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Quantitative determination of iron and folic acid in *Lactuca sativa* (Lettuce) plant

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ABSTRACT

Lettuce is one of the most widely used vegetable all over the world. Millions of people all over the world especially women and children suffer from neural tube and other diseases as a result of iron and folic acid deficiencies. The sole aim of this research therefore was to determine the level of iron and folic acid in lettuce plant. High performance liquid chromatography (BLC-20) and spectrophotometry were used for the determination of folic acid and iron respectively. Results from the current study suggest the level of folic acid to be 0.042 mg/L at 282nm and that of Iron 69.93 µg/g at 515nm. Women and children an indeed a great number of the Nigerian population is at risk of consequences attributable to deficiency of these important nutrients.

Keywords: *Lactuca sativa*, iron, folic acid

INTRODUCTION

Lactuca sativa commonly known as lettuce belongs to the Asteraceae family. Lettuce is considered as the most important vegetable in the group of leafy vegetables which is used as fresh vegetable in salads [1]. The *genus Lactuca* composes approximately 100 species. Lactuca *sativa* is an annual glabrous herb with a thin tap root and an erect stem 30-100cm tall, branched in the upper part. The leaves are spirally arranged, forming a dense rosette. The shape is oblong to transverse elliptic to triangular and undivided [2].

Traditionally, *Lactuca sativa* is used in folkloric medicine for the treatment of inflammation, pain, stomach problems which encompasses of indigestion and lack of appetite [2]. There are a number of bioactivities conducted to evaluate the therapeutic significance of *Lactuca sativa* including; anticonvulsant, sedative-hypnotic, antioxidant, analgesic and anti-inflammatory activities [3]. Extensive phytochemical studies on *Lactuca sativa* lead to the isolation of various classes of compounds such as sesquiterpene lactones [2].

Nutritional deficiency of folate is commonly associated with people consuming inadequate diet. Pregnant women are at high risk of folate deficiency because pregnancy significantly increases folate requirement, especially during periods of rapid foetal growth [4]. Folate deficiency during pregnancy can results in neural tube defects, NTDs [5]. A complete lack of dietary folate takes months before deficiency develops as normal individuals have about 500-20,000µg of folate in body stores [6]. Inadequate folate intake results in a decrease in serum folate concentration and decrease in erythrocyte folate concentration. This is followed by a rise in homocysteine concentration and megaloblastic changes in the bone marrow and other tissues with rapidly dividing cells resulting to macrocytic

anemia. Symptoms of folate deficiency include weakness, fatigue, difficulty in concentrating, irritability, headache, shortness of breath and atrophic glossitis. Folate deficiency in pregnancy may also increase the risk of preterm delivery, infant low birth weight and fetal growth retardation, as well as increasing homocysteine level in blood, which may lead to spontaneous abortion and pregnancy complication such as placental abruption and pre-eclampsia [7]. Deficiency of folate can also lead to the impairment of DNA synthesis and repairmen and this could lead to cancer development [8].

Iron deficiency (sideropenia or hypoferremia) is one of the most common nutritional deficiencies. Iron serve as a carrier of oxygen to the tissues from the lungs in the form of hemoglobin, it is also a transport medium for electrons within the cells in the form of cytochromes, and serves as an integral part of enzyme reactions in various tissues. Too little iron can interfere with this vital functions and lead to morbidity and death [9]. The eventual consequence of iron deficiency is anemia where the body's stores of iron get depleted rendering it unable to maintain levels of hemoglobin in the blood [10]. Children and pre-menopausal women are the groups most prone to the disease. Symptoms of iron deficiency includes; chronic bleeding, excessive menstrual bleeding, non menstrual bleeding, bleeding from the gastro intestinal tract (ulcers, hemorrhoids, ulcerative colitis etc) rarely, laryngological bleeding or from the respiratory tract, inadequate intake, substances (in diet or drugs) interfering with iron absorption, mal-absorption syndromes, inflammation where it is adaptive to limit bacterial growth and blood donation [11].

Sequel to the above, the aim of this research was to determine the levels of iron and folic acid in lettuce plant which in turn may serve as a good source of folate and iron.

MATERIALS AND METHODS

Equipments and Reagents

GBC UV-visible central 101/202/303/404 spectrophotometer was used for measuring the absorbance of the sample (iron determination). High performance liquid chromatography (BLC-20) centrifuging machine and mechanical shaker (folic acid determination). All chemicals and reagents used were of analytical grade.

Sample Preparation for folic acid determination

The sample (3g of jute leaves) were extracted with 50ml of 0.1mol/L phosphate buffer pH 7.0 and 0.1% (V/V) of 2-mercaptoethanol was added. The mixture was shaken for 30 minute in a vortex shaker, and centrifuged at 3500rpm for 15 minute and filtered through a Millipore filter paper before chromatography analysis.

Solid phase extraction

The stationary phase was flushed with 5mL methanol and 5mL deionized water to actuate the stationary phase, the sample extract was passed through with a flow rate of 2-3 drops and the sample was eluted with 5mL NaOH (0.005 mol/L) pH 10.0 prior to HPLC analysis. All samples were filtered through a Millipore filter and then injected into the chromatograph.

Procedure

The elute was passed through the column monitored with a photodiode array detector at 282nm for folic acid. The mobile phase (pH 7.0; 90:10 KH_2PO_4: Methanol) was filtered through a 0.5nm membrane and degassed before use. The flow rate was 0.7mol/min. The column was operated at room temperature**.**

Iron Determination

Preparation of reagents

- HCl: H_2O = 1:1 (36% HC1 and distilled water used for the preparation of 1:1 ratio).
- 10% NH_2OH.HCl solution: 10% NH_2OH. HCl was prepared by dissolving 25gm NH_2OH. HCl in 25ml distilled water.
- NH_4OH. H_2O = 1:1 (14.3N NH_4OH were used for the preparation of 1:1 ratio).
- Buffer solution: Buffer (pH=5) was prepared by water containing 15ml of 1M HCl and diluted with distilled water to 250mL.
- Orthophenanthroline solution: Orthophenanthroline solution was prepared by dissolving 0.13gm Orthophenanthroline powder in a 25mL volumetric flask and diluted up to mark with distilled water. The powder was fully dissolved by shaking.

- Congo red paper: 0.1gm congo red powder was dissolved in 10ml ethanol and dried.
- Ferric ammonium sulphate solution: 0.216gm A.R. ferric ammonium sulphate was dissolved in one liter volumetric flack with distilled water and 1.25ml conc. HCl was added. The total volume was made 250ml with distilled water in this solution 1mL contains = 0.1mg Fe^{3+} this solution was kept as stock solution and was used for the preparation of calibration curve.

Procedure

The level of iron was determined by Orthophenanthroline method. Two grams (2g) of the sample was dissolved in 2mL conc. HC1 in a 250m1 beaker and the solution was diluted with 100m1of distilled water. To the 20mL stock solution, 1mL of 10% $NH_2OH.HC1$ solution was added to reduce Fe^{3+}. 5mL orthophenanthroline solution (W/V) and one congored paper were added to the solution and the color of the paper changed from red to blue. NH_4OH solution was added drop wise until it turned alkaline i.e. congored paper becomes red. 5ml of Buffer (pH=5) solution was added to the solution and filtered using whatmann – 42 filter paper, the solution was diluted with 100ml distilled water. An orange red color developed and its absorbance was measured in spectrophotometer within 10 to 20 minutes at 515nm. A blank solution was prepared by using the entire reagent by similar procedure and was used for calibration of the spectrophotometer.

RESULTS AND DISCUSSION

The result of folic acid determination is presented in Table 1 and 2 and the level of iron in lettuce leaves is shown in Table 3.

Table 1: **Linearity and detection limits of folic acid**

Vitamin	Linear range (mg/L)	Detection limit (mg/L)
Folic acid	0.4 – 100	0.042

Table 2: Mean and standard deviation of folic acid (mg/l) in cabbage

Vitamin	N	Mean ±S.D (mg/L)
Folic acid	5	2.82±0.19

Table 3: **Amount of iron (Fe) (Microgram/g) in the lettuce leaves**

Sample	**Orthophenanthroline method**			
	A	B	C	**Absorbance**
Jute	69.8	70.4	69.6	515 nm
Mean	69.93			

Iron supplementation alone or in combination with folic acid has been associated with the well being of the mother and fetus. It leads to a significant reduction in anaemia incidence during pregnancy and, thus plays a vital role in reducing maternal, morbidity and mortality [12].

Folic acid and iron deficiency during pregnancy are risk of factors for anemia, preterm delivery, and low birth weight, and may increased maternal mortality [13], [14] (Gregory, 1989; Black, 2013). The WHO recommends supplementation of folic acid and iron for all pregnant women at risk of malnutrition to prevent anaemia and birth defects [15] (Gunaratna *et al*., 2015), however people can get rid of their diseases by consumption of lettuce leaves which is rich in folic acid and iron as suggested by the findings of this work.

CONCLUSION

The findings of this research indicates that lettuce leaves are enriched with folic acid and iron contents and can be a good source of these minerals.

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