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Effect of Maturity Stages on the Nutritional Content of *Hygrophila spinosa* and *Chenopodium album* Leaves

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Abstract

Urbanization has impacted the knowledge and use of traditional leafy vegetables. The present investigation is conducted to understand the importance of edible leaves and the variation in their nutritional content at different stages of maturity. *Hygrophila spinosa* and *Chenopodium album* leaves have been selected owing to their economic cost, accessibility, and utilization within the population. A leaf undergoes several physiological and metabolic changes during maturity, which may affect its biochemical content. Hence, the samples have been analyzed for their nutritional composition at distinct stages (I to IV) of maturity based on the length of the leaf post-germination. Results revealed that both samples possessed the highest content of nutrients, including carbohydrates, proteins, minerals, β carotene, and vitamin C at stage 1 compared to the older stages. Alteration in metabolic patterns and environmental influences during various phases may be responsible for this effect. Stage I manifested the notable existence of essential phytonutrients and the diminished presence of potent anti-nutrients. Moreover, a significant percentage of micronutrients are found to be available post *in vitro* gastrointestinal digestion. The study highlights the importance of consuming young edible leaves. Regular dietary incorporation of the same may lead to alleviation of nutrient deficiency disorders and food insecurity.

Keywords: Biochemical; Edible leaves; Maturity; Nutrients; RDA; Stages.

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1. Introduction

Green leafy vegetables are significant nutritional determinants. The Indian subcontinent is blessed with many natural surroundings and hosts one of the largest flora and fauna. The climatic and soil conditions permit the cultivation of a wide variety of edible leaves for domestic and industrial uses. These are rich in macro-nutrients, minerals, vitamins, dietary fiber, and antioxidants [1-3]. Moreover, they possess moderate calories and fat content. The nutritional composition of edible leaves can prevent several deficiency disorders, mainly due to their rich micronutrient profiles [4]. Consumption of the required nutrients according to the Recommended Dietary Allowance (RDA) is essential for maintaining the health and wellbeing of the population [5]. However, popular amongst ancient

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communities, the demand for edible leaves has shown a decline, mainly because of a steep rise in urbanization and lifestyle changes.

Kulekhara (*Hygrophila spinosa)* is a member of the Acanthaceae family. The parts of this plant have been widely used in traditional medicine to treat various disorders such as diseases of the urinogenital tract, hyperdipsia, vesical calculi, flatulence, diarrhea, and anemia [6,7]. Kulekhara hosts several biochemicals: phytosterols, fatty acids, polyphenols, amino acids, vitamins, minerals, and glycosides [8,9]. Bathua (*Chenpodium album),* on the other hand, is an underutilized nutritious and palatable wild weed possessing nutritional and functional benefits. It can provide significant minerals, fiber, vitamins, and essential fatty acids in a diet [10,11]. Indeed *C. album* has been re-established as a significant Asian food source considering its adaptability under adverse conditions like low precipitation, high elevation, thin cold air, blistering sun, and sub-frigid temperatures. Regular intake of Bathua leaves has been correlated with an improved nutritional and physiological status in humans [12]. Nonetheless, the above leaves' popularity is restricted due to the paucity of awareness regarding its manifold benefits.

The characteristics of edible leaves are often influenced by numerous factors like preharvest conditions, distinction in genes, stages of maturity, and handling procedures [13]. Previous studies have reported the biochemical content of green leaves to display variation with advancing maturity, thereby affecting their nutrient profile and sensory parameters [14-16]. The nutritional composition at a particular stage is dependent on several factors such as the type of plant, availability of raw materials, transport, biosynthesis, and utilization by plant tissues [17]. Furthermore, the quantity and quality of biomolecules are governed by the balance of biosynthetic and catabolic reactions for ensuring adequate growth and development of the plant [18,19]. Dynamic changes in lifestyle patterns and eating habits have moderately replaced the plant-based diet with coarse grains, meat products, and fat [20,21]. Moreover, consumers generally opt for leaves that are low in cost without consideration of the maturity stage. Therefore, the present study explores the variation in the nutritional content of the selected edible leaves at different levels of maturity to analyze the stage of the leaf that would provide optimum amounts of nutrients for conferring good health. Kulekhara and Bathua leaves were chosen owing to their low cost and ready availability. The research further aims to increase consumer awareness regarding the potential of the studied leaves for mitigation of nutritional and food insecurity. Consumption of leaves at the desired stage may satisfy the required dietary intake and help prevent and treat various deficiency diseases.

2. Materials and Methods

2.1. *Growth and harvest of edible leaves*

The edible leaves were grown using home gardening to enable efficient growth monitoring and accurate selection of leaf maturity stages. Seeds of Bathua leaves *(Chenopodium album)* and plant cuttings of Kulekhara leaves **(***Hygrophila spinosa*) were sprinkled all over the soil containing natural fertilizers such as cow dung and Neemkhal.

Plants were grown under moderate sunlight with regular watering. Leaves were harvested at four different levels of maturity, the stages being determined by the length of the leaves (Table 1). The time of planting the seeds or leaf cuttings was noted as day 1. The time gap between subsequent harvesting stages was approximately 14-15 days.

Table 1. Selection of leaf maturity stages.

Stage of Harvest	Kulekhara Leaves (*Hygrophila spinosa)*		Bathua Leaves *(Chenopodium album)*	
	Time of Harvest (Days)	Length (cm)	Time of Harvest (Days)	Length (cm)
Stage I	Day 15	4.0	Day 14	2.5
Stage II	Day 30	6.3	Day 29	4.3
Stage III	Day 46	10.2	Day 45	5.1
Stage IV	Day 61	15.0	Day 60	7.0

2.2. *Preparation of leaf extract*

The fresh leaves at different stages were harvested, followed by washing to remove impurities. These leaves were thereafter divided into two batches viz.: fresh and air-dried. The fresh leaves were procured, blotted to remove excess moisture, and analyzed. The other batch was air-dried, ground into a fine powder, sieved, and stored in air-tight sterile containers at room temperature. Sample extracts were prepared by soaking either the fresh or air-dried leaves in distilled water or ethanol followed by filtration and labeled as fresh (F) and air-dried (D), respectively. The extracts were stored at 4 °C under sterile conditions.

2.3. *Analysis of proximate composition*

The selected leaf samples were analyzed for their moisture, ash, protein, and carbohydrate contents at different maturity levels according to standardized protocols recommended by the Association of Official Analytical Chemists (AOAC). The moisture content was estimated by the thermogravimetric method. In contrast, the ash percentage was obtained using the dry ashing technique through incineration of the materials in a muffle furnace at 600°C. The carbohydrate content was determined via the method described by Yemm *et al*. with a slight modification [22]. D-Glucose at a concentration of 1 mg/mL was used as the standard. 1 mL of the standard or sample solution was mixed with 4 mL of Anthrone reagent (Lobachemie, India), incubated at room temperature for 10 min, followed by measurement of Optical Density (OD) at 620 nm using a spectrophotometer (Hitachi UV-VIS Spectrophotometer U2900, India). The protein content of the leaf extracts was determined by the Biuret method with respect to the standard curve. 1 mL of either the standard or test solution was mixed with 4 mL of Biuret reagent (Lobachemie, India), incubated at room temperature for 30 min, followed by measurement of OD at 550 nm [23].

2.4. *Estimation of micronutrients*

2.4.1. *Quantitative estimation of β carotene and ascorbic acid*

The concentration of β carotene was calculated using Lambert-Beer's law. Briefly, ethanolic extracts of the samples were centrifuged at 3000 rpm for 3-4 min. The supernatant was transferred to a separating funnel with 10-15 mL of petroleum ether to separate layers. The upper layer was collected in a volumetric flask; volume made up to 100 mL with petroleum ether followed by determination of absorbance at 452 nm [24]. The ascorbic acid content of the leaves was measured using the indophenol dye method with respect to the standard. 1 mL of 2,6-dichlorophenol indophenol dye was titrated with a 1:1 mixture of metaphosphoric acid: ascorbic acid or sample solution [25].

2.4.2. *Quantitative estimation of minerals*

The leaf samples were quantified for iron, calcium, and phosphorus contents at different stages of maturity. Iron was estimated by the Iron Assay Method (Iron TIBC kit, Microxpress, India). Calcium and phosphorus were estimated by the OCPC method (Calcium Reagent kit, CliniQuant-FSR, India) and the Molybdate UV method (Phosphorus kit, MicroXpress, India), respectively. Reagents were mixed according to the manufacturer's recommendation, incubated at room temperature for 5 min, followed by measurement of OD at the specified wavelengths. The concentrations of the minerals were calculated concerning their standards.

2.5. *Estimation of phytonutrients*

Kulekhara and Bathua leaf samples were qualitatively estimated for their phenols, flavonoid, saponin, tannin, anthraquinone, and alkaloid contents at the different maturity stages (I – IV) using standard procedures approved by Bhattacharyya *et al*. [26]. +++, ++, and + were used to denote very high, moderately high, and low abundance, respectively.

2.6. *Availability post gastrointestinal digestion*

The availability of micronutrients post gastrointestinal (GI) digestion was estimated using the *in vitro* static model enlisted by Wojtunik-Kulesza *et al*. with a slight modification [27]. Briefly, gastric juice was formulated using glucose (3.5 g/L), NaCl (2.05 g/L), KH_2PO_4 (0.60 g/L), $CaCl_2$ (0.11 g/L), and KCl (0.37 g/L), adjusted to pH 2.0 using 1 M HCl, and autoclaved at 121 °C for 15 min. Intestinal juice was simulated by using NaCl (125.0 mM), $CaCl_2$ (0.6 mM), $MgCl_2$ (0.3 mM), trypsin (11 U/mL), α-chymotrypsin (24 U/mL) and pancreatic lipase (590 U/mL). Bile solution was formulated by using Oxgall solution (Difco laboratories, India). Sample extracts were subjected to *in vitro* gastric and intestinal digestion for 30 and 60 min, respectively. The samples were evaluated for their micronutrient contents after the time points employed for gastrointestinal digestion and

represented as percentage (%) availability with respect to the total amount in the introduced sample.

2.7. *Statistical analysis*

Results were expressed as mean± standard error mean (SEM) of N≥3 experiments. p-value was calculated using a linear regression model (R studio, version 4.0.2, USA). Only $P \leq 0.05$ was considered significant with 95% confidence interval. Significance expressed as $P \leq 0.001$ = ***, $P \leq 0.05$= **; $P \leq 0.1$= *.

3. Results and Discussion

3.1. *Proximate composition of the edible leaves*

Table 2. Proximate composition of the edible leaves.

Sample	Stages of Maturity	Moisture[a] (%)	Ash[b] (%)	Carbohydrate (g/100 g)		Protein (g/100 g)	
				F	D	F	D
Hygrophila spinosa	I	38.58*	22.12*	19.98*	16.56***	9.12*	6.33***
	II	35.25*	18.31*	15.24**	14.32***	8.33*	5.06***
	III	29.82*	15.22*	11.03*	12.26***	6.05**	4.62***
	IV	27.34*	12.13*	10.86*	10.54***	2.99*	2.11***
Chenopodium album	I	36.22**	20.15**	10.65**	9.23***	8.98*	6.80***
	II	33.11**	16.33*	8.34*	7.03***	7.25*	5.65***
	III	29.74*	13.10*	7.86*	6.15***	7.02*	4.35***
	IV	26.31**	9.45**	6.83*	5.87***	4.12**	3.22***

[a] Value expressed as % wet weight.
[b] Value expressed as % dry weight.

The selected edible leaves were analyzed for their proximate composition, including moisture, ash, carbohydrate, and protein contents at four different stages of maturity, owing to the importance of these constituents in supporting normal homeostasis, metabolism, growth, and repair in human beings. Results revealed that both *Hygrophila spinosa* and *Chenopodium* album displayed the highest moisture percentages at stage I compared to the other stages (Table 2). Moreover, the above contents manifested a steady decrease with the enhancement of leaf maturity, their amounts at the final stage being 27.34 and 26.31 %, respectively. The altered respiration rates and metabolism in younger leaves versus their older counterparts may be responsible for this effect [28]. The proportion of ash also portrayed a steady decline with the increasing age of the Kulekhara and Bathua leaves, indicating a lowering of mineral content from stage I to stage IV (Table 2). Furthermore, it was observed that the carbohydrate concentration was largest during stage I of both *Hygrophila spinosa* (19.98 g/100 g) and *Chenopodium album* (10.65 g/100 g) leaves when quantified in freshly prepared concentrates (Table 2). This quantity displayed a steady decrease with an elevation in leaf maturity, their amounts at stage 4 being 10.86 g/100 g and 6.83 g/100 g, respectively. Besides, the concentration of this macromolecule was lower in air-dried leaf samples (D) compared to the fresh extracts

(F). Nonetheless, air-dried leaves also exhibited the highest carbohydrate concentration at stage I followed by stage II, III, and IV (Table 2).

The increased demand for energy during plant maturity may be responsible for higher usage of carbohydrates, thereby culminating in lower amounts available in the mature stages [29]. Additionally, the proportion of carbohydrate biosynthesis enzymes has also been reported to decline with increasing age, further contributing to low levels. *Hygrophila spinosa* and *Chenopodium album* are good candidates for locally available and inexpensive carbohydrates, with the former being a better alternative. The protein content of *Hygrophila spinosa* (9.12 g/100 g) and *Chenopodium album* (8.98 g/100 g) leaves was also found to be highest at stage I (Table 2). An increase in leaf maturity brought a gradual decrease in protein levels in both the samples as manifested by protein amounts of 2.99 g/100 g and 4.12 g/100 g at stage IV in the fresh extracts of Kulekhara and Bathua leaves, respectively. Similar to the carbohydrate amounts, the protein concentration in the air-dried samples (D), although lower in comparison to the fresh extracts (F), displayed a rapid lowering with advancing stages of maturity (Table 2). Alteration in the rates of protein synthesis during leaf maturity may be responsible for the observed decrease. Indeed, previous studies have documented the rate of protein biogenesis to be maximal in young leaves compared to their older counterparts [30]. Hence, both the above leaves can provide valuable sources of carbohydrates, proteins, and minerals, especially when consumed in the early stages. Additionally, being economical reservoirs of vegetarian protein, consumption of same can be preferred across communities and socio-economic groups of the population to meet the desired dietary requirements.

3.2. *Micronutrient content of the edible leaves*

3.2.1. *Ascorbic acid and β carotene content of the edible leaves*

The selected samples were studied for their ascorbic acid and β carotene contents to assess the variation, if any, at different stages of leaf maturity. Data portrayed *Hygrophila spinosa* leaves harvested at stage I to possess the highest vitamin C content (60.32 mg/100 g), followed by stage II (52.11 mg/100 g), stage III (48.77 mg/100 g), and stage IV (38.76 mg/100 g). Moreover, the vitamin C content of freshly procured *Chenopodium album* was also observed to be greatest at the first stage (88.26 mg/100 g) compared to 75.68 mg/100 g, 68.22 mg/100 g, and 55.08 mg/100 g at stage II, III, and IV respectively (Table 3). However, the ascorbic acid content in dried leaf samples displayed a lowered concentration versus the fresh extracts. Nevertheless, maximal content of this vitamin was observed at stage I of both Kulekhara (49.23 mg/100 g) and Bathua leaves (79.17 mg/100 g) compared to the older stages. Hence, the elevation of leaf maturity brought about a concomitant lowering in the ascorbic acid content in both the studied samples. Variations in the synthesis rates and utilization of this vitamin by the plant at the different stages may lead to the above difference.

Furthermore, vitamin C is susceptible to fluctuations in temperature, position changes, and mechanical bruising during harvest, handling, drying, and storage, thereby further resulting in reduced quantities [13]. Although both the above samples demonstrated significant amounts of ascorbic acid, *Chenopodium album* displayed a higher content of this vitamin than *Hygrophila spinosa*. Additionally, biochemical analysis revealed fresh Kulekhara leaves to possess the largest content of β carotene at stage I (4.40 mg/100 g). Advancement of leaf maturity was observed to diminish the above amounts as displayed by the presence of 1.52 mg/100 g of β carotene at stage IV (Table 3). Similarly, Bathua leaves also portrayed the maximum content of this biomolecule at stage I (8.83 mg/100 g) followed by stage II (7.34 mg/100 g), III (6.06 mg/100 g), and IV (3.87 mg/100 g) when quantified in freshly prepared concentrates (F). Although found to be lower in the air-dried samples, the content of this micro-nutrient manifested a steady decline from stage I-IV (Table 3). Hence, the ascorbic acid and β carotene percentages manifested a decrease with the advancing age of the leaf. Moreover, the nutrients' content was higher in the fresh leaf extracts versus their dried counterparts, owing to the sensitivity of the same towards temperature alterations and drying. Vitamin C is essential for a normal physiological state and defense against diseases due to its antioxidant actions. However, regular dietary supplementation is essential since it cannot be stored inside the human body. The RDA for this micronutrient is 40 mg/day for adults [5]. β carotene, on the other hand, is an important member of the carotenoid group, essential for the maintenance of cellular homeostasis. The human body converts β carotene into Vitamin A. Indeed, 4800 µg of β carotene has been recommended for daily consumption [5]. Unfortunately, developing nations have been observed to exhibit several instances of ascorbic acid and vitamin A deficiencies. During the initial stages of plant growth and development, biosynthetic reactions tend to outpace degradative ones, resulting in a net accumulation of nutrients. However, this trend has been observed to get reversed during maturity, resulting in lowering nutrients. Therefore, dietary incorporation of young edible leaves, especially *Chenopodium album* and *Hygrophila spinosa* may solve nutritional insecurity and deficiency disorders. Furthermore, the utilization of these leaves may be preferred due to easy accessibility, inexpensive cost, and regular availability.

3.2.1. *Mineral content of the edible leaves*

Kulekhara and Bathua leaves were further analyzed for their iron, calcium, and phosphorus contents from stage I to IV of leaf maturity. Both *Hygrophila spinosa* and *Chenopodium album* leaves showed a decline in iron amounts with advancing leaf age. The iron concentration in fresh Kulekhara leaves decreased from 12.03 mg/100 g to 5.88 mg/100 g from stage I to stage IV. Moreover, Bathua leaves also displayed a reduction in the content of this mineral with progressing age as manifested by amounts of 6.9 mg/100 g and 2.7 mg/100 g at stage I and IV, respectively (Table 3). Iron is a vital mineral required primarily for the exchange of gasses and other essential processes. Alarmingly,

India has been reported to harbor a significant percentage of iron deficiency anemia cases. The RDA for iron is 17 mg/day and 21 mg/day for males and females, respectively, with the requirements varying during pregnancy, lactation, and aging [31]. Dietary incorporation of Kulekhara and Bathua leaves may help in meeting the daily iron requirements.

Table 3. Micronutrient content of the edible leaves.

Sample	Leaf Stages	Ascorbic acid (mg/100 g)		β carotene (mg/100 g)		Iron (mg/100 g)		Calcium (mg/100 g)		Phosphorus (mg/100 g)	
		F	D	F	D	F	D	F	D	F	D
Hygrophila spinosa	I	60.32***	49.23**	4.40**	3.80*	12.03*	10.19***	70.22*	68.44***	116.12*	112.22***
	II	52.11**	45.73*	3.70*	3.18*	10.12*	9.24***	57.89*	55.56***	100.09*	98.33***
	III	48.77*	39.56**	2.75**	2.33**	8.02*	7.13***	52.10*	48.33***	95.06*	92.33***
	IV	38.76**	30.09*	1.52*	1.19**	5.88*	5.36***	46.23*	42.68***	89.05*	84.34***
Chenopodium album	I	88.26**	79.17*	8.83**	7.93*	6.90*	5.1***	321.31*	300.01***	115.87*	114.68***
	II	75.68**	70.11*	7.34*	6.88**	5.20**	4.22***	298.21*	270.02***	102.22**	100.33***
	III	68.22*	61.15*	6.06*	5.06***	3.60*	2.75***	267.71*	250.10***	95.66*	91.21***
	IV	55.08**	48.85*	3.87*	3.03***	2.70*	1.45***	241.08*	222.61***	85.96*	82.88***

In fact, Kulekhara leaves have been traditionally used for the prevention and treatment of anemia. The analysis results portray the leaves of *Hygrophila spinosa* as better sources of iron than *Chenopodium album.* Noteworthy, the above leaves carry the highest content at stage I. Moreover, increased amounts of Vitamin C at this level may further help in the assimilation of iron. Furthermore, biochemical estimations depicted the calcium levels as the highest in *Hygrophila spinosa* (70.22 mg/100 g) and *Chenopodium album* (321.31 mg/100 g) leaves when harvested during their first growth stages. The amount of this mineral displayed a marginal decrease with an increase in leaf maturity

from stage I to IV (Table 3). Interestingly, the calcium content of Bathua leaves was found to be significantly more compared to Kulekhara leaves, highlighting the former as a preferred source of this mineral. Fluctuations in temperature and changes in soil acidity may lead to a slight decrease in calcium levels in the advanced stages of the leaves [32]. Calcium is an essential micronutrient required for metabolism, homeostasis, and maintenance of bone health. An average adult is recommended a diet containing 600 mg/day of calcium. Regrettably, diets in the Indian subcontinent are usually deficient in calcium owing to lack of resources, disturbances in the economy, and paucity of knowledge. Moreover, the standard eating routine of the population basically comprises cereals and pulses that are poor sources of the above. Interestingly, regular consumption of young leaves, particularly *Chenopodium album* may aid in solving the above issue. Phosphorus is a basic auxiliary segment of cell membranes and nucleic acids and is associated with bone mineralization, vitality, and cell signaling. Inadequacies in phosphorus manifest as loss of hunger, muscle shortening, bone delicacy, and numbness. Plant sources can serve as significant reservoirs of phosphorus [33]. Hence, the two edible leaves were tested for their phosphorus contents from stage I to stage IV. *Hygrophila spinosa* revealed phosphorus amounts of 116.12, 100.09, 95.06 mg, and 89.05 mg/100 g of the leaves from stage I to IV, respectively. Similarly, the phosphorus content of fresh *Chenopodium album* decreased from 115.87 mg/100 g to 85.96 mg/100 g with advancing age (Table 3). Additionally, results revealed the mineral contents in the dried leaves (D) to be lower than the freshly procured ones (F). Nevertheless, the content of these minerals was found to be greatest in the first maturity stage compared to those harvested at later ages (Table 3). Decreased rate of growth at the later stages may be responsible for the detected variation. Men and women aged twenty years and above are recommended a daily dose of 1596 and 1189 mg of phosphorus. Dairy, meat, and fish are rich sources of phosphorus. However, regular consumption of these may be difficult for people belonging to lower socio-economic groups. Therefore, *Hygrophila spinosa* and *Chenopodium album* leaves may serve as attractive, low-cost, and readily available sources of the studied minerals. Moreover, dietary accommodation of these leaves, especially at stage I, may help fulfill the human body's micronutrient needs, thereby potentially aiding in the mitigation of mineral deficiency disorders.

3.3. *Phytonutrient composition of the edible leaves*

The *Hygrophila spinosa* and *Chenopodium album* leaves were analyzed for their phytonutrient composition at four different stages of maturity. Results revealed the significant presence of phenolic compounds in both the leaf samples, especially at the initial growth stages. Moreover, *C album* leaves portrayed increased amounts of flavonoids at stage I and II compared to the other stages (Table 4). However, *H spinosa* was found to be devoid of any flavonoid content. Phytochemicals are responsible for providing color, flavor, and aroma and protecting the human body against a plethora of chronic diseases. Additionally, they aid in protecting the plant against invasion, disease,

and infection. Interestingly, the selected edible leaves were observed to display the limited presence of tannins, alkaloids, and saponins, along with the absence of anthraquinones (Table 4). Previous studies have reported tannins, specific saponins, and a few members of the alkaloid group to function as anti-nutrients, thereby hindering the biological availability of essential nutrients [34]. Hence, the low abundance of major anti-nutrients may further increase the nutritional importance of these leaf samples in the alleviation of nutritional deficiencies.

Table 4. Phytonutrient composition of the edible leaves.

Leaf Stages	Phenols		Flavonoids		Saponins		Tannins		Alkaloids		Anthraquinones	
	H. spinosa	*C. album*	*H. spinosa*	*C. album*	*H. spinosa*	*C. album*	*H. spinosa*	*C. album*	*H. spinosa*	*C. album*	*H. spinosa*	*C. album*
I	+++	+++	-	++	+	+	+	+	+	+	-	-
II	+++	++	-	++	+	+	+	+	+	+	-	-
III	++	+	-	+	+	+	+	+	+	+	-	-
IV	+	+	-	_	+	-	+	+	+	+	-	-

3.4. *Availability post gastrointestinal digestion*

Since the selected leaf samples displayed maximum content of nutrients, especially minerals and vitamins, at stage I of maturity, these were thereafter analyzed for their availabilities post *in vitro* gastrointestinal digestion to determine the influence of the simulated GI environment on the respective components. Results revealed ascorbic acid from both *H. spinosa* (70.1 %) and *C. album* (75.2 %) to display the highest availability amongst all the nutrients followed by calcium and β carotene (Table 5). Iron showed a low availability post gastrointestinal digestion manifested by 11.5 % and 10.1 % in Kulekhara and Bathua leaves, respectively. Previous studies have also reported non-heme iron from vegetable sources to portray a low bioavailability [35]. However, the notable presence of vitamin C in the samples may aid in the absorption and utilization of this mineral. The amount of nutrients provided by food sources, especially edible leaves, are subjected to certain losses during their transit through the GI tract owing to influences of the digestive environment and interaction with other biochemicals. Moreover, the bioavailability may also be affected by age, health status, and genetic makeup of an individual. Therefore, rich dietary sources of micronutrients are required to provide adequate amounts of these constituents for maintaining normal physiology and metabolism in the human body. *Hygrophila spinosa* and *Chenopodium album* being significant sources of important micronutrients, may aid in supplementing the required doses of these vitamins and minerals, thereby helping in lowering the instances of deficiency disorders associated with the same.

Table 5. Availability of edible leaves post *in vitro* gastrointestinal digestion.

Sample	Iron (%)	Calcium (%)	Ascorbic acid (%)	β carotene (%)
H. spinosa	11.5	36.2	70.1	30.5
C. album	10.1	38.4	75.2	32.6

4. Conclusion

Green leafy vegetables are capable of conferring health advantages to the human population. The quality of these leaves is vulnerable to changes in maturity, environmental fluctuations, and harvest conditions. The present study explores the nutritional content of *Hygrophila spinosa* and *Chenopodium album* leaves at four different stages of maturity. The samples were selected based on their ease of availability and preference by the local population. Biochemical analysis revealed young Kulekhara, and Bathua leaves to possess maximum carbohydrates, proteins, ascorbic acid, β carotene, iron, calcium, and phosphorus compared to the older stages. Moreover, these nutrients were found to be more abundant in fresh leaves versus their dried counterparts. The content of the above nutrients manifested a gradual decline with advancing age, their amounts being the lowest at stage IV. The differential metabolic patterns, biosynthetic rates, and climatic fluctuations at the various stages may be responsible for this effect. Carbohydrates and proteins are major macronutrients required for optimum growth and metabolism. Both *Hygrophila spinosa* and *Chenopodium album* were observed to be significant reservoirs of these biomolecules, especially at stage I. Moreover, *Hygrophila spinosa* leaves were noticed to be a richer source of carbohydrate compared to *Chenopodium album.* The edible leaves were also perceived as good candidates for dietary ascorbic acid and β carotene. A regular supply of these nutrients is essential for the maintenance of normal physiology and protection against pathogens.

Interestingly, Bathua leaves were found to possess superior amounts of these compounds, mainly at stage I of leaf maturity. Furthermore, the edible leaves were also observed to possess significant amounts of iron, calcium, and phosphorus, which portrayed a steady decrease with an increase in levels of maturity. The iron and calcium content was detected to be the greatest during the first growth stage of Kulekhara and Bathua leaves, respectively, signifying their utility in solving anemia and calcium deficiency disorders. Although the content of the studied nutrients was lower in the dried leaves (D) compared to the fresh leaf extracts (F), they displayed a significant decrease with the advancing age of the leaf. Poverty and urbanization have together contributed to food insecurity, energy insufficiency, and nutritional imbalances. The majority of the population cannot satisfy the RDA due to unavailability of resources, lack of awareness, and associated expenses. Moreover, the selected samples displayed the limited presence of major anti-nutrients, including tannins, alkaloids, and saponins.

Furthermore, the initial growth stages of the leaves manifested significant amounts of phenols in both *H spinosa* and *C album* and flavonoids in *C album* leaves, thereby further highlighting their positive effects. Additionally, ascorbic acid displayed significant availability post GI digestion followed by calcium, β carotene, and iron. Therefore, the

studied edible leaves may help to replenish the human body's macro and micro nutritional requirements due to their abundant biochemical composition, mainly when consumed at the initial stages of leaf maturity. Moreover, being vegetarian and low in cost can be accessed by people across various socio-economic groups and communities. This study also highlights the importance of home gardening for obtaining good quality, inexpensive and optimum stages of edible leaves. Dietary incorporation of young Kulekhara and Bathua leaves may not only help in the reduction of food and nutritional insecurity but also promote good health. The present analysis aims to extend the knowledge about the nutritional importance of edible leaves and signify the benefits of consuming them at the initial stages of maturity.

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References

1. A. A. Akindahunsi and S. O. Salawu, Afr. J. Biotechnol. **4**, 497 (2005).
2. Y. A. Iyaka, S. Idris, R. A. Alawode, and B. U. Bagudo, Am. J. Appl. Chem. **2**, 42 (2014). https://doi.org/10.3746/pnf.2020.25.3.293
3. A. D Pal and T. M. Zakir, J. Sci. Res. **12**, 621 (2020). https://doi.org/10.3329/jsr.v12i4.45110
4. V. George and C. George, Int. J. Food Sci. Nutr. **2**, 126 (2017).
5. B. Srilakshmi, Dietetics (New Age International Publishers, New Delhi, 2019).
6. S. Bera, S. Das, and A. Roy, SATSA Mukhapatra- Annual Technical Issue **21**, 232 (2017).
7. A. D. Kshirsagar, K. G. Ingale, N. S. Vyawahare, and V. S. Thorve, Pharmacogn. Rev. **4**, 167 (2010). https://doi.org/10.4103/0973-7847.70912
8. H. N. Natesh, L. Abbey, and S. K. Asiedu, Horticult. Int. J. **1**, 58 (2017). https://doi.org/10.15406/hij.2017.01.00011
9. A. Patra, S. Jha, and N. P. Murthy, Pharmacogn. J. **1**, 82 (2009).
10. S. Pandey and R. K. Gupta, J. Pharmacogn. Phytochem. **3**, 1 (2014).
11. A. Poonia and A. Upadhayay, J. Food Sci. Technol. **52**, 3977 (2015). https://doi.org/10.1007/s13197-014-1553-x
12. K. D. Sharma, G. Bindal, R. Rathour, and J. C. Rana, Int. J. Food Sci. Nutr. **63**, 290 (2012). https://doi.org/10.3109/09637486.2011.624493
13. S. K. Lee and A. Kader, Postharvest Biol. Technol. **20**, 207 (2000). https://doi.org/10.1016/S0925-5214(00)00133-2
14. V. Khader and S. Rama, Asia Pac. J. Clin. Nutr. **12**, 45 (2003).
15. M.V. Flyman and A. J. Afolayan, J. Food Qual. **31**, 661 (2008). https://doi.org/10.1111/j.1745-4557.2008.00218.x
16. C. Weerakkody, W. Nayananjalie, R. Wathsala, and K. Jayasena, Int. J. Livest. Res. **8**, 71 (2018).
17. D. Diba, G. Bultosa, and G. N. Tolesa, Bots. J. Agric. Appl. Sci. **12**, 15 (2017).
18. B. Ncube and J. V. Staden, Molecules **20**, 12698 (2017). https://doi.org/10.3390/molecules200712698
19. M. A. Grusak, J. Am. Coll. Nutr. **21**, 178S (2002). https://doi.org/10.1080/07315724.2002.10719263
20. J. Kearney, Philos. Trans. R. Soc. B. **365**, 2793 (2010). https://doi.org/10.1098/rstb.2010.0180
21. N. W. Solomons. Asia Pac. J. Clin. Nutr. **9**, S41 (2000). https://doi.org/10.1046/j.1440-6047.2000.00165.x

22. E. W. Yemm and A. J. Willis, Biochem. **57**, 508 (1954). https://doi.org/10.1042/bj0570508
23. M. M. Hojjatie and D. Abrams, J. AOAC Int. **97**, 712 (2014). https://doi.org/10.5740/jaoacint.12-443
24. W. Horwitz and G. W. Latimer, Official Methods of Analysis of AOAC International, 18th Edition (M. D. Gaithersburg, Maryland, 2005).
25. H. A. Okeri and P. O. Alonge, Pak. J. Pharm. Sci. **19**, 39 (2006).
26. S. Bhattacharyya and S. Roy, Int. J. Pharm. Sci. Res. **9**, 3845 (2018).
27. K. Wojtunik-Kulesza, A. Oniszczuk, T. Oniszczuk, M. Combrzy´nski, D. Nowakowska, and A. Matwijczuk, Nutrients, **12**, 1401 (2020). https://doi.org/10.3390/nu12051401
28. K. Hardwick, M. Wood, and H. Woolhouse, New Phytol. **67**, 79 (1968). https://doi.org/10.1111/j.1469-8137.1968.tb05456.x
29. M. L. Salin and P. H. Homann, Plant Physiol. **48**, 193 (1971). https://doi.org/10.1104/pp.48.2.193
30. L. Li, C. J. Nelson, J. Trösch, I. Castleden, S. Huang, and A. H. Millar, The Plant Cell **29**, 207 (2017). https://doi.org/10.1105/tpc.16.00768
31. Institute of Medicine (US) Panel on Micronutrients, Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (National Academies Press, Washington DC, 2001).
32. P. J. White and M. R. Broadley, Calcium in plants. Ann. Bot. **92**, 387 (2003). https://doi.org/10.1093/aob/mcg164
33. V. Smil, Annu. Rev. Energ. Environ. **25**, 53 (2000). https://doi.org/10.1146/annurev.energy.25.1.53
34. A. Popova and D Mihaylova, Open Biotechnol. J. **13**, 68 (2019). https://doi.org/10.2174/1874070701913010068
35. J. R. Hunt, Am. J. Clin. Nutr. **78**, 633S (2003). https://doi.org/10.1093/ajcn/78.3.633S