Asian Plant Research Journal

8(3): 10-21, 2021; Article no.APRJ.73277
ISSN: 2581-9992

Phytochemical Screening, Antioxidant Potential and Nutritional Compounds of *Aframomum melegueta* and *Syzygium aromaticum* Seeds in Ibadan, Oyo-State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author MKO designed the study and interpret the results. Author FOI collected prepared the plants samples, carried out all laboratory experiments and wrote the manuscript. Author OAO interpreted the results and wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2021/v8i330176
Editor(s):
(1) Dr. Shiamala Devi Ramaiya, Universiti Putra Malaysia, Malaysia.
Reviewers:
(1) Syahran Wael, University of Patttimura, Indonesia.
(2) Kamran Ashraf, Universiti Teknologi MARA Shah Alam, Malaysia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/73277

Original Research Article

Received 24 June 2021
Accepted 04 September 2021
Published 18 September 2021

ABSTRACT

Consequents upon the efficacies of the local claims of *Aframomum melegueta* (*Ataare*) and *Syzygium aromaticum* (*Kanafuru*) in the treatment of respiratory infections and diseases in the study area, the present study was conducted to investigate the phytochemical, antioxidant capacity and nutritional composition in compounds of *Aframomum melegueta* and *Syzygium aromaticum* seeds to validates their local claims. The aqueous extracts of the plants seeds were obtained using standard procedures. The phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, terpenoids and phenols in the aqueous extracts of the plants seeds, while tannins was absent in the extract of *Aframomum melegueta.* Flavonoids and phenols revealed the highest antioxidant potential of the plants quantitatively at 0.1 g/m. The proximate contents of the plants

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seeds showed the level of crude contents ranging from moisture (7.34% ± 0.01 and 13.74% ± 0.03), fiber (28.33% ± 0.02 and 16.23% ± 0.02), protein (21.03% ± 0.02 and 10.79% ± 0.05), fat (7.13% ± 0.02 and 27.94% ± 0.10) and carbohydrates (32.76% ± 0.03 and 26.53% ± 0.02) respectively. The results also revealed the presence of potassium (63.50% ± 0.2 ppm and 64.20% ± 0.2 ppm), calcium (7.54% ± 0.2 ppm and 10.40% ± 0.2 ppm) and magnesium (9.05% ± 0.2 ppm and 9.11% ± 0.2 ppm) in the two plants seeds respectively. Therefore this study justifies the local use of *Aframomum melegueta* (*Ataare*) and *Syzygium aromaticum* (*Kanafuru*) as sources of medicine to manage and alleviate various symptoms associated with respiratory diseases and health conditions.

Keywords: Respiratory infection; plant extract; proximate; antioxidant; symptom.

1. INTRODUCTION

Plants are important in our everyday existence. They are sources of food, produce the air we breathe, and serve as raw materials for many industrial products such as clothes, foot wears, building purposes and so many others. Plants also provide raw materials for various construction purposes and in the manufacture of bio fuels, dyes, perfumes, pesticides and drugs [1].

Recently, there has been an upsurge of interest in the therapeutic potential of plants as well as their potentials as an antioxidants in reducing oxidative stress or injuries in tissues [2,3]. There are many plants that are used as herbs for the management of various diseases, and spices rich in flavonoids and phenolic compounds, that have been demonstrated to have anti-inflammatory, antiallergenic, antiviral, anti-aging, and anti-carcinogenic activities which can be attributed to their antioxidant properties [4-6]. Antioxidants prohibit free radical damage by scavenging radicals or preventing radical formation. There is search for natural food compounds with high therapeutic and oxidative activity in recent years, due to the resistance of allopathic drugs to disease caused organisms as against the use of herbal drugs treatment [7]. Though human body contains several enzymatic systems that scavenge free radicals, adequate diets from plant-derived food can also serve as reducing power, chelating activities and as an antioxidants to scavenge free radicals in the body [8]. Therefore, the higher intake of foods that have high level of antioxidants capacity the better its gaining importance [9,10].

Aframomum melegueta (*Ataare*) and *Syzygium aromaticum* (*Kanafuru*) are plants with medicinal properties that are locally used in alleviating various respiratory diseased conditions such as cough, throat infections, asthma, bronchitis, cold, catarrh, flu, tonsillitis etc. [11-20]. They are also used as spices in the preparation of different herbal soups and drinks. *Aframomum melegueta* and *Syzygium aromaticum Aframomum melegueta* (*Ataare*) and *Syzygium aromaticum* (*Kanafuru*) belong to the families Zingiberaceae and Myrtaceace respectively [21,22]. These plants were also reportedly having bioactive ingredients as potential that make them responsible for the management of various respiratory disorders [23,24]. Every human being requires good nutrition and sound health as bases for adequate development and well-being [25,26]. *Aframomum melegueta* and *Syzygium aromaticum* seeds are available, effective, less-toxic and are disease resistance. Incidentally, the bioactive ingredients in each of these plants that are responsible for the action they expressed when used traditionally have not been well assessed and documented in the study area. Therefore, information on the bioactive compositions of the seeds of these plants that justifies their nutritional value and health benefits is highly imperative.

2. MATERIALS AND METHODS

2.1 Study Area

The seeds of *Syzygium aromaticum* and *Aframomum melegueta* were purchased from Bode Herbal Market, Molete road, Ibadan, Oyo State, Nigeria. Ibadan, Oyo State is located at the latitude 7°22N and longitude 3°55E. The seeds were identified and authenticated in the herbarium unit of the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti.

2.2 Preparation of Aqueous Extract of the Plants Samples Seeds

The method of extraction was as described by [27]. The seeds of both plants were rinsed

thoroughly with distilled water and allowed to dry under shade. The dried samples were then pulverized and stored in an air tight container. A sample of 5 g of each powdered plant material was soaked in 100 ml of distilled water for 48 hours. The solution was filtered using approximately 11 cm diameter Whatman filter paper. The extract was subsequently collected after 24 hours and immediately used for phytochemical analyses [28].

2.3 Phytochemical Screening of the Aqueous Extracts of the Plants Samples Seeds

The aqueous extract of the *Aframomum melegueta* and *Aframomum melegueta Aframomum melegueta* (Alligator pepper) and *Syzygium aromaticum* (Clove) seeds were screened for the presence or absence of metabolites using standard phytochemical screening tests as described by [29]. The extract was tested for saponins, alkaloids, tannins, terpenoids, flavonoids and phenols. Detection of alkaloids. Wagners test: A fraction of the plants extracts was treated with Wagners reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of distilled water) and observed for the formation of reddish-brown precipitate. Detection of Phenolic Compounds. Ferric Chloride Test: A fraction of the extract was treated with 5% $FeCl_3$ (ferric chloride) solution and observed for the formation of deep blue colour.

Detection of Flavonoids. Concentrated H_2SO_4 Test: Concentrated H_2SO_4 (Sulphric acid) was added to a small fraction of the extract, and observed the orange colour formation.

Detection of Saponins. Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam. Test for Terpenoids: An amount of 0.8 g of selected plant sample was taken into a test tube, 10 ml of methanol was poured into it, shaken well and filtered to take 5 ml extract of plant sample. 2 ml of chloroform was then mixed with the extract of selected plant sample followed by 3 ml of sulphuric acid in the selected sample extract. Formation of reddish-brown color indicates the presence of terpenoids in the selected plants.

Test for Tannins. A fraction of the extract was put in a test tube. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.4 Determination of Total Phenol Content (TPC)

The Total Phenolic Content (TPC) was determined according to the Folin and Ciocalteaus method using gallic acid as standard. Concentrations of 0.01- 1mg/ml of gallic acid were prepared in methanol. Concentrations of 1mg/ml extracts of *Syzygium aromaticum* and *Aframomum melegueta* were also prepared in distilled water. A total of 0.5 mL of the sample was mixed with 2.5 mL of a ten-fold diluted FolinCiocalteau reagent and 2 mL of 7.5% sodium carbonate. The mixture was left undisturbed for 30 minutes at room temperature before the absorbance was read at 760 nm. All the determinations were performed in triplicates. The total phenolic content in the methanol extract was expressed in Gallic Acid Equivalents (GAE)

Determination of Total Flavonoids Contents. To 2 mL of the sample was added 2 mL of 2% $AlCl_3$ in methanol. The absorbance was read at 420 nm after incubation for 1 hour at room temperature. The concentration of 1 mg/ml of the extract in methanol was used, while quercetin concentrations ranging from 0.01 - 0.15 mg/ml were used to obtain the calibration curve. The total flavonoid content of the extract was expressed in Quercetin Equivalents (QE).

2.5 Proximate Analysis of the Plant Seeds Samples

Proximate analysis was carried out on the dried powdered plant seeds according to the procedure of Association of Official Analytical Chemists [30]. The percentages of moisture content, ash content, crude fibre, crude protein and carbohydrate were determined and calculated accordingly.

2.6 Determination of Crude Protein Content

Each of the plants sample of 0.5 g was accurately weighed using an analytical balance and wrapped in a filter paper. The weighed samples were dropped in a labeled Kjeldah flask. 6M H_2SO_4 (Sulphuric acid) was added to the content of the flask and a di-sodium sulphate was added to increase the boiling point of the H_2SO_4 and thus the digestion temperature. Copper catalyst was added. The mixture was heated until a clear coloured solution of the H_2SO_4 was observed and the flask was cooled for about 5 - 10 minutes. 10 ml of deionized

water was added to the cooled digest in the flask, the mixture was then made basic by adding concentrated sodium hydroxide which is denser than the digest through the wall of the flask to allow it to settle at the base of the digestion apparatus. An anti-bumping agent such as zinc was also added to prevent bumping. The content of the flask was mixed until it was basic to litmus paper. About 10 ml of 40% NaOH and 5 ml of deionized water was added into the distillation chamber. The mixture was then distilled into a 100 ml collection cup containing 30 ml of boric acid. The distillate was back titrated with a 0.1M sodium hydroxide (NaOH) in a burette using methyl orange as indicator.

The% Nitrogen for each sample was calculated from the titre value as:

$$\%N = \frac{V \times 0.1 \times 0.014 \times 100}{\text{Weight of sample}}$$

Where,

V = Titer value (volume) of NaOH used

The percentage protein was calculated by multiplying the percentage Nitrogen with a constant factor of 6.25.

2.7 Determination of Moisture Content

A clean crucible was dried to a constant weight in an air oven at 110°C, cooled in a desiccator and weighed (W_1). 2.0 grams of finely ground sample was accurately weighed into the previously labeled crucible and reweighed (W_2). The crucible containing the sample was dried in an oven to constant weight (W_3).The percentage moisture content was calculated thus:

$$\% \text{ Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

2.8 Determination of Ash Content

A porcelain crucible was dried in an oven at 100°C for 10 minutes, cooled in a desiccator and weighed (W_1). 2.0 grams of the finely ground sample was place into a previously weighed porcelain crucible and reweighed (W_2), it was first ignited and then transferred into a furnace which was set at 550°C. The sample was left in the furnace for 8 hours to ensure proper ashing. The crucible containing the ash was then removed; cooled in a desiccator and weighed (W_3).

The percentage ash content was calculated as follows:

$$\% \text{ Ash Content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

2.9 Determination of Crude Fat Content

A clean, dried 500 cm^3 round bottom flask containing few anti-bumping granules was weighed (W_1) with 300 cm^3 petroleum ether (40-60°C) for extraction poured into the flask fitted with soxhlet extraction unit. The extractor thimble containing 2.5 grams of the sample was placed inside the soxhlet unit. The round bottom flask and a condenser were connected to the Soxhlet extractor and cold water circulation was connected. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 hours. The solvent was recovered and the oil dried in an oven set at 70°C for 1 hour. The flask and oil was then weighed (W_2). The lipid content was calculated thus:

$$\% \text{ Crude Lipid content} = \frac{W2 - W1}{\text{Weight of sample}} \times 100$$

2.10 Determination of Crude Fibre Contents

Two grams of the ground sample was weighed into a round bottom flask, 100 cm^3 of 0.25M sulphuric acid solution was added and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100 cm^3 of hot 0.31M NaOH solution was added, the mixture boiled under reflux 30 minutes and filtered under suction. The residue was washed with boiling water until it was base free, dried to constant weight in an oven at 100°C, cooled in a desiccator and weighed (C_1). The weighed sample (C_1) was then incinerated in a muffle furnace at 550°C for 2 hours, cooled in a desiccator and reweighed (C_2). Calculation:

$$\% \text{ Crude fiber} = \frac{C_1 - C_2}{\text{Weight of sample}} \times 100$$

2.11 Determination of Carbohydrate Content

The total carbohydrate in the sample was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude

protein and crude fibre was subtracted from 100 [31] (Muller and Tobin, 1980). Calculation:

% Total Carbohydrate = 100 – (% Moisture +% Ash +% Fat +% Protein +% Fibre)

2.12 Determination of the Mineral Elements in the Plants Samples Seeds

The elemental analysis was carried out according to the procedures of Association of Official Analytical Chemists [30] (AOAC, 1990). The atomic absorption spectrophotometer (AAS) was used for the analyses of the following metals: Magnesium (Mg), Zinc (Zn), Iron (Fe), Cadmium (Cd), Cupper (Cu), Lead (Pb), Calcium (Ca) and Phosphorus (P) while the flame photometer was used in the analyses of Potassium (K) and Sodium (Na). The mineral elements were determined from the ash obtained during proximate analysis which was digested and dissolved with dilute hydrochloric acid solution using Atomic Absorption Spectrophotometer and flame Photometer. 1.0 g of each ground sample was put in individual crucibles and placed in a muffle furnace which was ashed at 500^0C after which were cooled in desiccators. (1) 3 ml of concentrated HCl was added to the ash and evaporated to dryness. (2) 20 cm^3 of 25% HCl was then added to the residue of the ash. (3) The resulting solution were quantitatively transferred to 100 cm^3 volumetric flask and made up to the mark with distilled water. (4) The individual solutions were then used directly for the elemental determination using the Atomic Absorption Spectrophotometer and flame Photometer (5). Stock standard solutions of the various metals were prepared. (6) Various standards of the individual metals were prepared from the stock solution for each mineral which were used and read in the equipment in concentration mode.

2.13 Statistical Analysis

The data collected were subjected to analysis of variance for evaluation and significant differences were separated by Duncans multiple range test [32]. Significance was accepted at the level of $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The result in Table 1 shows The result in Table 1 below shows the presence of saponins, alkaloids, terpenoids, flavonoids and phenols in the aqueous extracts of *Aframomum melegueta* and *Syzygium aromaticum* seeds. Tannins was absent in *Aframomum melegueta* extract.

All the phytochemicals screened were observed to be more concentrated in *Syzygium aromaticum than Aframomum melegueta*. The phytochemical composition of the plants seeds validates the medicinal importance of the plants. Therefore the plants seeds could be used for the management of respiratory diseases. Similar results on the phytochemical screening of *Aframomum melegueta* and *Syzygium aromaticum* seeds were reported by [33,34] respectively. Tannins have been reported to have healing effects on wounds. Alkaloids are organic compounds that contain nitrogen, and are physiologically active with sedative and analgesic properties for relieving pains, anxiety and depression. Saponins are reported to have the analgesic, anti-inflammatory, antioxidant activity, to impair the digestion of protein and to act as antifungal and antiviral agents [35-41]. Therefore, the presence of these bioactive compounds in the aqueous extracts of *Syzygium aromaticum* and *Aframomum melegueta* seeds in this study justifies their therapeutic claims.

Table 1. Phytochemical Screening of aqueous extracts of *Syzygium aromaticum* and *Aframomum melegueta* seeds

Phytochemicals	*Aframomum melegueta*	*Syzygium aromaticum*
Saponins	+	+
Alkaloids	+	+
Terpenoids	+	+
Tannins	-	+
Phenols	+	+
Flavonoids	+	+

Keys; - = Absent; + = Present

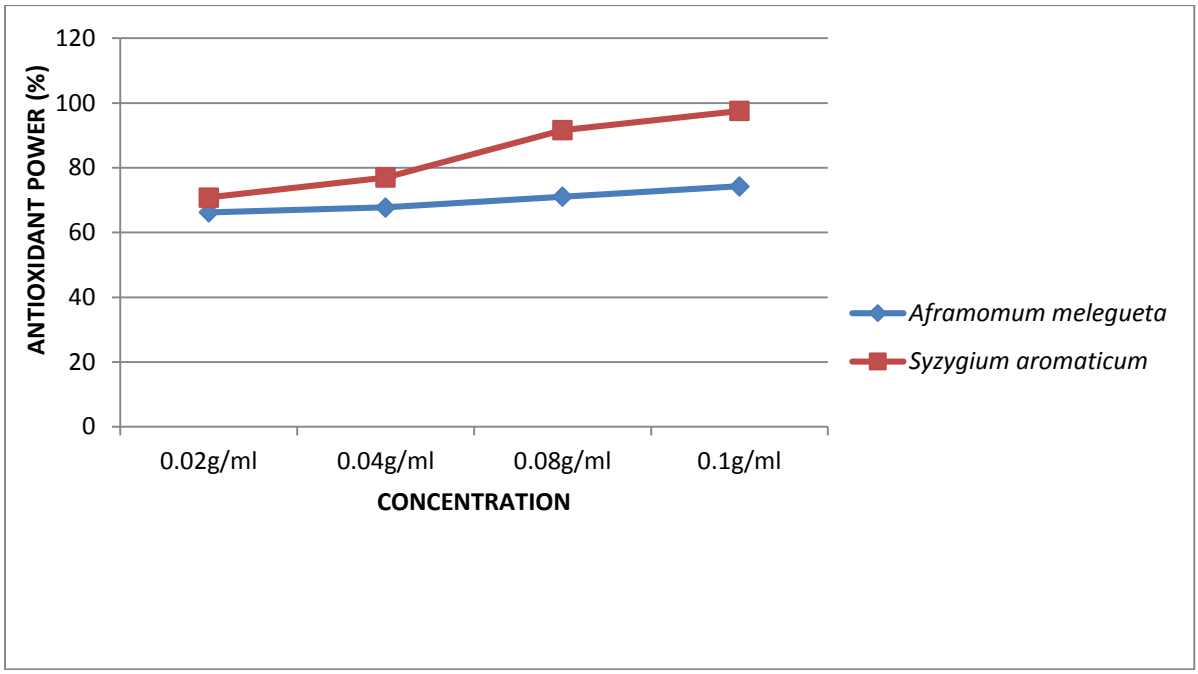

Fig. 1. Quantitative composition of flavonoids in *Syzygium aromaticum* and *Aframomum melegueta*

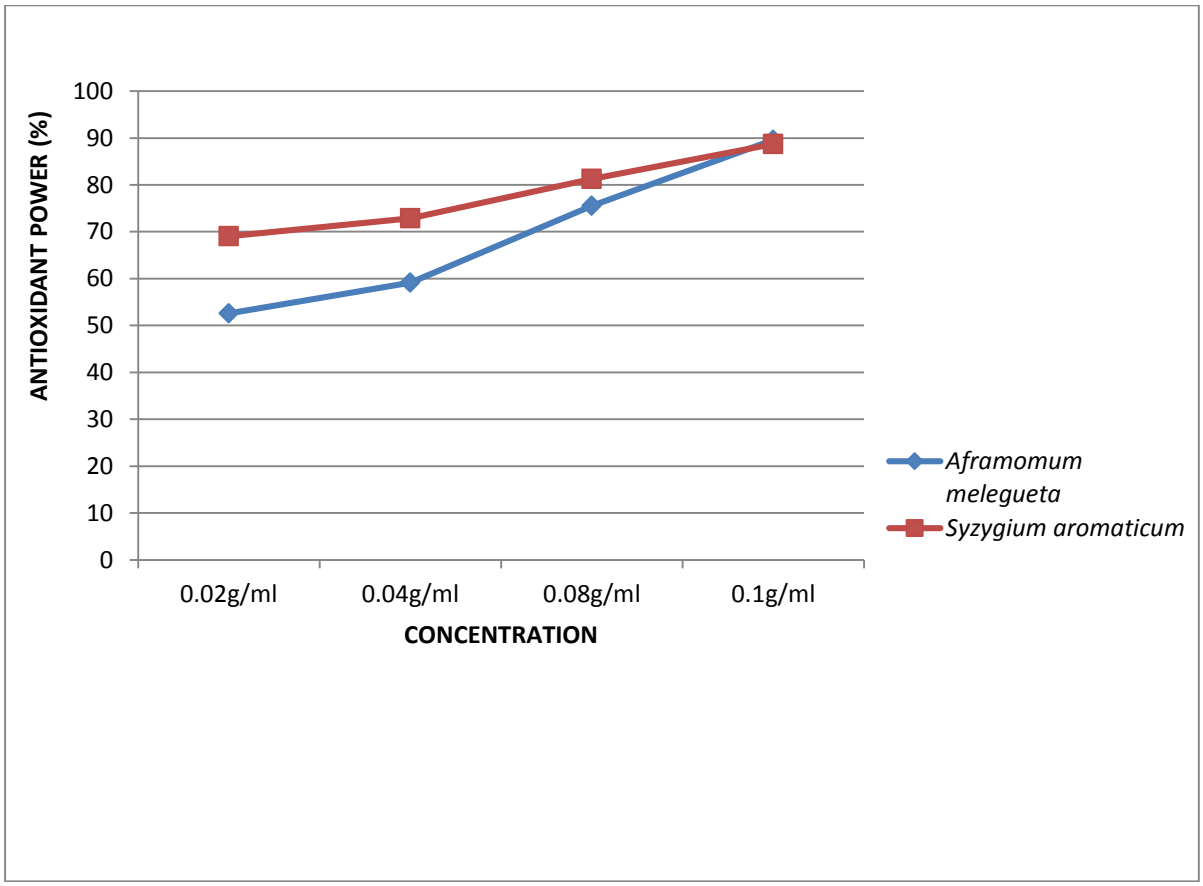

Fig. 2.Quantitative composition of phenols in *Syzygium aromaticum* and *Aframomum melegueta* seeds

The total flavonoids and phenolics contents of the aqueous extracts of the plants seeds at different concentrations (g/ml) shows the antioxidant power of the plants samples (Figs. 1and 2) respectively. Flavonoids are the most common group of poly-phenolic compounds in the human diet and are found ubiquitously in plants where they exhibit antioxidant activities. The lower the concentration of the plants samples extract, the lower the antioxidant power while the higher the concentration, the higher the antioxidant power of the plants samples. At the highest concentration of 0.1 (g/ml) of the aqueous extracts of *Syzygium aromaticum and Aframomum melegueta* the total flavonoids content was 97.56% ± 0.24 and 74.32% ± 1.88 respectively; and the total phenolics content was 88.72% ± 0.29 and 89.60% ± 2.51 respectively. This shows that, the content of flavonoids and phenols in the aqueous extracts of the seeds of the plants samples are significantly higher with the increase in concentration. Therefore, the therapeutic, antioxidant and scavenging ability in the plants samples could be attributed to the presence of flavonoids and phenolic compounds in the plants samples. This results corroborate the In-vitro Antioxidant Properties of these plants seeds as reported by [14,42-45]. Also, the effective role of these plants seeds in the inhibition of various conditions associated with respiratory disease is attributed to the presence of phenols and flavonoids in higher concentrations as earlier reported by [46-48]. Therefore, the plants seeds could be used as antioxidants to prevent the outbreak of respiratory diseases. Similarly, many researchers have reported that the antioxidant properties of plants are directly related to their contents of phenols and flavonoids, which have a tendency to chelate metals and scavenge active oxygen species [9,28,49,50].

The proximate composition of *Aframomum melegueta* and *Syzygium aromaticum* seeds were presented in Fig. 3, where it revealed the presence of varied proportions of moisture content, ash content, carbohydrate, protein, crude fiber and fat. Comparing the proximate contents of the plants samples, *Aframomum melegueta* has a relatively higher concentration of carbohydrates (32.76% ± 0.03), crude protein (21.03% ± 0.02) and crude fibers (28.33% ± 0.02) while *Syzygium aromaticum* has concentration of moisture content (13.74% ± 0.03), ash content (4.53% ± 0.02) and crude fat (7.94% ± 0.10). The availability of these nutrients in the extracts *Aframomum melegueta* and *Syzygium aromaticum* seeds suggests the relative importance of the plants as sources of food to provide energy, repair worn-out tissues, aid digestion, and provide minerals and vitamins to the body of man [51-53]. Thus, having these plants as spices in the food speaks volume on the nutritional quality of the food and could aids in the management of various symptoms that could lead to the existence of degenerative diseases in human body. Similar results were reported on the plants seeds by [43,54]. Ash contains inorganic materials of the plant because ashing destroys all the organic material present in the sample. Ash is also an indicative of high digestibility of the plant [55]. A strong correlation may be suggested between moisture contents and fiber, which could be of interest to human health as the fibrous foods are easily digested and disintegrated [51]. Fibers and moisture in the diet are necessary for digestion and for effective elimination of wastes. It can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, and diabetes and breast cancer [53]. The proximate composition of plants provides valuable information with regard to the nutritional quality of the plants [56].

It was also observed that various elements such as; Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Copper (Cu), Chromium (Cr), Iron(Fe), Manganese (Mn), Lead (Pb), and Zinc (Zn) were present in *Aframomum melegueta* and *Syzygium aromaticum* seeds in different quantities. The quantity of Calcium (Cu) was 10.40% ± 0.2 ppm and 7.54% ± 0.2 ppm; The quantity of Potassium (K) was 64.20% ± 0.2 ppm and 63.50% ± 0.2 ppm; The quantity of Sodium (Na) was 21.50% ± 0.2 ppm and 24.10% ± 0.2 ppm; The quantity of Magnesium was 9.11% ± 0.2 ppm and 9.05% ± 0.2 ppm; The quantity of Zinc was 1.42% ± 0.2 ppm and 1.04% ± 0.2 ppm amongst others Fig. 4. Interestingly, Iron (Fe), Copper (Cu), and Manganese (Mn), were relatively low in both plants seeds. These are trace element that are needed in low quantities. Also, chromium (Cr) and lead (Pb) were found in low quantities in *Syzygium aromaticum* and *Aframomum melegueta* seeds, they are harmful elements that as well require in low quantities [52]. *Syzygium aromaticum* and *Aframomum melegueta* plants seeds have the potentials for providing essential nutrients for human and animal diets since the nutritional activity of any plant is usually traced to the particular elements it contains [52,53].

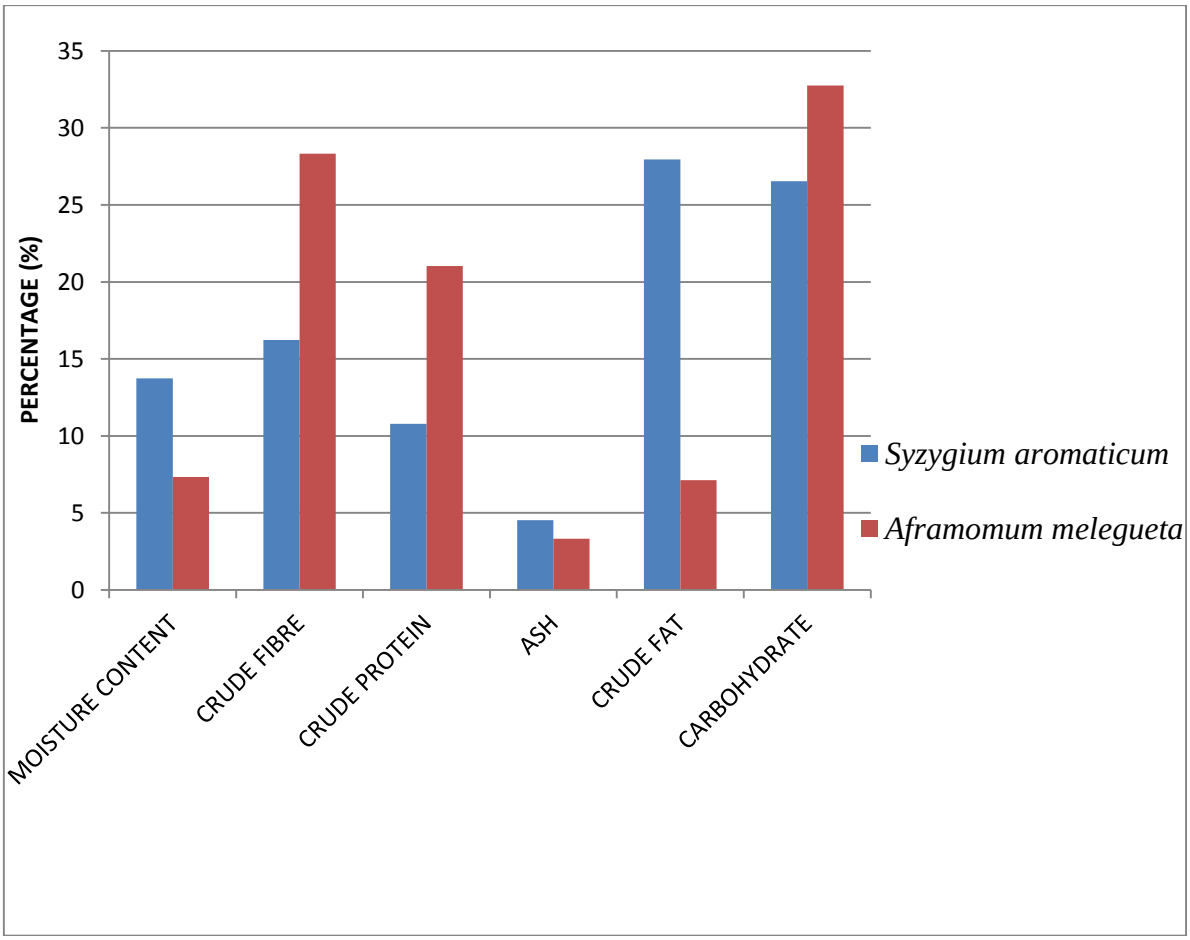

Fig. 3. Proximate composition of *Syzygium aromaticum* and *Aframomum melegueta* seeds

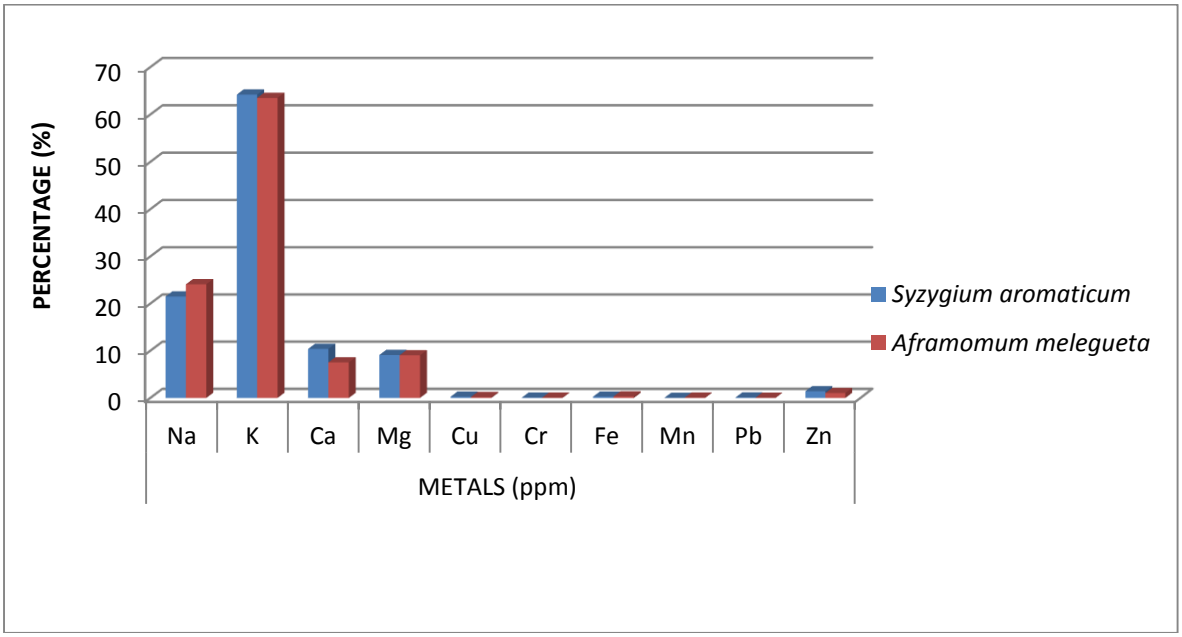

Fig. 4. Elemental composition of *Syzygium aromaticum* and *Aframomum melegueta* seeds

For example, calcium plays a fundamental role in the constitution of biological systems; its presence in bones provides man with the required rigidity and support [55]. Potassium exists primarily as an intracellular constituent in the body and hence could be of advantage to the improvement of healthy conditions of an individual [56]. The high content of potassium could be due to the intake of the element by the plant from the soil. Manganese acts as activator of many enzymes [57]. Zinc is involved in normal function of immune system. Iron is an essential element for human beings and animals and is an essential component of hemoglobin. [58-63]. It could thus be concluded that consumption of these plants parts might be safe. Similar results on the elemental composition of each plants seeds were also reported by [43,64].

4. CONCLUSION

The study revealed the presence of important phytochemicals such as alkaloids, terpenoids, saponins, tannins phenols and flavonoids; the proximate content includes moisture, carbohydrates, crude proteins, fibers and crude fat; and the elements such as calcium, potassium, magnesium and phosphorus; copper, zinc and sodium were also present in appreciable levels in the plants samples.

The results also showed that the plants seeds have a high antioxidant activity due to the phenolic and flavonoid contents in their extracts. They possessed excellent antioxidant properties and scavenging abilities. However, *Syzygium aromaticum* extracts exhibited better bioactive properties than *Aframomum melegueta*. The antioxidant properties of plants seeds validate the medicinal and nutritive potentials in the plant samples. Therefore *Aframomum melegueta* and *Syzygium aromaticum* could be said to possess potential and value necessary to maintain healthy life.

DISCLAIMER

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

ACKNOWLEDGEMENTS

The authors are grateful to those who contributed immensely to the success of this study. They appreciate the effort of the curator of the Department of Plant Science and Biotechnology Herbarium unit for plants authentication and assignment of voucher number.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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