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# Extraction and Determination of Piperine and Mineral Contents of Ethiopia and Alligator Peppers Grown in Nigeria

Onmeje Godwin Okibe* Ajibola Victor Olatunji, Agbaji Edith Bolanle

Department of Chemistry, Faculty of Physical Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
*Correspondence: okibego@gmail.com

**Abstract:** This study investigated the extraction and determination of piperine and mineral content in Ethiopia and Alligator pepper grown in Nigeria. Piperine contents of the raw samples and their extracts were determined spectrophotometrically. The concentration of piperine in mg/g for alligator pepper and extracts are: 16.241 ± 0.10, 8.494 ± 0.10, 8.759 ± 0.10, 7.153 ± 0.10, 6.879 ± 0.10 and 8.031 ± 0.10 while that of Ethiopian pepper and extracts are as follows: 16.865 ± 0.10, 7.766 ± 0.10, 7.936 ± 0.10, 7.578 ± 0.10, 7.020 ± 0.10 and 7.246 ± 0.10 mg/g. The mineral contents of the raw samples and their extracts were determined by atomic absorption spectrophotometry after wet ashing of the samples using Varian AA240FS Fast Sequential Atomic Absorption Spectrophotometer. While Sodium (Na) and potassium (K) were analysed by flame photometry using Essex PFP 7 Jenway model flame photometer. The results of the mineral contents for Alligator pepper seeds are: K:3.90, Na: 3.20, Ca:45.01, Mg: 58.85, Fe: 11.44, Zn: 3.95, Mn: 10.45, Cu: 0.09 and Ethiopian pepper are: K: 5.30, Na: 2.70, Ca: 34.71, Mg: 39.93, Fe:1.22, Zn: 0.72, Mn:1.35, Cu: 0.18. These values are in mg/kg. It can be concluded that, these two peppers and their extracts can be used as food supplements apart from serving as food flavour and the study has also revealed that they are good sources of piperine.

[Okibe GO, Ajibola V.O., Agbaji EB. **Extraction and Determination of Piperine and Mineral Contents of Ethiopia and Alligator Pepper Grown in Nigeria.** *Researcher* 2020;12(9):80-86]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). http://www.sciencepub.net/researcher. 9. doi:10.7537/marsrsj120920.09.

**Keywords:** Piperine, Alligator pepper, Ethiopia pepper, *Aframomum melegueta*, *Xylopia aethiopica*

## 1. Introduction

Black Peppers get their flavours mainly from piperine compound found in them. Although the solid crystalline piperine is tasteless, its alcoholic solution has a very sharp taste. Piperine is the major pungent principal of pepper, which probably comprises over 95% of the total pungent alkaloids present (Wattanathorn *et al*., 2008).

Piperine, along with its isomer chavicine, is the alkaloid responsible for the pungency of black pepper and long pepper. It has also been used in some forms of traditional medicine and as an insecticide. Piperine forms monoclinic needles, is slightly soluble in water (1 g/25 L (18 °C)), and is highly soluble in alcohol (1 g/15 mL), ether (1 g/36 mL) and chloroform (1 g/1.7 mL). The solution in alcohol has a pepper-like taste (Wattanathorn *et al*., 2008).

Determination of piperine content is a good measure of the pungency of the spice. The first generally accepted method for the analysis of piperine was based on a Kjeldahl nitrogen determination, from which the percentage of piperine was calculated. Other nitrogen containing substances, occurring naturally in pepper but not contributing to the pungency are all determined by this method as "piperine". More accurate methods based on ultra-violet spectroscopy and colorimetric analyses are developed subsequently. The method specified here is based on a number of international collaborative studies over a long period of time. Necessarily the method seeks to optimize a number of variables in an attempt to define procedures and provide a common measure of the pungency of pepper, especially the black peppers: *Xylopia aethiopica* (Ethiopia pepper*), Aframomum meleguata* (Alligator pepper) and *Piper nigrum L.* (Epstein *et al*., 1993). The pepper extract has a guaranteed capsaicin content of no less than 8%, in particular in the range 8% to 10%; the piperidides contain piperine and are obtained from an essential pepper oil; the essential pepper oil is obtained from a pepper from the piperaceae family, in particular a pepper from the group formed by: black or common pepper, the fruit of *Piper nigrum L*.; long pepper: *Piper longum L.* and *Piper officinarum; Betl-Piper Betle L*.; *Piper methysticum*; *Piper parthenium*; *Piper angustifolium*; *Aframomum meleguata* and *Xylopia aethiopica.*

The safety of synthetic flavour has previously been questioned, leading to a reduction in the number of permitted flavours. Artificial flavoring is known to cause many problems including: nervous system depression, dizziness, chest pain, headaches, fatigue, allergies, brain damage, seizures, nausea, kidney problem, high blood pressure and much more. Some of the popular flavours can also cause genetic defects, tumors, bladder cancer, and many other types of cancers.

Due to this limitation and worldwide tendency towards the consumption of natural products, the interest in natural flavour has increased significantly. Natural food flavours have the ability to serve as antioxidant, anti-inflammatory, anti-diabetic, anti-microbial, immunity-boosting, cancer and heart disease-protecting abilities. Hence the imperative of this research.

*Aframonum melegueta* and *Xylopia aethiopica* are widely used as natural flavour and colourants in Nigeria especially among the Hausas and the Yorubas for preparing local soups and stews. Therefore, there is a need to carry out their compositional analysis. Presently there is no stability study of possible extracts obtained from them. Hence, there is a need to carry out this study. It seems much related to Ginger and has many of the same bioactive characteristics in common. *Aframomum melegueta* (Alligator pepper) and *Xylopia aethiopica* (Ethiopia pepper) are well known spice/flavour and colourants which are added to a diet but there is overall lack of evidence to support its usage as a supplement and higher oral doses may still have some toxicity associated with them (Sugita *et al.,* 2013) which needs to be more thoroughly investigated. This study was therefore undertaken to determine the piperine and mineral contents of Ethiopian pepper and alligator pepper.

## 2. Materials and Methods

### 2.1 Purchase and authentication of Ethiopian and Alligator pepper

The Ethiopian and alligator pepper samples were purchased from Samaru market and taken to the Department of Botany, Ahmadu Bello University, Zaria where there identified and authenticated to be Ethiopian and alligator pepper.

### 2.2 Pulverized fruits and seeds

Ten grams (10 g) of ground pepper was soaked in 100 ml of ethanol for 3 days, filtered and concentrated. 5 ml of the filtrate was transferred into 100 ml volumetric flask and the flask was immediately filled up to volume with ethanol. The ultraviolet absorption at the maximum, 343 nm was read against a solvent blank in spectrophotometer. The piperine content was calculated from the molecular absorbances of the compound by a component equation (Chritiane *et al.*, 1963).

$$A_{343} = \frac{C_{\text{Piperine}} \times A_{343} \text{ of std.Piperine Solution}}{C_{\text{std.Piprine Solution}}}$$

Where **A** is the absorbance of sample at the wavelength indicated in nm in the subscript and **C** is the concentration of the indicated substance in grams per litre. The ratio of absorbance to concentration (0.1323) is constant because the solutions obey Beer's law over the range of concentrations studied (0 to 8 mg. per litre). The following constants, replacing these ratios. The equation above has been determined from measurements on a number of standard solutions of piperine, thus:

A343 = (C piperine) 0.1323

Hence, the concentration of piperine was determined by simple subject formula:

$$\text{C Piperine} = \frac{A_{343}}{0.1323}$$

The final formula after incorporating dilution and percentage factors for 3 ml of filtrate was determined using this relation:

% Piperine = (14.40 A343) X 1.042 for 3 ml used in the cuvette. The Committee on Quality (2008)

### 2.3 Alligator and Ethiopia pepper extracts

For the various extracts, 0.50 g of the prepared extracts was weighed and dissolved in 50 $cm^3$ ethanol and the solution filtered into a 100 $cm^3$ standard volumetric flask, beaker washed and filtered with successive 10 $cm^3$ portions of ethanol and finally made up to the mark. 5 $cm^3$ aliquot was diluted in 50 $cm^3$ standard volumetric flask with ethanol and the absorbance (A) of this solution was measured in the spectrophotometer at 343 nm using ethanol as a blank in 1 cm cells. The concentration of the piperine was determined using equations above (Chritiane *et al.*, 1963).

### 2.4 Mineral Contents

The mineral contents of the raw samples (seeds and fruits) and their extracts were determined by atomic absorption spectrophotometry after wet ashing of the samples. 0.50 g of each sample was digested using a mixture of nitric acid and hydrochloric acids (3:1) according to the method of Oshodi *et al*. (1999).

The samples were then diluted with distilled to 50 $cm^3$ volume and the mineral contents (Ca, Mg, Mn, Fe, Cu and Zn) of the solutions were determined by atomic absorption spectrophotometry using Varian AA240FS Fast Sequential Atomic Absorption Spectrophotometer. Sodium (Na) and potassium (K) were analysed using flame photometry (Essex PFP 7 Jenway model flame photometer).

## 3. Results

The findings of this study are presented in graphs and tables as follow:

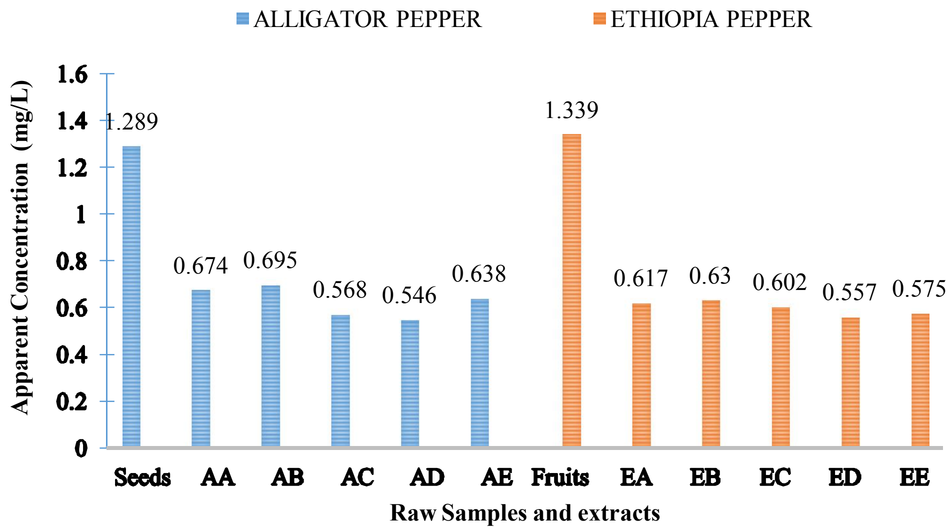


**Figure 1: Apparent Concentration of Piperine in mg/Litre**

(**Alligator pepper extracts:** AA Ethanol + 1.50M HCl Extract; AB Ethanol + 1% Citric Acid Extract; AC Acetone Extract; AD 2% Citric Acid Extract; AE Hexane Extract; **Ethiopia pepper extracts**: EA Ethanol + 1.5M HCl Extract; EB Ethanol + 1% Citric Acid Extract; EC Acetone; ED 2%Citric Acid Extract: EE Hexane Extract)

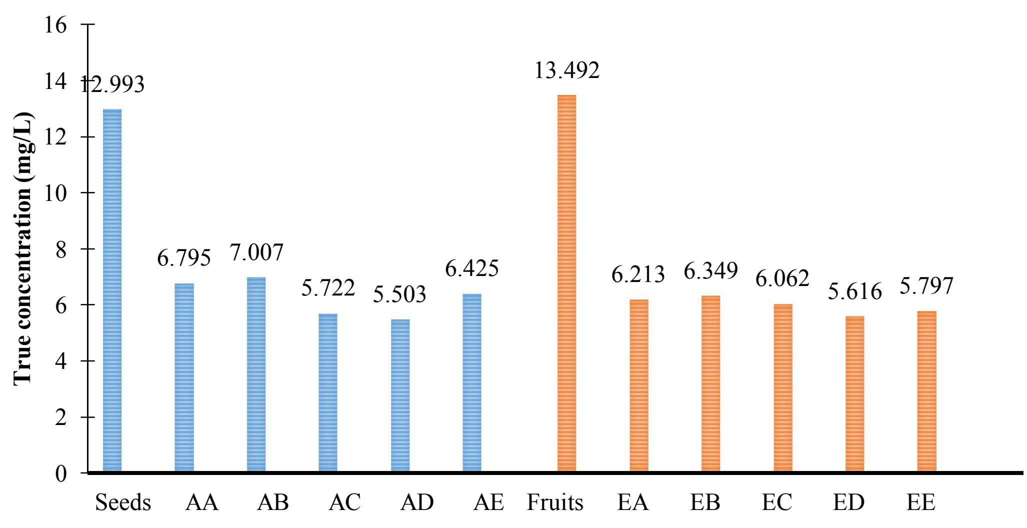


**Figure 2: True Concentration of Piperine in mg/Litre**

(**Alligator pepper extracts:** AA Ethanol + 1.50M HCl Extract; AB Ethanol + 1% Citric Acid Extract; AC Acetone Extract; AD 2% Citric Acid Extract; AE Hexane Extract; **Ethiopia pepper extracts**: EA Ethanol + 1.5M HCl Extract; EB Ethanol + 1% Citric Acid Extract; EC Acetone; ED 2%Citric Acid Extract: EE Hexane Extract)

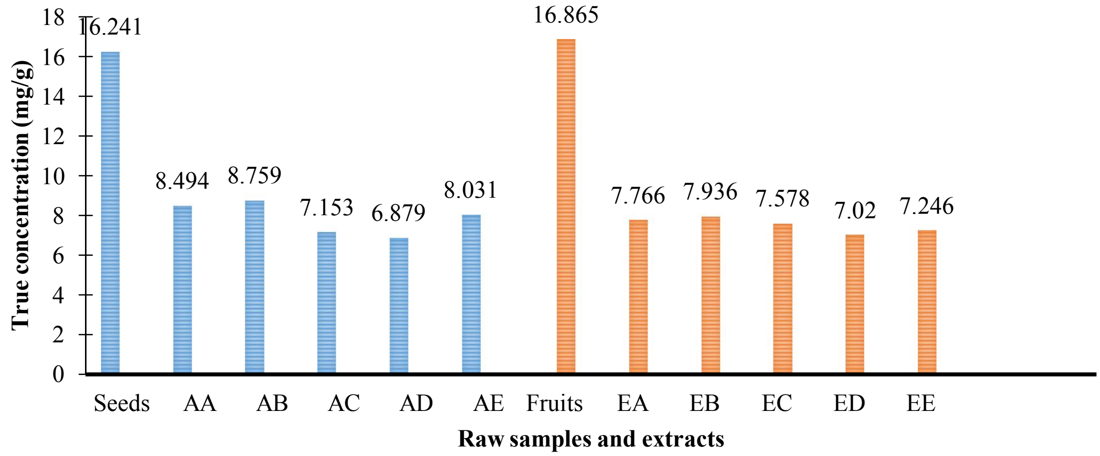


**Figure 3: True Concentration of Piperine in mg/g**

(**Alligator pepper extracts:** AA Ethanol + 1.50M HCl Extract; AB Ethanol + 1% Citric Acid Extract; AC Acetone Extract; AD 2% Citric Acid Extract; AE Hexane Extract; **Ethiopia pepper extracts**: EA Ethanol + 1.5M HCl Extract; EB Ethanol + 1% Citric Acid Extract; EC Acetone; ED 2%Citric Acid Extract: EE Hexane Extract)

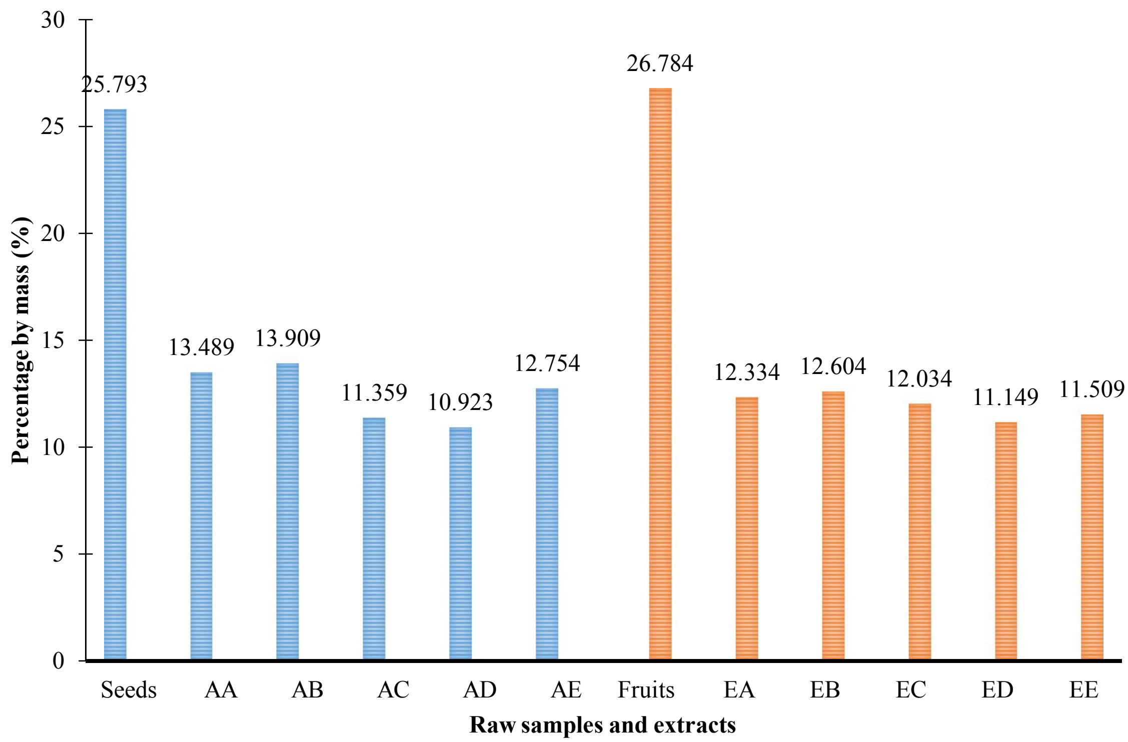


**Figure 4: Percentage by Mass of Piperine in Samples (%)**

(**Alligator pepper extracts:** AA Ethanol + 1.50M HCl Extract; AB Ethanol + 1% Citric Acid Extract; AC Acetone Extract; AD 2% Citric Acid Extract; AE Hexane Extract; **Ethiopia pepper extracts**: EA Ethanol + 1.5M HCl Extract; EB Ethanol + 1% Citric Acid Extract; EC Acetone; ED 2%Citric Acid Extract: EE Hexane Extract)

The result for mineral composition for Alligator pepper and its extracts is shown on Table 1 while the result for mineral composition for Ethiopia pepper and its extracts is shown on Table 2. The ratio of the mineral composition for sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) is shown on Table 3.

Table 1: Mineral Composition of the Flavourant Samples (Alligator Pepper and extracts)

| **Flavourant Samples** | **Mineral composition (mg/kg)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **K** | **Na** | **Ca** | **Mg** | **Fe** | **Zn** | **Mn** | **Cu** |
| **AP (Seeds)** | 3.90 | 3.20 | 45.01 | 58.85 | 11.44 | 3.95 | 10.45 | 0.09 |
| AA | 3.00 | 0.44 | 10.90 | 12.81 | 2.16 | 0.43 | 0.56 | 0.22 |
| AB | 0.69 | 0.25 | 4.48 | 10.95 | 0.99 | 0.52 | 0.08 | 0.15 |
| AC | 3.55 | 0.12 | 7.06 | 12.48 | 2.64 | 0.26 | 1.61 | 0.59 |
| AD | 1.65 | 0.90 | 11.17 | 15.41 | 0.93 | 1.08 | 3.15 | 0.13 |
| AE | 0.20 | 0.06 | 4.90 | 8.60 | 0.82 | 0.66 | 0.07 | 0.11 |

(AA Ethanol + 1.50M HCl Extract; AB Ethanol + 1% Citric Acid Extract; AC Acetone Extract; AD 2% Citric Acid Extract; AE Hexane Extract)

Table 2: Mineral Composition of Flavourant Samples. (Ethiopian Pepper and extracts)

| **Flavourant Samples** | **Mineral composition (mg/kg)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **K** | **Na** | **Ca** | **Mg** | **Fe** | **Zn** | **Mn** | **Cu** |
| **EP (Fruits)** | 5.30 | 2.70 | 34.71 | 39.93 | 1.22 | 0.72 | 1.35 | 0.18 |
| EA | 0.55 | 0.21 | 2.80 | 4.43 | 0.29 | 0.59 | 0.05 | 0.07 |
| EB | 2.10 | 2.75 | 2.75 | 6.26 | 0.61 | 0.45 | 0.05 | 0.13 |
| EC | 0.43 | 1.30 | 1.30 | 5.79 | 0.72 | 0.50 | 0.11 | 0.14 |
| ED | 2.30 | 11.97 | 11.97 | 14.97 | 0.74 | 0.59 | 1.48 | 0.13 |
| EE | 1.30 | 3.03 | 3.03 | 6.61 | 0.17 | 0.54 | 0.16 | 0.07 |

(EA Ethanol + 1.5M HCl Extract; EB Ethanol + 1% Citric Acid Extract; EC Acetone; ED 2%Citric Acid Extract: EE Hexane Extract)

Table 3: Ratios of Mineral Composition for Na, K, Ca and Mg

| | **MINERAL COMPOSITION** | | |
|---|---|---|---|
| **SAMPLES** | **Na/K** | **Ca/Mg** | **K/(Ca+Mg)** |
| AP (seeds) | 0.82 | 0.76 | 0.030 |
| AA | 0.15 | 0.85 | 0.126 |
| AB | 0.36 | 0.41 | 0.045 |
| AC | 0.03 | 0.57 | 0.182 |
| AD | 0.56 | 0.73 | 0.069 |
| AE | 0.30 | 0.57 | 0.004 |
| EP (fruits) | 0.51 | 0.87 | 0.071 |
| EA | 0.38 | 0.63 | 0.076 |
| EB | 0.13 | 0.44 | 0.233 |
| EC | 0.51 | 0.23 | 0.060 |
| ED | 0.20 | 0.80 | 0.085 |
| EE | 0.18 | 0.46 | 0.135 |

(**Alligator pepper extracts:** AA Ethanol + 1.50M HCl Extract; AB Ethanol + 1% Citric Acid Extract; AC Acetone Extract; AD 2% Citric Acid Extract; AE Hexane Extract; **Ethiopia pepper extracts**: EA Ethanol + 1.5M HCl Extract; EB Ethanol + 1% Citric Acid Extract; EC Acetone; ED 2%Citric Acid Extract: EE Hexane Extract)

## 4. Discussion

### 4.1 Piperine Concentration in Samples

Piperine is the active principle of black peppers, an ingredient of several polyherbal formulations. There are several methods reported for quantitation of piperine but there are no reported methods for quantitation of piperine from Alligator and Ethiopia peppers. This research work also investigated the quantitation of piperine concentration in the seeds and fruits of these peppers and their extracts as well. The various piperine concentration as investigated are presented on the Figures 1, 2, 3 and 4. These results show that these two peppers contain an appreciable amount of piperine in their seeds and fruits like other African black peppers which can be extracted for use.

### 4.2 Mineral Composition (Essential Element Analysis)

The values recorded for K and Na in all the flavourants (Tables 1 and 2) were not up to Recommended Daily Allowance (RDA) of 2,500 mg (Food and Nutrition Board, 2000) but were relatively high in this natural flavourants that can provide the needed nutrient to the body when consumed. Apart from giving the desirable flavour to the food, this natural flavourants can also add reasonable quantity of potassium (K) and Sodium (Na) that is required to maintain osmotic balance of the body fluid. Similar quantities of these minerals had earlier been reported in vegetable materials (Olaofe and Sanni, 1988; Oshodi *et al*., 1999; Aremu *et al*., 2005).

Though the concentration of iron (Fe) and zinc (Zn) were low with Fe ranging between 11.44mg/kg-0.17mg/kg and Zn between 3.95-0.26mg/kg, the quantities are available for biochemical functions. The daily recommended Fe requirements for humans are 10-15 mg for children, 18 mg for women and 12 mg for men. Copper (Cu) was detected in all the flavourant extracts with the least values ranging between 0.57-0.07mg/kg. Although the daily requirement is only 2 mg. Fe and Cu are present in cytochrome oxidase (enzyme) which is involved in energy metabolism. All the flavourants could supply the daily body requirement of Calcium (Ca) of 800 mg. Ca is a co-ordinator among inorganic elements, for example excess amount of K, Mg or Na in the body can be corrected by Ca and also adequate quantity of Ca in the diet assist in Fe utilization. The Magnesium (Mg) values in these flavourants are high as well. Mg is an activator of many enzymes systems which maintains the electrical potential in nerves (Shills, 1973; Shills and Young, 1992).

In Table 3, the Na/K, Ca/Mg and K/(Ca+Mg) ratios were also shown. Both K and Na are required for osmotic balance of the body fluid and the pH of the body, muscle regulation and nerve irritability, glucose absorption control and enhancement of normal retention of protein during growth (Food and Nutrition Board, 2000). Na/K ratio of less than one is the daily recommended dose (Nieman *et al*., 1991). All the flavourants have Na/K ratio less than 1, hence they all have capacity to hinder high blood pressure. The Ca/Mg ratio is also less than 1 as recommended (Food and Nutrition Board, 2000). The K/(Ca+Mg) for all the flavourants is between 0.0375-0.182 milliequivalent. To prevent hypomagnesaemia, the K/(Ca+Mg) must be less than 2.2 milliequivalent (Marten and Andersen, 1975), therefore all the flavourants are suitable to prevent hypomagnesaemia**.**

## 5. Conclusion

The percentage content of piperine in the various parts of *Aframomum melegueta* and *Xylopia aethiopica* should be investigated seasonally to establish the appropriate period of harvest that produces the highest levels of piperine. The stability of piperine in herbal aqueous extracts should be investigated to aid in the standardisation of herbal preparations containing extracts *of Aframomum melegueta and Xylopia aethiopica.* Stability studies with challenges such as heat on piperine should be performed to know the decomposition pattern. Suitable methods should be developed for detection of possible degradation products and acceptable limits set for any toxic components that are discovered.

**Corresponding Author:**
Mr. Onmeje Godwin Okibe
Department of Chemistry,
Ahmadu Bello University,
Zaria, 810107, Kaduna State, Nigeria
Telephone: +234 (0) 801-0161-6867
E-mail: okibego@gmail.com

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9/25/2020