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# Studies on Nutritional Composition and Efficacy of Selected Spices in Southern Nigeria against Some Food Spoilage Fungi

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author BNE designed the study. Author USU managed analyses of the study and performed the statistical analysis. Author NM managed literature searches and drafted the first manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

The efficacy of antifungal property of aqueous and ethanolic extracts of Alligator pepper (*Aframomum melegueta*), Aidan fruit (*Tetrapleura tetraptera*) and Black pepper (*Piper nigrum*) was tested against three fungal isolates implicated in food spoilage. The fungal isolates from stale bread, beans and spoilt onion are *Aspergillus* sp., *Mucor* sp. and *Penicillium* sp. Nutritional composition and antifungal efficacy of the spices was determined using conventional methods and poisoned food technique, respectively. Results obtained showed that the moisture content of the tested spices is low but they are all rich in carbohydrate. Fat content (12.70%), protein content (11.70%), carbohydrate content (62.94%) and energy value (372.20 Kcal) of Alligator pepper is higher and its fibre content is lower than that of the other two spices. Protein content (0.5%) of Black pepper is the lowest among the spices, but on the contrary in terms of ash content. Based on statistical analysis,

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the spices were significantly different at <0.05 probability. In terms of clear zone of inhibition, ethanolic extract of the three spices had more antifungal effect compared with the aqueous extract whereas the antifungal efficacy of the extracts increased with the increase in concentration. The highest mycelia growth inhibition (4.67 mm) was recorded by ethanolic extract (1.0%) of Aidan fruit tested against *Mucor* sp. Based on these findings, ethanolic extracts from the selected spices could be used as an alternative to chemical preservatives to prolong shelf life of foods.

*Keywords: Efficacy; fungi; spices; spoilage; extract; nutritional.*

## 1. INTRODUCTION

In recent times, there has been increasing consumer demand for reduction in quantity or total elimination of synthetic preservatives added to food because of certain illnesses linked with excess consumption of chemical preservatives. Concomitantly, consumers have also demanded wholesome foods from manufacturers. These two requirements are often contradictory [1,2,3].

Many attempts have been made in the search for natural antimicrobial compounds for the purpose of preventing microbial food spoilage that reduces shelf life of foods. This has become necessary because of health concern raised by consumers of foods that contain high-level chemical preservatives [4,5,6,7,8].

Spices are primarily added to food to function as flavouring agent since ancient times [9]. In recent decades, wider application of spices has been extended to medicine and food preservation. Clove, oregano, thyme, cinnamon and cumin are well-known spices that had demonstrated a reasonable level of efficacy as an antimicrobial agent both in the treatment of some infectious diseases and protection of food against spoilage organisms [10,11]. Spices which function as natural food preservatives when added to food could also influence its nutritional value [12].

According to Rawat [13], different categories of microorganisms are responsible for food spoilage. Fungal contamination of stored food commodities is one of the significant challenges facing farmers in tropical regions. *Aspergillus* and *Penicillium* are fungi genera highly implicated in contamination of stored food products. They often produce mycotoxins [14,15]. Mucor species have also been associated with food spoilage [16].

Nigeria is richly blessed with plants (spices) whose potentials have not been fully exploited. In Southern Nigeria, Alligator pepper (*Aframomum melegueta*), Aidan fruit (*Tetrapleura tetraptera*) and Black pepper (*Piper nigrum*) have been used extensively to spice food as well as in the treatment of several diseases [17]. The local names of the three spices are detailed by Adesina et al. [18] and Ogbunugafor et al.[11]. Alligator pepper, Aidan fruit and Black pepper grow well in the tropics. Black pepper has the shape of a heart. Aidan fruit tree produces fruits that hang at the ends of branches on stout stalks. When the fruit is not fully mature, it appears green but on maturity and ripening it becomes shiny, glabrous, dark-purple-brown [18-20].

Hamini-Kadar [21,22] and many other researchers have studied the antimicrobial properties of different spices, yet there is limited information regarding the antifungal properties of numerous spices against diverse fungi associated with food spoilage. Therefore, this study aimed to investigate the antifungal potential as well as the nutritional composition of three spices commonly used in Southern Nigeria as a strategy to reduce over-reliance on chemical food preservatives.

## 2. MATERIALS AND METHODS

Dried Alligator pepper (*Afromomum melegueta*), Black pepper (*Piper nigrum*) and fully mature Aidan fruit (*Tetrapleura tetraptera*) used in this study were purchased from retail spice-sellers in Akpan Andem Market Uyo, Akwa Ibom State, Nigeria and transported to the Analytical Laboratory, Department of Food Science and Technology, University of Uyo, Uyo for analysis. The three spices were authenticated by an expert in Plant Science and Biotechnology Department, University of Uyo.

### 2.1 Isolation and Identification of Test Fungi

*Aspergillus* sp., *Mucor* sp. and *Penicillium* sp. used in this work were isolated from spoilt food samples of stale bread, beans and spoilt onion using Potato Dextrose Agar (PDA) culture plates prepared according to standard procedures.

Identification of the isolates was carried out using standard manuals adopted by Pinto et al.[23], Ilhan et al. 24]. The isolated test organisms were maintained on PDA slant stored at 10°C. The isolates were subcultured regularly into freshly prepared PDA culture plates and stored appropriately.

## 2.2 Preparation of Extract

Each of the three spices were thoroughly washed under running tap water 2-3 times, then rinsed once with sterile water before each of them were transferred separately into an oven set at 50° C for drying that lasted for 48 hr. The dried spices were separately ground into fine powder using a manual grinder and separately stored inside airtight container kept in the refrigerator at 40° C. Sterile water and ethanol 95% (v/v) were separately used to obtain aqueous and ethanolic extract, respectively from each of the spices using a similar method described by Upadhyaya et al. [25].

### 2.2.1 Aqueous extraction

Twenty-five grams (25 g) of each powdered spice was separately dissolved in 100 ml sterile distilled water to constitute 25% (w/v). The mixture contained in sterile flask was properly covered with aluminium foil to avoid evaporation and left undisturbed at room temperature (28±2°C) for 24 hr followed by filtration using sterile Whatman No. 1 filter paper. After filtration, the aqueous extract was evaporated inside a water bath until 25 ml aqueous extract was left in the container.

### 2.2.2 Ethanol extraction

Accurately, 25 g of each powdered spice was weighed and dissolved in 100 ml of 95% (v/v) ethanol. The resulting mixture (25% w/v) was subjected to further extraction procedure similar to aqueous extraction.

## 2.3 Antifungal Activity Using Poisoned Food Technique

The antifungal activity of aqueous and ethanolic extracts of the three spices against selected food spoilage fungi was evaluated using a poisoned technique described by Balamurugan [26]. The test organisms were inoculated on PDA plates in triplicates and incubated at 25°C for 3-7 Days to obtain young, growing colonies of molds. 100 ml spice extract was mixed with 15 ml molten PDA which was allowed to cool to about 45°C. The mixture was poured on sterile Petri dish and allowed to solidify at room temperature (28±2°C) for 30 min. A mycelia disc 6 mm diameter cut out from a periphery of 3-7 Day old culture was aseptically inoculated into the agar plates containing the plant extract and incubated at 25°C. Agar plates inoculated with mycelia disc without plant extract added to serve as control. The diameter of each fungal growth was measured and recorded after 48 hr. Percentage mycelia growth inhibition was calculated as given below:

$$\% \text{ Mycelia growth inhibition} = \frac{(\text{Diameter of a fungal colony in control} - \text{Diameter of fungal colony with plant extract})}{\text{Diameter of fungal colony in control}} \times 100$$

## 2.4 Determination of Nutritional Composition

Proximate composition (%) of the dried spices in terms of moisture, ash, fat, crude protein and fibre content were determined using [27] methods described by Nwinuka et al. [28] while carbohydrate content was estimated by difference method. The energy value (Kcal) of the samples was also estimated using approved formula.

### 2.4.1 Determination of moisture content

The moisture content of the dried spice samples was determined based on thermal drying method. One gram of the dried sample was weighed in triplicates and placed inside a clean, dried and weighed crucible. The samples were placed inside an oven set at 105°C and allowed to dry for 3 hr. The dried hot sample was removed from the oven and transferred into a desiccator and left for 30 min to cool before the dried sample inside the crucible was reweighed. The moisture content was calculated using the expression below:

$$MC\ (\%) = \frac{Wo}{Wi} \times 100$$

Where: $W_o$ =loss in weight (g) on drying and $W_i$ = initial weight of the sample (g) before drying

### 2.4.2 Determination of ash content

Ignition method was used in determining ash content of the dried samples. Clean crucibles were pre-heated in a muffle furnace at 500°C and allowed to cool. From the dried sample used

for moisture content determination, 1 g was transferred into triplicate crucible that had been pre-heated, cooled and weighed. Gently, the lid of the crucible was placed on it and then returned to the oven that heat is yet to be applied. Temperature of the muffle furnace was allowed to rise to 500°C and then timed for 3 hr ashing. The crucible containing ashed samples were removed from the hot oven, placed inside desiccator for 30 min to cool and then reweighed. The expression below was used to calculate ash content of the samples:

$$\text{Ash (\%)} = \frac{Ma}{Ms} \times 100$$

Where: $M_a$ = Mass of ash (g) and $M_s$ =Mass of sample used (g)

### 2.4.3 Determination of fat content

Direct solvent extraction method using Soxhlet was the method adopted to determine fat content of the samples. Petroleum ether 40 – 60°C was the solvent used for fat extraction. In triplicates, 3 g of the dried sample was weighed and tightly closed inside Soxhlet extraction thimble with 60 ml petroleum ether and fat extraction lasted for 4 hr. On completion of fat extraction, the solvent was evaporated and the flask dried inside an oven set at 60°C for 1 hr. The flask was placed inside a desiccator, allowed to cool and reweighed. The expression used to calculate percentage fat is stated below:

$$\text{Fat (\%)} = \frac{Mex}{Ms} \times 100$$

Where: $M_{ex}$ = mass of extract (g) and Ms = mass of sample used (g)

### 2.4.4 Determination of crude protein content

The macro-Kjeldhal method which involves three stages was used to determine the total organic nitrogen and subsequent conversion into percentage crude protein using a conversion factor. In the digestion stage, 1 g sample of each of the dried spices was weighed in triplicates and gently poured into digestion flask. About 3-5 granules of anti-bump and 3 g copper catalyst mixture (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide) was added to each flask followed by 20 $cm^3$ concentrated sulphuric acid. The process of digestion started when the content of the flask was placed on a heating mantle which continued until a clear solution was obtained before the content of the flask was allowed to cool. Filtration of the digest was done and made up to 100 $cm^3$ using distilled water. A volume of 20 $cm^3$ of the diluted digest was pipetted into round-bottomed flask. Commencement of distillation stage involved placing the content of the round-bottomed flask on a heating mantle. Liebig condenser was used to connect the round-bottomed flask to a beaker (receiver flask) that contains 20 $cm^3$ of 2% boric acid as well as methyl red indicator. Using a Buchner funnel, the condenser was submerged in the boric acid. Ammonia is formed after 30 $cm^3$ of 40% sodium hydroxide was injected into the flask. Heating of the flask marked the commencement of ammonia distillation which lasted until boric acid solution had completely changed from purple to greenish-yellow. The last stage which is titration involved using boric acid mixture that contain ammonium borate complex to titrate against 0.1 N HCl until colourless end point was observed and the titre noted. The expression below was used to calculate total organic nitrogen in the sample.

$$\text{\% TON} = \frac{TV \times NE \times TVd}{Ms \times Vd} \times 100$$

Where TV = Titre value, NE = mg nitrogen equivalent to molarity of acid, $TV_d$ = total volume at which digest was diluted, $M_s$ = mass of sample (g), $V_d$ = volume of digest diluted.
% crude protein = % TON x 6.25

The general factor 6.25 is suitable for products that have proportion of specific proteins that are not well defined.

### 2.4.5 Determination of crude fiber content

Five gram sample ($W_1$) of each of the dried spices was gently boiled with 10 ml petroleum ether to dissolve fat followed by acid hydrolysis with 2 ml $H_2SO_4$. The residue obtained was boiled with dilute 4 ml NaOH and then with 4 ml HCl in order to remove all digestible matter. Alcohol and ether was used to wash the residue, then dried in an oven to a constant weight ($W_2$) and allowed to cool. Crude fibre is the dried residue which is non-digestible matter. The expression below was used to calculate percentage fiber.

$$\text{\% Crude fiber} = \frac{\text{Loss in weight on ignition}}{\text{Weight of dried spice sample}} \times 100$$

Where: loss in weight on ignition is ($W_1$) - ($W_2$); weight of dried spice sample is ($W_2$)

#### 2.4.6 Determination of carbohydrate content

The carbohydrate content of dried spice sample was estimated by subtracting the sum of moisture, protein, crude fiber, fat and ash content from 100%.

### 2.5 Determination of Energy Value

The method described by Akubugwo et al. [29] was used to estimate energy value (Kcal) of the spices. It was determined by using the factor 3.36, 8.37 and 3.6 to multiply percentage protein, fat and carbohydrate, respectively.

### 2.6 Statistical Analysis

Data obtained from the various tests in triplicates were subjected to statistical analysis using analysis of variance (ANOVA). In order to separate the means obtained from replicate analysis. Duncan multiple range tests (DMRT) in statistical package for the social sciences (SPSS) 20.0 software was used.

## 3. RESULTS AND DISCUSSION

The nutritional composition and antifungal efficacy of aqueous and ethanolic extract of Alligator pepper (*Aframomum melegueta*), Aidan fruit (*Tetrapleura tetraptera*) and Black pepper (*Piper nigrum*) against three fungal isolates implicated in food spoilage were determined. The cultural morphology of fungal isolates used in this study is presented in Table 1. The probable organisms identified based on cultural morphology are *Aspergillus* sp., *Mucor* sp. and *Penicillium* sp.

### 3.1 Antifungal Effect of Extracts from the Spices against the Fungal Isolates

This study revealed that ethanol extract of the three spices had more effective antifungal activity against the three fungal isolates compared with that of aqueous extract. It is suggestive that ethanol is a better extractant than distilled water considering the fact that ethanol extract of the spices resulted in a wider activity spectrum clear zone of inhibition than that of aqueous extract. In a similar study, [30-32] reported that extractable natural product obtained using ethanol had higher activity than that of aqueous extract.

Table 2 shows the effect of Alligator pepper (*Afromomum melegueta*) on mycelia growth of the fungal isolates after 48 hr incubation period. Interestingly, the result obtained from this study shows that antifungal effect of ethanolic extract 0.5 and 1.0% from the spices against mycelia growth of fungal isolates increased with increase in the concentration of the extract. Effiong and Sanni [33] made a similar observation in a related study. According to Odetunde et al. [34], Alligator pepper contains phytochemicals which give it antimicrobial properties. It is suggested that phytonutrients such as flavonoids, phenolic compounds, tannins, saponin, terpernoids, cardiac glycosides and alkaloids present in Alligator pepper gives it that ability to function as an antimicrobial agent. In addition to established antibacterial effect of Alligator pepper against *Escherichia coli*, *Salmonella* spp. and *Klebsiella* spp. by Effiong and Sanni [33], this study has further demonstrated that alligator pepper also possess antifungal property. According to Doherty et al. [20], the zone of inhibition (mm) of alcoholic extract of Alligator pepper tested against *Aspergillus* sp. after 24 and 48 hr incubation is 45 mm and 47mm, respectively. However, aqueous extract of the spice recorded lower zone of inhibition 40 mm and 45 mm after 24 and 48 hr incubation, respectively. Odetunde [34] reported that ethanolic extract of Alligator pepper exhibited fungitoxic effect against *Fusarium oxalicum*, *Botryodiplodica theobromae* and *Aspergillus niger* isolated from diseased cassava. The antifungal property of *Afromomum melegueta* against *Aspergillus* sp., *Mucor* sp. and *Penicillium* sp. corroborates the findings of a related study by Ikegbunam et al. [35].

The level of antifungal activity of Aidan fruit (*Tetrapleura tetraptera*) extracts on mycelial growth of *Aspergillus* sp., *Mucor* sp. and *Penicillium* sp. based on zone of inhibition after 48 hr incubation is shown in Table 3. The result revealed that aqueous extract at different concentrations had no significant effect on mycelia growth (clear zone was negligible) except 1.0% aqueous extract of *Tetrapleura tetraptera.* This could be as a result of low concentration of active ingredient(s) in the spice responsible for significant antifungal activity was released into the aqueous extract at 0% and 0.5% concentration compared with 1.0% concentration. According to Pundir and Jain [36], antifungal action of aqueous extracts could be ascribed to anionic components such as thiocyanate, nitrate, chloride and sulphates including other water soluble components which naturally occur in plant materials. The antimicrobial property of Aidan fruit could be attributed to phytonutrients present in the spice. Koma [37] reported that phylobatannins and

volatile oils present in *Tetrapleura tetraptera* are bioactive phyto-compounds responsible for inhibiting the activities of several pathogens. The phytochemical compositional values of saponin, alkaloids, tannins, phenols and sterols present in the fruit of *Tetrapleura tetraptera* were determined by Akintola et al. [38].

The efficacy of Black pepper (*Piper nigrum*) on mycelial growth of the three fungal isolates after 48 hr incubation period is presented in Table 4. Based on the result obtained, aqueous extract concentration 0%, 0.5% and 1% of Black pepper did not show observable inhibitory effect against the test organisms but ethanolic extracts at different concentration displayed inhibitory ability. However, [15] in a related study reported that antifungal activity of 10, 15 and 20% concentration of aqueous extracts of *Piper nigrum* against *Aspergillus* sp. increased with increasing extract concentration. Low aqueous extract concentration of Black pepper tested against *Aspergillus* sp., *Mucor* sp. and *Penicillium* sp. could be the reason no sign of inhibition was observed in this study. Although ethanolic extract proved to be a better extractant than distilled water, a higher concentration of aqueous extract than ethanolic extract could also demonstrate antifungal effect against some fungal isolates. Singh [39] were able to identify different components responsible for antifungal properties of *Piper nigrum*.

This study revealed that ethanolic extract from Aidan fruit at 1.0% concentration demonstrated highest mycelia growth inhibition of 4.67 mm against *Mucor* sp whereas the ethanolic extracts from the three spices against *Aspergillus* sp. had the least mycelia growth inhibition of 1.10 mm. This result implies that *Aspergillus* sp. is the most resistant fungi among the three fungal isolates used to determine antifungal efficacy of the three ethanolic spice extracts. Table 4 shows that aqueous extract of *Piper nigrum* at 0%, 0.5% and 1.0% concentration had zero mycelia growth inhibition but at 1.0% concentration, aqueous extract of Aidan fruit and Alligator pepper demonstrated some level of inhibition against mycelia growth from the three fungal isolates. This is an indication that aqueous extract of *Piper nigrum* is the least effective spice extract against the test organisms used in this study.

Although the effect of different solvents used for extraction as well as concentration of the spice extract on pathogenic microbes including food spoilage microorganisms had been studied extensively, barely had any of such studies been carried out to also determine the combined effect of geographical location, period of harvesting, age and freshness of spices despite being linked by some researchers as influential factors that could affect antimicrobial potential of the spices [35,40].

## 3.2 Proximate Composition and Energy Value of Selected Spices

The ash content of Alligator pepper (*Afromomum melegueta*), Aidan fruit (*Tetrapleura tetraptera*) and Black pepper (*Piper nigrum*) reported in this study is 4.35, 3.40 and 14.9 %, respectively. The ash content of Alligator pepper and Aidan fruit reported by Ibekwe and Orok [41] and Akin-Idowu et al. [42], respectively are comparable with the results obtained from this study. Ash content of the three spices is an indication of the level of minerals present in the spices. Consumption of foods rich in minerals helps the human body in maintaining water balance as well as play some useful role in bone and body metabolism [38]. However, the ash content of Black pepper reported in this study is higher than the value reported by Nwofia et al. [43] in a related study that involved nine accessions of *Piper nigrum* from Nigeria.

This study revealed that crude fiber content of Alligator pepper (*Afromomum melegueta*), Aidan fruit (*Tetrapleura tetraptera*) and Black pepper (*Piper nigrum*) is 2.61, 44.8 and 49.0 %, respectively. This result clearly shows that the three spices are rich in crude fiber content except Alligator pepper. The fiber content of Alligator pepper and Black pepper is not in agreement with findings by Alaje et al. [44] and Nwofia et al. [43], respectively but that of *Tetrapleura tetraptera* supports the findings of Uyoh et al. [45], Okwu [46]. According to Akintola et al. [38], fibre is considered as an essential nutrient for humans because of its role in lowering constipation, diabetes and high blood pressure as well as reduces the risk of developing cardiovascular disease and cancer.

The crude protein content of Alligator pepper (*Afromomum melegueta*), Black pepper (*Piper nigrum*) and Aidan fruit (*Tetrapluera tetraptera*) is 11.70, 9.63, and 0.5 %, respectively. The crude protein content of Black pepper reported in this study is not in agreement with the findings of Nwofia et al. [43]. According to Alaje et al. [44] and Uyoh et al. [45], the protein content of Alligator pepper and Aidan fruit, respectively is lower than the result obtained from this study.

**Table 1. Identification of fungal isolates by cultural morphology**

| Isolate code | Morphology | Nature of hyphae | Spore shape | Reproductive structure | Pigmentation | Probable organism |
|---|---|---|---|---|---|---|
| 1 | White colony | Long erect non-septate | Smooth and regular with sporangiophores | Globose vesicle | White | *Mucor* sp. |
| 2 | Aerial hyphae | Septate | Oval | Globose swelling | Black | *Aspergillus* sp. |
| 3 | Flat Sporangiophores | Septate | Oval | Long conidophores with chains of spore | Greenish blue | *Penicillium* sp. |

**Table 2. Effect of alligator pepper (*Afromomum melegueta*) extract on mycelia growth of (mm) fungal isolates after 48 hr incubation**

| Test organism | Ethanolic extract (0%) | Ethanolic extract (0.5%) | Ethanolic extract (1.0%) | Aqueous extract (0%) | Aqueous extract (0.5%) | Aqueous extract (1.0%) |
|---|---|---|---|---|---|---|
| *Mucor* sp. | 1.57±0.08$^{a}$ | 3.07±1.00$^{a}$ | 4.03±1.00$^{a}$ | 0 | 0 | 4.03±1.00$^{a}$ |
| *Aspergillus* sp. | 1.10±0.08$^{a}$ | 2.33±1.00$^{b}$ | 3.27±0.10$^{b}$ | 0 | 0 | 3.27±0.10$^{b}$ |
| *Penicillium* sp. | 1.17±0.08$^{a}$ | 1.80±1.00$^{c}$ | 3.00±0.10$^{a}$ | 0 | 0 | 3.00±0.10$^{a}$ |

*Values are means ±SD of triplicate determination. Means in the column with different superscript are significantly different at (P<0.05).*

**Table 3. Effect of aidan fruit (*Tetrepleura tetraptera*) extract on myclial growth (mm) of fungal isolates after 48 hr incubation**

| Test organism | Ethanolic extract (0%) | Ethanolic extract (0.5%) | Ethanolic extract (1.0%) | Aqueous extract (0%) | Aqueous extract (0.5%) | Aqueous extract (1.0%) |
|---|---|---|---|---|---|---|
| *Mucor* sp. | 1.93±1.00$^{a}$ | 2.80±0.13$^{a}$ | 4.67±1.00$^{a}$ | 0 | 0 | 4.60$^{a}$±1.00 |
| *Aspergillus* sp. | 1.10±0.15$^{b}$ | 3.13±0.13$^{a}$ | 3.90±0.11$^{b}$ | 0 | 0 | 0.23$^{b}$±0.097 |
| *Penicillium* sp. | 1.93±0.15$^{b}$ | 1.90±1.00$^{b}$ | 3.53±0.11$^{b}$ | 0 | 0 | 3.00$^{b}$±0.097 |

*Values are means ±SD of triplicate determination. Means in the column with different superscript are significantly different at (P<0.05).*

**Table 4. Effect of black pepper (*Piper nigrum*) extract on the mycelia growth (mm) of fungal isolates after 48 hr incubation**

| Test organism | Ethanolic extract (0%) | Ethanolic extract (0.5%) | Ethanolic extract (1.0%) | Aqueous extract (0%) | Aqueous extract (0.5%) | Aqueous extract (1.0%) |
|---|---|---|---|---|---|---|
| *Mucor* sp. | 1.83±1.00$^{a}$ | 3.47±0.13$^{a}$ | 4.43±0.08$^{a}$ | 0 | 0 | 0 |
| *Aspergillus* sp. | 1.10±0.24$^{b}$ | 2.43±0.25$^{b}$ | 3.77±0.25$^{b}$ | 0 | 0 | 0 |
| *Penicillium* sp. | 1.97±0.24$^{b}$ | 2.07±0.25$^{b}$ | 3.50±0.44$^{b}$ | 0 | 0 | 0 |

*Values are means ±SD of triplicate determination. Means in the column with different superscript are significantly different at (P<0.05).*

**Table 5. Proximate composition (%) of different plant spices**

| Selected spices | Moisture | Fat | Crude protein | Crude fibre | Ash | Carbohydrate |
|---|---|---|---|---|---|---|
| Alligator pepper | 5.7±0.20$^{a}$ | 12.7±0.20$^{a}$ | 11.70±0.20$^{a}$ | 2.61±0.02$^{a}$ | 4.35±0.02$^{b}$ | 62.94±0.02$^{a}$ |
| Aidan fruit | 5.90±0.20$^{b}$ | 3.4±0.20$^{b}$ | 9.63±0.03$^{b}$ | 44.81±0.01$^{b}$ | 3.40±0.32$^{a}$ | 32.86±0.20$^{b}$ |
| Black pepper | 2.56±0.03$^{c}$ | 2.15±0.20$^{c}$ | 0.5±0.01$^{c}$ | 49.0±0.01$^{c}$ | 14.9±0.02$^{a}$ | 30.89$^{c}$±0.02$^{c}$ |

*Values are means ±SD of triplicate determination. Means in the column with different superscript are significantly different at (P<0.05).*

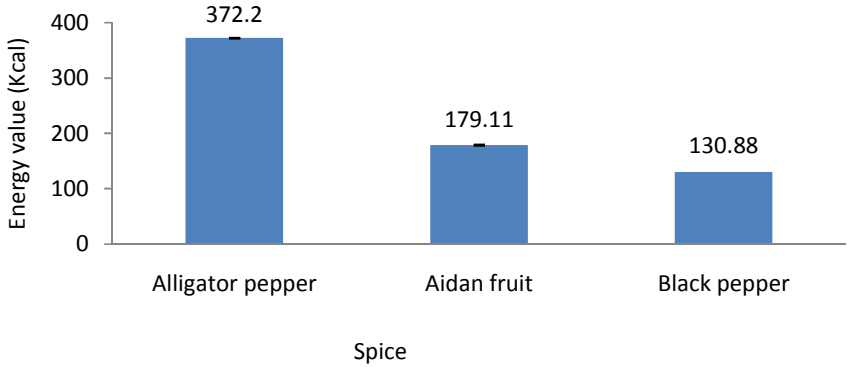


**Fig. 1. Energy value of alligator pepper, aidan fruit and black pepper**

Carbohydrate content of *Tetrapluera tetraptera* and *Afromomum melegueta* used in this study is approximate twice the result reported by Uyoh et al. [45] and Alaje et al. [44], respectively. Carbohydrate content of Black pepper is not in agreement with the findings [43]. However, the carbohydrate content of Alligator pepper reported by Okwu [46] is similar with the result obtained from this study. There is an indication that high carbohydrate content of the spices will help stabilize plasma level and thereby prevent body protein from being easily degraded to obtain energy [38].

Fat content of Alligator pepper, Aidan fruit and Black pepper reported in this study is 12.7, 3.40 and 2.15%, respectively. According to Akintola et al. [38], fat content in food is important because it serves as means of storage and transport of metabolic fuel in the human body. It also serves as electrical insulators for subcutaneous tissues and emulsifier for drug preparation. Since fat content of food substances gives it palatability [38], it could be that higher fat content (12.7%) of Alligator pepper compared with Aidan fruit and Black pepper contribute in the wide acceptability of Alligator pepper used as a custom among the Igbos to welcome a visitor into their home [47]. This result clearly shows that Aidan fruit and Black pepper are not rich sources of fat compared with Alligator pepper. The fat content of seeds of Black pepper reported by Nwofia et al. [43] is similar but that of leaves of Black pepper is higher than the result obtained from this study. Contrary to the result reported in this study, [44] revealed that fat content of Alligator pepper is 2.60%. Uyoh [45] reported that Aidan fruit of 20 accessions have relatively high fat content whereas [46] reported similar fat content with the result obtained from this study.

This study revealed that moisture content of the selected spices is low. The value for Alligator pepper, Aidan fruit and Black pepper is 5.7, 1.90, and 2.56%, respectively. Alaje [44,45] and [43] reported that Alligator pepper, Aidan fruit and Black pepper, respectively have higher moisture content compared with the results reported in this study. Generally, the moisture content of food gives an indication of its susceptibility to microbial spoilage. The low moisture content of the spices used in this study suggests that they are not easily degraded by microorganisms. Therefore, the shelf life of the three spices may not be considered as being very short [38].

Fig. 1 shows that energy value of Aidan fruit, Alligator pepper and Black pepper is 179.11, 372.20 and 130.88 Kcal, respectively. This study revealed that energy value of Alligator pepper is more than twice the value in Aidan fruit and Black pepper. The benefit of high energy value of Alligator pepper revealed from this study could also support its acceptability as a custom by the Igbos when it is served visitors that had travelled some distance to pay someone a visit [47]. In a related study, Nwofia et al. [43] reported higher energy value in the leaves (295.67-273.68 Kcal) and seeds (366.23-384.18 Kcal) of Black pepper collected from different locations compared with the result obtained from this study. Odebunmi [48] reported a lower energy value (310.54 Kcal) in Alligator pepper compared with 372.2 Kcal depicted in Fig. 1. According to Effiong et al. [17], the energy value of *Tetrapleura tetraptera* range between 234.42-379.48 g/cal is not in agreement with the finding from this study.

The variations in proximate composition and energy value of the three spices used in this study in relation with similar studies by other researchers could be as a result of agronomic factors that have to do with variety, maturity, processing conditions and cultural practices. However, there are limited studies to determine the effect of these factors on the proximate composition and energy value of the three spices [49,50].

## 4. CONCLUSION

This study has shown that Aidan fruit, Alligator pepper and Black pepper possess antifungal properties. The ethanolic extracts of the spices exhibited more effective antifungal property than the aqueous extract. Among the three fungal isolates, *Mucor* sp. and *Aspergillus* sp. are the most and least sensitive microorganism, respectively to ethanolic extract from the spices. Based on the proximate composition of the spices, its addition to food could also improve its nutritional value better than many chemical preservatives.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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