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Research Article

Antioxidant Activity of Iron Isolated from Petals of *Hibiscus Rosa Sinensis*

Sarojini Johnson, Sirajunnisa Abdul Razack*, Pavithra Sellaperumal, Geethalakshmi Ramakrishnan, Priyanga Jayakrishnan, Keerthana Sivanesan and Renganathan Sahadevan

Centre for Biotechnology, Anna University, Chennai, Tamilnadu, India

***Corresponding Author**: Sirajunnisa Abdul Razack, Centre for Biotechnology, Anna University, Chennai, Tamilnadu, India.

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Abstract

Iron is universally an essential element for mental and physical development of human beings and its presence in food products serve against anaemia. The present study is an attempt was made to isolate iron from petals of *Hibiscus rosa sinensis.* Lyophilised petal powder of the flower was used for the study. The iron content was determined quantitatively by isothiocyanate assay and inductively coupled plasma-omission electron spectroscopy. Isolation and partial purification of iron was carried out by TLC and column chromatography respectively. The study reported that the iron content in the lyophilized petal powder was 0.8 mg/g of sample through ICP-OES. The iron fraction was read spectrophotometrically and its presence was noted at 208 nm. Antioxidant activity of the isolate was performed and scavenging activity was found to be the maximum at a concentration of 1 mg/ml. This work suggested that the iron from hibiscus, generally non-edible, can be utilized for various biological activities and in food fortification.

Keywords: *Hibiscus rosa sinensis, Iron, Antioxidant*

Introduction

Iron is one of the globally significant inorganic elements required by the human body for its development. Iron is essential in various stages of life cycle and for the cells of central nervous system. It is the main necessity in various human metabolic activities and act as the co-factor of several enzymes playing roles in neurotransmitter synthesis, redox reactions and in myelin production [1]. Iron deficiency in food, most importantly during the first two years of life, causes severe problems in the brain development. Iron deficit leads to anemia exhibiting consequences like poor cognitive development, inadequate work productivity and high maternal mortality [2]. Anaemia is the most common nutritional deficiency in infancy and childhood and the only micronutrient deficiency in developed countries [1]. Iron has been thus understood as the most important nutritive mineral to be taken in with the food. If the foodstuff lacks iron, then it can be added externally and fortified.

Plants are generally referred to as the living creatures comprising of chemicals potential as drugs [3]. Several plant parts had been used in traditional medicine especially in India, Far East and Middle East countries. The plant sources can be used as therapeutic agents to isolate bioactive compounds, to utilize compounds as lead drugs in synthetic products, to use as a pharmacological tool and to use as herbal remedy [4]. Iron from non-edible sources is of great interest for research as it does not compete with food crops. Hibiscus is widely used in India as an ornamental plant and as a medicine in Indian system. This genus of family *Malvaceae* comprises about 275 species in the tropical and sub-tropical regions. The plant is a potent antifungal agent and has anticancer activity. The leaves and flowers of *Hibiscus rosa-sinensis* are used as antiseptic and antidiabetic [5]; [6]. Flowers, in specific, are used against epilepsy, bronchial catarrh, leprosy and used as hair growth promoters [7,8]. Species of Hibiscus like syriacus and rosa-sinensis are large resources of phytochemicals like flavanoids, phenolics and tannins, macromolecules like proteins and carbohydrates and trace elements like phosphorus, calcium and iron [9]. This article is an attempt to isolate iron from the petals of *H. rosa-sinensis*, characterize and partially purify the product and check its antioxidant property. Extensive literature survey revealed meagre research on isolating iron from the flower petals, hence the source was selected for this study.

Experimental Methodology

Sample collection

The stamens and calices were removed and the petals were left for shade drying until it became brittle. After drying it is ground in a blender into fine powder. The powder was frozen overnight for lyophilisation. Freeze drying was later carried out for 24h and collected in an air tight container until further use and analysis.

Analysis of iron content

Determination of Iron using isothiocyanate assay

10 ml of sample solution and 10 mL of each Fe^{2+} (Ferrous sulphate) standard solution were taken separately in a series of test tubes. 10 mL of 1 mol l^{-1} ammonium thiocyanate solution was added to each tube and were mixed gently. A stable red colour appeared over the next few minutes, nearly 15 min, which were read spectrophotometrically at an optical density of 490 nm.

Quantitative determination using ICP-OES

Freeze dried powder (4g) was sent for analysis by ICP-OES for detection and confirmation of iron content. The sample was analyzed at SGS India Private limited, Ambattur, Chennai, Tamilnadu, India.

FT-IR spectroscopic analysis

A quantity of 0.5 mg of sample with 150 mg of KBr was pressed into a disc and introduced into the sample holder to obtain the result as spectrum with FTIR spectroscope scanning the samples at wavelength ranging 8000 - 300 cm^{-1}.

Thin Layer Chromatography

TLC was performed according to Kastelan Macan M., *et al*. (1987). A strip of TLC sheet was spotted with sample and standard. Meanwhile, a volume of 20 ml solvent system was prepared and left for half an hour to saturate. The spotted plate was placed in solvent system until the mobile phase reached $3/4^{th}$ of the sheet. Then the plate was taken out and air dried. The dried plate was exposed to ammonia vapour. The presence of iron was detected by the appearance of dark brown coloured spot. The R_f value was calculated using this formula

$$R_f = \frac{Distance\ moved\ by\ sample(cm)}{Distance\ moved\ by\ solvent(cm)}$$

The brown spot was scraped off from the plate and collected in an eppendorf vial containing 500 µl of 10 mM Tris HCl buffer. This was used as the sample for column chromatography.

Partial purification using silica gel chromatography

A column was prepared in the laboratory using micropipette tip in which cotton layer was placed upon which 0.5 ml of silica gel homogenized in distilled water (1.5g of silica gel in 5 ml of distilled water) was added. The column was left at 4°C for 1 hour to set. Then the mobile phase (10M Tris buffer: Acetonitrile: 9:1) was first added to clear and stabilize the column. The sample at a flow rate of 100 µl / min was added. The total elution time was 60 min. The fraction was collected and measured using UV-Visible spectroscopy to confirm the sample from TLC as iron.

Antioxidant activity of the product

The antioxidant activity of the sample was evaluated on the basis of the free radical scavenging effect of 1, 1- diphenyl 2- picrylhydrazyl (DPPH) [10]. In brief, sample solutions at various concentrations of mg/ml were made upto to 1 ml with distilled water. 1 ml of DPPH solution (0.004% in methanol) was added to sample and standard solutions. After incubating the solutions for 30 min at dark, the absorbance was read at 517 nm. Vitamin C and distilled water with DPPH were used as the reference and blank respectively. Percent scavenging

ability was calculated using the formula:

Percent (%) scavenging activity = 1 – (A/B) x 100

Results and Discussion

The petals of *H. rosa-sinensis* were shade dried and used for the study. Sun drying was not performed in order to maintain the nutritive value of the sample of study. The shade dried petals were blended and lyophilized for 12h to obtain fine powder (Figure 1).

Figure 1: *(a) Shade dried hibiscus petals and (b) lyophilized powder.*

FT-IR was performed to detect the presence of functional groups. Sample was scanned in the wavelength ranging between 8000 cm^{-1} and 300 cm^{-1}. Figure 2 illustrates the recorded spectrum of hibiscus petal powder. An intense peak at 3396 cm^{-1} indicated the presence of -OH groups. A sharp peak at 2919 cm^{-1} denoted alkanes stretch and an absorption peak at 1725 cm^{-1} showed the presence of C=O group of aldehydes. An intense absorption peak was observed at 1623 cm^{-1} indicating the presence of primary amine, N-H. Different peaks denoting alkane groups of α-CH_2 stretch and α-CH_3 bending were found at 1442 cm^{-1} and 1371 cm^{-1} respectively. A short absorption peak was observed at 1265 cm^{-1} which demonstrated the presence of O-C group of acids. A sharp absorption peak at 1060 cm^{-1} denoted the presence of –C-N amine stretching and NH_2 and N-H wagging at 771 cm^{-1}. A characteristic peak for Fe^{3+} was observed at 619 cm^{-1}.

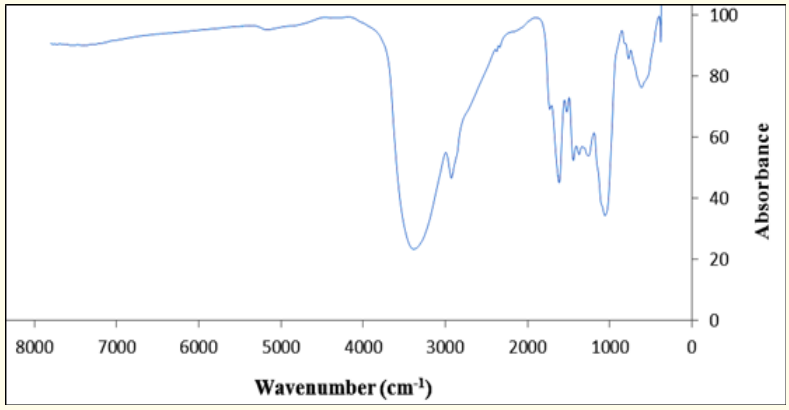

Figure 2: *FT-IR Spectra of Hibiscus petal powder.*

The freeze-dried powder was used for determination studies. Estimation of iron was performed by isothiocyanate assay (Figure 3) and absorbance was read spectrophotometrically at 490 nm against ferrous sulphate. The report showed that 1g of petal powder consisted of 4.5 mg of the trace metal, iron. A study carried out by Ferrante A., *et al*. (2011) reported that 14 mg of iron was present in petal of one flower [11]. Lower amount of the element was obtained in our study which might apparently due to the geographical conditions.

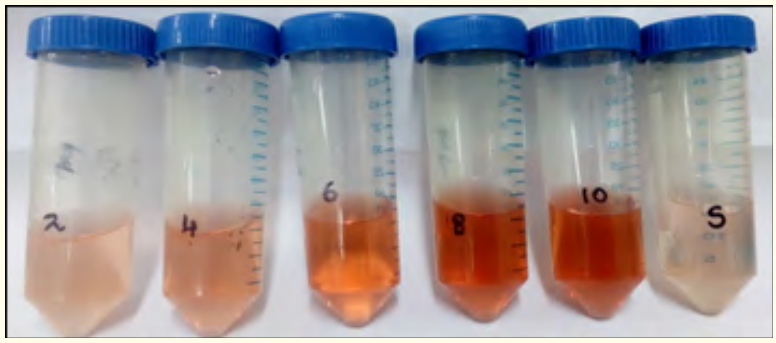

Figure 3: *Blood red colored complex formation based on the concentration.*

ICP-OES was carried out to quantitatively analyze and confirm the presence of iron in the petal powder sample. The report eventually indicated that the sample contained 0.8 mg of iron/ g of powder. The study showed that the sample consisted of adequate amount of iron to be extracted and utilized for fortification of edible material from this commonly non-edible source. The level of trace element was found to be higher in biochemical characterization by isothiocyanate assay than in ICP-OES because the former protocol had high interferences with other inorganic elements in the sample whereas the latter was used in particular for the specific component to be identified [12]. Hence this study suggested that ICP-OES was better for quantifying trace metals than biochemical assays (Figure 4).

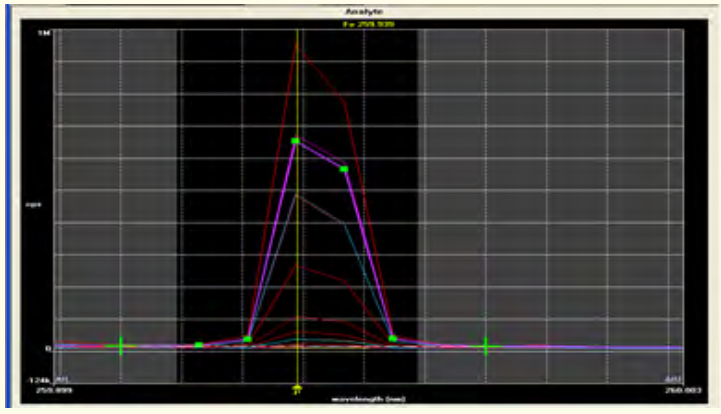

Figure 4: *Spectrum denoting the presence of iron in the sample.*

Thin layer chromatography was performed for isolating the trace metal from the petal powder (Figure 5A). A deep brown spot was observed which indicated and qualitatively confirmed the presence of iron in the sample. The R_f value was calculated to be 1. The result was completely in agreement with the work reported by M Kastelan Macan., *et al*. 1987 [13].

The brown spot was scraped off from the plate and stored in Tris buffer. The sample was added to the column and the fraction was collected (Figure 5B). The obtained fraction was collected from the column and detected by UV-Vis spectrophotometer scanning between 200 to 700nm (Figure 6). An intense peak was observed at 208 nm inferring iron. It was compared with the standard, ferrous sulphate, which was detected at 210 nm.

Figure 5: *(A) TLC plate showing spot of iron.*

Figure 5: *(B) Silica gel column with the sample being collected.*

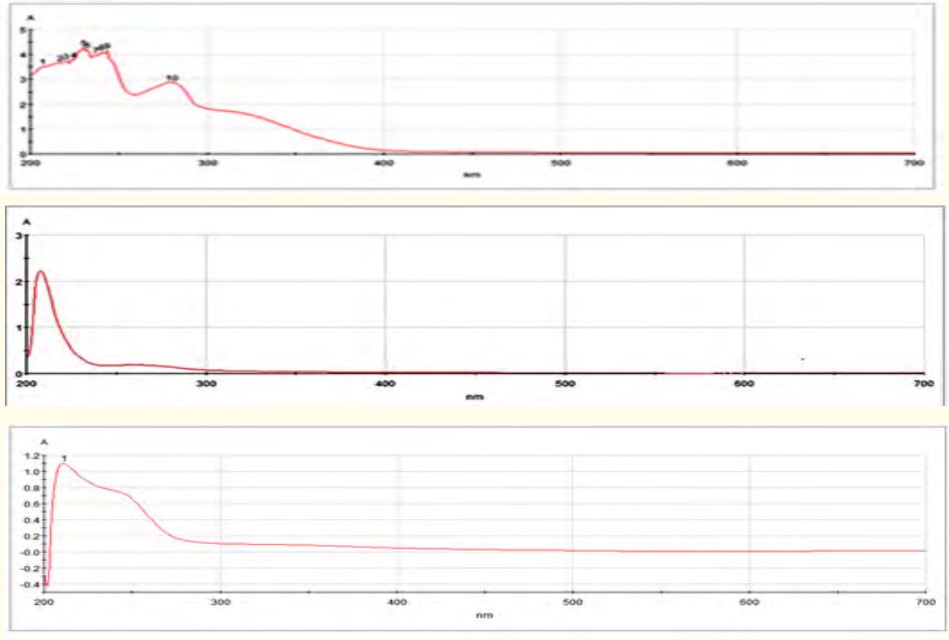

Figure 6: *Spectra of (a) hibiscus whole powder extract, (b) iron after extracting through column and (c) standard $FeSO_4$ obtained through UV-VIS spectrophotometer.*

Antioxidant activity of iron

DPPH assay was performed to check the free radical scavenging activity of iron isolated from dried petal powder. Antioxidants are those macromolecules that could scavenge the free radicals of reactive oxygen species, i.e. superoxide anions like hydrogen peroxide, singlet oxygen and free hydroxyl anions which are generated by transfer of one electron [14]. DPPH could be scavenged on accepting one hydrogen or an electron [15]. Thus, this activity results in the reduction of stable DPPH radical (purple) to non-radical DPPH- (yellow) form. The present study showed that the generated sample possessed good antioxidant activity at various concentrations (20 – 100 µg/ml). The maximum scavenging concentration was observed to be 1mg/ml and the percentage activity was 71.9% (Figure 7).

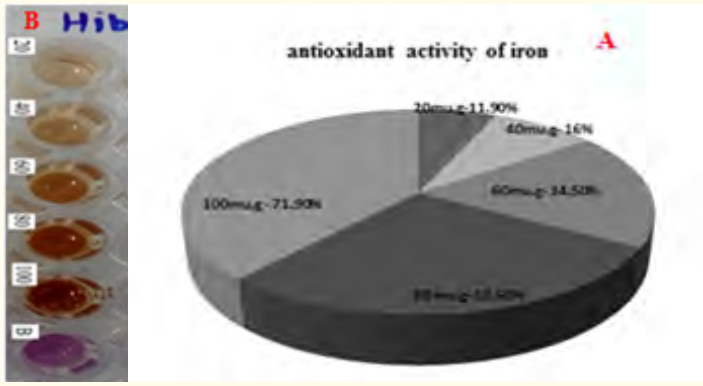

Figure 7: *Antioxidant activity of iron at varying concentrations.*

Conclusion

The present investigation was on extraction of iron from the petals of *Hibiscus rosa sinensis* which has been used in Indian system as native medicine. Anemia, due to iron deficiency, is one of the alarming illnesses endemically and pandemically. Iron has to be a main part of the diet as it is one of the most essential components in regulation of body functions. Hence this study was successful in identifying and isolating the compound of interest from dried powder of Hibiscus petals. The extract of petals was characterized using biochemical assays and iron was quantified using ICP-OES. Iron was partially purified using silica gel chromatography and confirmed its presence using UV-Vis spectrophotometer. The extracted element was assessed for its antioxidant property which showed that the activity was at its maximum at 1mg/ml. This study demonstrated that Hibiscus is a good resource of iron and can be utilized for various biological activities and food fortification. Further studies could be carried out in purifying the product and implementing the trace metal in food products.

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