Pharmacogn. Res., 2025; 17(1):53-59.
https://www.phcogres.com

Original Article

Profiling of Calcium Content among the Leafy Vegetables of West Bengal Using Atomic Absorption Spectroscopy

Durba Banerjee, Palash Mondal, Prerona Saha*

Department Pharmaceutical Chemistry, Guru Nanak Institute of Pharmaceutical Science and Technology, Panihati, Kolkata, West Bengal, INDIA.

ABSTRACT

Background: Leafy vegetables are essential for maintaining a balanced diet and are well known for their abundant amount of vitamins, minerals and dietary fibers. Leafy vegetables are significant dietary source of calcium, among other nutrients. It's important to maintain adequate calcium levels in the body for optimal health. A calcium deficiency can lead to conditions such as osteoporosis, osteopenia and osteomalacia. **Objectives:** This study aims to determine dietary calcium content among some leafy vegetables that are available in West Bengal using Atomic Absorption Spectroscopy (AAS), to provide valuable insights into their nutritional value. **Materials and Methods:** Eight leafy vegetables were selected, *viz.* bottle gourd leaf, onion leaf, mustard greens, pot-biroot leaf, wild celery leaf, garlic leaf, spreading hogweed leaf and chickpea leaf. Using Atomic Absorption Spectrometry (AAS), Ca content of the dried powdered samples was quantified by wet oxidation as well as by dry ashing method. **Results:** The highest amount of Ca is present in mustard greens that are 704.4±40.18 mg/100 g and 504.2±361.47 mg/100 g by wet oxidation and dry ashing respectively. **Discussion:** The best three dietary recommendations from selected leafy vegetables are mustard greens>bottle gourd leaf>chickpea leaf. **Conclusion:** All the selected leafy greens have a significant amount of Ca. Further study can be extended to investigate their bioavailability to confirm the most beneficial form of Ca for leafy vegetables.

Keywords: Atomic Absorption Spectrometry (AAS), Calcium, Leafy vegetables, West Bengal.

Correspondence:
Dr. Prerona Saha
Associate Professor, Department Pharmaceutical Chemistry, Guru Nanak Institute of Pharmaceutical Science and Technology, Panihati, Kolkata-700114, West Bengal, INDIA.
Email: prerona.saha@gnipst.ac.in

Received: 10-06-2024;
Revised: 25-07-2024;
Accepted: 04-09-2024.

INTRODUCTION

Leafy vegetables are consumed by the people of worldwide as a traditional food in the form of different dishes. They are commonly consumed by the people of West Bengal. Leafy vegetables are an excellent source of dietary nutrition; they are a packed source of essential vitamins and minerals, including vitamin A, C, K and folate, as well as calcium, magnesium and potassium. These nutrients are essential for maintaining good health and also in the prevention of different diseases.[1]

Calcium is a vital mineral for maintaining different physiological activities within the human body. It is crucial in bone and teeth development, muscle contraction, blood clotting, the transmission of nerve impulses, cell signalling, enzyme activation and hormone secretion.[2,3]

An adequate amount of calcium is thus essential for maintaining the general well-being of the human body and also to prevent Ca deficiency-related disorders. The most common functions of the Ca are represented in Figure 1.

Calcium provides structural support to bones by forming hydroxyapatite crystals, these crystals contribute to the hardness and strength of bones.[4,5] In muscle contraction, Ca plays an important role. Ca stimulates the actin filament and initiates the contraction of the muscle.[6-9] After any injury, blood coagulation occurs in three steps which include the activation of clotting factors, platelet aggregation and the formation of a stable blood clot.[10-12] In the different steps of blood coagulation, Ca is utilized. Ca acts as a secondary messenger for cell signalling. Ca helps in the release of neurotransmitters and leads to signal transmission. In some biochemical reactions enzymes that are present in their inactive form are activated by the Ca where Ca is used as a cofactor.[13-15]

Ca deficiency is known as hypocalcemia, which leads to various Ca deficiency symptoms. The body may experience several illnesses and symptoms if there is not sufficient amount of Ca present.[5] The common conditions linked to insufficient calcium within the body are osteoporosis, rickets, tetany hypoparathyroidism and so on (Figure 2).[16-20]

DOI: 10.5530/pres.20251752

Epidemiological study on Ca-related bone disorders revealed that around the world, approximately 200 million individuals experience osteoporosis annually, which is characterized by low bone density. The risk of osteoporosis is higher in women compared to men and in older adults compared to younger individuals. Statistics from World Health Organization (WHO) indicate that 30% of postmenopausal women have osteoporosis. In India, 61 million people are reported to have osteoporosis till now, with 80% of them being women. Statistical reports of 2021, show that osteoporosis affects one in five adults in India, while osteopenia (precursor to osteoporosis) is present in one in two adults. In terms of the elderly population, two in five older women and one in three older men have osteoporosis. Additionally, bone biopsy data revealed the occurrence of osteomalacia and osteoporomalacia (combination of osteoporosis and osteomalacia). These bone diseases have risen notably and are expected to increase further in the coming years and are a major public health issue around the world.

Ca deficiency leads to various complications and disorders, which are at present treated with some medications, however these are found to have different side effects.[21] Ca rich foods must be included in the daily diet to prevent Ca deficiency disorders. Leafy vegetables are one of the most available and affordable sources of Ca.[22] Ca content analysis has not been established for some of the leafy vegetables till date. This study aims to carry out the quantitative assessment of dietary calcium present in such eight leafy vegetables of West Bengal using an atomic absorption spectrometer, for whom calcium content is not yet determined by the AOAC (Association of Official Analytical Chemists) method of analysis.

MATERIALS AND METHODS

Collection of Plant samples: Following eight leafy vegetables that commonly consumed by the people of West Bengal were collected from sodepur area of West Bengal in august 2023 (Table 1).

Processing of the samples

The collected plant samples were shade dried and ground into fine powder. For Ca content analysis, powdered samples were then treated by wet oxidation and dry ashing methods as follows:

Wet oxidation

0.5 g of each powder sample was weighed and transferred to a 100 mL beaker, 10 mL of 65% v/v Nitric acid (HNO_3) was added to them and the mixtures were digested on a hot plate for 30 min at 55-70°C. After digestion, the mixtures were cooled at room temperature and 70-75% v/v 5 mL perchloric acid ($HClO_4$), was added. The mixtures were digested again on a hot plate until clear solutions were obtained. Following this, the mixtures were cooled and diluted with distilled water. The mixtures were then filtered using Whatman filter papers and the filtrates were then transferred to the respective 100 mL volumetric flasks and the volume of the samples were made up using distilled water.[23-28]

Dry ashing

5 g of each powder sample was weighed and transferred to the respective crucibles. The crucibles were then placed in a muffle furnace where they were subjected to a temperature of 500°C for 1 hr. The ashes of the powders were then digested with a mixture of hydrochloric acid (37% v/v) and nitric acid (65% v/v) in a 1:3 ratio for 30 min on a hot plate. Then the digested samples were diluted with distilled water. Following this, the samples were filtered and the filtrates were collected in the respective 50 mL

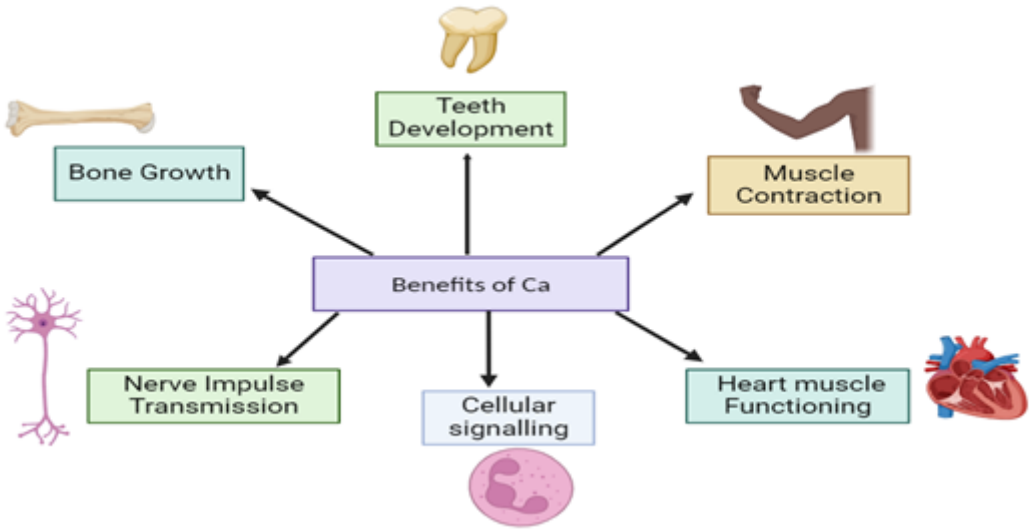

Figure 1: The most common functions of the calcium.

volumetric flask and the volume of the samples was made up using distilled water.[29-35]

Calibration of atomic absorption spectrometer

2% v/v HNO_3 was prepared and used as a blank and the calibration standards of 0.062, 3 and 5 mg/L Ca solutions were prepared using the stock solution of Ca (1.012 g/mL). After that by considering some factors like the wavelength of light: 422.67 nm, slit width: 0.7 nm, oxidant: Air, oxidant flow: 10 L/min and acetylene flow: 2.7 L/min, the calibration curve was prepared.[23-35]

Analysis for Ca

Each sample was analysed for Ca using the atomic absorption spectrometer Perkin (Elmer pinAAcle 500) using 2% v/v HNO_3 as a blank. The concentration of Ca was presented in mg/100 g (mean±SD) for the selected leafy vegetables.

Table 1: Eight selected leafy vegetables of West Bengal.

Sl. No.	Common Name	Scientific Name	Family name	Pictures
1	Bottle gourd leaf (Lau shak)	*Lagenaria siceraria*	Cucurbitaceae	
2	Onion leaf (Peyaj pata)	*Allium cepa*	Amaryllidaceae	
3	Mustard green (Sorse shak)	*Brassica juncea*	Brassicaceae	
4	Pot-biroot Leaf (Gima Shak)	*Glinus oppositifolius*	Molluginaceae	
5	Wild celery leaf (Radhuni pata)	*Trachyspermum roxburghianum*	Apiaceae	
6	Garlic leaf (Rosun pata)	*Allium sativum*	Amaryllidaceae	
7	Spreading hogweed leaf (Punarnava shak)	*Boerhavia diffusa*	Nyctaginaceae	
8	Chickpea leaf (Chhola shak)	*Cicer arietinum*	Fabaceae	

RESULTS

Calibration curve of AAS

After calibration, the calibration curve for Ca that is absorbance versus concentration was obtained (Figure 3).

Calcium content in selected leafy vegetables

Wet oxidation

Among the selected leafy vegetables, in the wet oxidation method mustard green showed the highest amount of Ca 704.400±40.18 mg/100 g, followed by bottle gourd leaf (573.800±36.240 mg/100 g), chickpea leaf (566.800±58.380 mg/100 g), wild celery leaf (351.400±31.300 mg/100 g), garlic leaf (192.280±9.810 mg/100 g), spreading hogweed leaf (137.340±6.083 mg/100 g), pot biroot leaf (81.060±8.768) and onion leaf (32.800±0.26 mg/100 g). Onion leaves were found to possess the least amount of Ca. The graphical representation is displayed in Figure 4.

Dry ashing

The estimated Ca content in the mustard green by dry ashing is 504.2±361.47 mg/100 g which is the highest amount among the selected leafy vegetables, followed by bottle gourd leaf (112.0±73.65 mg/100 g), chickpea leaf (70.24±0.09 mg/100g), wild celery leaf (60.980±23.030 mg/100 g), garlic leaf (46.690±24.800 mg/100 g), spreading hogweed leaf (9.975±0.680 mg/100 g), pot biroot leaf (7.368±0.524 mg/100 g) and onion leaf (1.366±0.046 mg/100 g). The graphical representation is displayed in Figure 5.

DISCUSSION

Among the wet oxidation and dry ashing, wet oxidation is preferred for the estimation of Ca content by the AAS method. During the processing of samples by wet oxidation method, acid like HNO_3 and heat are used to oxidize organic impurities present in the sample, resulting in more amount of Ca remaining as Ca^{++} form. On the other hand, in dry ashing only high temperature

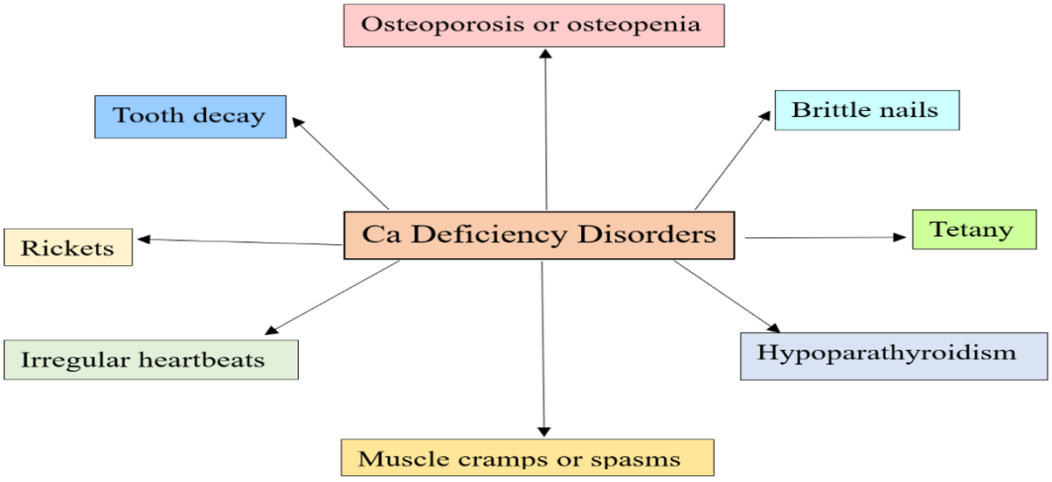

Figure 2: Calcium deficiency disorders.

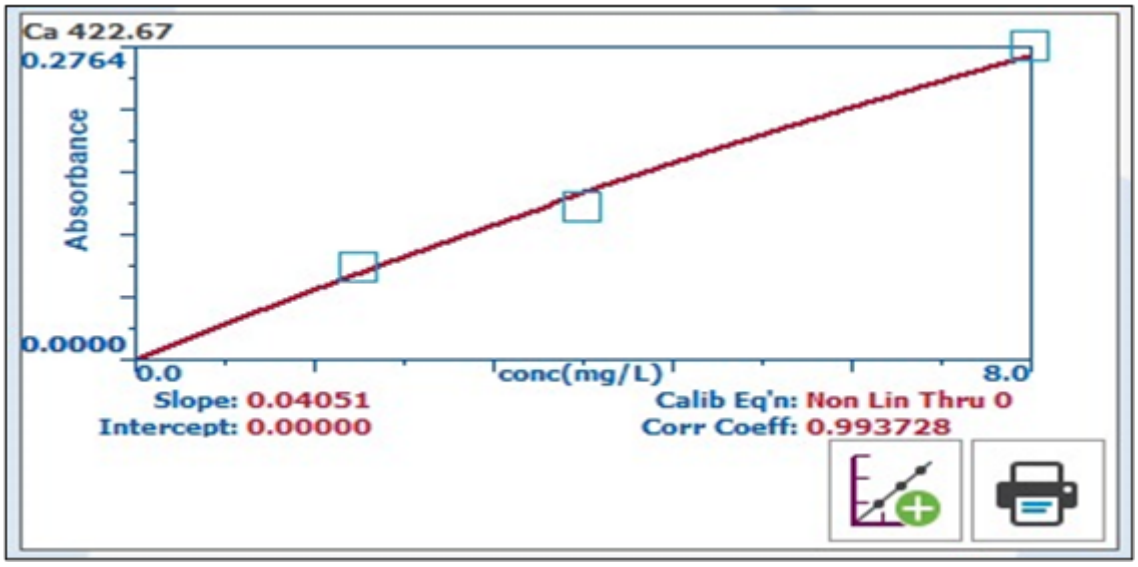

Figure 3: Calibration curve of calcium.

is used to remove organic impurities and comparatively less amount of Ca remains as Ca^{++} ion. Moreover, in dry ashing high temperature is required which is similar to the cooking process. However, the sequence for the amount of Ca present in the selected leafy greens is similar in both processes.

In both cases, of wet oxidation and dry ashing the estimated amount for Ca by AAS are in the sequence which is mustard greens>bottle gourd leaf>chickpea leaf>wild celery leaf>garlic leaf>spreading hogweed leaf>pot-biroot leaf>Onion leaf.

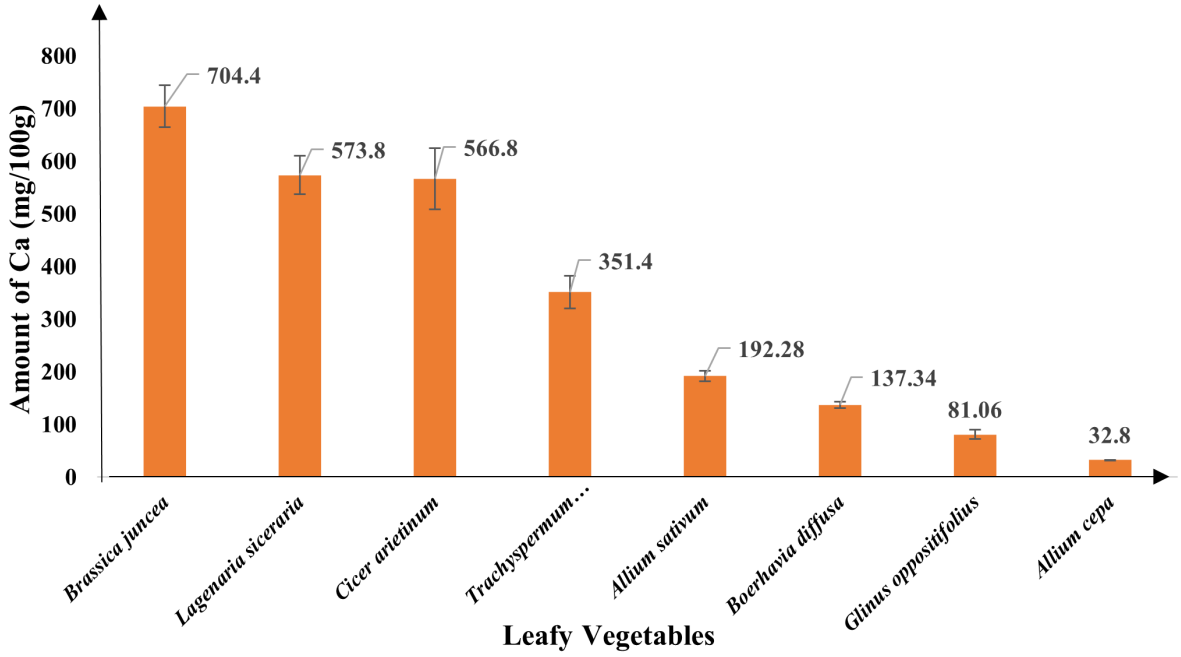

Figure 4: Amount of Ca (mg/100 g) in selected leafy vegetables by wet oxidation method.

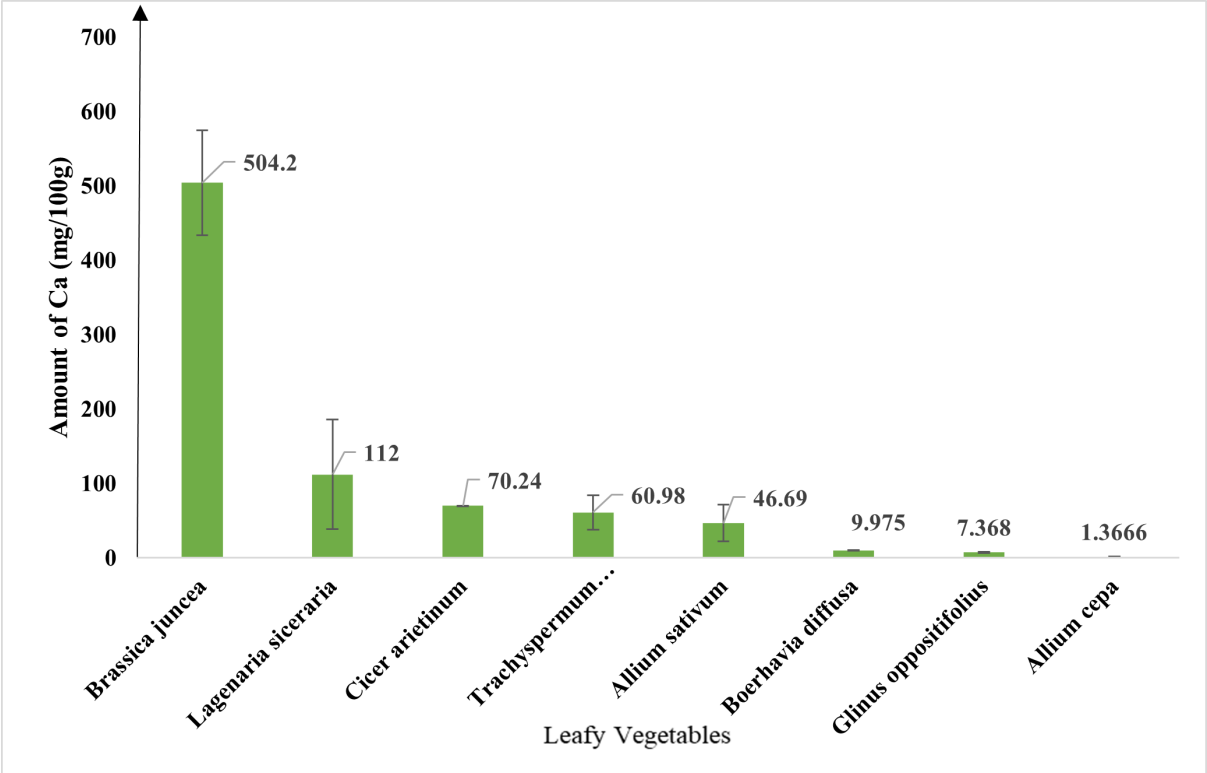

Figure 5: Amount of Ca (mg/100 g) in selected leafy vegetables by dry ashing method.

CONCLUSION

Ca plays a crucial role in different physiological activities as well as in preventing different diseases like osteopania, osteoporosis, osteomalacia, irregular heartbeats, muscle cramps and so on. Epidemiological studies demonstrate the worldwide increasing frequency of Ca deficiency bone disorders especially in women, elderly people and adolescents. To combat this situation Ca rich food plays a significant role. Leafy vegetables not only have low calories, but they also have a significant amount of dietary Ca and can be offered as a dietary recommendation to support bone health and overall well-being. The results of this study reveal the significant amount of calcium content among the selected leafy vegetables. This study also highlights the diversity in the amount of Ca content among the selected leafy vegetables of West Bengal. Certain varieties exhibit notably high levels of calcium, representing their potential role in promoting bone health, muscle function and overall well-being among the population. Depending on the amount of Ca estimated by the AAS method, the best three dietary recommendations from the selected leafy vegetables are mustard greens followed by bottle gourd leaf and chickpea leaf. This study focuses on their nutritional significance and potential health benefits.

Future research can be carried out on the effect of climate change, soil composition and available nutrients. Ca level among these selected leafy greens, as well as the bioavailability of Ca from the selected leafy greens considering the effect of anti-nutrients like oxalates, tannins, phytate etc on the it. Moreover, the research can further focus on the development of genetically modified varieties of leafy vegetables with higher calcium content, especially for people suffering from Ca deficiency disorders like osteoporosis, tetany, rickets and cardiovascular disorders.

ACKNOWLEDGEMENT

The authors are thankful to the "Acharya Prafulla Chandra Roy Advanced Research Laboratory", of Guru Nanak Institute of Pharmaceutical Science and Technology (GNIPST) for providing the laboratory facilities required for the completion of research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Durba Banerjee: Conception and design, acquisition, analysis and interpretation of data, statistical expertise, drafting of article. Palash Mondal: Acquisition of data, statistical expertise. Prerona Saha: Conception and design, critical revision, final approval, administrative support, data assembly.

ABBREVIATIONS

AAS: Atomic Absorption Spectroscopy; **Ca:** Calcium.

REFERENCES

1. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants: a review. Afr J Food Sci. 2010;4(5):200-22.
2. Pravina P, Sayaji D, Avinash M. Calcium and its role in human body. Int J Res Pharm Biomed Sci. 2013;4(2):659-68. doi: 10.7897/2230-8407.04235.
3. Theobald HE. Dietary calcium and health. Nutr Bull. 2005;30(3):237-77. doi: 10.1111/j.1467-3010.2005.00514.x.
4. Tamimi I, Cortes AR, Sánchez-Siles JM, Ackerman JL, González-Quevedo D, García Á, *et al.* Composition and characteristics of trabecular bone in osteoporosis and osteoarthritis. Bone. 2020;140:115558. doi: 10.1016/j.bone.2020.115558, PMID 32730941.
5. Vieira E, Silva M, Maia-Filho A, Ferreira D, Figuerêdo-Silva J, Rovaris K, *et al.* Effect of cerium-containing hydroxyapatite in bone repair in female rats with osteoporosis induced by ovariectomy. Minerals. 2021;11(4):377. doi: 10.3390/min11040377.
6. Szent-Györgyi AG. Calcium regulation of muscle contraction. Biophys J. 1975;15(7):707-23. doi: 10.1016/S0006-3495(75)85849-8, PMID 806311.
7. Johansson B. Calcium and regulation of contraction: a short review. J Cardiovasc Pharmacol. 1987;10; Suppl 1:S9-13. doi: 10.1097/00005344-198710001-00003, PMID 2442527.
8. Sanders KM. Regulation of smooth muscle excitation and contraction. Neurogastroenterol Motil. 2008; 20; Suppl 1: 39-53. doi: 10.1111/j.1365-2982.2008.01108.x, PMID 18402641.
9. Sommerville LE, Hartshorne DJ. Intracellular calcium and smooth muscle contraction. Cell Calcium. 1986;7(5-6):353-64. doi: 10.1016/0143-4160(86)90038-2, PMID 3545487.
10. Ferguson JH. The blood calcium and the calcium factor in blood coagulation. Physiol Rev. 1936;16(4):640-70. doi: 10.1152/physrev.1936.16.4.640.
11. Lansdown AB. Calcium: a potential central regulator in wound healing in the skin. Wound Repair Regen. 2002;10(5):271-85. doi: 10.1046/wrrd.1524.2002.10503. PMID 12406163.
12. Periayah MH, Halim AS, Mat Saad AZ. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. Int J Hematol Oncol Stem Cell Res. 2017;11(4):319-27. doi: 10.15171/ijhpm.2017.67, PMID 29340130.
13. Shi J, Kim KN, Ritz O, Albrecht V, Gupta R, Harter K, *et al.* Novel protein kinases associated with calcineurin B–like calcium sensors in Arabidopsis. Plant Cell. 1999;11(12):2393-405. doi: 10.1105/tpc.11.12.2393, PMID 10590166.
14. Tandogan B, Ulusu NN. Importance of calcium. Turk J Med Sci. 2005;35(4):197-201. doi: 10.3906/sag-0504-2.
15. Carmeliet G, Van Cromphaut S, Daci E, Maes C, Bouillon R. Disorders of calcium homeostasis. Best Pract Res Clin Endocrinol Metab. 2003;17(4):529-46. doi: 10.1016/j.beem.2003.08.001, PMID 14687587.
16. Walker AR. Osteoporosis and calcium deficiency. Am J Clin Nutr. 1965;16(3):327-36. doi: 10.1093/ajcn/16.3.327, PMID 14263053.
17. Glorieux FH, Pettifor JM. Vitamin D/dietary calcium deficiency rickets and pseudo-vitamin D deficiency rickets. BoneKEy Rep. 2014;3:524. doi: 10.1038/bonekey.2014.19, PMID 24818008.
18. Edmondson JW, Brashear RE, Li TK. Tetany: quantitative interrelationships between calcium and alkalosis. Am J Physiol. 1975;228(4):1082-6. doi: 10.1152/ajplegacy.1975.228.4.1082, PMID 236662.
19. Fujita T, Palmieri GM. Calcium paradox disease: calcium deficiency prompting hypoparathyroidism and cellular calcium overload. J Bone Miner Metab. 2000;18(3):109-25. doi: 10.1007/s007740070013.
20. Shlisky J, Mandlik R, Askari S, Abrams S, Belizan JM, Bourassa MW, *et al.* Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes. Ann N Y Acad Sci. 2022;1512(1):10-28. doi: 10.1111/nyas.14758, PMID 35247225.
21. Straub DA. Calcium supplementation in clinical practice: a review of forms, doses and indications. Nutr Clin Pract. 2007;22(3):286-96. doi: 10.1177/011542650702200 3286, PMID 17507729.
22. Rau YV, Murty VV. The utilisation of calcium from green leafy vegetables. Ann Biochem Exp Med. 1942;2(2):87-100.
23. Sahito SR, Memon MA, Kazi TG, Kazi GH, Jakhrani MA, Haque QU, *et al.* Evaluation of mineral contents in medicinal plant *Azadirachta indica* (Neem). J Chem Soc Pak. 2003;25(2):139-43.
24. Parnami M, Varma K. Nutritional composition of dried curry leaf powder (*Murraya koenigii*). Int J Emerg Technol Innov Res. 2019;6(6):409-12.
25. Linkon KM, Satter MA, Jabin SA, Abedin N, Islam MF, Lisa LA, *et al.* Mineral and heavy metal contents of some vegetable available in local market of Dhaka city in Bangladesh. IOSR J Environ Sci Toxicol Food Technol. 2015;9:01-6.
26. Pankaja N, Prakash J. Availability of calcium from kilkeerai (*Amaranthus tricolor*) and drumstick (*Moringa oleifera*) greens in weanling rats. Nahrung. 1994;38(2):199-203. doi: 10.1002/food.19940380212, PMID 8196748.

27. Manivannan C, Viswanathan G, Sundaram KM. Calcium bioavailability in leafy vegetables and medicinal plants. Int J Health Sci. 2022;6(2):88. doi: 10.18502/ijhs.v6iS2.9577.
28. Bhowmik S, Datta BK, Saha AK. Determination of mineral content and heavy metal content of some traditionally important aquatic plants of Tripura, India using atomic absorption spectroscopy. J Agric Technol. 2012;8(4):1467-76. doi: 10.23986/afsci.5416.
29. Bhattacharjee S, Dasgupta P, Paul AR, Ghosal S, Padhi KK, Pandey LP. Mineral element composition of spinach. J Sci Food Agric. 1998;77(4):456-8. doi: 10.1002/(SICI)1097-0010(199808)77: 4<456::AID-JSFA55>3.0.CO;2-M.
30. Kaya I, Incekara N. Ingredients of some weeds consumed as food in Aegean region. Turk Herboloji Derg. 2000;3(2):56-64.
31. Divrikli U, Saracoglu S, Soylak M, Elci L. Determination of trace heavy metal contents of green vegetable samples from Kayseri-Turkey by flame atomic absorption spectrometry. Fresenius Environ Bull. 2003;38(2):199-203.
32. Bozokalfa MK, Yağmur B, Ascıogul TK, Eşiyok D. Diversity in nutritional composition of Swiss chard (*Beta vulgaris* subsp. L. var. cicla) accessions revealed by multivariate analysis. Plant Genet Resour. 2011;9(4):557-66. doi: 10.1017/S1479262111000876.
33. Zoroddu MA, Aaseth J, Crisponi G, Medici S, Peana M, Nurchi VM. The essential metals for humans: a brief overview. J Inorg Biochem. 2019;195:120-9. doi: 10.1016/j.jinorgbio.2019.03.013, PMID 30939379.
34. Sarkar T, Salauddin M, Roy S, Chakraborty R, Rebezov M, Shariati MA, *et al.* Underutilized green leafy vegetables: frontier in fortified food development and nutrition. Crit Rev Food Sci Nutr. 2022:1-55. doi: 10.1080/10408398.2022.2078311.
35. Shukla S, Bhargava A, Chatterjee A, Srivastava J, Singh N, Singh SP. Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). Plant Foods Hum Nutr. 2006;61:21-6. doi: 10.1007/s11130-006-0017-0.

Cite this article: Banerjee D, Mondal P, Saha P. Profiling of Calcium Content among the Leafy Vegetables of West Bengal Using Atomic Absorption Spectroscopy. Pharmacog Res. 2025;17(1):53-9.