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Quantitative Assessment of the Ascorbic Acid Contents of *Musa acuminata*, *Malus domestica* and *Citrus paradisi via* Dichlorophenolindophenol and Spectrophotometric Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. Author AOO designed the study, performed laboratory analyses and wrote the first draft of the manuscript. Author IRA managed the literature searches. Authors IRA and TON wrote the second draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Fresh fruits are main sources of daily requirements for ascorbic acid (Vitamin C). Despite the wide varieties of local and introduced fruit types grown in Nigeria, more than 30% of the population often suffers vitamin C deficiency. The main aim of this study was to determine the ascorbic acid (AA) contents in fruit pulp of *Musa acuminata*, *Malus domestica* and *Citrus paradisi*.
Place and Duration of Study: Fresh fruit samples of *Musa acuminata* (banana), *Malus domestica* (apple) and *Citrus paradisi* (grape fruit) were purchased from local markets in Benin City on March 28, 2017. At the time of purchase, the fruits had excellent general appearance
Methodology: Aqueous extracts of the fruits were first screened for acidity and analyzed by the 2,6-dichlorophenolindophenol (DCPIP)/spectrophotometric method.
Results: The ascorbic acid content of the aqueous extracts obtained ranged from 6.63±0.2126 mg /

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100 g to 28.53±1.2269 mg / 100 g. The lowest content of AA was found in *Malus domestica* with a concentration of 6.63±0.2126 mg / 100 g, followed by *Musa acuminata* with a concentration of 8.90±0.2160 mg / 100 g. The highest concentration of AA content (28.53±1.2269 mg / 100 g) was recorded for *Citrus paradisi*.

Conclusion: This study is useful in determining the fruit types to consume in addressing vitamin C deficiency that is prevalent in many tropical and sub-tropical countries of Africa.

Keywords: Ascorbic acid; Citrus paradisi; Malus domestica; Musa acuminata; dichlorophenolindophenol.

1. INTRODUCTION

Ascorbic acid is a water-soluble antioxidant and the most bioactive form of vitamin C. It is a strong antioxidant, reacting with singlet oxygen and other free radicals to relieve oxidative stress, reduce the risk of atherosclerosis and some forms of cancer, thereby imparting health benefits on humans [1]. Ascorbic acid plays an important role in diverse metabolic processes in the human body, from enhancement of neuro-physiological functions to acting as co-substrate for several enzymes [2]. It is important in collagen formation, a protein that gives structure to bones, cartilages, muscles, and blood vessels. Ascorbic acid also aids in the reduction of plasma cholesterol levels, improvement of the immune system, wound healing, absorption of inorganic iron and maintenance of capillaries, bones, and teeth [3]. Skeletal muscles (35 mg/kg ascorbate), liver (125 mg/kg), brain (140 mg/kg ascorbate) and adrenal glands (550 mg/kg ascorbate) have the highest concentrations of ascorbic acid (ascorbate) in the human body [4]. Rich sources of ascorbic acid from literature include blackcurrant, citrus fruits, leafy vegetables, tomatoes, green and red peppers [5]. Recommended daily intake (RDId) for ascorbic acid is 75 mg/day for adult women, 90 mg/day for adult men and 45 mg/day for children of 9–12 years of age [6].

Methods for ascorbic acid assessment involve titration with an oxidant solution; dichlorophenol indophenol (DCPIP), potassium iodate or bromate [7,8]. Chromatographic methods with electrochemical detection, particularly High Performance Lipid Chromatography (HPLC) is also used but has been reported to be quite selective and sensitive for ascorbic acid assessment in foodstuffs and biological fluids [9]. Other methods are fluorimetric, volumetric and UV-VIS absorbance-based determinations. Volumetric techniques can suffer from lack of specificity which limits their use to samples not containing other reducing agent.

Optical methods for vitamin C estimation include spectrophotometrical determination of iodine reacted with ascorbic acid and chemiluminescence. More recently, a spectrophotometric method was proposed based on ascorbic acid oxidation to dehydroascorbic acid, by using the Cu(II)-neocuproine complex, which is reduced to Cu(I)-*bis* (neocuproine), with the absorbance of the latter being determined at 450 nm [10].

This study aims to determine the concentrations of ascorbic acid present in *Musa acuminata*, *Malus domestica* and *Citrus paradisi*. The specific ascorbic acid content determination is necessary to understand the link between dietary intake of ascorbic acid and human health.

2. MATERIALS AND METHODS

2.1 Collection of Fruits

Ten fresh fruit samples each of *Musa acuminata*, *Malus domestica* and *Citrus paradisi* were purchased from local markets in Benin City, Nigeria. At the time of collection, the general appearances of the fruits were excellent.

2.2 Sample Preparation

The fruit samples were thoroughly cleaned using deionized water to remove any adhering contaminants present. The fruits epicarps (outer layer of the fruits) were peeled, the flesh cut into small pieces and freeze dried. The different fruits were then transferred separately into an electric blender and homogenized into a pulp/paste. The homogenized samples were transferred into a jar of known weight and made up to 1 L with distilled water. The slurry was then centrifuged at 10,000 rpm for 15 minutes and the supernatants collected. Aliquots (5 ml) of the supernatants were preconditioned with 2 ml acetone followed by 5 ml distilled water, the mixture was filtered via a 0.22 µm membrane filter and the filtrates obtained were stored in the fridge at 4°C to be used for further analysis.

2.3 Determination of pH of the Fruit Filtrates

Exactly 30 ml of each fruit filtrate was transferred into a clean 50 ml beaker. A pH electrode was immersed into the beaker and the reading was allowed to stabilize for about 5 minutes before the final pH value was recorded [11]. Triplicate determinations were done for each sample and the average pH was calculated and recorded.

2.4 Determination of Ascorbic Acid Content

2.4.1 Extraction of ascorbic acid

Each of the freeze-dried fruit samples (0.5 g) was extracted using 20 ml of 3% (w/v) metaphosphoric acid, followed by shaking at 300 rpm for 30 minutes. The extract was centrifuged at 4000 rpm for 10 minutes. The supernatant was collected and used for ascorbic acid assay.

2.4.2 Assay for ascorbic acid

Ascorbic acid was quantitatively determined using 2,6 dichlorophenolindophenol (DCPIP) with spectrophotometric analysis (B.BRAN Scientific and Instrument Company, England. No: 722S10159). A standard curve with a series of known ascorbic acid solutions was prepared in 3% (w/v) metaphosphoric acid to establish a calibration curve. Exactly 1 ml of sample extract was added to 3 ml of 0.2 mM DCPIP and the spectrophotometer reading was taken immediately after mixing for 15 seconds at 515 nm [12]. The results were expressed in mg ascorbic acid per 100g dry weight (mg/100g DW). A sample with blue color is indicative of low concentration of AA, while a clearer solution is indicative of high AA. The experiment was done in triplicates and the percentage absorbance was calculated as;

$$\%Abs = \frac{A_o - A}{A_o} X \quad 100\%$$

Where A_o= Absorbance of blank
A= Absorbance of a known concentration and
% Abs = Percentage Absorbance

3. RESULTS AND DISCUSSION

Result obtained for the AA calibration curve is shown in Table 1 and Fig. 1. The % absorbance increased with increasing AA concentration. The plot of the concentration of AA against its % absorbance yielded an R^2 value of 0.966 and gave a linear equation of:

$$y = 2.065x - 1.565$$

Where 2.065 and -1.565 represent the values of the slope and y- intercept respectively. This data is in comparable range with that reported by other spectrophotometric studies on ascorbic acid assessment [13-16], indicating the validity, reproducibility and acceptability of the method.

3.1 pH Measurement

The acidity of the fruit pulp was determined prior to AA assay to ascertain the presence of acid containing substance (preliminary examination). pH values obtained for *Musa acuminata* was highest, followed by *Malus domestica* and lastly, *Citrus paradisi*. These pH values were within the normal pH range from literature expected for the fruits (Table 2).

The acidity of the fruits studied can in part, be attributed to their ascorbic acid contents. Since pH is a direct measure of acidity, it is the most commonly used analytical measurement in industrial processing as it clearly plays an important role in food production, processing, storage and quality [17]. A pH value of 2.5 to 5.5 prolongs the shelf life of fresh fruit by hindering the growth and multiplication of non-acidophilic micro-organisms. From the result obtained, the three fruit extracts had pH values within this range.

3.2 Ascorbic Acid Contents of the Fruit Extracts

Table 3 depicts the concentrations of AA in the fruit extracts. AA concentration obtained for *Malus domestica* was lowest, followed closely by *Musa acuminata*. *Citrus paradisi* had the highest concentration of AA.

Results of the determination of the ascorbic acid content of the fruit extracts by spectrophotometric method show that the fruits studied had ascorbic acid present in the following increasing order; *Malus* domestica<*Musa acuminata*<*Citrus paradisi*. *Malus domestica contains* about 6.63 mg of ascorbic acid, accounting for 7.4%, 8.8% and 14.7% of the recommended daily intake of ascorbic acid for adult men, adult women and children respectively. Also, *Musa acuminate* contains

Table 1. Data obtained for AA calibration curve

Concentration of AA (µg/ml)	Absorbance	Blank absorbance	% absorbance
0 (blank)	0.427	0	0
1	0.425	0.002	0.47
2	0.420	0.007	1.64
5	0.415	0.012	2.81
10	0.375	0.052	12.18
20	0.218	0.209	48.95
25	0.173	0.254	59.48
50	0.020	0.407	95.32

Table 2. pH of the various fruit samples

Fruit sample	Normal pH range	pH value of fruits
Musa acuminata	4.0-6.5	4.60
Malus domestica	3.35-4.0	3.53
Citrus paradisi	2.0-4.0	2.75

Table 3. Ascorbic acid content of the fruit extracts

Fruit	Ascorbic acid content (mg/100g)
Musa acuminata	8.90±0.21
Malus domestica	6.63±0.21
Citrus paradisi	28.53±1.22

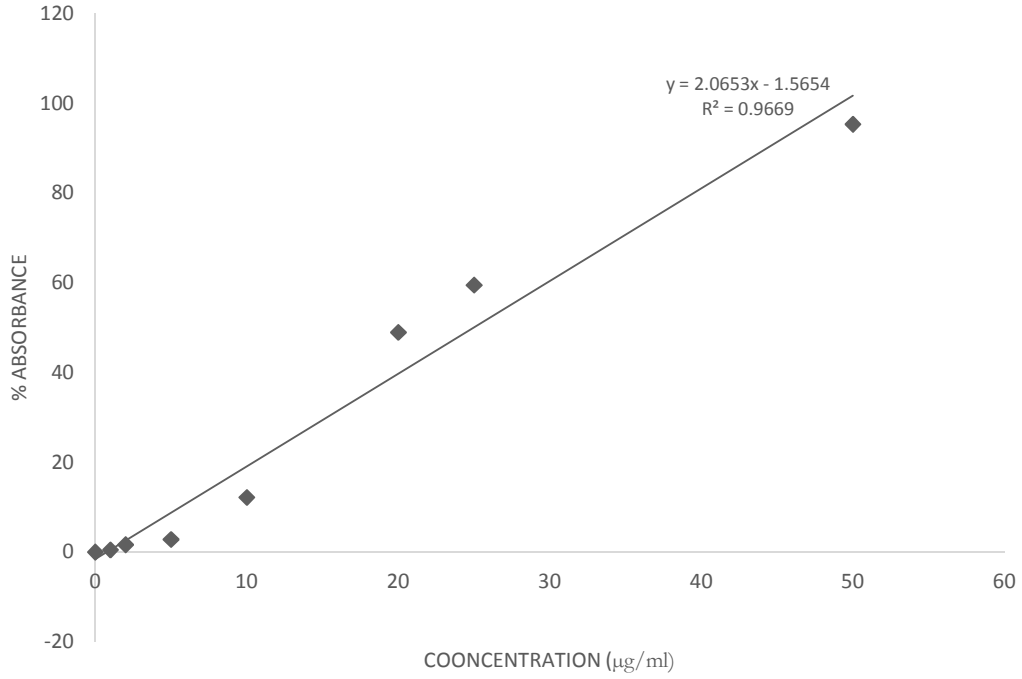

Fig. 1. Ascorbic acid calibration curve

about 8.9 mg ascorbic acid, accounting for 9.9%, 11.9% and 19.8% of the recommended daily intake of ascorbic acid for adult men, adult women and children respectively. *Citrus paradisi* with the highest amount of ascorbic acid (28.53 mg) in this study contributes 31.7% in adult men,

38.4% in adult women and 63.4% in children of the recommended daily intake (RDId) for ascorbic acid.

The concentration of AA reported in this study is lower compared to a concentration reported by Deekshika et al. [18], who found 20.13 ± 1.54 mg/100g to 54.78 ± 2.19 mg/100 of AA in mango and banana extracts, but higher compared to AA concentration of 4.939 ± 0.00080 mg/100g obtained for banana extracts by Mohamad et al. [19].

4. CONCLUSION

Based on the result of this study, *Musa acuminata* (banana), *Malus domestica* (apple) and *Citrus paradisi* (grape fruit) are good sources of ascorbic acid. These fruits are recommended for the daily intake of AA for adults and children.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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