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Comparative Analysis of Calcium Oxalate Accumulation in Indian Vegetables and Napier Grass Versus Spinach

Rafia Firdous and Sumera Nazneen*

Department of Botany, Anwarul Uloom College, New Mallepally, Hyderabad, Telangana, 500001, India

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Corresponding Author: **Sumera Nazneen** | E-Mail: **(sumeranaazneen2008@gmail.com)**

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ABSTRACT

The calcium and oxalate levels in 14 different plant species—including radish, beetroot, onion, and Napier grass—sold in local markets were examined. Analyzing the variations in the oxalate content of these 14 leafy and root vegetables gathered from different local markets in the Telangana state twin towns of Hyderabad and Secunderabad was also deemed relevant in this context. Numerous leafy and root vegetables, as well as grazing crops like Napier grass, create and collect oxalates. Despite the fact that oxalate can be a significant component of plants, significant details about its production, accumulation, and catabolism remain unclear. Fourteen Indian-grown leafy and root vegetables were tested for water-soluble oxalate concentration. The water-soluble oxalates in these 14 leafy vegetables were divided into three groups: Low (0 to 4.0%), Medium (4.1 to 8.0%), and High (>8.1%). The amounts of aqueous oxalates in four leafy plants (colocasia, purple and green amaranth, and spinach) varied from 8.115 to 12.580%. The medium water soluble oxalate content of two more leafy vegetables (cabbage and onion stalks) varied from 4.275 to 5.330%, while the low group's eight vegetables (beetroot, cabbage, coriander, curry leaf dill, drumstick, fenugreek, Napier grass, and taro) ranged from 0.332 to 2.50. Of the total calcium, the proportion of calcium bound in the water-soluble oxalate component of the leafy vegetables varied from 5.2% to 332.1% in spinach and onion stalks, respectively. These leafy and root crops' calcium binding capacities varied from 1.02 to 40.20% for spinach and radish, respectively.

Keywords: *Calcium Oxalates, Napier grass, Salinity, Sodium Chloride, Soluble Oxalate, Total Oxalate, Leafy Vegetables, Spinach, root vegetables, oxalate, quantification, soybean, Napier grass*

1. Introduction

A balanced diet that includes plant-based foods has been shown to have several health advantages, however they also contain many anti-nutrients [1]. It is common to overlook the existence of substances like oxalate, cyanogenic glycosides, lectins, protease inhibitors, and phytates [2]. A ubiquitous plant metabolite, oxalic acid is a dicarboxylic acid with a variety of uses, such as maintaining pH and metal ion homeostasis and aiding in plant defence. Strong chelating properties and a detrimental impact on the absorption of dietary minerals have been demonstrated for oxalic acid [3]. Leafy greens and root vegetables, which commonly available in Indian marketplaces, are known to collect oxalates to varied degrees. In addition to differing amongst species, this accumulation is also influenced by environmental variables including climate and farming practices [4]. This study focuses at the levels of calcium and oxalate in 14 plant species that were obtained from local markets in Hyderabad and Secunderabad, Telangana state, India. These species include typical vegetables like radish, beetroot, and onion, as well as Napier grass, a common grazing crop. According to earlier research, the amount of water-soluble oxalates in leafy and root vegetables can vary greatly, with certain plants having greater quantities than others [5].

Depending on the amounts and kinds of oxalates present, the build-up of oxalates in these plants may affect calcium bioavailability, which may be advantageous or disadvantageous to consumers [6]. Due to heredity and environmental influences, the soluble oxalates, which are frequently measured using techniques like HPLC, tend to differ greatly among plant species, affecting the oxalate concentrations in these foods that are sold in local markets. Comprehending how much oxalate is in certain plants is important for dietary recommendations, particularly for people who are at risk of kidney stones. Overconsumption of oxalate can cause calcium oxalate kidney stones because the body is unable to metabolize it, which causes it to crystallize in the kidneys [7]. Therefore, determining the amount of oxalate in vegetables—especially those that are often consumed in areas like Telangana, India—can aid in developing dietary recommendations to reduce these risks [8].

2. Materials and Methods

A range of plant species, including 14 leafy and root vegetables as well as one Napier grass, were purchased at the local markets in the Telangana state twin towns of Hyderabad and Secunderabad. The EDTA method was used to determine the calcium and magnesium content of each plant species, while the water soluble oxalate content was determined using standard chemical techniques. These 14 leafy vegetables, root vegetables, and napier grass were processed and their neutral normal ammonium acetate was prepared with distilled water.

2.1 Sample Materials

The twin cities of Hyderabad and Secunderabad's wholesale marketplaces provided raw materials used in this investigation. India's Telangana State. They were picked in rural areas up to 200 kilometers away from urban marketplaces, and they were sold around 24 hours after harvest. The leafy vegetable components were purchased, cleaned, allowed to air dry at room temperature, and then processed into a powder.

These dry ingredients were used for all extractions. As explained in detail by Savage et al. (2000) [9], the calcium and magnesium contents of 1 g of dried leafy vegetable samples were extracted chemically, together with the water-soluble oxalates. By using difference, the amount of insoluble oxalate (calcium oxalate) was determined [10]. Every sample was examined four times, and the results are shown as a mean of mg oxalate/100 g DM.

Table:1 English, Indian and Latin names of plant species tested for oxalates

S.No.	English Name	Indian Name	Scientific Name	Plant Part
1	Amaranthus Green	Chauli, chavleri	*Amaranthus viridis*	Leaves
2	Amaranthus Purple	Chauli, chavleri	*Amaranthus cruentus*	Leaves
3	Beet Root	Beet, or Beetroot	*Beta Vulgaris*	Leaves
4	Cabbage	Cabbage	*Brassica Oleracea*	Leaves
5	Coriander	Dhania Patta	*Coriandrum sativum*	Stalks
6	Curry	Curry Patha	*Murraya koenigii*	Leaves
7	Dill	Shepu	*Peucedanum graveolens*	Leaves
8	Drumstick	Seeng	*Moringa oleifera*	Leaves
9	Fenugreek	Methi	*Trigonella foenum-graecum*	Leaves
10	Pusa Giant Napier grass (PGN)	Napier Grass	*Pennisetum purpureum Schumach*	Leaves
11	Onion Stalk	Kanda Pyaaz	*Allium cepa*	Leaves
12	Radish	Mooli	*Raphanus sativus*	Leaves
13	Spinach	Palak	*Spinacia oleracea*	Leaves
14	Taro	Arbi	*Colocasia esculenta*	Leaves

2.2 Determination of Calcium and Magnesium by Versenate (EDTA) method

In order to extract the calcium and magnesium found in plant species' leaves, neutral normal ammonium acetate solution is used. This exchanges ammonium ions with Ca^{+} and Mg^{++} ions that are adsorbed on soil colloids, allowing the Ca and Mg ions to enter the extracting solution. By titrating against 0.01 N EDTA, which measures the strength of Ca^{+} and Mg^{++} ions present in the leaf sample, the concentration of these ions is ascertained by Gupta,1999 [11]. Five ml of 16% sodium hydroxide and two to three carbomate crystals were added to ten milliliters of plant tissue extract. About 40 mg of muroxide (ammonium purpurate) indicator was added to this combination. After that, a 0.01 N EDTA solution was used to titrate this mixture until the color changed from orange red to reddish violet (purple). Eriochrome black T indicator Versenate (EDTA) titration is the standard technique for estimating Ca^{+} and Mg^{++} ions [12].

2.1.1 Determination of magnesium by Versenate (EDTA) method: (Gupta 1999)

In a mechanical shaker, weigh 5 grams of a homogeneous plant leaf sample, add 25 milliliters of Normal Neutral Ammonium Acetate, mix for 10 to 15 minutes, and then filter through Whatman Conical flask with No. 1 filter paper. Ten milliliters of plant tissue (leaves) extract were pipetted out, to which two to five carbamate crystals and five milliliters of ammonium chloride-ammonium hydroxide buffer were added. Three to four drops of Eriochrome Black- T were then added as an indicator. The combination was titrated using a 0.01N EDTA solution until the end result was brilliant blue (the wine red tint completely vanished).

Calculations:

From the volume of 0.01 N EDTA solution reguired for titration, concentration of Ca^{+} and Mg^{++} ions is directly obtained in m.e./litre as follows:

$$\text{Conc. Of } Ca^{+}Mg^{++} = \frac{\text{Volume of EDTA consumed X Normality of EDTA soln.}}{\text{Volume of water sample taken}} \text{X 1000}$$

Calcium (in gms/litre) = Normality of EDTA X volume of EDTA X Eq. Wt. Of calcium in (20/ml of aliquot taken

(Ca + Mg) gm/litre = Normality of EDTA X Vol. of EDTA X Eq.Wt. of calcium + Magnesium(32.196) ml. of aliquot taken

2.2. Estimation Of Oxalates In Leaf Samples

Potassium permanganate (KMn04), is a potent oxidizer. The hue of permanganate, MnO_4^-, is a deep, dark purple. At the equivalency point, the solution will change from dark purple to a light pink hue due to the reduction of the purple permanganate ion to the colourless Mn^{+2} ion. For this titration, no further indicator is required. Permanganate reduction depends on very acidic conditions.

The proportion of water-soluble oxalates was calculated using the Abaza *et al.* (1967) [13] technique. Moreover, Dewey and Lu (1959) evaluated yield [14].

2.2.1. Water soluble Oxalate content: A porcelain dish 12-15 cm in diameter is filled with 25-50 mL of distilled water and 1-2 g of a representative sample of healthy leaves that have been finely powdered. The mixture is then left for an hour, stirring regularly. Fill the burette with 0.1 N potassium permanganate. Note the burette's initial reading. The top of the meniscus may be read rather than the bottom due to the vivid hue of the KMn04 solution. Titrate the contents of the china dish until a persistent light pink or violet coloration develops. Note the final value of Titer.

Calculations:

$$\text{Oxalates\% in Meq/100g} = \frac{\text{(FTV of Leaf Sample-ITV of Leaf Sample) X 0.1 N KMNO4}}{\text{Weight of Leaf sample Taken i.e 1 or 2}} \text{X 100}$$

2.2.2. Hot water soluble Oxalates: 5 grams of distilled water are added to a 5-gram representative sample of healthy, undamaged leaf samples, which are then cooked in a water bath at 56+1 degrees Celsius for precisely 30 minutes. After this period, the leaves were taken out of the tubes, mashed for five minutes in a blender, and then the entire amount was placed in a china dish. Rinse and transfer from blender to china dish using as little water as possible. Use 0.1N KMn04 to titrate the contents as described above until a light pink or violet hue is permanently present. Note the final value of Titer.

Calcium Oxalate molecular weight

Molar mass of CaC204 = 128.097 g/mol

Molecular weight of Oxalate: 88.018 g/mol

Convert grams Calcium Oxalate to moles or moles Calcium Oxalate to grams

Molecular weight calculation:

40.078 + 12.0107*2 + 15.9994*4

Table:2 Percent composition by element

Element	Symbol	Molecular Mass	Number of Atoms	Mass Percentage
Calcium	Ca	40.078	1	31.287%
Carbon	C	12.0107	2	18.753%
Oxygen	O	15.9994	4	49.960%

Calcium bound oxalates (Calculated)

$$= \frac{\text{Oxalate Mass Percent}}{\text{Calcium Mass Percent}} = \frac{68.713}{31.287} = 2.196$$

Calcium Bound Oxalates %

$$= \frac{\text{Oxalates/Calcium Ratio}}{\text{Calcium Bound Oxalates}} \times 100$$

$$= \frac{\text{Oxalates/Calcium Ratio}}{2.196} \times 100$$

Calcium Binding Capacity %

$$= \frac{\text{Water soluble Oxalates}}{\text{Calcium Mass Percent (31.287)}} \times 100$$

3. Results and Discussions

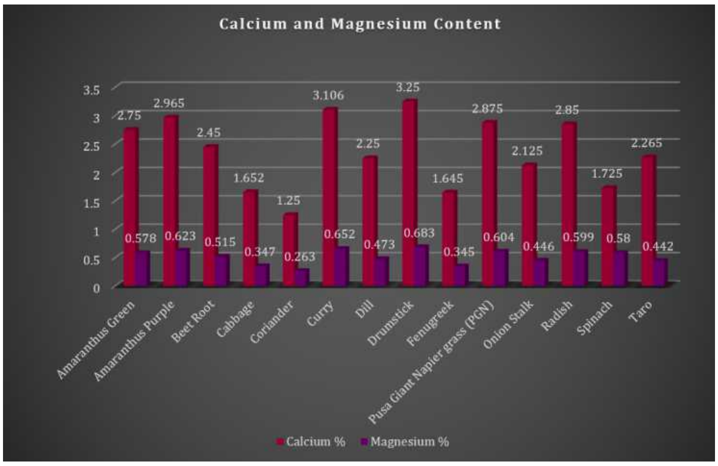
3.1 Calcium and Magnesium contents in Leafy and root vegetables

Table 3 and graph 1 & 2 show the calcium and magnesium contents that can be extracted using EDTA, as well as the calcium + magnesium contents of the 14 leafy vegetables, root vegetables, and Napier fodder grass that were studied. According to the findings, the calcium level of these root and leafy vegetables ranged from 1.250 to 3.106%, with a mean of 1.278%. Curry leaves had the greatest calcium content, while coriander leaves had the lowest. The calcium level of the root vegetables ranged from 2.125 to 2.850%, with onions having the lowest amount and radish having the highest. The trend of magnesium content in these root and leafy vegetables was comparable to that of calcium content. The magnesium content of curry leaves ranged from 0.263 to 0.652%, with a mean of 0.458%. The leafy vegetable coriander had the lowest magnesium content. The magnesium concentration of root vegetables ranged from 0.446 to 0.599%, with onions having the lowest level and radish having the highest.

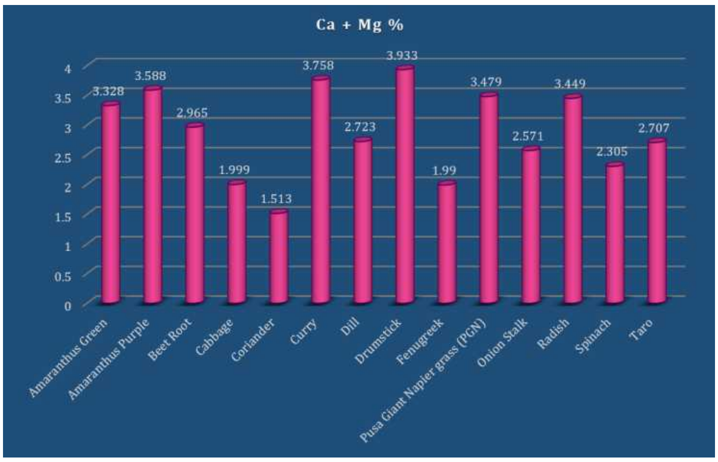
Table: 3. Calcium and Magnesium in Leafy vegetables, Root vegetables, Onion and Napier Grass

S.No.	Vegetable Name	Calcium %	Magnesium %	Ca + Mg %
1	Amaranthus Green	2.750	0.578	3.328
2	Amaranthus Purple	2.965	0.623	3.588
3	Beet Root	2.450	0.515	2.965
4	Cabbage	1.652	0.347	1.999
5	Coriander	1.250	0.263	1.513
6	Curry	3.106	0.652	3.758
7	Dill	2.250	0.473	2.723
8	Drumstick	3.250	0.683	3.933
9	Fenugreek	1.645	0.345	1.990
10	Pusa Giant Napier grass (PGN)	2.875	0.604	3.479
11	Onion Stalk	2.125	0.446	2.571
12	Radish	2.850	0.599	3.449
13	Spinach	1.725	0.580	2.305
14	Taro	2.265	0.442	2.707

Graph:1 Calcium and Magnesium content in vegetables



Graph: 2 Calcium + Magnesium percentage



3.2 Water Soluble Oxalates, in Leafy vegetables, Root vegetables, Onion and Napier Grass

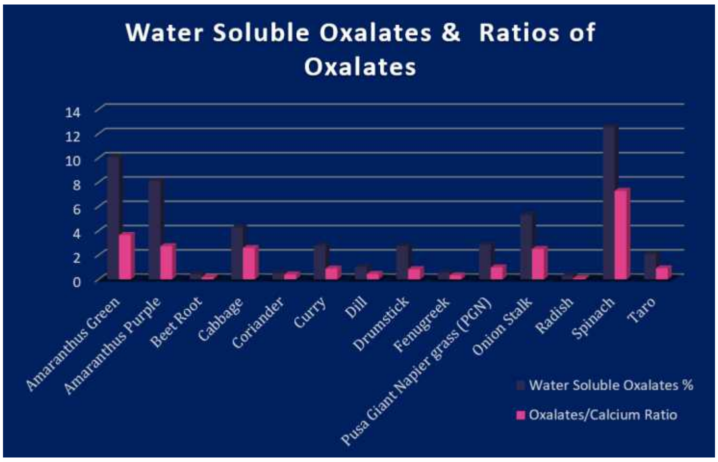
Fourteen Indian-grown leafy and root vegetables were tested for water-soluble oxalate concentration. The water soluble oxalates in these 14 leafy vegetables were divided into three groups: Low (0 to 4.0%), Medium (4.1 to 8.0%), and High (>8.1%). The amounts of aqueous oxalates in four leafy plants (colocasia, purple and areen amaranth, and spinach) varied from 8.115 to 12.580%. Eight vegetables (beetroot, cabbage, coriander, curry leaf dill, drumstick, fenugreek, Napier grass, and taro) were in the low group, with a range of 0.332 to 2.50%, while two additional leafy vegetables (cabbage and onion stalks) had medium water soluble oxalate, ranging from 4.275 to 5.330%.

This was consistent with previous studies [15] & [16]. Eleven commercial cultivars were screened for oxalate concentration as part of Beiquan Mou's (2008) evaluation [17] of the entire USDA spinach (*Spinacia oleracea*) germplasm collection (338 accessions). The genotypes evaluated showed significant differences in oxalate concentration, ranging from 5.3% to 11.6% on a dry weight basis. Foods can be categorized into three classes based on the ratio of total oxalate to total calcium in mEq, according to Noonan and Savage (1999). This ratio is intriguing since it illustrates how this diet and other foods ingested concurrently affect calcium availability. With a ratio of 4.58, spinach was the only sample that was placed in group 1. With ratios of 1.22 and 1.62, purple and green amaranth leaves were categorized as belonging to group 2. Group 3 included the remaining eight samples, whose ratios for colocasia and radish leaves ranged from 0.03 to 0.98, respectively [18] [19] [20] [21] [22].

Table :4 Water Soluble Oxalates, Ratios of Oxalates : Calcium and Calcium : Magnesium in Leafy vegetables, Root vegetables, Onion and Napier Grass

S.No.	Vegetable Name	Water Soluble Oxalates %	Oxalates/Calcium Ratio	Ca/Mg Ratio
1	Amaranthus Green	10.065	3.66	4.758
2	Amaranthus Purple	8.115	2.737	4.759
3	Beet Root	0.456	0.186	4.757
4	Cabbage	4.275	2.588	4.761
5	Coriander	0.513	0.41	4.753
6	Curry	2.785	0.897	4.764
7	Dill	1.024	0.455	4.757
8	Drumstick	2.75	0.846	4.758
9	Fenugreek	0.55	0.334	4.767
10	Pusa Giant Napier grass (PGN)	2.85	0.991	4.76
11	Onion Stalk	5.33	2.508	4.765
12	Radish	0.312	0.109	4.758
13	Spinach	12.58	7.293	2.974
14	Taro	2.075	0.916	5.124

Graph:3 Water Soluble oxalates and ratios of oxalates



3.3 Ratio of Water soluble Oxalates/Calcium in Leafy vegetables, Root vegetables, Onion and Napier Grass

Water-soluble oxalate/calcium ratios for leafy, root, onion, and napier grass are shown in Table 4 and illustrated in graph 3. The findings showed that the ratio of calcium to water-soluble oxalates ranged from 0.186 to 7.293, with a mean of 3.740. Beetroot had the lowest ratio, at 0.186, while spinach had the highest, followed by amaranthus green (3.660) and amaranthus purpurple (2.737). According to earlier studies, such as those conducted by Radek and Savage (2008) on Oxalates/Calcium ratios 11 in Indian leaf vegetables [23]. Marina et al. (2002) also reported similar results in tea and herbal tea leaves [24], and Murali Krishna and Riazuddin Ahmed (2010) in their study on hybrid Napier grass, the ratio of water soluble Oxalate content to EDTA extractable Calcium content showed that the Hybrid Napier NB-21 and CN-46 genotypes had the highest ratio at 40" and 80 hours after harvesting, respectively, while IGFRI-7 and BN-9201 genotypes showed the lowest values at 40" and 80 hours after harvesting [25].

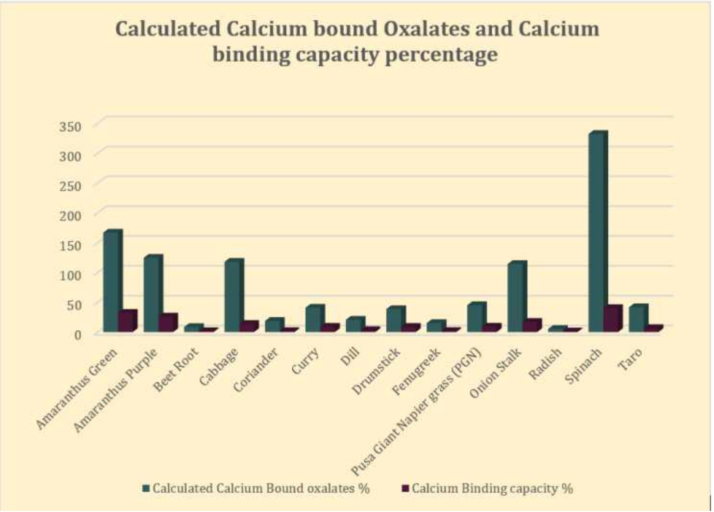
3.4 Calculated Calcium bound Oxalates and Calcium binding capacity percentage in Leafy vegetables, Root vegetables, Onion and Napier Grass

The quantity of calcium that could bind to the soluble oxalate was computed after the concentration of soluble oxalate in the extracted leafy vegetables, root vegetables, and Napier grass was established. The calcium binding capacity % and calculated calcium bound oxalates in leafy, root, onion, and hybrid napier grass are shown in graph. 4 and Table 5. The findings showed that the mean value of calcium-bound oxalates was 168.65%, with a range of 5.2 to 332.1%. The greatest ratio was recorded by spinach, followed by amaranthus green (166.7%) and Amaranthus purpurple (124.6%). Radish had the lowest ratio, at 5.2, closely followed by beets (8.5%). The calcium-binding ability of various root and leafy vegetables showed a similar pattern. The findings showed that the calcium binding capacity had a mean value of 20.61% and varied from 1.02 to 40.2%. The greatest percentage of calcium binding capacity was recorded by spinach (40.2%), followed by amaranthus green (32.17%) and amaranthus purpurple (24.93%). Radish (1.02%) had the lowest calcium binding capacity, closely followed by beets (1.46%).Of the root vegetables, beetroot had a calcium binding capacity of 1.46% and onions 17.03%. The findings were consistent with those of Radek and Savage (2008), who looked at the ratios of oxalates to calcium in Indian leaf vegetables.

Table:5 Calculated Calcium bound Oxalates and Calcium binding capacity percentage

S.No.	Vegetable Name	Calculated Calcium Bound oxalates %	Calcium Binding capacity %
1	Amaranthus Green	166.7	32.17
2	Amaranthus Purple	124.6	25.93
3	Beet Root	8.5	1.46
4	Cabbage	117.9	13.66
5	Coriander	18.7	1.64
6	Curry	40.8	8.9
7	Dill	20.7	3.27
8	Drumstick	38.5	8.79
9	Fenugreek	15.2	1.76
10	Pusa Giant Napier grass (PGN)	45.1	9.11
11	Onion Stalk	114.2	17.03
12	Radish	5.2	1.02
13	Spinach	332.1	40.2
14	Taro	41.7	6.63

Graph: 4 Calculated Calcium bound Oxalates and Calcium binding capacity percentage



4. Conclusion

Calcium and Oxalate contents were investigated in various plant species of 14 leafy vegetables and root vegetables including onion, beetroot, radish and Napier grass available in the local markets. In this context, it was also felt pertinent to analyze the variation in the Oxalate content of these various 14 leafy vegetables and root vegetables collected from various local markets of Hyderabad and Secunderabad twin cities in Telangana state.

The results of EDTA extractable Calcium and Magnesium and Calcium + Magnesium contents indicate that of the tested 14 leafy vegetables and root vegetables and Napier fodder grass are Calcium content in these leafy and root vegetables were ranged from 1.250 to 3.106% with a mean of 1.278%, the highest was recorded by curry leaves and lowest was recorded by coriander leafy vegetable. Among root vegetables Calcium content was ranged between 2.125 to 2.850%, highest recorded by radish and the lowest by onion. Magnesium content in these leafy and root vegetables also followed similar trend to that of Calcium content. The Magnesium was ranged between 0.263 and 0.652% with a mean of 0.458%, recorded by curry leaves and lowest was recorded by coriander leafy vegetable. Among root vegetables Magnesium content was ranged between 0.446 to 0.599%, highest recorded by radish and the lowest by onion.

The results indicated that that ratio of water soluble oxalates to calcium was ranged between 0.186 and 7.293 with a mean value of 3.740. Spinach recorded highest ratio followed by Amaranthus green (3.660) and Amaranthus purpurple (2.737) and lowest ratio was recorded by beetroot of 0.186.

Oxalates, particularly water-soluble oxalates, are commonly found in a wide variety of leafy and root vegetables, as well as pasture crops like Napier grass. These compounds can contribute to both the nutritional value and potential health risks of these plants, as high oxalate content can interfere with calcium absorption and lead to the formation of kidney stones in susceptible individuals. In a study conducted on 14 leafy and root vegetables grown in India, the water-soluble oxalate content was measured and categorized into three distinct groups: Low (0–4.0%), Medium (4.1–8.0%), and High (>8.1%). Among the 14 vegetables tested, four—spinach, purple amaranth, green amaranth, and colocasia—were found to have high levels of water-soluble oxalates, with values ranging from 8.115% to 12.580%. Two other vegetables, cabbage and onion stalks, contained medium levels of oxalates, with values between 4.275% and 5.330%. The remaining eight vegetables, including beetroot, cabbage, coriander, curry leaf, dill, drumstick, fenugreek, Napier grass, and taro, had lower oxalate concentrations, ranging from 0.332% to 2.50%. Additionally, the calcium binding capacity of the water-soluble oxalate fraction varied significantly across the vegetables, ranging from a low of 1.02% in radish to a high of 40.20% in spinach. The calcium bound in the oxalates ranged from 5.2% in onion stalks to 332.1% in spinach, indicating that the oxalate content can influence the bioavailability of calcium in these foods. This variability highlights the importance of considering oxalate content when evaluating the nutritional profiles of these plants.

An ongoing debate in oxalate research centers around the extent to which dietary oxalates hinder calcium absorption. Calcium is a mineral that can be somewhat challenging for the body to absorb from food sources. Even with low dietary intake levels, where one might expect an increase in absorption, calcium is typically absorbed at a rate of about 35%. However, this absorption rate can vary significantly depending on the specific food, and the presence of oxalates can further complicate this process. Oxalates form insoluble calcium oxalate salts when combined with calcium, reducing its bioavailability and inhibiting absorption.

Despite these concerns, there are two important considerations that help mitigate widespread concerns regarding the interference of oxalates with calcium absorption. First, public health guidelines already account for these variations in calcium absorption. For instance, the World Health Organization (WHO) recommends a daily intake of 1,000 milligrams of calcium, which is based on the average amount of calcium absorbed from various foods, including those like spinach, which is high in oxalates. This recommendation reflects the understanding that not all calcium from food sources will be absorbed, but still provides sufficient levels to meet nutritional needs. Therefore, while oxalates do reduce calcium absorption to some extent, they do not negate the overall importance of including calcium-rich foods in the diet, as the recommendations are designed to account for such factors.

Second, research on different population groups that consume a variety of plant and animal foods has shown that individuals who follow predominantly plant-based diets, such as vegetarians, do not exhibit a higher incidence of calcium deficiency or osteoporosis. This challenges the assumption that foods high in oxalates would lead to significant calcium absorption issues and associated health risks. Despite the fact that calcium is absorbed less efficiently from oxalate-containing foods compared to those without oxalates, the overall impact on calcium status does not appear to be detrimental. The intake of oxalate-rich foods, such as leafy greens, does not make them irrelevant or counterproductive in maintaining adequate calcium levels. In fact, vegetarians often achieve similar or even higher calcium intake from plant sources, demonstrating that oxalate-containing foods can still play a role in meeting calcium needs.

Third, studies have demonstrated that the overall combination of foods consumed in a single meal can significantly influence the bioavailability of soluble oxalates. The presence of other foods in the meal, particularly those that contain compounds like calcium, magnesium, or fiber, can reduce the amount of oxalates available for absorption in the digestive tract. For example, consuming calcium-rich foods alongside oxalate-containing foods can help bind some of the oxalates, making them less likely to be absorbed and reducing their potential interference with calcium absorption. This highlights the importance of dietary patterns and meal composition in determining how oxalates affect mineral absorption, rather than focusing solely on individual food components.

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