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***Journal of Scientific and Engineering Research*, 2019, 6(12):149-156**

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Customary processing methods (boiled paste, shade dried and sun dried) of three leafy vegetables (*Moringa oleifiera*, *Gongronema lanfolium* and *Ocimum grarissimum*) consumed by people of southern Cross River State, Nigeria

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Abstract This study investigated the customary processing methods (boiled paste, shade dried and sun dried) of three leafy vegetables (*Moringa oleifiera*, *Gongronema lanfolium* and *Ocimum grarissimum*) consumed by people of southern Cross River State, Nigeria on the nutrient and non-nutrient composition. Antimicrobial and antiplasmodial activities of the cold and hot extracts of the vegetables were also investigated. The vegetables used for the study were obtained from the University of Calabar staff quarters in Calabar. The leaves were divided into gin: each for study at four different levels of preparation/processing viz. unprocessed, i.e. dried and sun dried. Nutrients and non-nutrients were analyzed using standard methods of the Association of Official Analytical Chemists (AOAC). The kirby-bauer disk diffusion susceptibility test was used for antimicrobial studies, while trophozoite count was utilized for antiplasmodial activity. The results showed that total proteins and carbohydrates were significantly higher in the three processed samples compared to unprocessed samples unlike case with the moisture content of the samples. Similarly, vitamin and mineral levels were significantly reduced ($P < 0.05$) by the various processing methods. Vitamin B2 found in trace quantity (0.01 =1: 0.00 mg/100g) in the unprocessed samples was found to be absent in the boiled and sun dried samples. There was a significant ($P < 0.05$) reduction in the antinutrient (oxalale, cyanate and phytate) composition of the boiled and sundried samples compared to unprocessed and shade dried samples. The traditional processing methods also reduced ($P <0.05$) saponins, flavonoids, polyphenols, alkaloids, total carotenoids and total phenolics while total iranthonoids and volatile organic acids were not significantly affected ($P > 0.05$). The
Sensitivity test revealed that both the cold and hot aqueous extracts of the vegetables had antibacterial and antifungal activities. In addition, *Moringa oleifera* and *Gongronema latifolium* leaf extracts also showed antiplasmodial activity but *Ocimum gratissimum* did not. The three leafy vegetables studied are rich sources of nutrients. While the customary preparation methods are not completely deleterious to the proximate composition of the vegetables, there are significant losses of some essential vitamins and minerals. Also, the processed methods may offer some nutritional and health benefits to the consumers with respect to the non-nutrients such as o ate, pytate, cyanate which were substantially reduced in the processed samples. Additionally, the vegetables had appreciable levels of some phytochemicals such as flavonoids, saponins, phenolics and polyphenols which may have been responsible for their antimicrobial and antiplasmodial activities.

Keywords Customary processing methods, *Moringa oleifiera*, *Gongronema lanfolium*, *Ocimum grarissimum*

Introduction

Malnutrition is a global problem that manifest in various ways in different parts of the world. It is highly prevalent in developing countries where it presents mainly in the form of under-nutrition, due primarily to

poverty [1]. However, recent findings have shown that over nutrition is also becoming a public health problem in many developing countries including Nigeria [2]. In the countries, epidemics of non-communicable diet-associated ailments e.g. cancer, atherosclerosis, obesity, diabetes mellitus, hypertension and heart disease have been emerging. These problems are increasingly being associated with excessive consumption of one or more nutrients. The knowledge of the nutritional, preventive and therapeutic qualities of food eaten in a country is of utmost importance in solving the problems of malnutrition and diseases.

Plant foods make up a larger percentage of foods consumed in developing countries, because animal foods are often expensive and not easily affordable. Thus, the need to explore and utilize plant material as a viable source of food and medicine has become inevitable.

In Nigeria, a number of leafy vegetables are used as food in the traditional treatment of many ailments including malaria, diabetes mellitus, cancer, hypertension, as well as external injuries or wounds but little work has been done especially on plants form the southern part of Cross River State. More so, studies carried out on vegetables are mostly on the raw or dried forms, whereas in Nigeria, vegetables are usually subjected to many other forms of traditional processing before consumption.

Objective of the study:

1. Identify and quantify the vitamins in the vegetable samples (*Moringa oleifera*, *Ocimum gratissimum*, *Gongronema latifolium*) namely A, D, E, K, riboflavin, thiamine, niacin, pantothenic acid vitamin B_5, B6 and folic acid.

Literature Review

Hussain et al [3] observed that vegetables are laden with proteins, carbohydrate and to a small extent fats – essential nutrients of diets for humans. Moisture, fiber and ash contents of individual's vegetables are important parameters that can provide valuable information to the betterment of human health [4-5]. Hart et al. [6] in their study reported that edible plants or vegetables increase the nutritive value of diets owing to their rich content of minerals and vitamins especially pro-vitamin A (i.e. β- carotene), iodine, riboflavin, calcium, ascorbic acid and iron. They also observed that vegetables offer small amount of energy, thus accounting for their importance in energy restricted diets. The component of fiber in vegetables is reputed to have advantageous effect on the level of cholesterol in blood and to guard against the incidence of bowel.

Related diseases, whereas in diabetic patients, it improves the tolerance of glucose. Fiber cleans the digestive tract by removing potential carcinogens from the body. The carbohydrate in vegetables consists mainly of indigestible fibrous materials such as cellulose, hemicelluloses, and lignin, in addition to glucose, fructose, sucrose and starch. The proportion of the fibres in the vegetables depends on the stage of maturity while the turgidity or rigidity of the vegetables depends on their water content which may vary between 75 percent and 95 percent [7].

Mensah et al [8] had shown that vitamin C found in vegetables is an anti-oxidant that aids the body in its protective mechanisms against degenerative diseases like arthritis, cancer and type 2 diabetes mellitus. Furthermore, vegetables strengthen the body's immune or defense system, while plants or leaves with much content of protein are recommended for people suffering from diseases that are linked to protein deficiency. They possess dietary iron (Fe) required for haemoglobin synthesis.

Materials and Methods

Vegetables' Collection

Fresh leafy vegetables of *Ocinum grotisimum*, *Gongronema latifolum* and *Moringa oleifera* were harvested from the garden of Staff quarters, University of Calabar, Calabar. All the vegetables were obtained in the morning of the same day and taken to the herbarium in the department of Botany, University of Calabar in order to identify and authenticate them. Thereafter, they were taken to the scientific research Laboratory in the Department of Biochemistry, University of Calabar for processing and chemical assays.

Preparation of samples for analyses

The leaves were carefully hand-picked to remove inedible parts and all extraneous materials. Each leaf type was washed thoroughly with running clean tap water, subsequently rinsed using distilled water and allowed to drain. Two thousand five hundred (2500 g) of each vegetable was shared into five portions of 500 g each for laboratory analyses based on the usual form in which they are used in Calabar and surrounding communities. These are: unprocessed, paste, shade dried and sun dried.

Determination of proximate Composition

The proximate analyses was carried out on the unprocessed, boiled paste, shade dried and sun dried samples of *Moringa oleifera*, *Ocimum gratissimum* and *Gongronema latifolium*. The samples were each analyzed for the various proximate constituents: moisture, ash, fat, crude protein, crude fibre and Carbohydrate as stated below.

Determination of vitamins

The spectrophotometric method of Toro and Akerman [10] was used. Vitamin A in the was extracted by treating a 3.0ml portion ol the sample powder with 3.0 ml of absolute ethanol followed by 6.0 ml of pure hexane. Alter centrilugation, the upper hexane layer was used for analyses as described in appendix two.

Determination of vitamin K

Five grams (5.0 g) samples which were tested for vitamin K activity were first reduced to slurry in a Warring Blender with a minimum amount of water and then freeze-dried to remove the moisture. In freeze drying the slurry was first frozen for eight hours at 180 F. This frozen slurry placed in a pre-cooled freeze dryer and the chamber evacuated to 5.0 mm of mercury. After about five min the plate was warmed to I009 F with circulating water. The low vacuum caused the moisture to sublime without thawing the slurry. The material was left in the dryer 15 to 18 hr depending on the thickness of the layer. The best drying was obtained with a layer ½ inch thick. The dried material was powdered to increase the surface available for interaction with the extracting solvent. The powder was extracted with a 25 percent aqueous solution of propylene glycol to which 0.15 percent sodium meta-bisulfite was added as a preservative. The extraction process continued in a Soxhlet apparatus for three to four hr was sufficient to extract the vitamin K present in the sample. The sodium pentacyanoamineferrorate reagent was introduced into the aqueous solution of propylene glycol for the colour development to occur after sometime. The amount of vitamin K present was then determined by taking an optical density reading on the spectrophotometer and determining its corresponding concentration value using the standard curve. The solution was cooled prior to the addition of the sodium pentacyanoamineferroate reagent since the colour complex formed is unstable to heat, and the colour diminished rapidly. This was determined by heating a solution in which the colour had already developed. After five min of heating, the colour intensity began to reduce and the colour disappeared entirely within 15 min leaving a pale orange solution. In the case where pigments might increase the reading on the spectrophotometer due to their absorbance at the same wavelength, 1.0 ml of the aqueous propylene glycol solution was removed prior to the addition of the sodium pentacyanoamineferroate reagent. This portion was also read at 650mm and its reading, if any, subtracted from that of the sample to which the sodium pentacyanoamineferroate reagent had been added. Chlorophyll is the pigment most likely to cause interference since it absorbs in the same region as the colour complex. In some cases had been added to give a reading, so it was diluted in the ratio 1.0.0:10 and the optical density reading adjusted accordingly. The adjusted optical density readings found in the table represent the readings that would be obtained on a 100 ml sample which was read with no dilution. The adjustment was necessary so that the values could be compared to those on the standard curve, and these latter optical density reading were obtained from 100ml solutions read without dilution.

Determination of Vitamin B complex

The determination and qualification of the B complex vitamins was done following the standard method as elaborated by Sheher et al [9]. Appropriate measurements were done and the stipulated time observed. The details are explained in appendix two.

Estimation of Vitamin C

Determination of ascorbic acid (or Vitamin C) was done using the spectrophotometric method of Toro and Ackerman [10]. 5.0g of the stored powdered samples were weighed, and dissolved in water and transferred into a 100ml volumetric flask. Later it was filtered for analysis, 2.0 ml of sample (filtrate) and 3.0ml of meta-phosphoric acid solutions were mixed and then centrifuged. The supernatant (2.0ml) thereafter was treated with 0.15 M Na-citrate (0.5ml) solution (see appendix two for further details).

Antimicrobial studies

Extraction of the vegetable samples

Extraction was done using the Soxhlet apparatus. This assay involved the division of each vegetable type into two equal portions. Cold distilled water was used in the extraction of the first portion, whereas the second portion was extracted with hot distilled water. For the extractions, 100g of the sample was extracted with water in Soxhlet extractor for 48hr. The extract was concentrated under reduced pressure and preserved in the refrigerator at – 40 ^{o}C.

Preparation of four (4) different concentration of the vegetable extracts

One thousand (1000) ug of each crude extract was dissolved in 10ml of distilled water thus making a solution of 100 ugml w/v. The 100 ugml w/v of solution was then serially diluted produce 50 ug/ml and 12.5 ug/ml solutions.

Collection of test organisms and viability test

Three clinical microbial isolates-The bacteria species gram negative Escherichia coli and gram positive *Staphlococus aureus*, and the fungus species Candida albiocans collected from the University of Calabar Teaching Hospital, were used for the study. They were identified properly by subjecting them to several tests. At the end of incubation, the clinical bacterial isolates were characterized using gram reaction, motility, calalase, oxidase, coagulate, indole, methyl red, vogesproskauer, citrate utilization, hydrogen sulphide production, acid/gas production tests while sugar fermentation, lactophenol blue and catalase tests to confirm the Candida albicans isolate. Prior to the various aforementioned tests, the isolates were subjected to viability test thus: Nutrient agar (1.5g) and potato dextrose agar (2.0g) were dissolved separately in distilled water (50ml) in 250ml conical flasks, the contents of the flasks were thoroughly shaken and mixed well. Next, the media were sterilized at 121 ^{o}C for 15 min. following which the media were allowed to cool for 45 min and later 20 ml of each media was placed in properly petri-dishes. The media were further allowed to cool for 30 min in order to solidify, after solidification of the media the stock cultures of the clinical isolates were spread on the freshly prepared plates and then incubated at 37 ^{o}C for 24hr. After these confirmatory tests, the isolates were stored in bijou bottles and kept in the refrigerator in readiness for the susceptibility (sensitivity) tests.

Determination of susceptibility/ sensitivity

The screening for anti-microbial properties in the aqueous extracts of each of the vegetables was done using the method of agar diffusing technique and disc diffusion assay (Kirby-Bauer disc diffusion method, 1961). Ninety (90) glass petri-plates previously sterilized in a hot-air oven at 100 0c for an hour were first labelled accordingly, each plate carrying labels of the various dilutions of the Sugar cane extract at specific locations with their respective clinical isolates, i.e. plate 1, for instance carried 100 percent, 50 percent, 25 percent, 12.5 percent and the extract labels were placed at specific locations around the petri-plates. The labeling followed a clock-wise direction. One (1.0) ml each of the clinical isolates were placed in their respective plates, afterwards Mueller-Hinton agar (20ml) sterilized and held at 45-55 ^{o}C was added to each plate, which was then swirled to allow for thorough mixing. The plates were kept for 30 - 45 min for the agar to solidify. Thereafter, the dried crude extract impregnated filter paper discs (carrying various concentrations of the extract) were placed in the six petri-plates according to their respective concentrations using a sterilized forceps after which they were incubated at 37 ^{o}C for 24 - 48 hrs. All tests were performed in triplicates. After the incubation period, the diameter of zone inhibition in milimeters (mm) was measured using a transparent ruler to ascertain sensitivity of the isolates to the various crude extract dilutions. This zone of inhibition was compared with the diameter of zone of inhibition of ampicillin (or amphotencin B) and fulcin which served as controls for the bacterial and

fungal isolates respectively (see appendix two for Table 4). Hot and cold distilled water were also used as negative control.

Agar Diffusion (well method) technique: In this technique, the procedure for the disc diffusion method was followed except after overlaying 1.0ml of the clinical isolate with 20 ml sterilized Mueller-Hinton agar, followed by solidification of the agar, a cork borer was used to prepare wells at specific locations on the agar surface each well representing the specific dilution or concentration of the extract. Thereafter, 0.1 ml of the particular concentration of the extract was transferred (with a dropping pipette) onto its corresponding well.

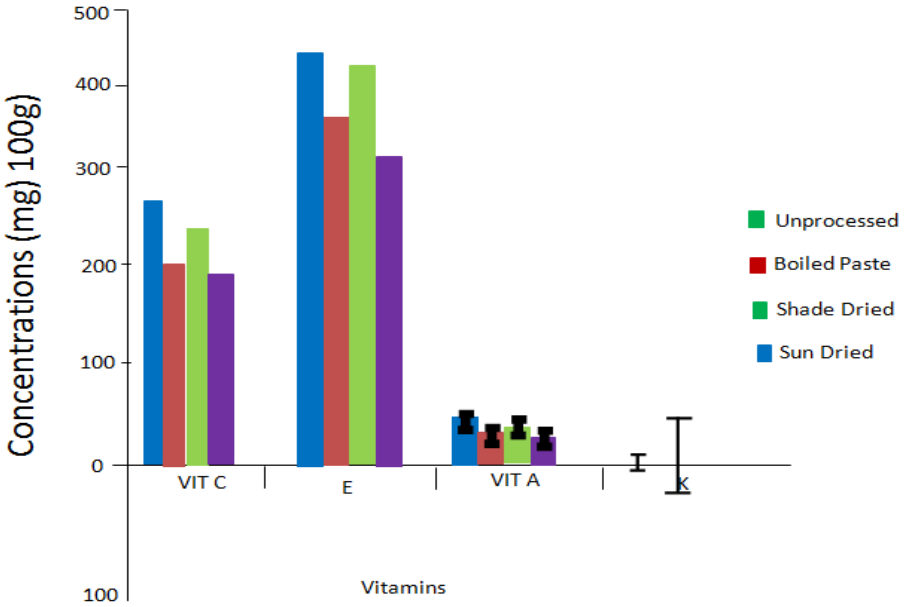

Figure 1: vitamin compositions of Moringa oleifera

Values are expressed as mean SD n = replicate of three (3) per sample

** Significant vs unprocessed at p< 0.05, a significant vs Boiled past at p< 0.05, b significant vs shade dried at p< 0.05*

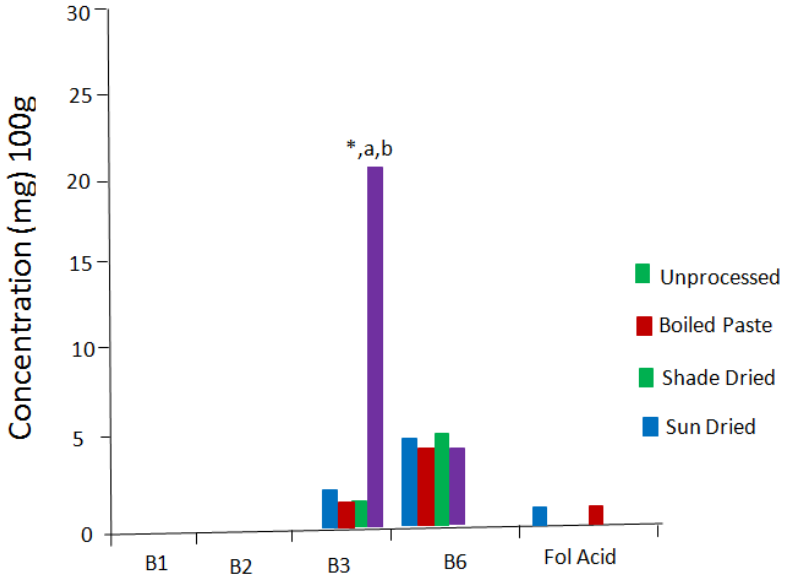

Figure 2: Vitamin compositions of Moringa oleifera

Valuees are expressed as mean ⊥SD n = replicate of thre (3) per sample

* Significant vs unprocessed at *p*< 0.05, [a] significant vs Boiled past at *p*< 0.05, [b]significant vs shade dried at *p*< 0.05

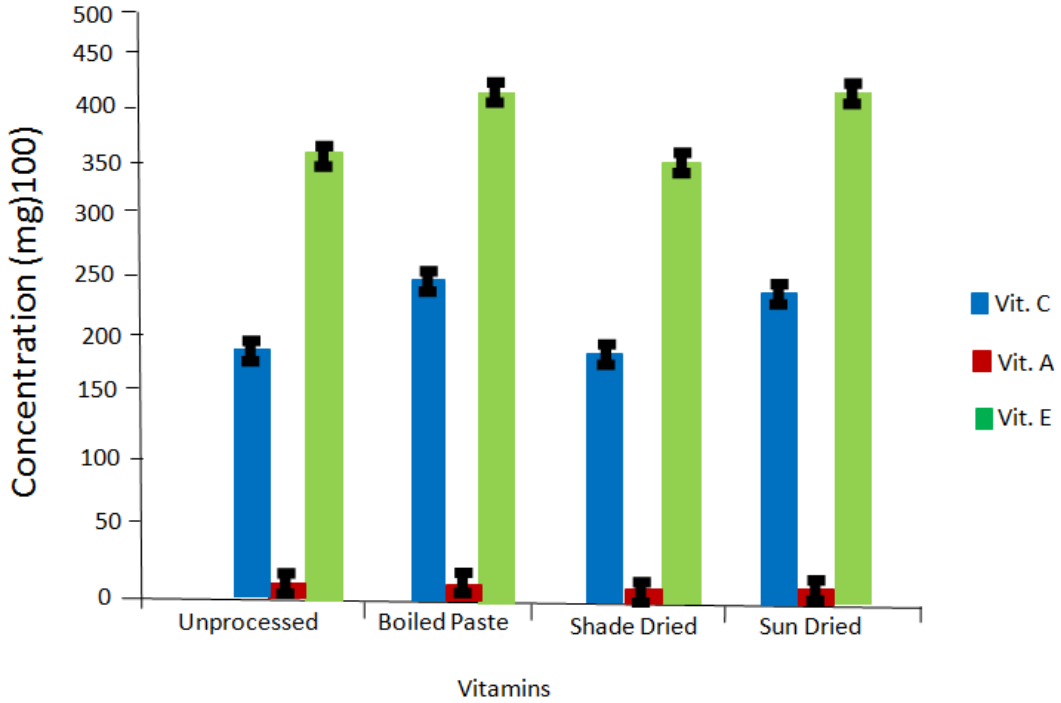

Figure 3a: Vitamin Composition of Ocimum gratissimum
Values are expressed as mean SD. N= replicate of three (3) per sample
There were no significant difference at p>0.05

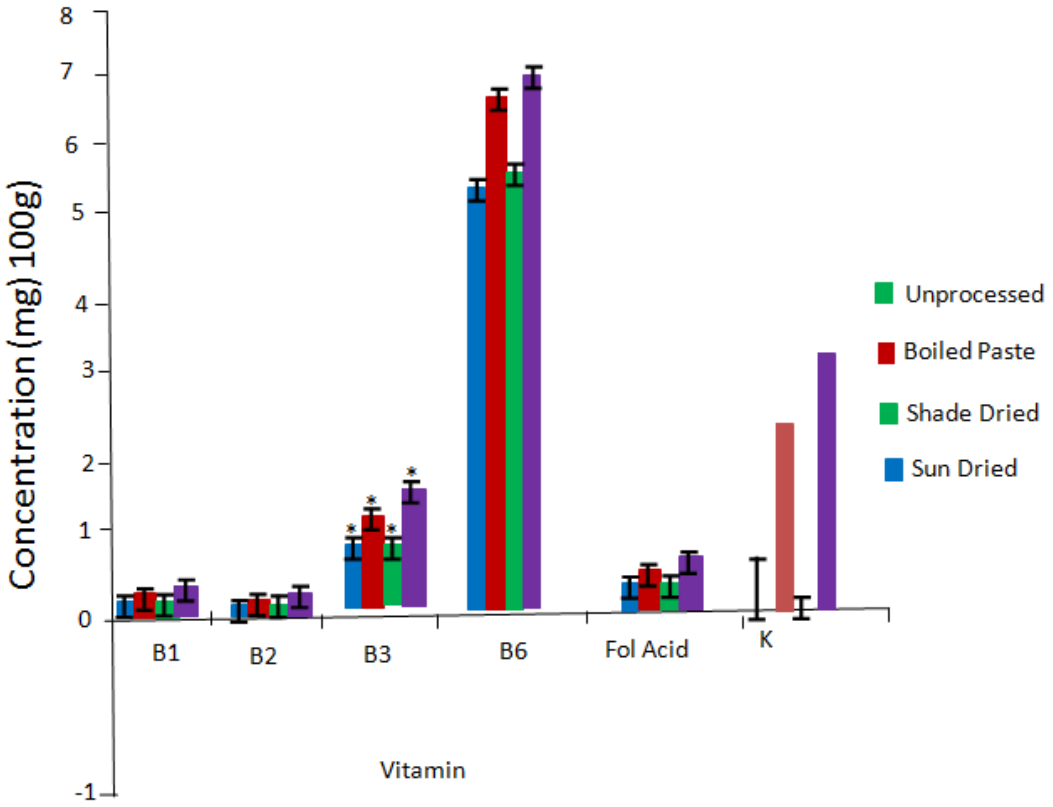

Figure 3b: Vitamin Composition of Ocimum gratissimum
Values are expressed as mean SD n= replicate of three (3) per sample fol. cid is folic acid
** Significant vs Unprocessed at p<0.05*

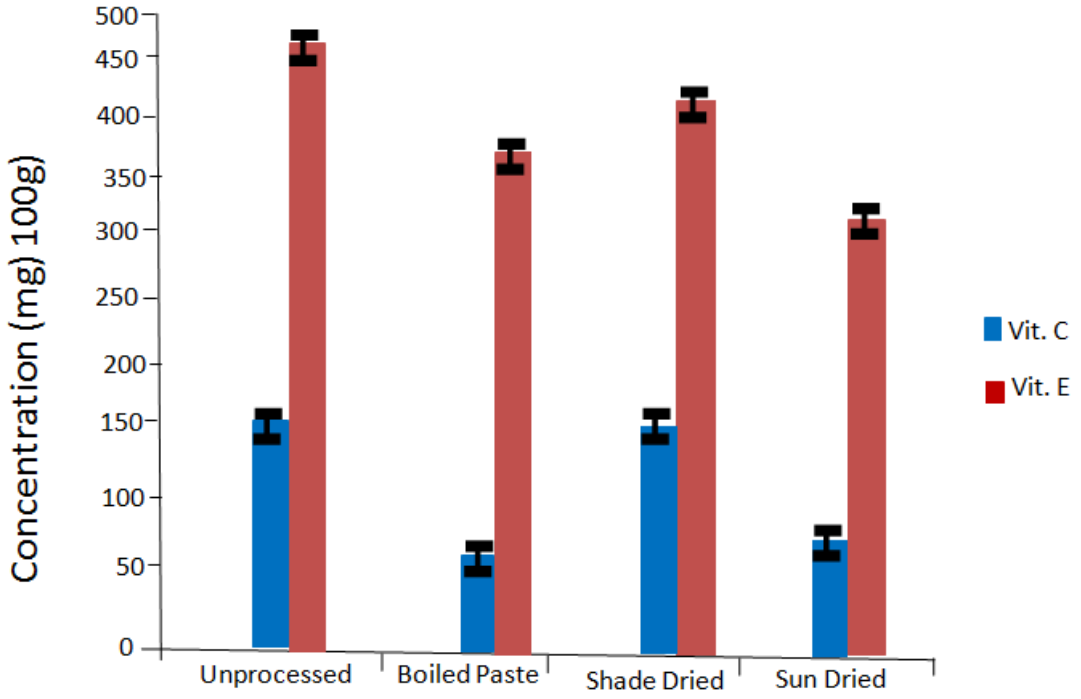

Figure 4a: Vitamin Composition of Gongronema latifolium
Values are expressed as mean SD n= replicate of three (3) per sample
**significant vs Unprossed at p<0.05*

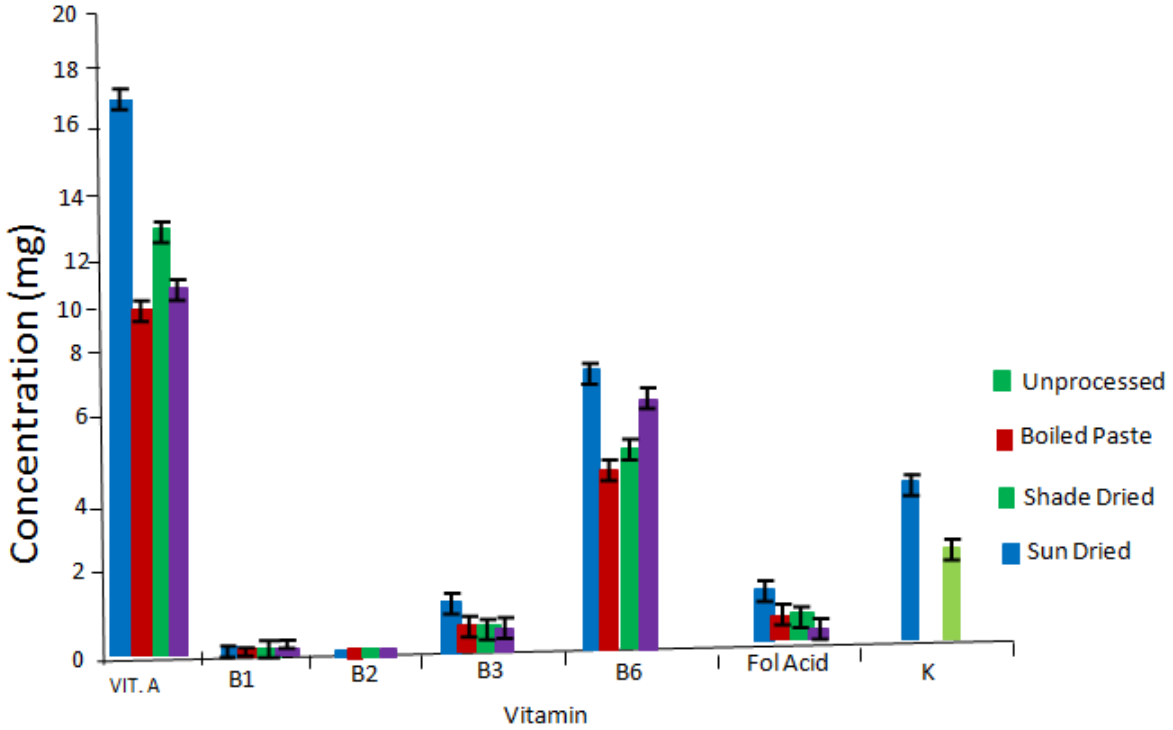

Figure 4b: Vitamin Composition of Gongronema latifolium
Values are expressed as mean SD. n= replicate of three (3) per sample
** Significant vs unprocessed at p< 0.05*

Discussion

The vitamins composition of these vegetables was significantly high in unprocessed samples, however, these vitamins reduced significantly following processing showing an adverse effect of both sun drying and boiling on these nutrients. Also, the minerals were less affected except iron, selenium and potassium whose levels reduced significantly following processing. A similar reduction in iron and calcium contents of processed green cowpea was reported by Deol and Bains [11] following boiling. However, is this study, the loss in Calcium content of these vegetables was not significant. It is important to note that the vitamin C, E and A content of *Moringa oleifera*, *Ocimum gratissimum* and *Gongronema latifolium* was high and although sun drying and boiling reduce its level significantly, these processing methods still leave a substantial level of the vitamins in the vegetables.

Recommendations

Given the high economic value and shelf life of dry vegetables;

1. Possible ways of packaging of these vegetables should be investigated
2. Since most of the vitamins and minerals are lost during processing, these nutrients should be supplemented by other dietary sources.

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