

## A STUDY ON THE DEVELOPMENT AND EFFECTIVENESS OF IRON-RICH BALLS FROM UNDERUTILIZED LEAF SOURCES FOR ANAEMIA MANAGEMENT IN CHILDREN

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### ABSTRACT:

The present study aimed to develop an iron-rich product from underutilized leaves and see its supplementation effect on anaemic subjects. Four underutilized leaves were selected: amaranth, guava, beetroot, and wheatgrass. All four leaves were dried and ground to form, making iron-rich powder (55g iron/ 100g powder). Iron Balls were prepared by using dried leaf powder as they fulfilled the iron needs completely and can be easily consumed by any age group of the population. The intervention of 45 days was done on 20 anaemic subjects (10 experimental groups and 10 control groups) i.e., children 5-11 years of age group. Age, Gender, Education, Eating Habits, Food Consumption, Number of meals and likes and dislikes were collected from the parents of subjects. Haemoglobin estimation and Height and Weight measurements were done for the subjects' pre- and post-supplementation following standard procedures. The experimental subjects were served with 10 Iron Balls each day and control subjects were not provided any supplementation. Intermediate checking whether side effects are seen or not was done. The results of the intervention can be concluded as a significant difference was found in Haemoglobin values of the experimental group before and after 45 days of intervention (as the p-value was <0.05); whereas no significant difference was found in the values of the control group. Hence, the product can be used as an iron supplement to meet the daily iron needs of children.

**KEYWORDS:** - Underutilized leaves, Iron-rich, Iron Balls, Supplementation, Intervention, Proximate analysis

### 1. INTRODUCTION

In anaemia, the number of red blood cells will be lower than the normal value. Haemoglobin is required to carry oxygen. Due to a lack of haemoglobin, the body has decreased capacity of the blood to carry oxygen to the tissues in the body. WHO has declared anaemia as a serious global public

health problem, especially in pregnancy and childhood. According to WHO, 42 % of children under 5 years and 40 % of pregnant mothers worldwide are anaemic. In India, the prevalence of anaemia in women of reproductive age is 53%, which is the fifth highest globally (WHO, 2023). According to NFHS prevalence of anaemia in children between 6 months to 59 months is 58.6% in India (NFHS-5, 2019-21). According to another study, the prevalence of Iron deficiency anaemia is higher in children between 1 to 4 years and adolescent girls, but it is lower in children between 5 to 9 years and adolescent boys (Kulkarni et al., 2021).

Anaemia is a serious public health problem in India affecting all segments of the population. The vulnerable groups are infants, young children, adolescent boys and girls, women of childbearing age and pregnant women. Low consumption of green leafy vegetables is known to be a major cause of iron deficiency anaemia (Joshi and Mathur; 2010). Development of Low-cost value-added products is needed to treat this problem at large (Alvarez-Uria et al., 2014). In India majority of the population are Vegetarian, NIN recommends the consumption of green leafy vegetables at least 4-5 times a week can improve the iron status and thus also help to improve the haemoglobin level (National Institute of Nutrition, 2011). There are several nutritional and non-nutritional factors that are the main cause of Anaemia. Iron, vitamin A, folate, vitamin B12, ascorbic acid and zinc deficiencies are related to nutritional factors. The deficiency of these nutrients may occur due to inadequate dietary intake and poor bioavailability of these micronutrients. The bioavailability of iron from a plant-based diet in India is assumed to be 8 per cent which was also considered while calculating the RDA for Indians. (RDA 2020)

The National Nutrition Monitoring Bureau (NNMB) reported that consumption of adequate amounts of iron daily in the diet can also cause iron deficiency anaemia. Thus, it can be concluded that only the consumption of an adequate amount of iron is not important, but its bioavailability also plays a vital role in Combating anaemia. The inclusion of green leafy vegetables with vitamin C rich food in our daily diet ensures better absorption of Iron from GLVs (National Institute of Nutrition, 2011).

In India, green leafy vegetables are known as the best inexpensive source for most of the vital nutrients. Green Leafy vegetables are appreciated because they not only supply protective nutrients but also add variety to a monotonous diet with their alternative taste, pleasing appearance and aroma. Among all the foods green leafy vegetables occupy an important place because they contain an adequate amount of nutrients which are essential for humans. Green leafy vegetables are rich sources of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorous (Opazo-Navarrete et al., 2021). Besides this, the Green leafy vegetables are highly seasonal and are available in plenty at a particular season of the year. Abundant supply during the season results in spoilage of large quantities. There are many underutilized greens available in the market with promising nutritive value, which are good for human consumption. Many of them can tolerate adverse climatic conditions. Many of them can be raised at lower management cost as their handling

is easy and they can be grown in adverse climatic conditions. Due to a lack of knowledge, most of them are underutilized. In recent times underutilized foods are becoming more popular to increase the per capita availability of foods. Amaranth leaves are one of the most consumed green leafy vegetables (GLV) in households. Amaranth leaves have higher amounts of protein, dietary fibers, calcium, iron, manganese, and magnesium and are resistant to climate change (Soriano-García et al., 2018). Wheatgrass juice has been known for its therapeutic properties for years because of its medicinal value. Wheatgrass juice contains a significant amount of chlorophyll, Micronutrients, and Iron. The intervention of Wheatgrass increases significant haemoglobin levels and helps to cure anaemia (Joshi and Mathur; 2010). Guava leaves are widely used as a folk medicine. Due to high Vitamin C content, guava leaves play an important role in improving our immunity and keeping the small blood vessels healthy. Guava leaves contain Vitamin B complex which helps in improving blood circulation to the brain, stimulating cognitive function, and relaxing the nerve; high concentration of calcium and phosphorus which helps against diseases such as osteoporosis, hypocalcemia, hypophosphatemia etc. The vitamin C content of Guava leaves helps to increase collagen activity (Kumar et al., 2021). The nutritional value of Beetroot leaves is more in comparison to their roots. Beetroot leaves are rich in carbohydrates, protein, fiber, minerals like iron potassium, magnesium, copper, calcium, vitamins like A, B6, E (Tocopherol), and C (Ascorbic acid) and natural antioxidants like  $\beta$ -carotene and vitamin A (Retinol) (Dey et al., 2022). Beetroot leaf powder may have a beneficial effect on anaemia by enhancing iron absorption and increasing haemoglobin production (Gheith & El-Mahmoudy, 2018).

India is a country of greens. The diet consumed in developing countries is known to lack lots of micronutrients. Many different micronutrients are known to be deficient in diets in developing countries. In our country, green leafy vegetables are well-known as the most affordable source of various essential nutrients. Several studies have been done on the nutritional value of many green leafy vegetables and it has been established that their consumption on a regular basis may result in the reduction of micronutrient deficiencies leading to improvement in the nutrition and wellbeing of humans. Green leaves are available seasonally for a short period of time, so they can be dried to preserve and can be used for a longer time. Out of ignorance and lack of processing techniques, several green leafy vegetables rich in nutrients are often discarded intentionally, wasted, or used for animal feed. Thus, there is a need to identify the untapped vegetables, preserve them and utilize them. As dried leaves contain a higher proportion of nutrients than fresh leaves because of the concentration of the nutrient upon moisture evaporation, the present investigation made use of dried powder of underutilized leaves to develop and standardize iron-rich value-added products as the low-cost iron-rich value-added product is needed to treat iron deficiency anaemia.

## 2. Research Methodology:

### Design:

The study was planned to investigate the relationships between value-added iron-rich food product incorporation into the daily diet and changes in haemoglobin status in Anaemic children during the 45-day intervention period. The formulation of the product was done according to FFSI guidelines. As the subjects selected were children; hence informed consent form was elaborated to their parents and guardians and written consent was collected from the guardians of all participants.

**Participants:**

A total of 20 participants from the slum area of Ahmedabad were included in the study and showed their interest. The eligibility of the participants was done by a screening questionnaire. Data like Education; Eating Habits; Food Consumption; Number of meals and likes and dislikes were collected from the parents of subjects. The inclusion criteria were age 5-11 years, the child is not severely anaemic, and no medication or nutrient supplement is taken by the child for treatment of anaemia.

**Anthropometric Assessment:**

The height of experimental and control subjects was measured using a plastic Stadiometer in a standing position. The weight of experimental and control subjects was measured using an electronic bathroom weighing scale in an erect position. BMI was also estimated according to the Indian Academy of Pediatrics Growth Chart (IAP 2020)

**Haemoglobin estimation:**

Haemoglobin test was done by Haemoglobinometer (Fully Cell-Counter Automatic) and its cutoff points were 12-16mg/dl. Blood samples for the estimation of blood serum hemoglobin were obtained and direct estimation was done by the instrument.

**Collection of leaves:**

A list of underutilized plant leaves with rich iron content was prepared using food consumption, Indian Food Composition Tables (IFCT) and exhaustive literature and the conclusion was that Amaranth leaves, Guava leaves, Beetroot leaves and Wheatgrass are rarely consumed in the daily diet by individuals. Hence these are selected to make an iron-rich product.

**Preparation of leaf powder:**

Freshly green undamaged amaranth leaves and beetroot leaves were selected and collected from the farms to produce the best quality dehydrated powder. Guava leaves were collected from the trees of guava farms. Wheatgrass was grown by tray method.

Amaranth, Beetroot and Guava leaves were sorted and cleaned to remove the adhering dust and impurities and washed with clean water 2-3 times. Even wheat grass was washed similarly.

↓

Lemon juice was applied to all the washed leaves and wheat grass.

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Leaves and grass were kept in the shade in a ventilated room for drying for several days on a clean dry cotton cloth till the leaves turned crisp and brittle and their moisture level reduced.



A natural current of wind /air was used for shade drying of leaves and the room was insect, rodent and dust proof.



Dried leaves were milled to homogenous powder using an electric mixer or grinder and were passed through fine mesh sieve plates.



The powder was stored in airtight containers and zip-lock pouches separately for future use.

### Steps of preparing dry leaf powder



Figure 1: Leaves collection, cleaning, separation, washing, and drying

### Powder Formulation:

The selection of leaves and their ratio for the formulation of powder was done according to its iron content which is mentioned below in Table 1. The formulation ratio was 5:3:1:1 for beetroot leaves; wheatgrass; guava leaves and amaranth leaves. According to the formulation ratio, the powder of leaves and grass was mixed properly and greenish-brown iron-rich powder was developed.

Name of Ingredient	Iron Content (mg/100g)	Source
Amaranth leaves	71.85	Peter.,2014

Guava leaves	13.50	Thomas.,2017
Beetroot leaves	83.6	Pallavi and Beena.,2010
Wheatgrass	17.6	Chouhan S.K and Mogra R.,2014

Table 1: The Iron content of powder of leaves



Figure 2: Dried leaf powder wheatgrass powder and developed Iron -Rich powder

**Criteria used for the formulation of the product were: -**

Recipes that can easily be consumed by children, recipes that require fewer ingredients to reduce the interference in iron absorption, and the iron content of the developed powder. Based on the above criteria, the Iron balls were formulated to combat anaemia in children.

**Method of preparing Iron balls:****Ingredients: -**

- Powdered sugar -30gm
- Edible gum/acacia gum/gum Arabic /gum sundai/Indian gum-30gm
- Leaf powder-100gm
- Nimboo satva powder /citric acid/ tartaric/ nimboo ke phool-5gm

**Method:**

- Add powdered sugar and nimboo satva powder to the sample powder and mix thoroughly.
- Take edible gum in a big vessel and add water to it. Heat the vessel and stir the mixture until edible gum melts and mixes completely with water. Add melted gum gently to the sample mixture and blend the mixture simultaneously till the mixture is of dough consistency.
- Make small balls from the dough by using your hands and roll them into powdered sugar (to increase shelf life). Spread the balls on plates or plastic sheets and let them dry at room temperature for 24-48 hours in an insect, rodent and dustproof room. After drying the iron balls are ready to



Figure 3: Developed Iron-Rich balls

**Statistical Analysis:**

Mean and standard deviation were calculated for all variables and the significance between means of the group was studied by using paired t-test by using a standard statistical package (SPSS 20.0) and the significance of difference was defined at ( $P < 0.05$ )

**3. Result and Discussion:****Characteristics of Participants:**

Table 2 shows the characteristics of the participants. The mean age of the experimental and control was 9.1, and 9.3, The weight of all the subjects of both groups was within the normal range. The mean value of height for both groups of participants was 122 and 119 respectively. The mean value for the Haemoglobin was 7.9 (experimental group) and 9.05 for the experimental group. Although the sample size was very small ( $n=10$ ) the statistical test shows no significant difference between all the parameters for the experimental and control groups.

S.NO.	Experimental group				Control group			
	Age	HB	Height	Weight	Age	HB	Height	Weight
1	10	6.3	130	25	9	7.2	121	20
2	11	8.1	123	20	9	8.8	112	24
3	9	7.3	113	22	8	8.5	107	21
4	9	8.6	127	27	11	9.3	117	23
5	10	7.4	130	23	10	10.1	128	26
6	7	7.3	105	22	11	9.8	125	27
7	7	9.9	121	23	10	7.4	127	23
8	9	5.6	131	39	10	10.9	124	35
9	9	9.6	119	28	8	11.2	114	18
10	10	9.2	123	22	7	7.3	115	26
<b>MEAN±SD</b>	9.1±1.2	7.93±1.4	122±8.2	25.1±5.4	9.3±1.3	9.05±1.4	119±7.0	24.3±4.7
	P=0.73	P=-0.099	P=0.36	P=0.73				

Table 2: Characteristics of Participants:

**Changes in the Haemoglobin level after intervention:****Preparation of supplementation:**

The raw material was collected and prepared according to the 10 experimental subjects and 45 days duration of intervention. RDA of Iron, Vitamin C and Sugar was focussed while preparing the supplementation i.e., Iron Balls for experimental subjects.

**Pre-supplementation phase:**

The height of experimental and control subjects was measured using a plastic Stadiometer in a standing position. The weight of experimental and control subjects was measured using an electronic bathroom weighing scale in an erect position. As the subjects selected are children; hence informed consent form was elaborated to their parents and guardians and their confirmation was taken.

**Supplementation phase:**

The experimental subjects were supplemented with 10 Iron Balls a day i.e., 5 balls both times i.e., morning and evening with a glass of lemon water without sugar. Subjects were checked for side effects every 15 days. Control subjects were not provided with any supplementation. No side effects were seen in the subjects during the intervention course.

**Post-supplementation phase:**

After 45 days of supplementation, Haemoglobin values, Height and Weight of experimental and control subjects were done to check the changes.

Below graph and Table below show the changes in Haemoglobin values of experimental subjects before and after 45 days. As the p-value is 0.000432 i.e.,  $<0.05$ , there is a significant difference in Haemoglobin values. There is a significant increase in the Haemoglobin values of the anaemic subjects leading them to the non-anaemic category.

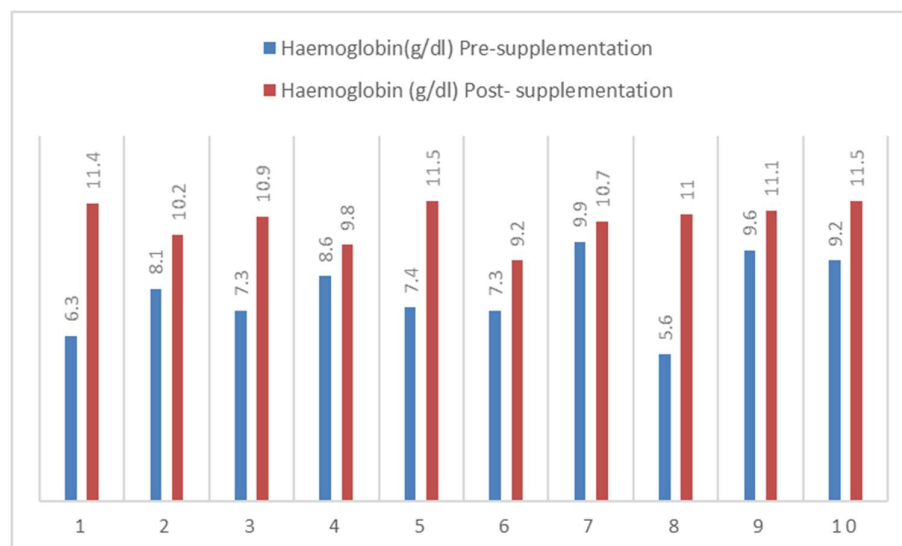


Figure 4: Comparison of Haemoglobin values of Experimental Subjects Pre- Supplementation and



Experimental subject code	Haemoglobin(g/dl) Pre-supplementation	Haemoglobin (g/dl) Post- supplementation
1	6.3	11.4
2	8.1	10.2
3	7.3	10.9
4	8.6	9.8
5	7.4	11.5
6	7.3	9.2
7	9.9	10.7
8	5.6	11
9	9.6	11.1
10	9.2	11.5
<b>MEAN±SD</b>	7.93	10.73
<b>P VALUE</b>	.000*	

Table 3: Experimental Subjects (Haemoglobin Pre supplementation and Post Supplementation)

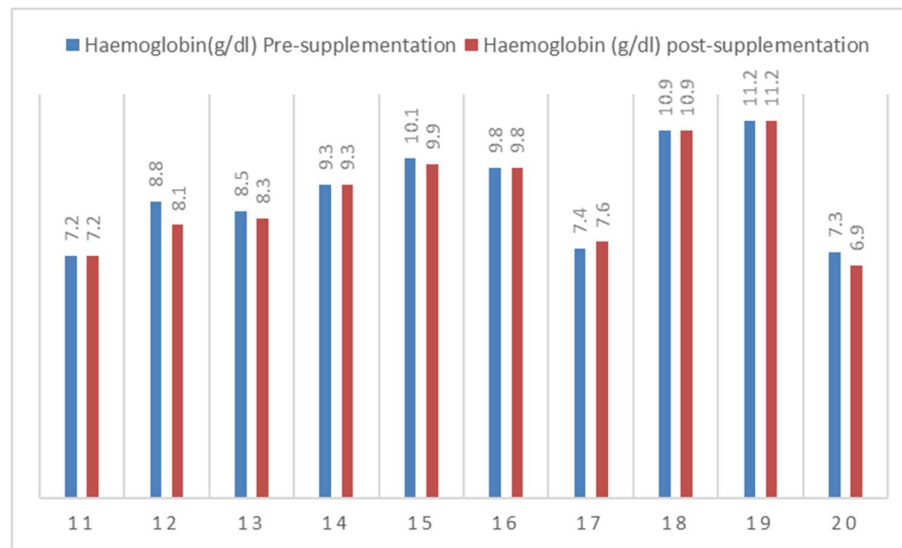


Figure 5: Comparison of Haemoglobin values of Control Subjects Pre-Supplementation and Post Supplementation

Control subject code	Haemoglobin(g/dl) Pre-supplementation	Haemoglobin (g/dl) post-supplementation
11	7.2	7.2

12	8.8	8.1
13	8.5	8.3
14	9.3	9.3
15	10.1	9.9
16	9.8	9.8
17	7.4	7.6
18	10.9	10.9
19	11.2	11.2
20	7.3	6.9
<b>MEAN±SD</b>	9.0	8.9
<b>P VALUE</b>	.146	

TABLE 4: Comparison of Haemoglobin values of Control Subjects Pre-Supplementation and Post Supplementation

Table 4 shows the changes in Haemoglobin values of control subjects before and after 45 days. As the p-value is 0.14 i.e., >0.05, there is no significant difference in Haemoglobin values. As Figure 3 shows, no changes were found in the Haemoglobin values of control subjects.

#### 4. Key findings of the study:

**Baseline characteristics of the subjects:** The participants in both the experimental and control groups had similar baseline characteristics in terms of age, weight, height, and initial haemoglobin levels.

**Formulation of Iron-Rich Powder:** The iron-rich powder was formulated by drying and milling the selected leaves and grass according to a specific ratio. The formulation aimed to create a greenish-brown iron-rich powder.

**Supplementation Phase:** During the 45-day intervention period, the experimental group received supplementation with 10 Iron Balls per day, while the control group did not receive any supplementation. No side effects were observed in the experimental group throughout the intervention.

#### Changes in Haemoglobin level:

**Experimental Group:** After 45 days of supplementation, there was a significant increase in the haemoglobin levels of the anaemic children in the experimental group. The p-value was less than 0.05, indicating a statistically significant difference.

**Control Group:** In contrast, there were no significant changes in the haemoglobin levels of the control group after the 45-day period. The p-value was greater than 0.05, signifying no statistically significant difference.

#### Conclusion:

The study's findings suggest that the incorporation of the iron-rich value-added food product,

consisting of dried underutilized leaves, into the daily diet of anaemic children resulted in a significant increase in their haemoglobin levels. This intervention was effective in improving the haemoglobin status of the experimental group, leading them out of the anaemic category.

In contrast, the control group, which did not receive the supplementation, did not experience significant changes in their haemoglobin levels during the same period.

These results highlight the potential of utilizing underutilized greens with high iron content to combat iron deficiency anaemia, especially in resource-constrained settings. Further research with larger sample sizes and longer intervention periods could provide more insights into the long-term effectiveness and sustainability of such interventions. Additionally, monitoring and evaluation of the broader nutritional impact of these interventions on the overall health and well-being of participants would be valuable.

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