How to Cite:
Manivannan, C., Viswanathan, G., & Sundaram, K. M. (2022). Calcium bioavailability in leafy vegetables and medicinal plants. *International Journal of Health Sciences*, *6*(S2), 8802–8810. https://doi.org/10.53730/ijhs.v6nS2.7303

Calcium bioavailability in leafy vegetables and medicinal plants

C. Manivannan
Department of Chemistry, Bannari Amman Institute of Technology, Sathyamangalam
Corresponding author email: cmanivannan89@gmail.com

Gopalan Viswanathan
Department of Chemistry, Bannari Amman Institute of Technology, Sathyamangalam

K. Meenakshi Sundaram
Department of Biotechnology, Research and Publication Wing, Bharath Institute of Higher Education and Research, Selaiyur, Chennai – 600073

***Abstract*---**Large quantities of fluoride intake are the primary reason for the prevalence of dental and skeletal fluorosis. Absorption of fluoride is 100% when it is administered through drinking water without food. But the fluoride absorption reduced from 50% to 80% when it is administered along with diet. Mostly diets with high calcium with rich in bioavailability reduce the fluoride absorption from the gastrointestinal tract by forming calcium fluoride insoluble complex. Apart from drinking water, milk and milk products; commonly available leafy vegetables and medicinal plants are the good sources for dietary calcium. Hence this study is proposed to account the total and bioavailable calcium level in various leafy vegetables and medicinal plants. For this purpose, eighteen leafy vegetables and five medicinal plants are collected and analyzed for total and bio-available calcium levels. Total and bio-available calcium levels are determined through wet digestion and *in-vitro* miller method respectively by the use of atomic absorption spectrophotometer. Among the selected leafy vegetables dwarf-copper, block-nightshade, curry, cabbage, drumstick and mint leaves contain high total calcium with rich in bioavailability. Moreover the medicinal plants such as *Cassia tora, Cassia alata* and *Cassia angustifolia* are shown high total and bio-available calcium level. Hence, the frequent usage of these plant materials through daily diet can reduce the risk of fluorosis.

***Keywords*---**fluoride, fluorosis, bioavailability, calcium, leafy vegetables, medicinal plants.

Manuscript submitted: 9 March 2022, Manuscript revised: 18 April 2022, Accepted for publication: 1 May 2022

Introduction

Prolong intake of fluoride more than the recommended level through water and food may cause dental and skeletal fluorosis (WHO, 2002). Dental fluorosis is characterized by lusterless, opaque white patches in the enamel, which may become stained yellow to dark brown, and in severe forms cause marked pitting and brittleness of teeth. Skeletal fluorosis is associated with limited movement of the joints, skeletal deformities, and intense calcification of ligaments, muscle wasting and neurological deficits (Susheela, 2003).

Fluoride is almost completely absorbed from drinking water in gastro-intestinal tract in the absence of Ca, Mg and Al (Jackson et al., 2002). But, the bioavailability of fluoride from various food items is reported that, it varies from 2% to 79%. Fluoride bioavailability was governed by various factors such as pH and mineral content of the food. Calcium intake reduces the absorption of fluoride from gut, by forming calcium-fluoride insoluble complex (Cerklewski, 1997). Some of the earlier studies reported that the serum fluoride level is greatly decreased when sodium fluoride is administered with high-calcium-diet (Harrison et al, 1984; Ekambaram & Paul, 2001). Adequate intake of calcium reduces the risk of fluorosis in children than the children intake inadequate calcium. Therefore, the intake of food items with high calcium with more bioavailability helps to reduce the absorption of fluoride and minimize the fluorosis risk (Ekambaram & Paul, 2001). Moreover, calcium is essential for normal growth and development of the skeleton as well as for teeth, nerve, and muscle and enzyme functions.

Apart from milk, dairy products and drinking water, some of the commonly available leafy vegetables and medicinal plants are the good and cheapest sources of dietary calcium. Also the intake of leafy vegetables and medicinal plants through diet can impart various health benefits. Calcium in plant materials is primarily complexes with some anti-nutrients available in plants such as oxalate, phytate, fiber, and other phenolic compounds. This reduces the solubility of calcium in the gastrointestinal tract and turn it indigestible (*Titchenal and Dobbs, 2007).* These complexes must be broken down, and the calcium released in a soluble form for better absorption. Acidic medium in stomach can increase the solubility of calcium complexes (*Mahoney, Holbrook & Hendricks, 1975).*

The calcium bioavailability level varies greatly among every plant materials may due to the presence of various levels of anti-nutrients. Hence, it is necessary to estimate the total as well as bio-available calcium level in commonly available leafy vegetables and medicinal plants, in order to find out the suitable plant sources to compete against the fluoride toxicity. *In-vitro* digestion experiment is conducted to estimate the bioavailability of calcium by simulate the human digestive system *via* a two-step digestion that includes a gastric digestion and intestinal digestion. Estimation of bioavailable calcium level from the *in-vitro* analysis through miller method is an accepted procedure and has advantages of cost, speed, reduced variability, and correlates well with human *in-vivo* studies. This method has advantage that, it includes enzymatic digestion of a plant sample under simulated gastrointestinal condition, followed by measuring soluble calcium which diffused from the sample (Miller et al, 1981; Narasinga Rao & Prabhavathi, 1978).

Materials and Methods

Collection of leafy vegetables and medicinal plant samples

About 200 g of fresh and raw leafy-vegetables were procured from markets in three different areas of Tirunelveli District and about 200 g of fresh and raw leaves of five cassia species such as c*assia tora, cassia alata, cassia angustifolia, cassia auriculata, cassia fistula* were collected in three different areas of Tirunelveli District using clean polyethylene bags. All the collected samples were cleaned with double-distilled water and stored in refrigerator for about 10°C.

Estimation of total calcium level

About 4 g to 5 g of accurately weighed edible portion of homogeneous plant sample was taken into a 250 ml long-neck conical flask. About 5 ml of concentrated sulphuric acid was added to the sample and it was heated to boiling. After allowing the solution to cool, 5 ml of concentrated nitric acid was added to the solution and again allowed to boil. Then the heating was interrupted and the solution was allowed to cool. Further, about 5 ml of 60% perchloric acid was then added and the content was heated on the hot plate till the brown fumes are completely expelled out. After the solution was cooled, about 20 ml of double-distilled water was added to the content. Then the solution was filtered using Whatman No.42 filter paper and make up to 100 ml. Calcium level in the sample solution was measured through atomic absorption spectrophotometer. The optimum conditions for the estimation of calcium in Atomic Absorption Spectrophotometer were, the wave length set at 422.7 nm, slit width 0.7 nm, relative noise 1.0 and oxidizing lean, blue flame obtained from air-acetylene mixture was used for the aspiration of samples. In order to reduce the ionization interferences and chemical interferences 0.1% of KCl and 0.1% of $LaCl_3$ were used respectively. Then the sample solution was introduced into Perkin-Elmer Flame Atomic Absorption Spectrophotometer (Perkin Elmer AA – 100, USA), after the calibration of instrument using appropriate calcium standards and control. Calcium level of the unknown sample was directly read out from the digital display of the meter. The level of calcium in sample was calculated from the reading shown in digital display of the meter by multiplying with appropriate dilution factor (Perkin Elmer, 2000).

Estimation of bio-available calcium level in five *Cassia* species

About 4 g to 5 g of accurately weighed leafy vegetable or leafy portion of medicinal plant sample was homogenized and mixed with 25 ml of pepsin-HCl (0.5 g of pepsin in 100 ml of 0.1 N HCl) solution. The acidity of the mixture was adjusted to pH 2 with distilled HCl and incubated in a 250 ml conical flask at 37°C in a metabolic shaker water bath for 2 hours. Then the content was kept in ice-bath for 10 min in order to stop the pepsin digestion. Thereafter, 5 ml of pancreatin-bile extract mixture (4 g of pancreatin and 25 g of bile-extract were dispersed in 0.1 M $NaHCO_3$ and the mixture was brought to 1 L with 0. 1 M $NaHCO_3$) was added and incubated at 37°C in a metabolic shaker water bath for 2 hours. Then the pH was gradually adjusted to 7.5 by treating with 0.5 M NaOH. Then the content was incubated at 37°C for 30 min. After the incubation period, the supernatant was filtered through Whatman no. 44 filter paper and the filtrate was made up to 50 ml. Then the solution was used for the determination of bio-available calcium by using Perkin-Elmer Flame Atomic Absorption Spectrophotometer (Perkin Elmer AA – 100, USA) calibrated by using appropriate calcium standards and control (Perkin Elmer, 2000). The amount of soluble calcium, expressed as the percentage of bio-available calcium present in the selected leafy vegetable or medicinal plant sample.

Statistical analysis

Relationship between the total and bio-available calcium was calculated through regression equations, correlation coefficient and significant level (Table 2). SPSS version 6 was used for the statistical calculation.

Results

Total and bio-available calcium level in medicinal plants and leafy vegetables

The levels of total and bio-available calcium in commonly available medicinal plants are shown in Table 1. When compared to all the selected plant materials *cassia tora* shows the highest total calcium level (3412 mg/100g). It is worth mentions here that among all the plant materials reported in the literature till date *cassia tora* is found to possess the highest total calcium. Levels of total and bio-available calcium in the selected medicinal plants and the percentages of calcium bioavailability in the selected medicinal plants are shown in Fig.1.

Tirunelveli senna (c*assia angustifolia*) shows the maximum calcium bioavailability (about 23%) among the selected medicinal plants. Even though coffee cassia (*Cassia tora* - usei-thagarai) contains a large amount of total calcium, its bioavailability level is small. Even this small amount of bioavailability of calcium is more than that of consuming a cup of milk. Ringworm-senna (c*assia alata* – seemai agathi) and avaram senna (c*assia auriculata* - avarai) are show considerable level of total and bioavailable calcium. Table 2 shows the high linear relationship between the total and bio-available calcium from the selected medicinal plants. Regression equation shows the quantitative increment of bio-available calcium level from the increase of each unit of total calcium.

The levels of total and bio-available calcium in leafy vegetables commonly used in diet and chosen for the present study are shown in Table 3. Bakphul, curry leaves, cauliflower leaves, punarnava leaves and dwarf-copper show the total calcium level more than 500 mg/100g. Even though tropical amaranth, coriander, spinach, red spinach, spiny pigweed and common purslane show considerable level of total calcium, the bioavailability levels are very low. The percentages of calcium bioavailability in the selected leafy vegetables are shown in Fig.2.

From the selected leafy vegetables, dwarf-copper (ponnanakanni) and block-nightshade (Manathakkali keerai) shown above 40% of bio-available calcium. Furthermore, curry, cabbage, drumstick and mint leaves also contain high total bioavailable calcium level. Even though bakphul and common purslane show rich total calcium level, their calcium bioavailability levels are very low.

Discussion

Commonly, plant materials are the good source of calcium and also for vitamin A, C and K. Consumption of plant sources high calcium with rich in bioavailability is the suitable way to minimize the risk of fluorosis. Frequent intake of dietary calcium through leafy vegetables and medicinal plants helps to get required amount of calcium for reducing the risk of fluorosis and for maintaining good health. Calcium is absorbed from the small intestine by both active and passive mechanisms. At low and moderate intakes of calcium, calcium is absorbed *via* active transfer. Active transfer needs the action of the active-form of vitamin D (*Gueguen* & Pointillart, 2000). Normally, calcium is soluble from calcium complexes during digestion and is released probably in ionized form for absorption. If calcium intake level increase then the active transfer mechanism becomes saturated and an increasing proportion of calcium is absorbed via passive diffusion (Allen, 1982).

The selected leafy vegetables and medicinal plants show high level of total calcium, but their bio-available calcium levels are inappropriately varied from their total calcium levels. Calcium in plants may primarily complexes with oxalate, phytate, fiber, lactate, fatty acids, protein and other compounds. Oxalate is present in large amounts in most of the commonly consumed plant materials (Ismail et al., 2005; *Brzezinski et al., 2002).* Calcium complexes with oxalate are unavailable for the absorption from the gut (*Brzezinski et al., 2002).* Soluble oxalates in medicinal plants and other consumed foods can also reduce the absorption of minerals. The insoluble form of calcium (calcium-oxalate) may not absorb through any absorption process.

It has been suggested that the intake of dietary calcium through leafy vegetables like black night shade, dwarf copper, curry, cabbage, drumstick and mint can also impart some other important beneficial effects to human health. Also these leafy vegetables are commonly used as diet components frequently. Intake of medicinal plants like *cassia tora* of high in total calcium with rich bioavailability through diet can deliver more calcium to minimize the absorption of fluoride through the intestinal tract. Moreover, it has been suggested that the intake of dietary calcium from plant materials consists of high bio-available calcium with low anti-nutrient level, especially oxalate reduce the risk of kidney stone formation (*Brzezinski et al., 2002).*

Conclusion

Among the selected medicinal plants *cassia tora*, c*assia alata* and *cassia angustifolia* show considerable level of total and bio-available calcium. Also, the intake of leafy vegetables like black night shade, dwarf copper, curry, cabbage, drumstick and mint of high total calcium with rich bioavailability through diet can deliver more calcium to minimize the absorption of fluoride through the intestinal tract.

Hence, it is recommended to the people residing in fluoride endemic areas to intake these plant sources frequently as diet component to get more bio-available calcium and reduce the risk of fluorosis. Also, it is recommended to avoid plants high calcium level with low bio-availability. Because these plants may contain considerable level of anti-nutrients such as oxalate, phytate, tannin and polyphenols may creates some health defects and inhibits the absorption of calcium and other essential minerals for human health. Subsequent intake of leafy vegetables and medicinal plants through diet may additionally useful to get essential vitamins for other beneficial health effects.

References

Allen, L.H. (1982). Calcium bioavailability and absorption: a review. *American Journal of Clinical Nutrition,* 35, 783 – 808.

Brzezinski, E. (2002). Durning, A.M., Grasse, B., Fusselman, E and Ciaraldi, T., University of California, 17-29.

Cerklewski, F.L. (1997). Fluoride bioavailability, *Nutrition Research,* 17(5), 907–929.

Ekambaram, P. Paul, V. (2001). Calcium preventing locomotor behavioral and dental toxicities of fluoride by decreasing serum fluoride level in rats. *Environmental Toxicology and Pharmacology*, 9, 141-146.

Gueguen, L., Agr, M and Alain Pointillart, D.V.M., (2000). Mineral bioavailability and bone mineral contents in pigs given calcium carbonate postprandially. *American Journal of Clinical Nutrition,* 19, 119-136.

Harrison, P.T.C. (2005). Fluoride in water: a UK perspective. *Journal of Fluorine Chemistry,* 126(11–12), 1448–1456.

Ismail, A., Horner, H.T., Palmer, R.G., Reddy M.P and Massey, L. (2005). Oxalate level in vegetables. *Journal of Agricultural and Food Chemistry*, 53, 5670-5674.

Jackson, P.J., Harvey, P.W and Young, W.F. (2002). *Chemistry and bioavailability aspects of fluoride in drinking water.* Report No.05037, Henley Road, Medenham, Marlow, Bucks, SL7 2HD: WRC-NSF Ltd 27, 5-9.

Mahoney, A.W., Holbrook, R.S & Hendricks, D.G. (1975). *Nutrition metabolism,* 18, 310-317.

Miller, D.D., Schricker, B.R., Rasmussen, R.R & Campen, D.V. (1981). An in vitro method for estimation of iron availability from meals. *American Journal of Clinical Nutrition,* 34, 2248–2256.

Narasinga Rao., B.S & Prabhavathi, T. (1978). An in vitro method for predicting the bioavailability of iron from foods. *American Journal of Clinical Nutrition*, 31, 169-175.

Norwalk, CT.

Perkin Elmer. (2000). *Manual of Perkin Elmer Atomic Absorption Spectrophotometer* AA100.,

Susheela, A.K. (2003). Treatise on fluorosis, 2nd edn. *Fluorosis Research & Rural Development Foundation*, Delhi, India, 137.

Titchenal, C.A & Dobbs, J.C., (2007). *Journal of Food Composition and Analysis*, 20, 717–724.

WHO (World Health Organization), (2002). *Environmental Health Criteria*. 227: Fluorides, Geneva, 1-251.

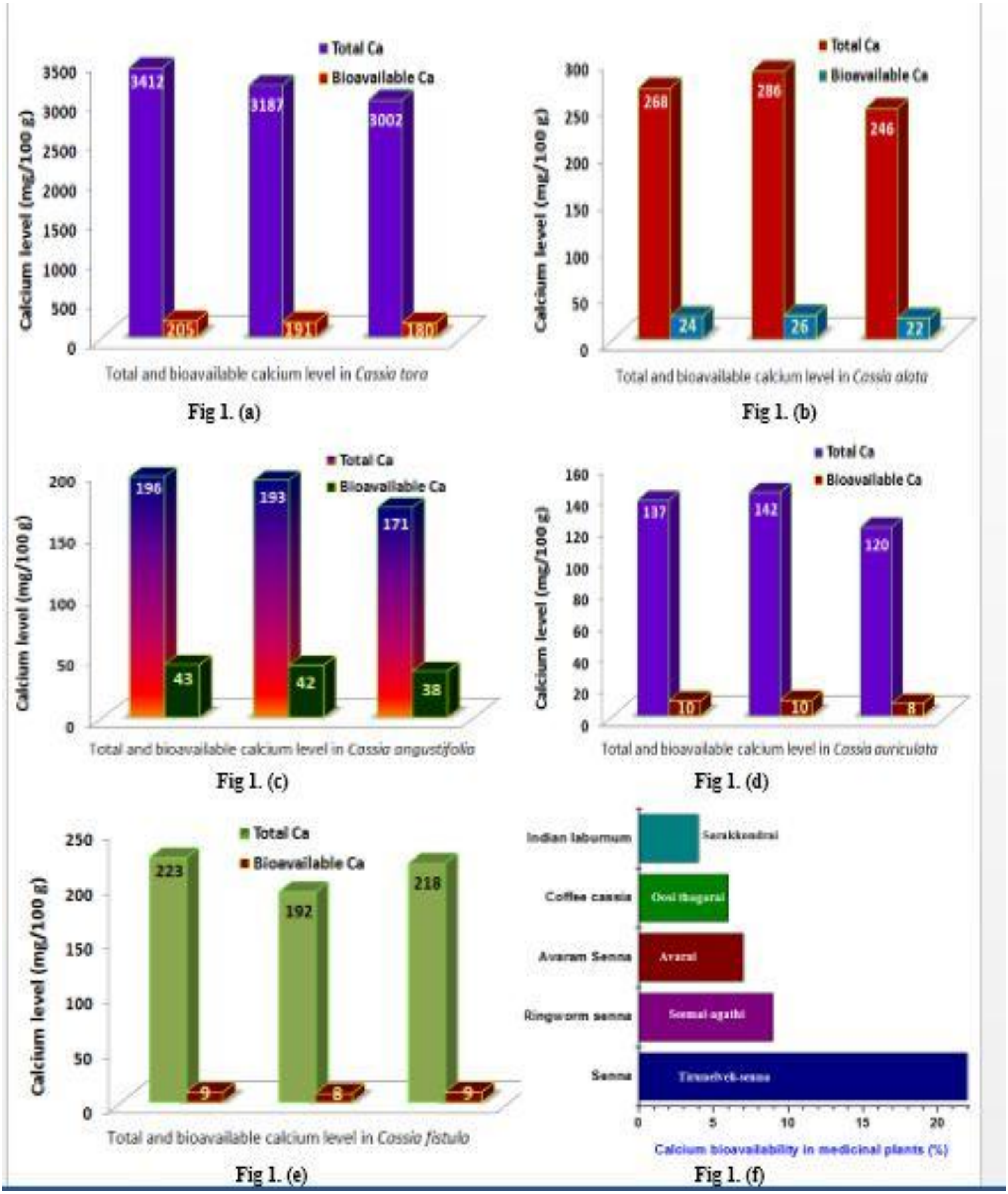

Fig.1. Level of total and bioavailable calcium level in medicinal plant

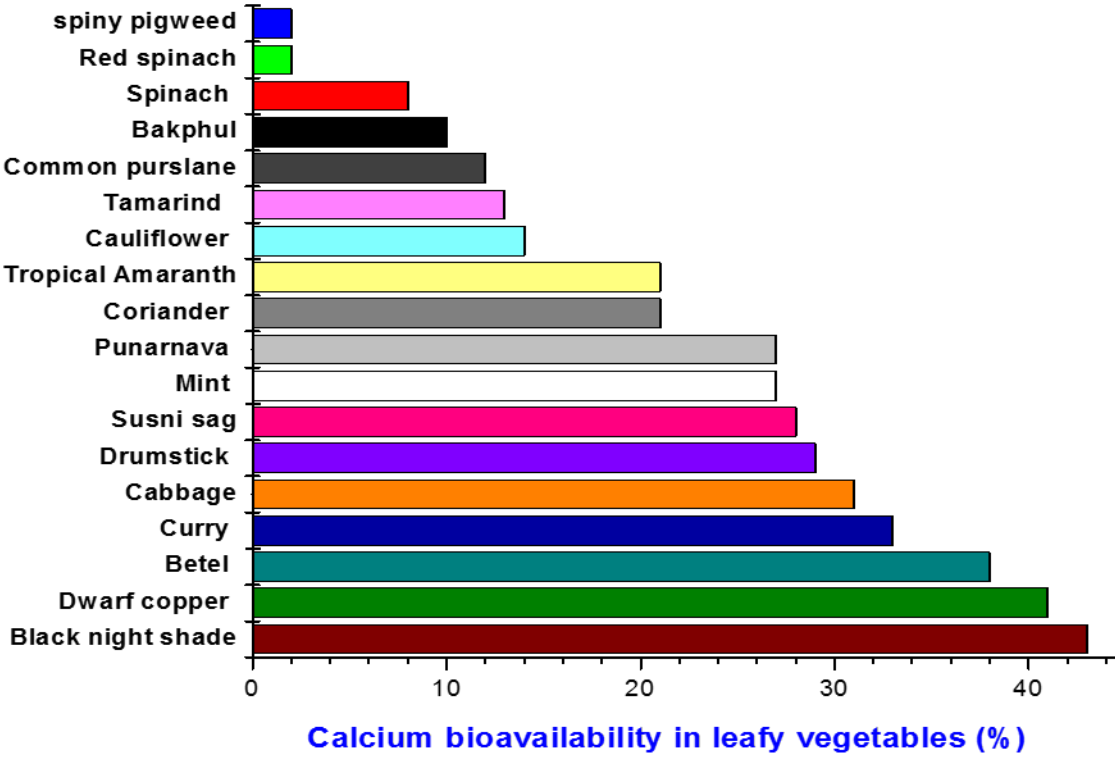

Fig.2. Percentages of calcium bioavailability in leafy vegetables

Table 1. Total and bioavailable calcium level in medicinal plants
Values represent mean level with standard deviation

Vernacular name	Common name	Botanical name	Total calcium (mg/100 g)	Bioavailable calcium (mg/100g)
Usei-thagarai	Coffee cassia	*Cassia tora*	3200 ± 205	192 ± 12
Seemai-agathi	Ringworm senna	*Cassia alata*	267 ± 20	24 ± 2
Tirunelveli-senna	Senna	*Cassia angustifolia*	187 ± 14	41 ± 3
Avarai	Avaram senna	*Cassia auriculata*	133 ± 12	9 ± 1
Sarakkondrai	Indian laburnum	*Cassia fistula*	211 ± 17	8 ± 1

Table 2. Relationship between total and bioavailable calcium level in selected medicinal plants

Name of the medicinal plant	Regression equation	Correlation coefficient (r)	Significant level (p)
Cassia tora	Y = 54.35+16.4 X	0.9999	0.00822
Cassia alata	Y = 26.67 + 10 X	0.9983	0.03671
Cassia angustifolia	Y = -24.2 + 5.1 X	0.9968	0.05094
Cassia auriculata	Y = - 3.67 + 0.1X	0.9762	0.13911
Cassia fistula	Y = 1.43 + 0.03 X	0.9886	0.09599

Table 3. Total and bio-available calcium level in leafy vegetables

Common name	Botanical name	Total Ca	Bio-available Ca
Curry leaves	*Murraya koenigii*	824 ± 42	272 ± 14
Cauliflower leaves	*Brassica Oleracea*	615 ± 32	86 ± 4
Tropical Amaranth	*Amaranthus polygonoides*	248 ± 23	52 ± 5
Bakphul	*Sesbania grandiflora*	1152 ± 53	115 ± 5
Coriander	*Coriandrum sativum*	158 ± 21	33 ± 4
Black night-shade	*Solanum nigrum*	417 ± 30	179 ± 13
Mint	*Mentha spicata*	204 ± 16	55 ± 4
Dwarf copper	*Alternanthera sessilis*	517 ± 31	212 ± 13
Spinach	*Spinacia oleracea*	131 ± 11	10 ± 1
Tamarind	*Tamarindus indica*	101 ± 12	13 ± 2
Punarnava	*Boerhavia diffusa*	637 ± 36	172 ± 10
Red spinach	*Amaranthus gangeticus*	366 ± 24	7 ± 1
Spiny pigweed	*Amaranthus viridis*	327 ± 19	7 ± 1
Betel	*Piper betle*	221 ± 21	84 ± 8
Common purslane	*Portulaca oleracea*	112 ± 12	13 ± 1
Drumstick leaves	*Moringa Olifera*	443 ± 30	128 ± 9
Susni sag	*Marsilea minuta*	367 ± 27	103 ± 8
Cabbage	*Brassica oleracea*	42 ± 4	13 ± 1

Values are in mg/100 g and represent mean level with standard deviation