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Spectrophotometric Comparison of Iron (III) in the Fruit Samples of Gooseberry and Bilimbi

Shubha S. Kumar[1], Sherin G. Thomas[2], Shiny P. Laila[2], P.R. Paulcy Rani[3], R.S. Sreepriya[4] 10.18805/ajdfr.DR-2280

ABSTRACT

Background: The fruit samples of *Phyllantus emblica* (Indian gooseberry) from *Phyllanthaceae* family are rich in iron content, known for centuries. The fruit samples of *Averrhoa bilimbi* (Bilimbi) of *oxalidaceae* family are not much investigated for the iron content. Since these traditional fruits are consumed for a long time, their iron contents are not yet compared by any means.

Methods: The comparison of iron rich gooseberry with bilimbi was done by employing the spectrophotometric method. The extraction of iron from the samples was done by acid digestion and the quantification done spectrophotometrically by thiocyanate method. The calibration plot constructed by recording the optical density of standard iron (III) solutions was linear.

Result: The iron content of gooseberry is found to be 2.5 times more than that of bilimbi.

Key words: Bilimbi, Gooseberry, Iron content, Regression analysis, Spectrophotometer.

INTRODUCTION

Iron is an essential element which holds significant biological importance and plays a vital role in various physiological processes. The most well-known function of iron is its role in oxygen transport within the body. Iron is the metal component of haemoglobin, the pigment protein in red blood cells responsible for carrying oxygen from the lungs to various tissues and organs. This process is essential for cellular respiration, energy production and overall metabolic functions. Iron also act as a cofactor for numerous enzymes involved in important biochemical reactions such as DNA synthesis, energy metabolism and detoxification of harmful substances. Iron plays a critical role in the proper functioning of the immune system. The immune cells, such as lymphocytes and macrophages, require iron for their proliferation, differentiation and optimal function. Iron deficiency compromise immune responses, making individuals susceptible to various infections. Adequate iron levels are crucial for proper brain development and function. Iron is necessary for the synthesis of neurotransmitters like dopamine, serotonin and norepinephrine, which are essential for mood regulation, cognition and emotional well-being. Iron is involved in DNA synthesis, helping in the replication and repair of genetic material. This is essential for cell division, growth and overall tissue maintenance. Iron is an essential component of the electron transport chain, a series of reactions that occur in the mitochondria to produce the energy currency of cells, adenosine triphosphate (ATP). The energy production would be compromised without adequate iron, leading to reduced physical performance and fatigue. Myoglobin, a protein found in muscles, also contains iron and facilitates the storage and transport of oxygen within muscle cells. This is crucial during periods of increased physical activity when oxygen demand in muscles rises. Iron participates in the degradation of certain toxic substances in the body, contributing to detoxification processes and protecting cells from oxidative damage. Maintaining adequate iron levels is essential for overall health and well-being. Iron deficiency can lead to anaemia which is a condition characterized by a decrease in the number of red blood cells or insufficient haemoglobin production. Anaemia can cause fatigue, weakness, difficulty concentrating and other serious health issues if left untreated. One of the primary sources of dietary iron is food. Iron mainly exists in complex forms bound to protein (hemoprotein) as heme compounds (haemoglobin or myoglobin), heme enzymes, or non-heme compounds (flavin-iron enzymes, transferrin and ferritin). About 60% of iron present in human body is found with haemoglobin, circulating erythrocytes, 25% is stored in a readily mobilizable form and the remaining 15% is bound to myoglobin in muscle tissues and in a range of enzymes involved in important cell functions including oxidative

[1]Government Women's Polytechnic College, Kaimanam, Thiruvananthapuram-695 040, Kerala, India.
[2]University College, Palayam, Thiruvananthapuram-695 034, Kerala, India.
[3]Government College For Women, Vazhuthacaud, Thiruvananthapuram-695 014, Kerala, India.
[4]Christian College, Kattakada, Thiruvananthapuram-695 572, Kerala, India.

Corresponding Author: Sherin G. Thomas, University College, Palayam, Thiruvananthapuram-695 034, Kerala, India.
Email: sheringthomas34@gmail.com
ORCID: 0000-0003-4902-783X

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metabolism. Living body requires adequate amount of iron for the synthesis of oxygen transport proteins, particularly haemoglobin and myoglobin and for the formation of heme related enzymes and other iron-containing enzymes involved in electron transfer reactions and oxidation-reduction reactions.

Knowing the iron content in different foods helps individuals ensure that their diet meets their daily iron requirements. In the present work, we compare the iron content in the locally available fruit samples of bilimbi and gooseberry (Barthakur *et al.,* 1991). Bilimbi (*Averrhoa bilimbi*) belongs to the family of *oxalidaceae*, native of Indonesia, grows to 15 m high with fairly cylindrical fruits produced in clusters. Bilimbi fruits are externally green with five broad rounded longitudinal lobes which changes to yellow when ripened and usually sour in taste. Often these fruits add a tangy flavour to various culinary dishes, especially in Southeast Asian and Indian cuisines. Additionally, bilimbi is extensively used for its medicinal properties (Jaiswal *et al.,* 2022) and as a natural cleanser. Gooseberry commonly known as amla (*Phyllantus emblica*) is the most important Indian medicinal plant (Oladeji *et al.,* 2020; Oladeji *et al.,* 2021) of the family *Phyllantaceae* which grows up to 8-18 m height. The fruits are bulbous and globular shaped, fleshy and smooth striated with an obovate-obtusely triangular six-celled nut. The seeds are 2-3 mm wide and 4-5 mm long and placed centrally. The fruits are mainly of culinary use and are widely used in making pickles, juices, chutneys and as a vegetable in various dishes. The antihypertensive activity, anti-diabetic activity and body weight studies of amla has been well investigated and reported so far (Elobeid *et al*., 2013; Khanna *et al.,* 2016; Mishra *et al.,* 2022). Amla also possesses antimicrobial (Ahmad *et al.,* 1998; Al-Gbouri *et al.,* 2018; Philip *et al.,* 2012), antidiabetic (Mehta *et al.,* 2009; Akhtar *et al.,* 2011; Nain *et al.,* 2012; Baliga *et al.,* 2013; Sharma *et al.,* 2020; Variya *et al.,* 2020; Gantait *et al.,* 2021), antiulcerogenic (Sairam *et al.,* 2002; Jaijoy 2011), antioxidant (Li *et al.,* 2020; Sheoran *et al.,* 2019; Prakash *et al.,* 2012; Chatterjee *et al.,* 2011), antimutagenic (Kaur *et al.,* 2002), anti-inflammatory (Golechha *et al.,* 2011), immunomodulatory (Zeng *et al.,* 2017), antipyretic (Perianayagam *et al.,* 2004), analgesic (Lim *et al.,* 2016), antitussive (Nosalova *et al.,* 2003), antiatherogenic (Santhoshkumar *et al.,* 2013), adaptogenic, snake venom neutralizing (Alam *et al.,* 2003), gastroprotective, antianemic, antihypercholesterolemic, hypolipidemic (Mathur, 1996), wound healing (Sumitra *et al.,* 2009; Saini 2022), antidiarrheal (Mehmood 2011), antiatherosclerotic (Antony *et al.,* 2006), hepatoprotective (Baliga 2019), nephroprotective (Malik *et al.,* 2016) and neuroprotective properties (Sarmah *et al.,* 2022)

The micronutrients of the food sample can be estimated by a number of means. The iron content of a set of 92 rice genotypes was estimated in the aliquot of seed extract by using an Inductive Coupled Plasma-Optical Emission Spectrophotometer (Swapan, 2021). The Iron content was estimated by Bhattacharjee et al using Atomic Absorption Spectrophotometer after acid digestion of the sample (Bhattacharjee *et al.,* 2020). As Iron forms a colored complex with ammonium thiocyanate, its content can also be quantitatively estimated spectrophotometrically (Ivsic, 2003) (Martins, 2005).

MATERIALS AND METHODS

The fresh fruit samples were collected from different plants of same species randomly in sufficient quantities (2 kg) to prepare representative samples. All chemicals used were purchased from commercial sources and were of AR grade. The quantitative analysis was done with the help of Perkin Elmer Lambda 25 UV-Vis spectrophotometer using a quartz cuvette. The experiment was done at University College, Kerala during the year 2024.

Preparation of fruit samples

The samples were thoroughly washed with water to remove soil particles and rinsed with distilled water, cut into small pieces, air-dried for two weeks, powdered and sieved as shown in Fig 1.

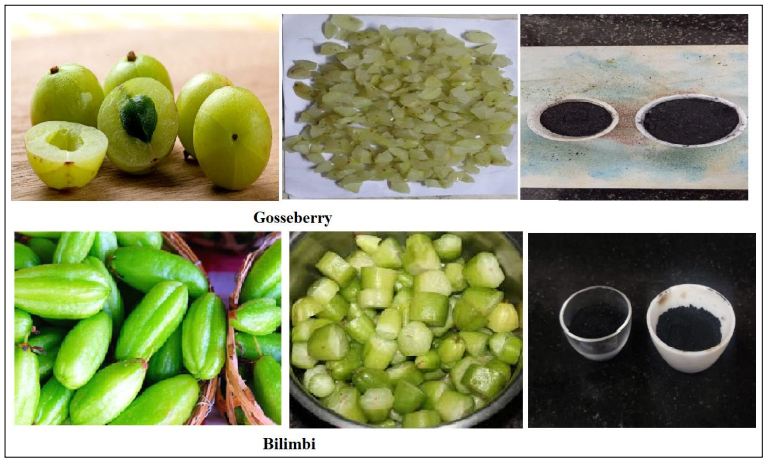


Fig 1: Photographs of raw and powdered samples.

Preparation of the extract

Iron in the fruit samples was extracted by following the acid digestion procedure: About 2 g of the dried fruit samples were accurately weighed into a crucible. The crucibles were heated in a muffle furnace at 450°C for one hour to reduce the samples completely to ashes. Further, the ashes were cooled and crushed to fine powders and digested with 10 mL of 1 M hydrochloric acid. 5 mL of distilled water was added and filtered the solution into a 100 mL conical flask.

Preparation of standard iron solution

Accurately weighed 2.41 g of powdered ferric ammonium sulphate [$Fe(NH_4)(SO_4)_2.12H_2O$] is added into a 100 mL beaker and added 20 mL of concentrated sulphuric acid, magnetically stirred for one hour, until the powder has fully dissolved. The solution was quantitatively made up to a 500 mL with distilled water. 20 mL of ferric ion solution was quantitatively made up to 200 mL with distilled water to prepare 0.001 M [Fe^{3+}] solution. Further dilutions were made to prepare standard solutions of varying concentrations (1, 2, 3, 4 and 5 $\times$ 10^{-5} M).

Sample analysis

Accurately 10 mL of the sample solution was transferred to clean and dry boiling tube. 10 mL each of the standard Fe^{3+} solutions were transferred into separate boiling tubes. 10 mL of 1M ammonium thiocyanate solution added to each iron solution in sequence, with a time gap of 2 minutes between each addition. The additions were carefully timed so that all the samples get the same period of time for the reaction. Mixed the solutions thoroughly, so that blood red colour developed. Absorbance at wavelength of 490 nm was measured for all the reference standards and the unknown samples. The measurements were made sequentially, with a two minute interval for every sample, reflecting the time of thiocyanate additions. Calibration curves were plotted and subsequently used for the determination of iron in fruit samples. The sample analyses were done in triplicate and the limit of detection (LOD) and the limit of quantification (LOQ) calculated.

RESULTS AND DISCUSSION

Iron plays a pivotal role in the production of haemoglobin, the protein responsible for carrying oxygen to cells. Gooseberries are well-rounded superfood in which the iron content packs a punch. Gooseberries on a single serving provide a significant portion of daily iron needs. The iron content in the locally available fruit samples of gooseberry and bilimbi were determined by spectrophotometric method and the results are tabulated as Table 1. The method is based on the reaction of iron(III) with thiocyanate to give a blood red coloured complex which shows maximum absorbance at 490 nm. The extract of gooseberry samples produced more intense colour when treated with the colouring agent.

The calibration plot was constructed by plotting the absorbances of standard Fe(III) solutions against concentration (Fig 2), linear regression analysis performed and limit of detection and limit of quantification calculated.

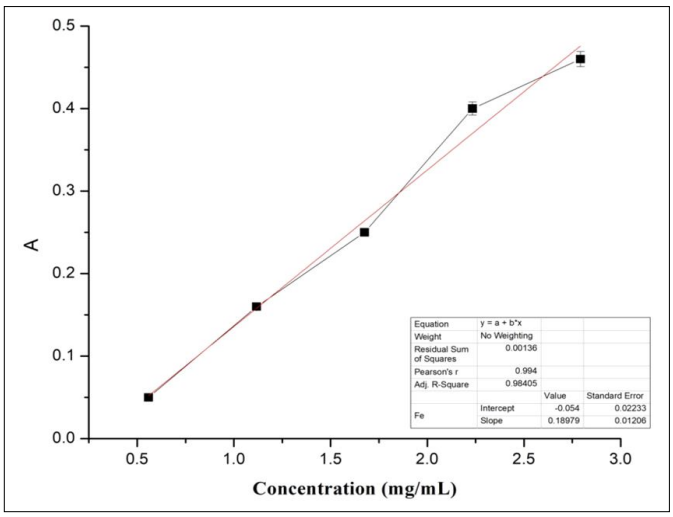


Fig 2: Plot of Fe (III) concentration vs absorbance.

Table 1: Results showing the concentrations of iron in fruit samples studied.

Gooseberry			Bilimbi		
Trial	Absorbance	Iron concentration (mg kg^{-1})	Trial	Absorbance	Iron concentration (mg kg^{-1})
1	0.2093	16.7948±0.10	1	0.0852	6.6125±0.10
2	0.2090	16.7702±0.11	2	0.0860	6.6732±0.10
3	0.2095	16.8093±0.10	3	0.0875	6.7895±0.10

LOD is the lowest concentration of Fe (III) in the sample that can be detected, but not necessarily quantified. LOQ is the lowest concentration of Fe (III) in the sample that can be determined with acceptable precision and accuracy (Shrivastava, 2011).

The equation obtained was y = 0.18979x +0.0540 with a correlation coefficient R^2 = 0.99405 indicating good linearity. The detection limit was found to be 0.8682 mg/mL and the limit of quantitation 2.630 mg/mL. The absorbances of the samples were recorded with the blank solution as the reference solution. The exact concentrations of the extracts were determined from the plot and the amount of iron (III) quantified. The method suggests that the iron (III) available after HCl digestion of the dried gosseberry and bilimbi samples are 16.7±0.1 mg kg^{-1} and 6.6±0.1 mg kg^{-1} iron respectively. It is observed that the gooseberry fruits are richer in iron content compared to the bilimbi fruits.

CONCLUSION

Fruits serve as an important source of iron, but the bioavailability of iron varies from fruit to fruit. Iron may be present in plant parts in inorganic or organic forms. The iron content in the acid extract of two fruit samples- gooseberry and bilimbi- were determined by spectrophotometric method using thiocyanate reagent. The results showed that iron is present in the range of 16.7±0.1 mg kg^{-1} and 6.6±0.1 mg kg^{-1} respectively in gooseberry and bilimbi and is about 2.5 times more in gooseberry than bilimbi.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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