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

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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 (Oludiran, 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, betacarotene, 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).

Corchorus 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$_2$O$_2$), 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 synthesize 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 formulations in the pharmaceutical and cosmetic industries (Marshall et al., 1995). This research was undertaken to

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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
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 Bibbysterting 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**

<table>
<thead>
<tr>
<th>Injection Volume(μl)</th>
<th>Actual Conc. (mg/ml)</th>
<th>Peak Area</th>
<th>Wavelength (nm)</th>
<th>Retention Time(mins)</th>
<th>Calculated Conc. (mg/ml)</th>
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CALIBRATION CURVE OF VITAMIN A

\[
y = 9E+06X + 1604, \quad R^2 = 1
\]

Fig 1: Calibration Curve of Vitamin A

**DRIED CORCHORUS OLITORIUS IN VIT A STD 0.0667mg/ml 23/11/2016**

Retention Time = 4.113 minutes
Wavelength of absorption = 325 nm
Conc of vit A = 0.162mg/ml
Area = 1457907

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

<table>
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<tr>
<th>Injection Volume(μl)</th>
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<th>Peak Area</th>
<th>Retention Time(mins)</th>
<th>Wavelength (nm)</th>
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Fig 4: Calibration Curve of Vitamin C

\[ y = 2E+08x + 13599 \]
\[ R^2 = 1 \]

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

PK AEA = 42185567

CONC. VIT C = 0.211mg/ml

Area

WAVELENGTH OF ABSORPTION = 267 nm
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.

REFERENCES


