FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 3 No. 2, June, 2019, pp 376 - 384

DETERMINATION OF VITAMIN A AND VITAMIN C IN *CORCHORUS OLITORIUS* (BUSH OKRA) USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT
Vitamins A and C are essential to humans as they play important roles in general metabolic processes and maintenance of good health conditions. Their essentiality is based on the fact that they cannot be synthesized by humans but must be consumed from plant based foods. *Corchorus olitorius* (Jew's mallow) being a widely cultivated and consumed vegetable either fresh or dried in Nigeria would serve as a reliably cheap and quantitative source of vitamins A and C. It has been used for healing benefits as natural medicinal herb as it has been used to treat and manage many diseases. This study was carried out to determine the vitamins A and C content in the fresh and dried leaf of *Corchorus olitorius* by using high performance liquid chromatography (HPLC). The fresh sample was found to contain 0.056 mg/ml of vitamin A and 0.108 mg/ml of vitamin C while the dried leaf was found to contain 0.162 mg/ml of vitamin A and 0.211 mg/ml of vitamin C. The result shows that the dried leaf of *Corchorus olitorius* contains higher concentration of vitamins A and C than the fresh vegetable. This is an advantage especially for people living in the rural areas as it can be dried and stored for future use with the vitamin content still maintained.

Keywords: *Corchorus olitorius*, Green Vegetable, HPLC, Vitamin A, Vitamin C

INTRODUCTION

Corchorus olitorius (Jew's mallow) is a vegetable that is widely grown in both dry and semi-arid regions and in the humid areas of Africa, because of its benefit in providing the body with good nutrients (Oladiran, 1986). Most of the species are consumed as a vegetable of which *Corchorus olitorius* is the most cultivated specie. *Corchorus olitorius* is used in the treatment of fever, gonorrhea, chronic cystitis and tumors (Zeghichi *et al.*, 2003). The crop is grown extensively in Nigeria as a wild plant throughout the year except for the periods of low temperature. The production in Nigeria is usually for local consumption. *Corchorus olitorius* is an important green vegetable of the Middle East, Egypt and Nigeria as well as parts of tropical Africa. Jew's mallow contains many active nutrients and compounds which includes protein, fat, carbohydrate, fiber, ash, calcium, potassium, vitamins, iron, sodium, phosphorous, beta-carotene, thiamine, riboflavin, niacin, ascorbic acid (Islam, 2010). Solvent extracts of *Corchorus olitorius* showed that it contains medicinally important bioactive compounds which justify the use of the plant species as traditional medicine for treatment of various diseases (Patil and Jain, 2019). It is popularly known as ewedu and lalo in the South- western and northern parts of Nigeria respectively, where the boiled and mashed fresh leaf is a delicacy (Makinde *et al.*, 2009).

Corchorius olitorius has been found to contain high concentrations of antioxidant compounds (Giro and Ferrante, 2018). The small amount of protein present is rich in methionine. The leaves are used in the treatment of chronic cystitis, gonorrhea, dysuria, and toothache (Hillocks, 1998). A cold infusion is used as a tonic to restore appetite and strength. The leaves have also been found to suppress elevation of post prandial blood glucose levels in rats and humans (Innami *et al.*, 2005).The leaves are widely used as a leafy vegetable in many Asian, African and European countries (Oyedele, 2006). In native medicinal practice C. *olitorius* is involved in the treatment of several diseases. Various parts of these plants: leaves, roots leafy stems, and seeds are used to prepare medicated recipes (Adebo *et al.*, 2018). The seeds are used for treatment of fever and as a purgative; they possess broad antibacterial properties (Pall *et al.*, 2006).

Vitamin A is an essential human nutrient that is important in vision, regulation of gene expression, immunity and growth and development. The use of high performance liquid chromatographic techniques for the analysis of vitamin A constituents has advantages over alternative assay methods. In cells, one important role of Ascorbic Acid is to reduce hydrogen peroxide (H_2O_2), which preserves cells against reactive oxygen species. Synthesized nanoparticles of *Corchorus olitorius* displayed strong to moderate cytotoxic activities against three cancer cell lines (HCT-116, HepG-2, and MCF-7) (Ismail *et al.*, 2018). Primates and several other mammals are not able to synthesis ascorbic acid. The only way humans can obtain ascorbic acid is via food. Currently, the estimated average requirement and recommended dietary allowance of ascorbic acid are 100 and 120 mg per day, respectively (Englard and Seifter, 1986).Analytical techniques such as sensors and biosensors have been suggested for the detection of ascorbic acid in various types of samples (Wang *et al.*,2008; Yogeswaren *et al.*, 2008). One of the most frequently used method is based on the reduction of the blue dye 2,6-dichlorophenolindophenol by ascorbic acid (AOAC, 1999). HPLC is considered a sensitive and selective method and therefore suitable for active substance determination; it is also suitable for the evaluation of stability in formulations in the pharmaceutical and cosmetic industries (Marshall *et al.*, 1995). This research was undertaken to

evaluate the content of Vitamin A and Vitamin C in *Corchorus olitorius* using HPLC.

MATERIALS AND METHODS

Apparatus and Chromatographic Condition

A Merck-Hitachi liquid chromatography system (Merck, Darmstadt, Germany) equipped with a model L-2130 pump, model L-2300 column oven, model L-2200 Autosampler, model Dell Monitor + CPU and model L-2455 Diode Array Detector were used. The chromatographic column was LiChrospher 100 RP-18, 125 x 4mm i.d, particle size 5µm membrane and degassed by sonication prior to use. Ultrasonic cleaner model D-5409, water distiller model W-4000 (made in UK by Bibbysteriling LTD), Eppendof centrifuge model 5804, and Sartorius weighing balance model LA-3105.

Reagents and Standards

Methanol and Acetonitrile were of HPLC-grade from Merck. A standard vitamin mixture was prepared by taking 2ml of each stock solution to a final volume of 100ml with methanol, stock solution was freshly prepared, and used immediately afterwards.

Area of Study

Gadau farm is in Itas/Gadau Local Government of Bauchi State, Nigeria. It is located at the north eastern part of Nigeria and the economic activities in the area are farming and fishing.

Collection of Samples

Fresh leaves of *Corchorus olitorius* were collected from the Gadau farmers and were air dried at room temperature for about two weeks.

Sample Preparation

The sample solutions for fresh and dried *Corchorus olitorius* were prepared and analyzed in the same manner as a standard solution. Preparation of the sample solutions was done by preparing five (5) different standard solutions of concentrations ranging from 5 – 25 µl for both fresh and dried samples. The standard solutions of the vitamins were prepared from a stock solution and were stored in a refrigerator. Working standard solutions were prepared immediately prior to usage. Aliquots of the solutions were then treated as samples in which the peak areas obtained were plotted against concentration for the calibration curves.

Method

Determination of Vitamins A and C Content of *Corchorus olitorius*

Vitamin A and vitamin C content of *Corchorus olitorius* leaves extract were determined using the procedure described by Benderitter *et al.* (1998).

Extraction of Samples

Both fresh and dried samples of *Corchorus olitorius* were extracted using the mobile phase acetonitrile and methanol (75:25). Fresh and dried samples of *Corchorus olitorius* were accurately weighed (1.5g), diluted in eight milliliters (8ml) of mobile phase and sonication for the extraction was allowed to take place for 30 minutes (sonication time). The samples were centrifuged and supernatant was collected for injection into the HPLC system.

RESULTS

Peak areas corresponding to injections made in the eluting solvent showed retention times independent of the injection volume but the variances increased along with the volume that was injected (Table 1). Although minor variations were observed in the retention times which may have resulted from temperature fluctuations, but the elution sequence was unaltered. The calculated concentration of vitamin A in mg/ml obtained for the fresh and dried *Corchorus olitorius* were calculated from the calibration graphs (Fig. 1) that were obtained by measuring the peak areas of vitamin A for the fresh and dried samples which was linear for the injected volume between the ranges of 5 - 25 µl.

Table 1: Injection Volume, Actual Concentration, Peak Area, Wavelength, Retention Time and Calculated Concentration of Vitamin A

Injection Volume(µl)	Actual Conc. (mg/ml)	Peak Area	Wavelength (nm)	Retention Time(mins)	Calculated Conc. (mg/ml)
5	0.2	460133	326	4.120	0.050
10	0.2	918766	325	4.120	0.100
15	0.2	1378870	325	4.120	0.151
20	0.2	1832177	325	4.113	0.200
25	0.2	2282473	326	4.140	0.249

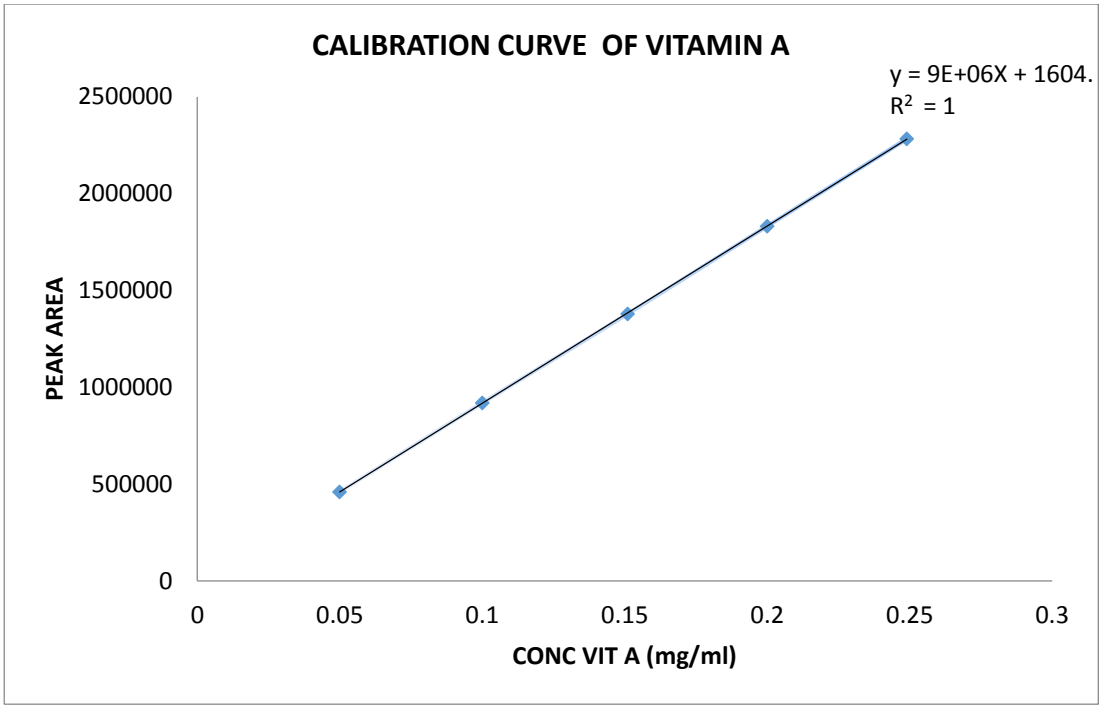

Fig 1: Calibration Curve of Vitamin A

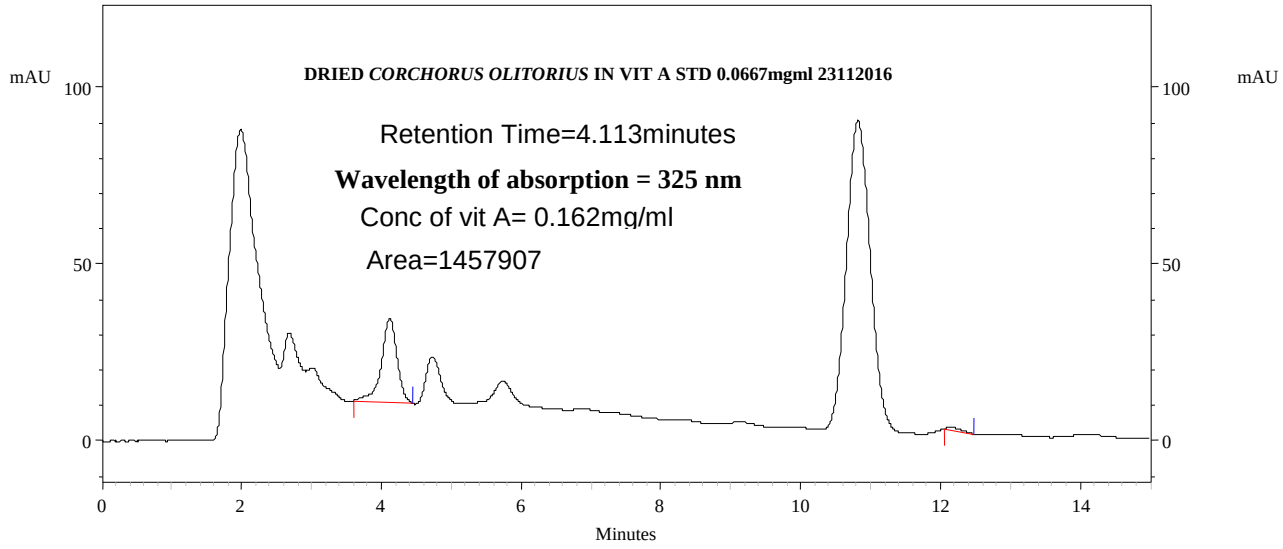

Fig. 2: Chromatogram of Vitamin A in Dried *Corchorus olitorius*

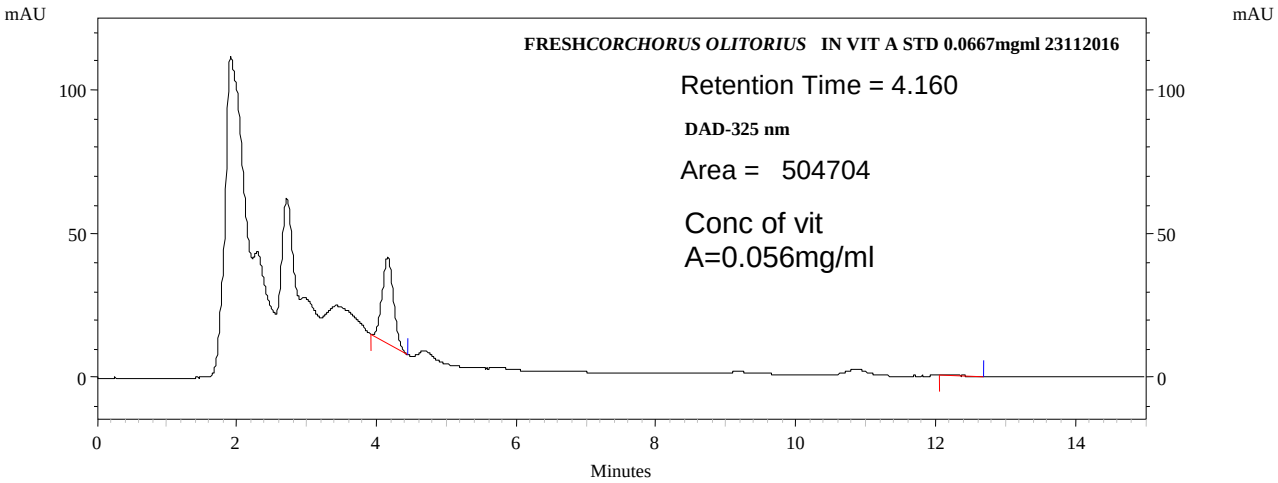

Fig. 3: Chromatogram of Vitamin A in Fresh *Corchorus olitorius*

Peak areas corresponding to injections made in the eluting solvent showed retention times independent of the injection volume but the variances increased along with the volume that was injected (Table 2). Although minor variations were observed in the retention times which may have resulted from temperature fluctuations, but the elution sequence was unaltered. The calculated concentration of vitamin C in mg/ml obtained for the fresh and dried *Corchorus olitorius* were calculated from the calibration graphs (Fig. 2) that were obtained by measuring the peak areas of vitamin C for the fresh and dried samples which was linear for the injected volume between the ranges of 5 - 25 µl.

Table 2: Injection Volume, Actual Concentration, Peak Area, Wavelength, Retention Time and Calculated Concentration of Vitamin C

Injection Volume(µl)	Actual Conc. (mg/ml)	Peak Area	Retention Time(mins)	Wavelength (nm)	Calculated Conc. (mg/ml)
5	0.2	9980163	0.507	254	0.061
10	0.2	7629878	0.553	247	0.046
15	0.2	21143094	0.520	260	0.129
20	0.2	32827332	0.480	267	0.200
25	0.2	39921543	0.533	263	0.243

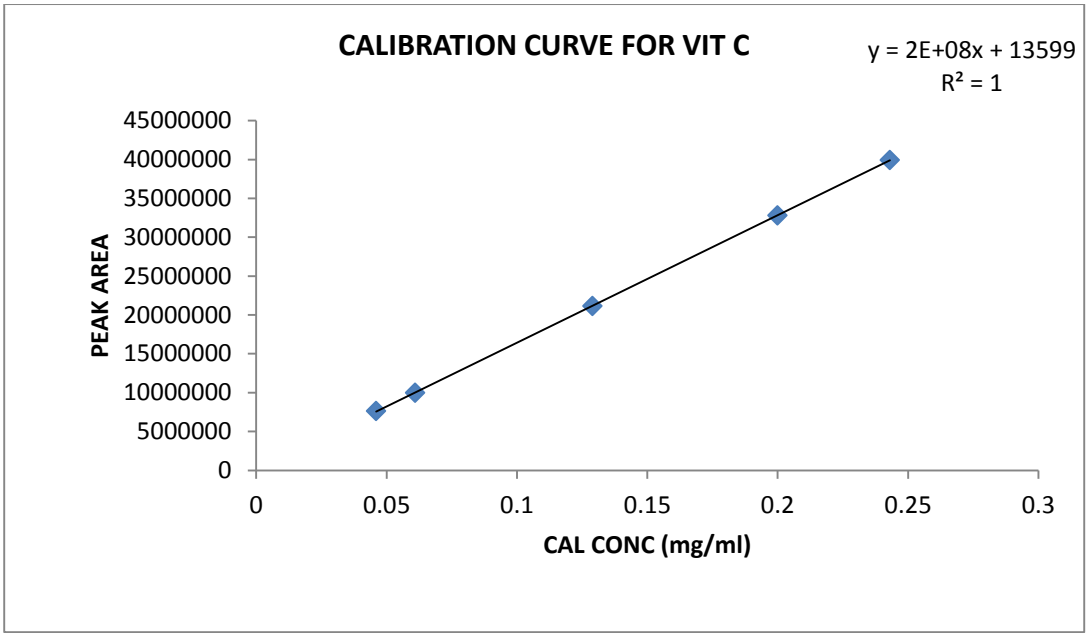

Fig 4: Calibration Curve of Vitamin C

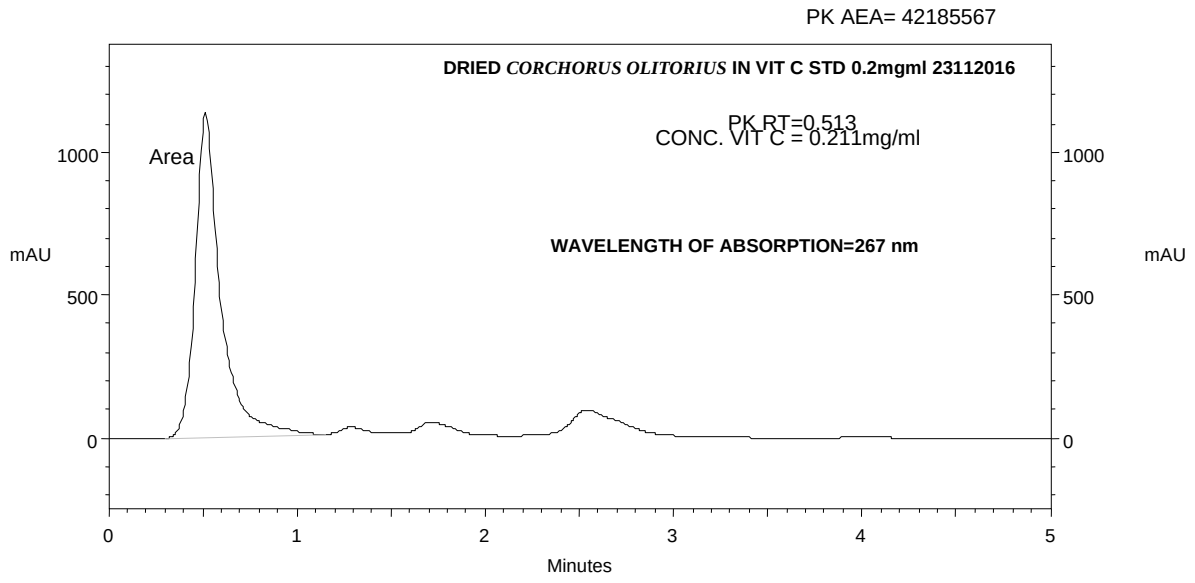

Fig. 5. Chromatogram of Vitamin C in Dried *Corchorus olitorius*

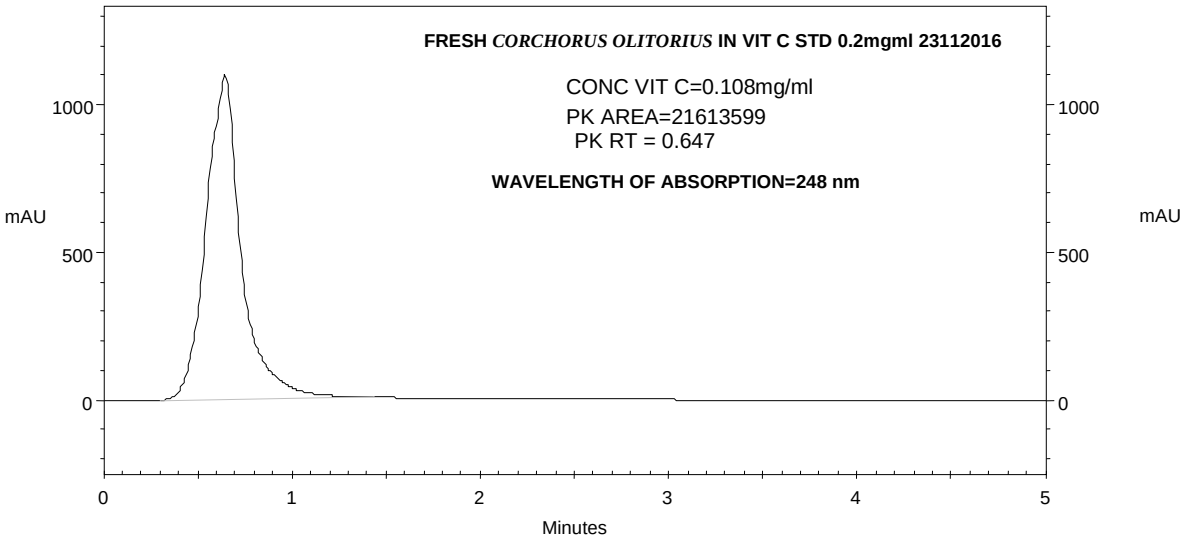

Fig 6. Chromatogram of Vitamin C in Fresh *Corchorus olitorius*

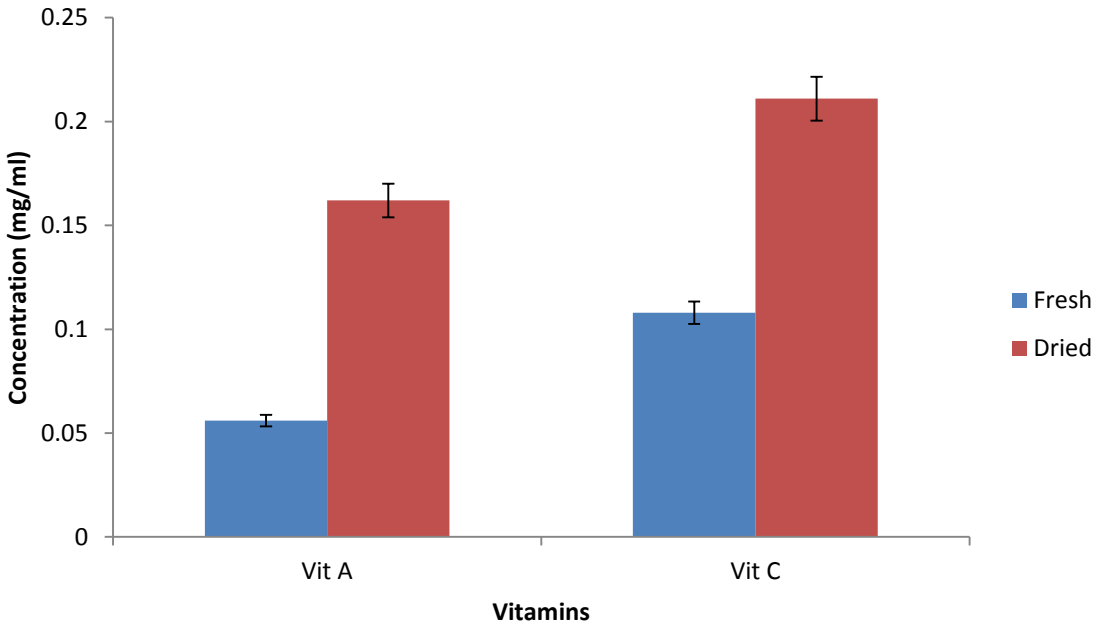

Fig. 7: Concentration of Vitamin A and Vitamin C in Both Fresh and Dried Samples of *Corchorus olitorius*.

DISCUSSION

Several analytical methods have been developed in the last decades to determine vitamin A and vitamin C content in *Corchorus olitorius* (Chatzimichalakis *et al.*, 2004; Moreno and Salvador, 2000). However, these analytical methods of vitamin A and vitamin C in most cases are based on outdated procedure, which are complicated, time consuming, inaccurate and do not allow the simultaneous determination of vitamins. In this research the current chromatographic technique was the modification of many techniques reported by many authors which was found to be rapid, valid, accurate, time-saving, low cost, and modern method to evaluate vitamin A and vitamin C composition. In this analysis for the determination of vitamin A and vitamin C content in the fresh and dried samples of *Corchorus olitorius*, it was found out that the concentration of vitamins A and C in dried sample was higher than in fresh sample. This may be due to the high moisture content of the fresh samples over the dried samples. The leaves of *Corchorus olitorius* have been reported to have high moisture content (Ekpo *et al.*, 2019; Dappah *et al.*, 2018). Temperature and time are the most important parameters that affect vitamin C degradation and as such the

retention of these vitamins in fruits and vegetables is largely dependent on these parameters during drying. Also the area of exposure to the drying conditions and concentration of oxygen in the drying atmosphere influences the final content in the dried products (Santos and Silva, 2008). The dried leaves were prevented from direct sunlight during preparation. This may have prevented loss of the nutrient as shade drying has been reported to decrease and prevent direct loss of nutrients (Traore *et al.,* 2017). The leaves of *Corchorus olitorius* have been reported to have various health benefits as it has been determined to contain ample amount of protein, fiber, vitamin (A, C and E), thiamine, riboflavin, and is rich in mineral nutrient like calcium and iron. (Dansi *et al.,* 2008).

To obtain a suitable and robust HPLC method for the determination of vitamin A and Vitamin C, different mobile phases, methanol and acetonitrile were used in the mobile phase. The mobile phase acetonitrile and methanol in the ratio of 75:25 at a flow rate of different injection volume and retention time gave sharp peaks with minimum tailing and good resolution. Vitamin A and Vitamin C were eluted at retention time around 4.20 and 0.60 respectively with symmetric peak shape.

For dried *Corchorus olitorius* (Fig. 2), at an absorption wavelength of 325 nm, the concentration of vitamin A, based on the peak, was found to be 0.162 mg/ml. The retention time for vitamin A peak of the dried sample was 4.113 minutes. The results obtained in this study was found to be less compared to that reported by Mitic *et al.* (2011)

For fresh *Corchorus olitorius* (Fig. 3), at an absorption wavelength of 325 nm, the concentration of vitamin A, based on the peak area was 0.056 mg/ml. The retention time for vitamin A peak of the fresh sample is 4.160 minutes. This result is similar to that reported by Steyn *et al.* (2001).

For dried *Corchorus olitorius* (Fig. 5), in the peak identification of vitamin C, based on the retention time of 0.513 minutes and absorption wavelength of 267 nm, the concentration of vitamin C, based on the peak area was 0.211 mg/ml.

For fresh *Corchorus olitorius* (Fig. 6), in the peak identification of vitamin C, based on the retention time of 0.647 minutes and absorption wavelength of 248 nm, the concentration of vitamin C, based on the peak area was 0.108 mg/ml. *Corchorus olitorius* is effective in preventing obesity, hyperlipidaemia, steatosis and insulin resistance (Gomaa *et al.,* 2019), this may be due to the presence of vitamin A, vitamin C and also flavonoid contents (Adeosun *et al.*, 2016). The rich mineral, vitamins and nutritional composition of this vegetable confers important nutritional needs and medicinal properties which serves to replenish the body and provides protection against many disease conditions (Ojiewo *et al.,* 2013; Bailey, 2003). *Corchorus olitorius* have been found to be low in crude fat but very good in unsaturated fatty acid and is virtually devoid of sterols and phospholipids which will therefore be good for people with heart diseases (Adeyeye *et al*., 2016).

The chart (Fig. 7) shows that *Corchorus olitorius* contains more vitamin C than vitamin A in both the dried and fresh samples, although the dried sample contains a higher concentration of both vitamins A and C than the fresh samples.

CONCLUSION

HPLC is a reliable and convenient analytical method for the quantification of vitamin A and vitamin C in food samples such as *Corchorus olitorius.* The concentration of Vitamin A and vitamin C in dried samples were found to be higher than that of the fresh samples of *Corchorus olitorius.* Results in this study have shown that *Corchorus olitorius* is rich in vitamins A and C and can serve as a good and cheap source of these vitamins to many people in Nigeria and other developing countries that may not be able to afford other expensive sources of vitamins.

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