Folic Acid Contents of Selected Commonly Consumed Vegetables in Southern Nigeria: Implications on Pregnant Women Attending Antenatal Clinics.

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Abstract- ***The present study aimed at determining the folic acid contents of selected commonly consumed vegetables (Jatropha tanjorensis, Talinum triangulaire, Vernonia amygdalina, Ocimum gratissimum and Telfairia occidentalis) in Southern Nigeria and their implications on pregnant women attending antenatal clinics. The determination of folate in the vegetable samples passed five main processes: extraction, deconjugation using chicken pancreas deconjugase, transformation, purification, and quantification. Through this process, all folates formed in the deconjugated sample extracts were converted to 5-methyltetrahydrofolic acid (THF-5CH3) monosodium glutamate and/or diglutamate. Purification was done by affinity chromatography with Folate Binding Protein and high-performance liquid chromatography (HPLC) equipped with fluorimetric detection was used to determine the total folate content. The five vegetables had total folate contents between 58.57 to 253.02 µg/100g. Jatropha tanjorensis had the highest total folate content and contributed as much as 63.3% of the 400 µg recommended dietary allowance (RDA) of folate for women of reproductive age while Vernonia amygdalina had the least total folate content. Based on the result of the study, pregnant women attending antenatal clinics are encouraged by the researcher to consume the leaves of Jatropha tanjorensis (Hospital Too Far) followed by Telfairia occidentalis (Fluted pumpkin leaves) in higher amounts, as they contained higher amounts of folic acid than the other vegetables studied since they are at high risk of folate deficiency, which causes pregnancy complications like neural tube defects, preterm delivery, infant low birth weight, foetal growth retardation, spontaneous abortion, placental abruption, and preeclampsia.***

Indexed Terms- ***Folate, Folate Deficiency, Vegetables, Pregnancy, Neural Tube Defects***

I. INTRODUCTION

Folate in the form of folic acid, also called vitamin B_9, is needed by the body during DNA and RNA synthesis and also in the metabolism of amino acids necessary for cell division [2, 23]. Folic acid is an essential vitamin, which occurs naturally in many foods and vegetables [18]. It acts as a coenzyme in the metabolism of amino acids, especially in the conversion of homocysteine to methionine during the synthesis of S-adenosyl-methionine (SAM) [21].

A defect in this metabolic reaction initiates a process that can cause megaloblastic anemia. Megaloblastic anemia is one of the characteristics of folate deficiency. It has been reported that folic acid deficiency, apart from causing megaloblastic anemia, also causes neural tube defects to occur [1, 10].

Some of the symptoms of folate deficiency are fatigue, weakness, difficulty concentrating, severe headache, shortness of breath, atrophic glossitis, and so on [12]. Folates are commonly found in vegetables, fruits, legumes, and cereals [9] and are vital in hematopoietic processes [15, 16].

In many countries including Nigeria, the intake of folate has been observed to be below the recommended daily intake [5], hence the Centre for Disease Control and Prevention (CDC) recommends a daily amount of 400 micrograms of folic acid for the prevention of folic acid deficiency, which causes megaloblastic anemia and neural tube defects [3].

Vegetables are the edible parts of plants that are consumed wholly or in parts, raw or cooked as part of main dish or salad. The constituents of a vegetable are leaves, stems, roots, flowers, seeds, fruits, bulbs and tubers [7, 20]. Green leafy vegetables and fruits have been reported to be the rich sources of folic acid in the form of 5-methyl tetrahydro folate [5-8; 11, 13]. However, low consumption of green leafy vegetables in diets is a major contributor to folic acid deficiency. Folic acid cannot be produced by animals and must be provided from plants. There is little information on the folate contents of commonly consumed vegetables in Southern Nigeria.

Hence, the present study aimed at determining the folic acid contents of selected commonly consumed vegetables in Southern Nigeria and their implications on pregnant women attending antenatal clinics. The data provided in this study will be useful in educating the public, food industries, pharmaceutical industries, and the ministry of health about the vegetables rich in folates in order to encourage their consumption.

Table 1**:** Selected commonly consumed vegetables in Southern Nigeria studied.

S/No	Scientific name	Common name
1	*Jatropha tanjorensis*	Hospital too far
2	Talinum triangulaire	Water leaf
3	*Vernonia amygdalina*	Bitter leaf
4	*Ocimum gratissimum*	Scent leaf
5	*Telfairia occidentalis*	Fluted pumpkin leaf

II. MATERIALS AND METHODS

- Sample Collection

Five different leafy vegetables were collected from Big Tree Market, Rumuolumeni, Port Harcourt, Rivers State, Nigeria and were identified by Dr. O.A. Wokoma of the Biology Department, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria.

- Preparation of Samples

The vegetable leaves used for the study were freshly harvested, destalked, washed with clean running tap water and room dried for three (3) weeks. After drying, the leaves were pulverized using an electric blender, sieved and stored in air-tight containers under refrigerated temperature for use.

Quantitative Determination of Folic Acid

Folate extraction and estimation were done following the method of Delchier et al. [5] with slight adjustments.

i. Principle of estimation: Folate is mainly found in vegetables as polyglutamate. The analysis of the samples followed the process of extraction, deconjugation, transformation, purification and quantification.
ii. Extraction: 5g of the sample was extracted using 15ml of 0.1M phosphate buffer (P^H 7.0) containing 1% ascorbic acid and boiled in a water bath at 100^0C for about 10 minutes. After cooling for about 15 minutes, the volume was adjusted to 50ml 0.1M phosphate buffer (P^H 7.0) containing 1% ascorbic acid and centrifuged for 10 min at 5000 rpm.
iii. Deconjugation: This is done using chicken pancreas conjugase. 1 ml of chicken pancreas was added to 10 ml of the extract and incubated for 2 hours at a temperature of 37^0C.
iv. Transformation of Folates: Here, all the folates present in the deconjugated sample extracts are transformed into 5-methyl tetrahydrofolic acid monosodium glutamate series of chemical reactions. 5 ml of 40% ascorbic acid P^H buffer, 15 ml of Tris buffer, 1 ml of 2-octanol, and 10 ml of sodium tetrahydroborate ($NaBH_4$) were carefully added into the beaker containing the sample extract, stirred gently and allowed to stand for 10 minutes. The P^H of the sample solution in the beaker was adjusted to 7.4 by adding 5M acetic acid, 80 µl of 37% formaldehyde and 10 ml of sodium tetrahydroborate ($NaBH_4$) and stirred continuously. The P^H was drastically reduced to a value less than 1.0 by gradually adding 37% HCl. The mixture was allowed to stand for another 10 minutes to prevent the formation of bubbles. The P^H of the sample solution in the beaker was adjusted to 5 by adding 5M NaOH and 10 ml of $NaBH_4$.

The solution was allowed to stand for 20 minutes and later transferred into the 100 ml volumetric flasks. Tris

buffer was added to the volumetric flasks to make it to the meniscus mark. The volumetric flasks were covered with parafilm and mixed very well by shaking. The solution was filtered with a 10 ml syringe attached to the 80 micron filters that have cellulose acetate membrane into 15 ml falcon tubes and labeled.

v. Purification of folates: Purification by affinity chromatography with folate binding protein followed the process of column conditioning using 0.1M phosphate buffer (P^H 7.0). 10 ml filtered sample was passed through the column gel and elution of folates was done with 8 ml eluent solution consisting of 0.02 M DL-dithiothreitol (DTT) and 0.02 M trifluoroacetic acid (TFA) into a beaker containing 40 µl of 60% NaOH and 200 µl of 25% amino acid. The volume was adjusted to 10 ml with eluent solution.

vi. Quantification of Folates: The amount of folates present in the sample is determined by using high performance liquid chromatography (HPLC) equipped with fluorimetric detection as reported by Ejoh et al. [6].

Statistical analysis

The determination of total folate was conducted in triplicates. Data were summarized using means and standard deviations in statistical package for social science (SPSS) windows, version 20.0.

III. RESULTS AND DISCUSSION

The results of folate analysis are presented in Table 2. The predominant form of folate in all the selected commonly consumed vegetables in Southern Nigeria analyzed was 5-methyl tetrahydrofolate. The five vegetables (*Jatropha tanjorensis, Talinum triangulaire, Vernonia amygdalina, Ocimum gratissimum* and *Telfairia occidentalis*) had total folate contents between 58.57 to 253.02 µg/100g, *Jatropha tanjorensis* having the highest while *Vernonia amygdalina* having the least. From the results, *Jatropha tanjorensis* was observed to have the potential of contributing as much as 63.3% of the 400 µg recommended dietary allowance (RDA) of folate for women of reproductive age in Nigeria and other countries [3, 6].

Table 2: Total folate content of selected commonly consumed vegetables in Southern Nigeria.

S/No	Name of vegetable	Total folate (µg/100g)
1	*Jatropha tanjorensis*	253.02 ±6.77
2	Talinum triangulaire	75.03± 13.35
3	*Vernonia amygdalina*	58.57 ±12.48
4	*Ocimum gratissimum*	66.23± 2.41
5	*Telfairia occidentalis*	189.16 ± 3.01

- IMPLICATIONS OF FOLIC ACID ON PREGNANT WOMEN ATTENDING ANTENATAL CLINICS

Pregnancy is the period during which foetal development occurs in the uterus [17] and this requires proper antenatal care, in which folate intake is not an exception.

Nutritional deficiency of folate in pregnancy is commonly associated with pregnant women consuming inadequate diet rich in folates [12]. Pregnant women are at high risk of folate deficiency because pregnancy increases folate requirement at a significant level, especially during the periods of rapid growth of the foetus [14]. Folate deficiency during pregnancy has been reported earlier as a major contributor to neural tube defects [22], preterm delivery, infant low birth weight and foetal growth retardation [19].

Ibrahim and Yusuf [12] also observed that folate deficiency increases the blood homocysteine level in pregnant women, which in turn leads to spontaneous abortion, placental abruption and preeclampsia. Based on this, pregnant women attending antenatal clinics are also encouraged to consume the leaves of Jatropha tanjorensis (Hospital Too Far) followed by Telfairia occidentalis (Fluted pumpkin leaves) in higher amounts, as they contained higher amount of folic acid than the other vegetables studied.

CONCLUSION

Five selected commonly consumed vegetables in southern Nigeria (*Jatropha tanjorensis*, *Talinum triangulaire*, *Vernonia amygdalina*, *Ocimum gratissimum* and *Telfairia occidentalis*) were observed to contain folic acid as earlier revealed in literature for green leafy vegetables and fruits. Green leafy vegetables and fruits have been reported to be the richest sources of folic acid. *Jatropha tanjorensis* was observed to have the highest total folate content followed by *Telfairia occidentalis*. A good combination of the two in diets could contribute more than 65% of the 400 µg recommended dietary allowance (RDA) of folate for women of childbearing age. Hence, pregnant women attending antenatal clinics are encouraged to include *Jatropha tanjorensis* and *Telfairia occidentalis* in their diets in addition to their daily intake of folic acid tablets, as this could help prevent folate deficiency that may result in neural tube defects, preterm delivery, infant low birth weight, spontaneous abortion, placental abruption, preeclampsia and foetal growth retardation.

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