huang, H. P., Yang, M. Y., & Wang, C. J. (2005): Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Food chemistry*, ***91*(4)**: 601-607.

[15] Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000): The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological reviews*, ***52*(4)**: 673-751.

[16] Prior, R. L. (2003): Fruits and vegetables in the prevention of cellular oxidative damage. *The American journal of clinical nutrition*, ***78*(3):** 570S-578S.

[17] Saleem, A., Husheem, M., Härkönen, P., & Pihlaja, K. (2002): Inhibition of cancer cell growth by crude extract and the phenolics of Terminalia chebula retz. fruit. *Journal of Ethnopharmacology*, ***81*(3):** 327-336.

[18] Zhang, Y., Vareed, S. K., & Nair, M. G. (2005): Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. *Life sciences*, ***76*(13)**: 1465-1472.

[19] Cieslik, E., Gręda, A., & Adamus, W. (2006): Contents of polyphenols in fruit and vegetables. *Food chemistry*, ***94*(1)**: 135-142.

[20] Qian, J. Y., Liu, D., & Huang, A. G. (2004): The efficiency of flavonoids in polar extracts of Lycium chinense Mill fruits as free radical scavenger. *Food Chemistry*, ***87*(2)**: 283-288.

[21] Sass-Kiss, A., Kiss, J., Milotay, P., Kerek, M. M., & Toth-Markus, M. (2005): Differences in anthocyanin and carotenoid content of fruits and vegetables. *Food Research International*, ***38*(8)**: 1023-1029.

[22] Trappey, A., Bawadi, H. A., Bansode, R. R., & Losso, J. N. (2005): Anthocyanin profile of mayhaw (Cretaegus opaca). *Food Chemistry*, ***91*(4)**: 665-671.

[23] Lin, J. Y., & Tang, C. Y. (2007): Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food chemistry*, ***101*(1):** 140-147.

[24] Hasler, C. M. (1998): Functional foods: their role in disease prevention and health promotion. *Food technology-champaign then chicago-*, ***52***: 63-147.

[25] Steinmetz, K. A., & Potter, J. D. (1996). Vegetables, fruit, and cancer prevention: a review. *Journal of the American Dietetic Association*, ***96*(10)**: 1027-1039.

[26] Steinberg, D. (1991): Antioxidants and atherosclerosis. A current assessment. *Circulation*, ***84***: 1420-1425.

[27] Block, G., Patterson, B., & Subar, A. (1992): Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutrition and cancer*, ***18*(1)**: 1-29.

[28] Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993): Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences*, ***90*(17)**: 7915-7922.

[29] Hertog, M. G., Feskens, E. J., Kromhout, D., Hollman, P. C. H., & Katan, M. B. (1993): Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*, ***342*(8878)**: 1007-1011.

[30] Byers, T., & Guerrero, N. (1995): Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. *The American journal of clinical nutrition*, ***62*(6)**: 1385S-1392S.

[31] Knekt, P., Järvinen, R., Seppänen, R., Heliövaara, M., Teppo, L., Pukkala, E., & Aromaa, A. (1997). Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *American journal of epidemiology*, ***146*(3)**: 223-230.

[32] Elliott, J. G. (1999): Application of antioxidant vitamins in foods and beverages: Developing nutraceuticals for the new millenium. *Food Technology*, ***53*(2)**: 46-48.

[33] Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables–the millennium's health. *International journal of food science & technology*, ***36*(7)**: 703-725.

[34] Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999): [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, ***299:*** 152-178.

[35] Zhishen, J., Mengcheng, T., & Jianming, W. (1999): The determination of flavonoid contents in mulberry and their scavenging

effects on superoxide radicals. *Food chemistry*, ***64*(4)**: 555-559.

[36] Benzie, I. F., & Strain, J. J. (1999): [2] Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in enzymology*, ***299****:* 15-27.

[37] Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995): Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, ***28*(1)**: 25-30.

[38] 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*(9)**: 1231-1237.

[39] Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001): Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of agricultural and food chemistry*, ***49*(10)**: 4619-4626.

[40] Mizushima, Y., & Kobayashi, M. (1968): Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology*, ***20*(3)**: 169-173.

[41] John, J. A., & Shahidi, F. (2010): Phenolic compounds and antioxidant activity of Brazil nut (Bertholletia excelsa). *Journal of Functional Foods*, ***2*(3)**: 196-209.

[42] Koley, T. K., Kaur, C., Nagal, S., Walia, S., & Jaggi, S. (2011): Antioxidant activity and phenolic content in genotypes of Indian jujube (Zizyphus mauritiana Lamk.). *Arabian Journal of Chemistry*.

[43] Liu, R. H. (2004): Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of nutrition*, ***134*(12)**: 3479S-3485S.

[44] Liu, R. H. (2007): Whole grain phytochemicals and health. *Journal of Cereal Science*, ***46*(3)**: 207-219.

[45] Dykes, L., & Rooney, L. W. (2007): Phenolic compounds in cereal grains and their health benefits. *Cereal foods world*, ***52*(3)**: 105-111.

[46] Budrat, P., & Shotipruk, A. (2008): Extraction of phenolic compounds from fruits of bitter melon (Momordica charantia) with subcritical water extraction and antioxidant activities of these extracts. *Chiang Mai J Sci*, ***13***: 123-130.

[47] Le Marchand, L. (2002): Cancer preventive effects of flavonoids—a review. *Biomedicine & pharmacotherapy*, ***56*(6)**: 296-301.

[48] Sreelatha, S., & Padma, P. R. (2009): Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. *Plant foods for human nutrition*, ***64*(4)**: 303.

[49] Siddhuraju, P., & Becker, K. (2003): Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*moringa oleifera* lam.) leaves. *J. Agric. Food Chem*, ***51****:* 2144-2155.

[50] Huang, D., Ou, B., & Prior, R. L. (2005): The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, **53**: 1841–1856.

[51] Umukoro, S., & Ashorobi, R. B. (2006): Evaluation of anti-inflammatory and membrane stabilizing property of aqueous leaf extract of Momordica charantia in rats. *African Journal of Biomedical Research*, ***9*(2)**: 119-124.

[52] Lin, W. C., & Lin, J. Y. (2010): Five bitter compounds display different anti-inflammatory effects through modulating cytokine secretion using mouse primary splenocytes in vitro. *Journal of agricultural and food chemistry*, ***59*(1)**: 184-192.

[53] Kobori, M., Nakayama, H., Fukushima, K., Ohnishi-Kameyama, M., Ono, H., Fukushima, T., ... & Deguchi, T. (2008): Bitter gourd suppresses lipopolysaccharide-induced inflammatory responses. *Journal of agricultural and food chemistry*, ***56*(11)**: 4004-4011.

[54] Milind, P., & Kaur, S. (2011): Is bottle gourd a natural guard. *Int Res J Pharm*, *2*(6): 13-17.

[55] Ghule, B. V., Ghante, M. H., Upaganlawar, A. B., & Yeole, P. G. (2006): Analgesic and anti-inflammatory activities of Lagenaria siceraria Stand. fruit juice extract in rats and mice. *Pharmacognosy magazine*, ***2*(8)**: 232.

[56] Sulaiman, M. R., Zakaria, Z. A., Bujarimin, A. S., Somchit, M. N., Israf, D. A., & Moin, S. (2008): Evaluation of Moringa oleifera aqueous extract for antinociceptive and anti-inflammatory activities in animal models. *Pharmaceutical biology*, ***46*(12)**: 838-845.