Evaluation of phytochemicals and anti-nutritional profile in underutilised green leafy vegetables

Namrata Sethi[a], Prerna Gupta[b], Jasvinder Singh Bhatti[c], Priyanka Kundu[b]
[a] Department of Food and Nutrition, Government Home Science College, Chandigarh India
[b] Department of Food Technology and Nutrition, Lovely Professional University, Phagwara, Punjab India
[c] Department of Human Genetics and Molecular Medicine, School of Health Sciences, Central University of Punjab, Bhatinda India

Abstract

Due to diverse climate in India, wide variety of green leafy vegetables can be found which are still unexplored and underutilized. In addition to being resilient and adaptive, these vegetables also demand low cost plantation and harvesting. Demand for sufficient food and nutrition has led to scout more of these vegetables. A study was conducted to assess the anti-nutritional and phytochemicals profile of some underutilized and commonly consumed green leafy vegetables (GLV) to increase their acceptability in the daily diet. Amaranth (Amaranthusviridis), cauliflower leaves (Brassica oleracea var. botrytis), radish leaves (Raphanus raphanistrum subsp. sativus), spinach (Spinacia oleracea) and lettuce leaves (Lactuca sativa) were collected locally. The leaves were washed and tray dried in a hot air oven at 60°C for 2-3 h and ground into powder. They were tested for presence of phytochemicals and anti-nutritional components phytic acid, nitrate, oxalate, tannin, and alkaloids. Among the GLV cauliflower greens had the highest content of oxalate (97.78±0.02 mg/100 g) followed by radish leaves (84.5±0.02) and spinach (72.51±0.02), respectively. Higher levels of phytic acid occurred in lettuce (26.89±0.02 mg/100 g), amaranth (25.79±0.02), and cauliflower greens (25.50±0.02). Tannin content was in cauliflower (37.79±0.03 mg/100 g), spinach (39.89±0.02), radish (42.54±0.02), amaranth (41.26±0.02), and lettuce (43.7±0.02). No differences occurred in nitrate content of all GLVs (cauliflower (12.44±0.01 mg/100g), radish (9.8±0.02), spinach (7.9±0.28), amaranth (11.86±0.01), lettuce (12.76±0.02). Alkaloid content of the GLVs was highest in cauliflower (72.81±0.01 mg/100 g) followed by radish (68.09±0.02) and lowest in lettuce (48.8±0.02). The

GLVs offer cheap, rich sources of several phytochemicals. Phytochemicals are considered to be new form of medicine inducing health. The anti-nutritional content is of no consequence and may be reduced by various processing and cooking methods. Without any interference from anti nutritional factors, greens can be tapped to their maximum potential. These underutilised greens can be developed as value-added food products but mostly greens serve as fodder for animals and is wasted. This study proves that the beneficial extracts from these underutilised green leafy vegetables can be used as rich sources of micronutrient and phytochemicals in our daily diet.

Keywords: Vegetables, antioxidants, micronutrients, phytochemicals, therapeutic

Introduction

Emerging as Nutraceuticals, Phytochemicals are bioactive compounds which are obtained from plants including luteins, diallylsulfides, isothiocyanates, antioxidants polyphenols, carotenoids, vitamins, phytoestrogens, glucosinolates, anthocyanins and flavonoids. Mostly found in vegetables, they have many potential health benefits (Carbonell-Capel et al., 2013; Gil-Chávez et al., 2013; Yalsan and Capar, 2017). Along with these phytochemicals, some vegetables also contain a certain amount of the anti-nutritional substances like oxalates, saponins, tannins phytic acid, and alkaloids (Begum and Manjula, 2015; Aguilar et. al., 2008) known to cause gastrointestinal disturbances and have an adverse effect on the absorption of calcium and iron. These side effects can be minimised by post-harvest heating and drying, canning, freezing, or by breeding varieties having lower anti-nutritional factors. (Ranum, et. al., 2014 and Gupta et.al., 2011). Housing these both come underutilised green leafy vegetables (GLVs) which not only increase iron and serum retinol concentrations but also help in lowering cholesterol and control blood glucose levels (Balakrishnan et.al., 2009). There are green leafy vegetables which are underutilized in many locations of India and can be exploited for natural antioxidants and anti-nutritional factors (Subhashree et. al., 2009; Sheela et. al., 2004). The study was conducted to assess the anti-nutritional and phytochemical profile of some commonly consumed underutilized green leafy vegetables to determine their suitability for consumption.

Materials and methods

Five leafy vegetables viz., amaranth (*Amaranthusviridis)*, cauliflower leaves (*Brassica oleracea var. botrytis*), radish leaves (*Raphanusraphanistrum subsp. Sativus*), spinach *(Spinaciaoleracea*) and lettuce leaves (*Lactucasativa*) were purchased from the local markets of Chandigarh. On removing leaves and stalks , they were washed under running cold water. After washing, surface drying of the leaves was done and then shifted to hot air oven operated at 60˚C for 2-3h. Fine powder was made of the leaves after drying and stored in an airtight container for further use.

Phytic acid was determined according to the method as suggested by Sadasivam and Manickam, 2007. About 0.5g sample was extracted with 20 ml of 0.5M nitric acid for 3-4 hours with continuous shaking. The final intensity of the colour so formed was checked at 465nm by using a spectrophotometer against a blank of amyl alcohol. The amount of phytic acid can be calculated from the standard curve. Tannins were determined by the Folin-Ciocalteu method and the absorbance was read at 760nm (Saeed et. al., 2012). All the solutions were made fresh and diluted to volume with water.0.5gm of the sample was transferred to a 50ml volumetric flask containing 30ml 0.25 N HCl for the estimation of oxalates. The mixture was boiled for 15minutes. Blank was prepared by using 2 ml of 1 N H_2SO_4 (Naik et. al., 2014). The absorbance was measured at 525nm. According to Chukwuma et. al.,2016 alkaloids were determined by using 2.5gm of the dried sample in which 200ml of 10% acetic acid solution was added and allowed to stand for 4 hours. The extract was then concentrated and 15 drops of concentrated ammonium hydroxide was added. After 3 hours, the supernatant so formed were discarded and the precipitates were treated with 20ml of 0.1M ammonium hydroxide and then dried. The final percentage of the alkaloids can be expressed by taking the weight of the alkaloid over the weight of the sample used into 100. Nitrates were determined according to the method used by Gaya et. al., 2006 and the final absorbance was read at 407nm.

Results and discussion

Table 1 shows the quantitative measurements of the anti-nutritional factors and phytochemicals of the selected underutilized greens. It is well established that GLVs have an important place in our diet due to their high micronutrient content. The presence of anti-nutritional factors should not hinder their consumption. It was observed in the following study that the amount and types of anti-nutritional factors found were in limited amounts and not harmful for consumption. All the anti-nutritional factors assessed have little or no impact on the absorption of nutrients and in turn, they help enhance the phytochemical profile of the vegetables. Different processing techniques have an impact on the anti-nutritional factors in foods and thus can be utilised to decrease the amounts of undesirable factors found (Natesh et. al.,2017).

The content of phytic acid in storage organs is much higher than the amount found in leaves. (Lott et. al., 2005; Raboy 2003). The phytic acid content of the leaves ranged from 21.67-26.89mg/100gm (Figure 1) with radish leaves having the least amount and maximum levels were found in lettuce leaves. Alkarawi et.al 2014 studied the presence of phytic acid in green leaves and found that apart from roots, (Campbell et al., 1991), a sufficient amount of phytic acid was also found in tubers (Samotus &Schwimmer 1962) and leaves (Bentsink et al . 2003). De Turk 1933 established that phytic acid levels are lower in the leafy part of the vegetables. Winkler & Zotz (2009) was one of the first to indicate a potentially important ecological role of phytic acid in leaves as herbivore deterrent. Otitujo et.al.,2014 observed that the phytochemical contents of the vegetables are affected by processing. The phytochemical contents of most of the vegetables in their raw forms increased when shade dried and decreased when cooked.

The nitrate content of the selected green leafy vegetables ranged from 7.9-12.76mg/100gms (Figure 2) with spinach having the lowest content and lettuce the maximum. Due to their role in nitrogen cycle and growth and development of plants, nitrates are considered an important natural constituent of plants (Lucariniet al., 2012; Boink, 2001; EFSA, 2008). A study conducted by Lammarino, 2014, also found that lettuce contains the highest concentrations of nitrate. A vegetable-rich diet is known to lower the risk of developing cardiovascular and other gastric complications (Naseem, 2005, Larsen, 2005, Webb, 2008; Bahra, 2012, Tang, 2011, Bondonono, 2016). Thus, the presence of nitrates in small amounts in the green leafy vegetables is considered safe and beneficial for human consumption.

The tannin contents were similar in all the vegetables included in this study (Figure 3). The cauliflower contains the least amount (37.795mg/100gm) whereas lettuce has maximum amount of tannins (43.7mg/100gm). Several studies report the health benefit of tannins (Kumari et. al., 2012). However, the literature on tannins composition in vegetables, particularly in leafy vegetables is scanty. Rao and Prabhavati, 1982 reported that a daily intake of tannin between 1.5 – 2.5g is safe for consumption and does not cause any side effects. In turn, tannins are known to act as phytochemicals and help in the prevention of degenerative and lifestyle diseases (Tuli et. al., 2017). None of the GLVs undertaken in the present study reported high levels of tannins and can safely be consumed in the daily diet.

The oxalate content was found highest in cauliflower leaves (97.78mg/100gm) and lowest in lettuce leaves (54.9mg/100gm). In spinach specifically, the oxalate is present in soluble form. This binds with the calcium present in food and thereby reduces its ability to be absorbed (Brorgren et. al., 2003). Cooking methods especially boiling markedly reduced soluble oxalate content by 30-87% (Chai et.al.,2005)

Alkaloids are naturally occurring compounds having anti-microbial properties. The types of alkaloids found in green leafy vegetables have no adverse effects on human health and can be considered safe for consumption (Ranjitha et al., 2005). Thus, the alkaloid content found in the green leafy vegetables tested was highest in cauliflower greens (72.81mg/100gm) and least in lettuce (48.8mg/100gm). The alkaloid content was higher in raw vegetables as compared to boiled ones (Onyeka et al., 2007). The alkaloid content of cauliflower leaves is known to have herbicidal and anti-microbial effects. Furthermore, the anti-nutritional factors present in them serve as phytochemicals and help to alleviate several diseases (Saadet.al.,2017).

Table 1: Anti-nutritional and phytochemical profile of selected GLVs

Name of leafy greens	Phytic acid (mg/100 g)	Oxalate (mg/100 g)	Nitrate (mg/100 g)	Tannin (mg/100 g)	Alkaloid (mg/100g)
Cauliflower	25.50±0.02*	97.78±0.02	12.44±0.01*	37.79±0.03*	72.81±0.01*
Radish	21.67±0.02 *	84.5±0.028	9.8±0.02*	42.54±0.02*	68.09±0.02*
Spinach	22.42±0.02 *	72.51±0.02	7.9±0.28*	39.89±0.02*	53.22±0.01*
Amaranth	25.79±0.02 *	65.79±0.02	11.86±0.01	41.26±0.02*	57.88±0.01*
Lettuce	26.89±0.02 *	54.9±0.02	12.76±0.02 *	43.7±0.028*	48.8±0.028*

All the values are represented as mean±SD.

*The mean difference is significant at the 0.05 level using one way ANOVA followed by Dunnet's t test

Figure 1: Phytic acid content of the greens/100 g.

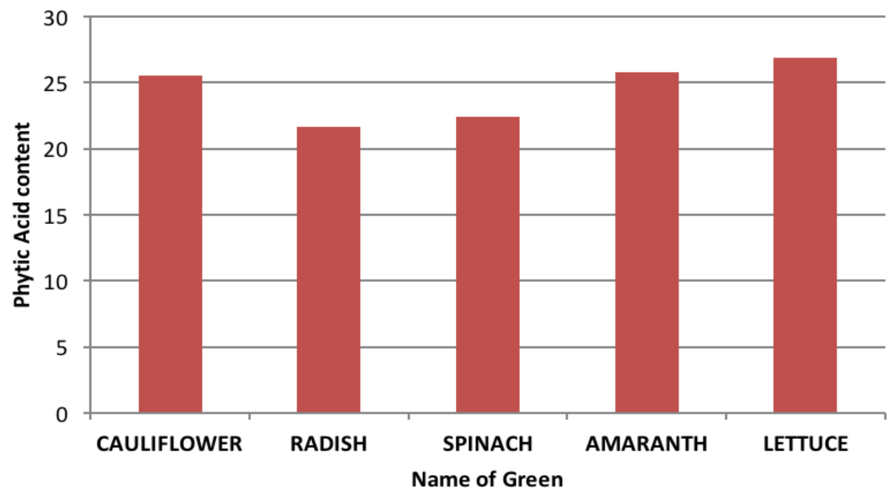


Figure 2: Nitrate content of the greens/100 g

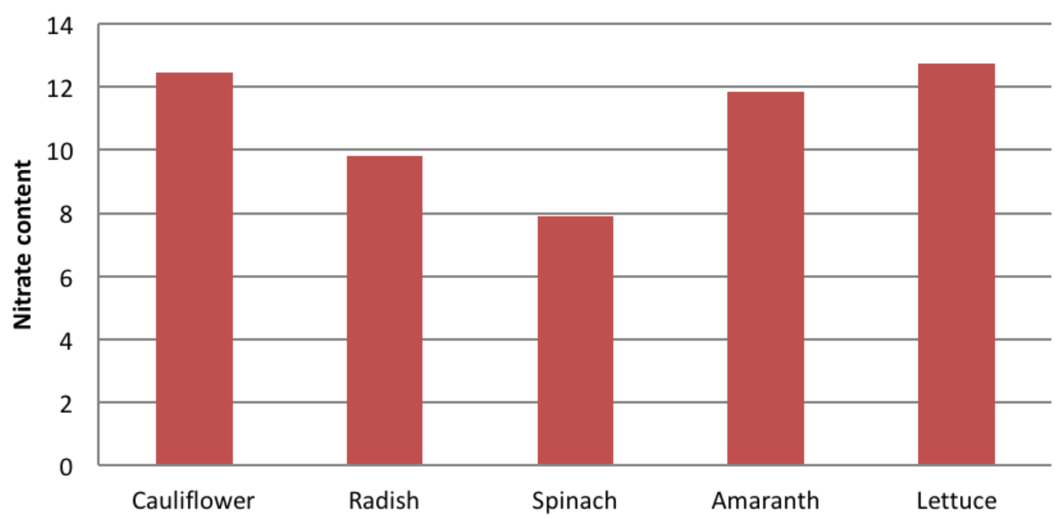


Figure 3: Tannin content of the greens mg /100 g

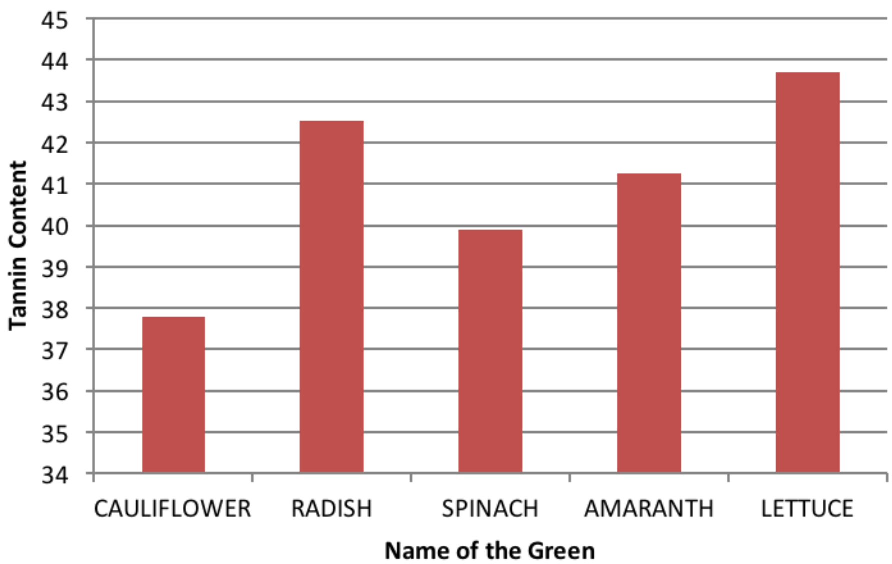


Figure 4: Oxalate content of the greens mg/100 g

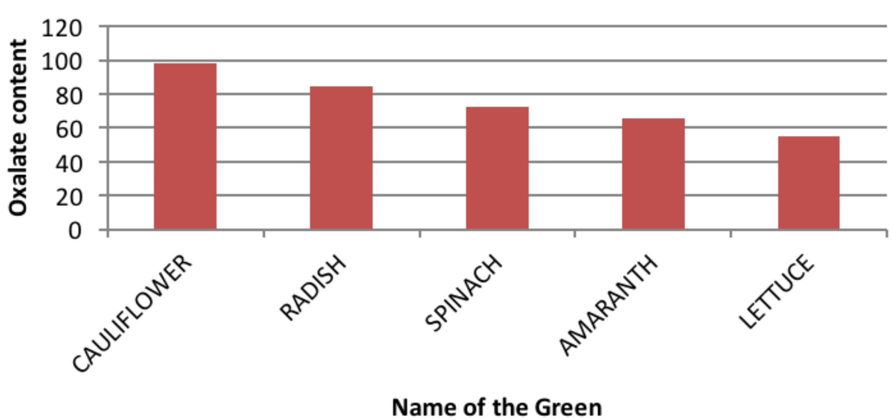
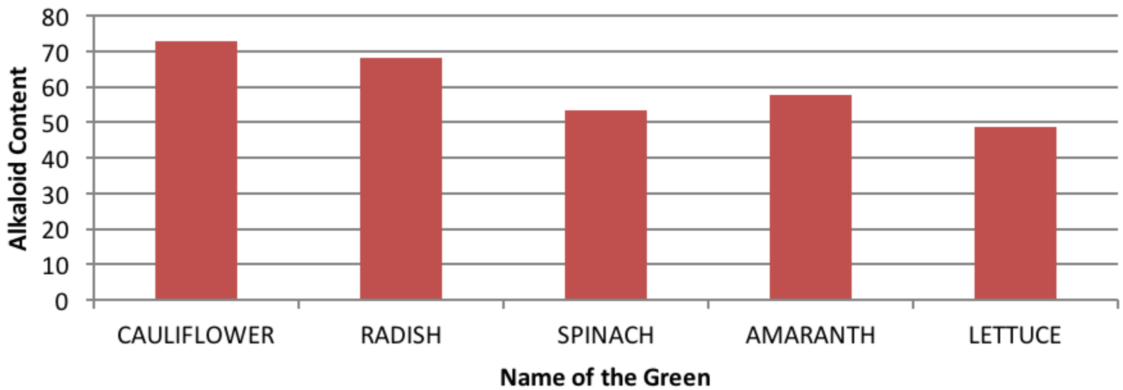


Figure 5: Alkaloid content of the greens mg /100gms



Conclusion

This study emphasize on use of underutilized and unexploited green leafy vegetables dense in health imparting phytochemicals. Various tests conducted on GLF attempts to ward off any myths surrounding the consumption of green leafy vegetables and their avoidance due to their anti-nutritional content. The daily consumption of green leafy vegetables shows many beneficial effects that may tend to overcome the anti-nutritional components present in them. Not forgetting the important implementation of basic household techniques like washing, blanching etc. The amount of green leafy vegetables consumed, and the percentage of anti-nutrients present in them, are well below the toxic levels for consumption by humans. Thus, efforts must be made to include green leafy vegetables as an integral part of our daily diet as they are a storehouse of nutrients and help in alleviating various health related complications especially micronutrient deficiencies. Bringing these crops into cultivation and consumption will also provide much needed nutritional security to general populace.

References

1. Aguilar, G., Sánchez, M., Martinez-Téllez, Miguel, Olivas, Guadalupe, Alvarez-Parrilla, Emilio, De la Rosa&Laura. (2008). Bioactive compounds in fruits: Health benefits and effect of storage conditions. Stewart Postharvest Review. 4. 1-10. 10.2212, 3.8.
2. Bagepalli, S., Rao, N and Tatineni, P (1982). Tannin content of foods commonly consumed in India and its influence on Ionisable Iron. Journal of the Science of Food and Agriculture. 33 (1).
3. Bahra, M., Kapil, V., Pearl, V., Ghosh, S.A., &Ahluwalia, A. (2012). Inorganic nitrate improves vascular compliance but does not alter flow mediated dilatation in healthy volunteers. Nitric Oxide 26:197–202.
4. Balakrishnan, S., Bhaskar, R., Keerthana, L.R., &Lijina, S. R., &Rajasekaran, P. (2009). Evaluation of antioxidant potential in selected green leafy vegetables. Food Chemistry. 115. 1213-1220. 10.1016/j.foodchem.
5. Bentsink, L., &Koomneef, M. (2003). Seed dormancy and germination. The arabidopsis book, 6, e0119.
6. Boguslaw, S., &Schwimmer, S. (1962). Effect of Maturity & Storage on Distribution of Phosphorus among Starch & Other Components of Potato Tuber. Plant Physiology, 37(4), 519-522. Retrieved from http://www.jstor.org/stable/4259957
7. Boink, A., &Speijers, G. (2001). Health Effects of Nitrates And Nitrites, A Review. Acta Horticulturae. 29-36. 10.17660/ActaHortic.563.2.
8. Campbell, M., Dunn, R., Ditterline, R., Pickett, S., Raboy, V. (1991). Phytic acid represents 10 to 15% of total phosphorus in alfalfa root and crown, Journal of Plant Nutrition, 14:9, 925-937.
9. Carbonell-Capella, J. M., Barba, F. J., Esteve, M. J., &Frígola, A. (2014). Quality parameters, bioactive compounds and their correlation with antioxidant capacity of commercial fruit-based baby foods. Food science and technology international 20(7), 479–487.
10. Catherine, P., Bondonno, Lauren, C.,Blekkenhorst, Richard, L., Prince, Kerry, L., Ivey, Joshua, R., Lewis, Amanda, Richard, J., Woodman Jon, O., Lundberg, Kevin, Croft,

Peter, Thompson, Jonathan, M., Hodgson(2016). Association of Vegetable Nitrate Intake With Carotid Atherosclerosis and Ischemic Cerebrovascular Disease in Older Women. Stroke; 48:1724-1729.

11. Chai, W., & Leibman, M. (2005). Effect of Different Cooking Methods on Vegetable Oxalate Content. Journal of agricultural and food chemistry. 53, 3027-30.
12. Charlie, J., Andrea, D., Kevin, P., Irene, O., Victor, R., John, N.A., &Lott (2005). The concentrations and distributions of phytic acid phosphorus and other mineral nutrients in wild-type and low phytic acid Js-12-LPA wheat (Triticumaestivum) grain parts. Canadian Journal of Botany, 83:1599-1607.
13. Chukwuma, S., Ezeonu, Chigozie, M., &Ejikeme, (2016). "Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods," New Journal of Science, Article ID 5601327: 9.
14. DE Turk, EE (1933). Chemical transformation of phosphorus in the growing corn plant, with results on two first-generation crosses, Journal of Agricultural Research 46, 121-141.
15. EFSA, (2008). Nitrate in vegetables Scientific Opinion of the Panel on Contaminants in the Food chain. The EFSA Journal 689, 1-79.
16. Gaya, U.I., &Alimi, S. (2006). Spectrophotometric Determination of Nitrate in Vegetables Using Phenol. J. Appl. Sci. Environ. Mgt.10 (1) 79 – 82.
17. Gowthami, R., Prakash, B.G., Raghavendra, K.V., Brunda, S.M. and Niranjana Kumara, B. (2019). Role of underutilised leafy vegetables to attain nutrition security. Acta Hortic. 1241,583-588.DOI:10.17660/ActaHortic.2019.1241.86.
18. Gupta, S., & Prakash, J. (2011). Nutritional and sensory quality of micronutrient-rich traditional products incorporated with green leafy vegetables, International Food Research Journal; 18: 667-675.

19. Hazra, D.K., Sharma, R. D. Sarkar, A., Mishra, B., Singh, J.B., Maheshwari, B. B. (1996). Toxicological Evaluation of Fenugreek Seeds: A Long Term Feeding Experiment in Diabetic Patients, Phytotherapy Research 1996-02-01 10(6): 519-520.
20. Iammarino, M., Di Taranto, A., & Chistino, M. (2014). Monitoring of nitrites and nitrates levels in leafy vegetables (spinach and lettuce): a contribution to risk assessment. J Sci Food Agriculture 15; 94(4):773-8.
21. Joana Gil-Chávez G, Villa JA, Fernando Ayala-Zavala J, Basilio Heredia J, Sepulveda D, Yahia EM, González-Aguilar GA (2013) Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. Compr Rev Food Sci Food Saf 12:5–23
22. Kumari, M., Jain, Shashi. (2012). Tannin: An Antinutrient with Positive Effect to Manage Diabetes. Research Journal of Recent Sciences. Vol I.
23. Larsen, FJ.,Ekblom, B., Sahlin, K., Lundberg, JO., Weitzberg, E. (2005). Effects of dietary nitrate on blood pressure in healthy volunteers. N Engl J Med, 355, 2792–2793.
24. Lucarini, M., D’Evoli, L., Tufi, S., Gabrielli, P., Paoletti, S., Di Ferdinandob, S., Lombardi-Bocciaa, G. (2012). Influence of growing system on nitrate accumulation in two varieties of lettuce and red radicchio of Treviso.
25. Madelene, B., & Geoffrey, P.S. (2003). Bioavailability of soluble oxalate from spinach eaten with and without milk products. Asia Pacific J ClinNutr ;12 (2): 219-224
26. Naik, V.V., Patil, N.S., Aparadh, V.T., & Karadge, B.A. (2014). Methodology in determination of oxalic acid in plant tissue: A Comparative Approach.
27. Naseem, KM. (2005). The role of nitric oxide in cardiovascular diseases. Mol Aspects Med, 26:33–65.
28. Nath, S.K.G., D, K.V., Yankanchi, M., Patil, G.B., Roopa. (2004). Proximate Composition of Underutilized Green Leafy Vegetables in Southern Karnataka. J. Hum. Ecol. 15. 227-229.
29. Nitesh, HN., Abbey, L., Asiedu, SK. (2017). An overview of nutritional and anti-nutritional factors in green leafy vegetables. HorticultIntJ ;1(2):58–65.

30. Onyeka, Uloma, Nwambekwe, I.O. (2007). Phytochemical profile of some green leafy vegetables in South East, Nigeria. Nigerian Food Journal, Vol 25.
31. Otitoju1, J.U., Nwamarah1, O., Otitoju2, E.C., Odoh1Iyeghe1, L.U. (2014). Phytochemical composition of some underutilsed green leafy vegetables in nsukka urban Lga of Enugu State G.T.O. Journal of Biodiversity and Environmental Sciences, Vol. 4, No. 4, p. 208-217.
32. Palanisamy, A., Kannan, D., Kavitha, M., Ravindran, C. and Rajangam, J. (2019). Potential vegetable crops - need for exploration and conservation for nutritional security for rural livelihoods. Acta Hortic. 1241, 69-74 DOI: 10.17660/ActaHortic.2019.1241.11
    Parveen Begums S and Manjula K., (2015). Bio active components in fruits and vegetables – potential sources for food to food fortification. International Journal of Nutrition and Agriculture Research. 2(1), 19 - 24
33. Raboy, V. (2003). myo-Inositol-1,2,3,4,5,6 hexakisphosphate, Phytochemistry, vol. 64,1033-1043.
34. Ranjitha, D., K, Sudha. (2005). Alkaloids In Foods International Journal Of Pharmaceutical, Ch0emical And Biological Sciences,5(4), 896-906.
35. Ranum, P., Peña-Rosas, J. P., Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. Annals of the New York Academy of Sciences, 1312(1), 105–112.
36. Saad, I., Rinez, I., Ghezal, N., and Haouala, R. (2017). Chemical composition and herbicidal potent of cauliflower (Brassica oleracea var. botrytis) and cabbage turnip (Brassica oleracea var. gongylodes). Tunisian Journal of Plant Protection 12: 95-113.
37. Sadasivam, S., Manickam, A. (2007). Biochemical Methods. New Age International (P) Limited.
38. Saeed, N., Khan, M.R., Shabbir, M. (2012). Antioxidant activity, total Phenolic and Total Flavonoid contents of whole plant extracts Torilisleptophylla L. BMC Complementary and Alternative medicine; 12:221.

39. Shrilakshmi, B. (2004). Food Science. New Age International Publishers. New Delhi; 401
40. Slavin, L., Lloyd, B. (2012). Health Benefits of Fruits and Vegetables. AdvNutr. Jul; 3(4): 506–516.
41. Subhasree, B., Baskar, B., Keerthana, R.L., Susan, R.L., Rajasekaran, P. (2009). Evaluation of antioxidant potential in selected green leafy vegetables, Food Chemistry, Volume 115, Issue 4,1213-1220.
42. Tang, Y., Jiang, H., Bryan, NS. (2011). Nitrite and nitrate: cardiovascular risk-benefit and metabolic effect. CurrOpinLipidol, 22(1):11-5.
43. Tuli, R.T., Rahma, M.M., Abdullah, A, T., Akhtauzzaman, M., NazrulIslam, S. (2017). Phytochemicals - Tannins in some Leafy Vegetables of Bangladesh. Indian Journal of Nutrition, Volume: 3.
44. Webb, AJ., Patel, N., Loukogeorgakis, S., et al. (2008). Acute blood pressure lowering, vasoprotective, and anti-platelet properties of dietary nitrate via bioconversion to nitrite. Hypertension, 51:784–790.
45. Winkler, U., Zotz, G. (2009). Highly efficient uptake of phosphorus in epiphytic bromeliads, Annals of Botany, vol. 103, 477-484.
46. Yalcin, H., Çapar, T.D. (2017). Bioactive Compounds of Fruits and Vegetables.
47. Yildiz, F., Wiley, R. Minimally Processed Refrigerated Fruits and Vegetables. Food Engineering Series. Springer, Boston, MA.