

Toxic chemicals and the microbiota of regular food seasonings

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Abstract

The occurrence of toxic substances in some regular food seasonings was studied as well as their microbial loads and antimicrobial activities. Five branded seasonings were used including: Benny, Curry, Thyme, Onga and Kitchen glory. Anti-nutrients content, heavy metal contents and microbiological assessments were done. Results obtained shows that there were some toxic substances in the seasonings at varying concentrations. Alkaloid was in the range of 0.04% (Kitchen glory) to 0.07% (Thyme), Phenol ranges from 0.04% (Kitchen glory) to 0.36% (Thyme) while others ranges from 0.12% (Benny) to 0.25% (Curry) and from 0.11% (Benny) to 0.36% (Thyme) respectively. Hydrogen cyanide was detected at very low concentration ranging from 0.56mg/kg in Curry to 7.96mg/kg in Kitchen glory. The variation in the anti-nutrients of the seasonings were found to be significantly different ($p < 0.05$). Other toxic substances detected in the seasonings were heavy metals at different levels including Iron (0.07 to 1.92mg/100g), Zinc (2.23 to 12.7mg/100g), Copper (0.07 to 0.69mg/100g), and Manganese (0.01 to 0.67mg/100g) and lead (0.00 to 0.27mg/100g). The microbial load of the seasonings varied significantly ($p < 0.05$) from 8.67×10^3 cfu/g to 17.00×10^3 cfu/g of bacteria while the fungal load varied from 0.00 to 2.00×10^1 cfu/g. The extracts of the seasonings shows varying levels of antimicrobial activity against some bacteria and fungi. Inhibition of bacteria by the seasoning extracts had diameters of inhibition zone ranging from 0.00mm (no inhibition) by Onga extracts to 11.33mm by extract of Thyme on *Staphylococcus aureus*, inhibition of *Bacillus Subtilis* was in the range of 8.00mm (Onga) to 13.67mm (Thyme) while *Pseudomonas aureginosa* was inhibited from 6.67mm (Onga) to 13.33mm (Thyme). Yeast inhibition was highest with Curry extract (10.33mm) and least with Onga extract (6.67mm). The corresponding values for *Aspergillus niger* was 11.67mm (Thyme) and 6.33mm (Onga) and Kitchen glory. It was concluded that the toxic substances in the regular seasonings included anti-nutrients and heavy metals as well as some bacteria and fungi with potentials of toxic production and some health hazard. There were significant variation ($p < 0.05$) in the levels of the toxic substances in the seasonings.

Keywords: toxic chemicals/substances, microbiota, regular food seasonings

Introduction

Seasonings are ingredients which are added to foods to enhance their flavor. These ingredients include salts, herbs, such as chili powder, condiments such as mustard, vinegar. Apart from adding flavors to foods, some seasonings also contain medical and health benefits (Herndon, 2013) [10]. Seasonings which are also referred to as additives has been defined by Food and Agriculture Organization (FAO, 2000) [8] as non-nutritive substances added intentionally to food, generally in small quantities to improve its appearance, flavors, and texture or storage properties. Spices and herbs have long been used as flavouring agents in foods and are believed by many people to possess medical properties (Kaeter and Milner, 2008) [13]. The leaves used in food preparation contain aromatic oils which releases flavors and scents when the leaves are crushed or chopped.

Herbs can be used raw or processed depending on the variety or uses (Anita, 2003) [3]. U.S.A, Food and Drugs Administration (FDA) describes a spice as an aromatic vegetable substance in whole, broken or ground form, whose function is for seasoning rather than nutritional purposes (Hulse, 1996). Also, spices have been used throughout history all over the world and for many centuries have been an important trading commodity (Joseph, 2012) [12]. Seasonings play an important role in production, like processing aids and flavor enhancer because of these, its important cannot be underestimated. As food, seasonings

have been shown to be sources of nutrient (Idris, 2002) [11]. Wide varieties in concentrations of trace metals have been reported in seasoning (Onimawo, 2010) [15]. Heavy metals contamination in the food chain is caused by environmental pollution. Lead (pb) and copper (cu) have aroused considerable concern. In Nigeria, seasonings are manufactured as bouillon cubes and other well packaged powdery forms with different brand names. They contain salt, sugar, monosodium glutamate (MSG) and other ingredients and are used extensively in food preparation in most homes and restaurants.

Spices and herbs may be contaminated because of conditions in which they were grown and harvested. Spores of both *Clostridium perfringens* and *Bacillus aureus* have been found to be present in spices and herbs. Contaminated spices have been reported to be one of the major causes of foodborne illness and spoilage. Fewer microorganisms are present in spices with higher antimicrobial activity such as sage, cloves and oregano. The conditions of imported spices in Nigeria may not be free from microbial contamination and can be sources of contamination to food and food stuffs. Foodborne diseases (also referred to as food poisoning) is the illness resulting from the consumption of contaminated food pathogenic bacteria, viruses, or parasites that contaminate food. Apart from monosodium glutamate present in seasoning, commercial seasonings have been criticized of possessing heavy metals such as lead and

copper. Unhygienic practices during production could introduce bacterial contamination in these seasonings. Therefore, this study will be justified by identifying some toxic chemicals, heavy metals and pathogenic bacteria in the samples assayed. Also, this will help to regulate human ingestion of these harmful chemicals and pathogenic bacteria in food seasonings.

Materials and Methods

Sources of Materials

The seasonings; thyme, curry, benny, kitchen glory, onga were procured from different markets and shops within and around Umuahia and Laboratory analysis were performed at National Root Crop Research Institute (NRCRI), Umudike.

Samples/Media Preparation

The samples were collected aseptically and taken to the laboratory without delay for analysis. The media used include Nutrient agar, MacConkey agar and Sabouraud's dextrose agar (SDA). All the media were prepared according to the manufacturer's instruction while aseptic principles were ensured to control possible contamination.

Determination of Antinutrient

Phytates, HCN, Phenol, Flavonoid, Alkaloid and Tannins will be determined by the standard method of AOAC (1991) [2] as described by (Onwuka, 2005) [16].

Determination of Phytate

Phytate was determined by the method described by Onwuka (2005) [16]. Two grams (2g) of seasoning was mixed with 0.2 NHCL to form phytate extracting solution. Exactly 0.5mls of the solution was mixed with 1mla of ferric solution (0.2g ammonium ion (iii) sulphide in 2NHCL made up to 1000ml distilled water) in a test tube. The mixture was heated in water bath for 30minutes, cooled and centrifuged for 30mins. 1ml of the supernatant was mixed with 1.5ml of 2,2-bipyridine solution (10g 2,2-bipyridine with 10ml thioglycolic acid and made up to 1liter). The absorbance was measured at 519nm in a spectrophotometer and recorded. A standard phytate solution was also analyzed. The Dhytate content was calculated thus:

$$\% \text{ Phytate} = 100/W \times A_v/A_s \times C \times V_f/V_a$$

Where:

W = Weight of sample

A_μ = absorbance of samples

A_s = absorbance of standard phytate solution

C = concentration of standard phytate solution

V_f = total extract volume

V_a = volume of extract analyzed

Determination of Cyanogenic Glycoside (HCN)

The alkaline picrate colorimetric method described by (Balagopalan *et al*, 1988) [5] was used. Stripes of filter paper were cut from Whatman No. 1 filter paper. The alkaline picrate solution was prepared by dissolving 1g of picrate and 5g of sodium carbonate in a small volume of minimally warm water and made up the volume to 200ml with distilled water. The picrate paper was prepared by dipping rectangular pieces of filter paper in picric acid solution and dried. Each test sample (1g) was dispersed in 200ml of distilled water in a 250ml conical flask. An alkaline picrate

paper was suspended inside the flask and held in place with the stopper used to cork the flask. Care was taken to ensure that the picrate paper did not touch the surface of the mixture in the flask. These were incubated at room temperature for 18hrs (overnight) and then each picrate paper was carefully removed and eluted in 60ml of distilled water. A standard cyanide solution was prepared (0.05M). The absorbance of the standard solution prepared was read first before each of the samples replicates was read. The absorbance of the sample solutions as well as those of the standard were measured spectrophotometrically at 540nm using the reagent blank to set the instrument at zero. The cyanide content was determined by calculation as shown below:

$$\text{HCN mg/kg} = 1000/W \times A_\mu/A_s \times C$$

Where:

W = weight of sample

C = concentration of standard cyanide solution.

At this point, the total filtrate resulting from digestion of 2g of sample flour was made up to 300ml aliquots of 125ml of the filtrate was heated until near-boiling and then titrated against 0.05m standardized KMnO4 solution to a faint pink color which persisted for 30s. The calcium oxalate content was calculated using the formula:

$$\% \text{ Oxalate} = 100/W \times E_w \times T$$

Where:

W = weight of sample analyzed

E_w = equivalent weight of KMnO4

T = titre value

Determination of Phenols

The Follins method described by (Pearson, 1976) [17] was used. Exactly 0.2g of the dried ground sample was dispersed in 10ml/s of methanol and shaken. The mixture was allowed to stand for 30min at room temperature before it was filtered through Whatman filter paper to exactly 1ml of the extract was placed in a test tube and 1ml of Follins reagent was added to it with 5ml of distilled water. The color was allowed to develop for about 3 to 4hrs at room temperature. The absorbance of the developed color was measured at 760nm wavelength. The procedure was repeated two more times to get an average. The phenol content was calculated thus:

$$\text{Pheno1} = 100/W \times A_\mu/A_s \times C/1000 \times V_f/V_a \times D$$

W = weight of sample analyzed

A_μ = absorbance of test sample

A_s = absorbance of standard solution

C = concentration of standard in mg/ml

V_f = total filtrate volume

V_a = volume of filtrate analyzed

D = dilution factor were applicable

Determination of Flavonoid

This was determined gravimetrically using the method described by (Harborne, 1973) [9]. A measured weight, 5g of the samples was boiled in 100ml of 2M of HCL solution for 30mins. The boiled mixture was allowed to cool and then filtered through Whatman No.42 filter paper. The filtrate

was treated with ethyl acetate starting with drop wise addition until in excess. The precipitated flavonoid was recovered by filtration using a weighed filter paper and dried in an oven at 80°C, cooled in a desiccator and reweighed. The difference in weight gave the weight of flavonoid which was expressed as a percentage of a sample weight analyzed. Given by the formula:

$$\% \text{Flavonoid} = W_2 - W_1 / W \times 100/1$$

Where:

W = weight of sample

W₁ = weight of empty filter paper

W₂ = weight of filter paper + flavonoid precipitate

Determination of Alkanoids

This was done by the alkaline precipitation gravimetric method described by (Harborne, 1973) [9]. A measured weight of the sample (5g) was dispersed into 50mls of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand at room temperature for 4hrs. It was later filtered via Whatman No.42 grade of filter paper. The filtrate was concentrated to a quarter of its original volume by evaporation over a steam bath. Alkaloid in the extract was precipitated by drop wise addition of NH₄OH until full turbidity was obtained. The alkaloid precipitate was recovered by filtration using a weighed filter paper and washed with 1% ammonia solution (NH₄OH), dried in the oven at 80°C for an hour. It was cooled in a desiccator and reweighed. By weight difference, the weight of alkaloid was determined and expressed as a percentage of the sample.

$$\% \text{ Alkaloid} = W_2 - W_1 / W \times 100/1$$

Where:

W = weight of sample

W₁ = weight of empty filter paper

W₂ = weight of filter paper + alkaloid precipitate

Determination of Tannins

The Follin-Dennis spectrophotometric method as described by Pearson (1976) [17] was used. A measured weight of each sample (5g) was dispersed in 50mls of distilled water and shaken. The mixture was allowed to stand for 30mins at room temperature, being shaken every 10mins. At the end of 30mins, the mixture was filtered through Whatman filter paper and the filtrate was used for the experiment. A portion, 2mls of the extract was measured into a 50ml volumetric flask. Similarly, 2mls of standard tannic acid solution and 2mls of distilled water were measured into separate flasks to serve as standard and blank respectively. They were also diluted with 35mls of distilled water separately. 1ml of Follin-Dennis reagent was added to each of the flask, followed by 2.5mls of saturated sodium carbonate solution (Na₂CO₃). The content of each flask was made up to mark (50mls) with distilled water and incubated for 90mins at room temperature. The absorbance of the developed color was measured at 620nm wavelength in a spectrophotometer. Readings were taken with a reagent blank at zero. The experiment was repeated two more times to get an average. The tannin content was calculated as shown below:

$$\% \text{ Tannin} = 100/W \times A_{\mu}/A_s \times C/1000 \times V_f/V_a \times D$$

Where:

W = weight of sample analyzed

A_μ = absorbance of the test sample

A_s = absorbance of the standard tannic solution

C = concentration of standard solution in mg/ ml

V_f = total volume of extract

V_a = volume of extract analyzed

D = dilution factor where applicable

Determination of the Toxic Elements/chemicals

An atomic absorption spectrophotometer (AAS) was used to determine the concentration of iron (a trace element/ metal). The solution (digest) from the ash was used. Solutions containing metal ions were aspirated into a flame in which they were converted to a free atom vapor. A monochromatic light source was directed through the flame, and the amount of radiation of a specific energy absorbed by the solution was recorded.

A calibrated graph was then prepared for the element and from this, the amount of the element present in each sample was read.

A general formula as shown below was read:

$$\text{Emg}/100\text{g} = 100/W \times X/100 \times D$$

Where:

W = weight of sample analyzed

X = equivalent concentration (in ppm) done from standardized curve

D = dilution factor.

Determination of Microbial Load

(Total bacterial and fungi count, *staphylococcus* and *staphylococcus* — *shigella* counts)

The spread plate technique (ICMSF-Cheesbrough, 2000) [6] was used. Each test sample was surface disinfected with cotton wool soaked in 70% ethanol and then homogenized in a surface disinfected porcelain laboratory mortar and pestle. A unit weight (1g) of each sample was diluted by mixing with 9ml of sterile distilled water in a test tube (10⁻¹). Subsequently, 1ml portion of the diluent was mixed with 9mls of sterile distilled water in a different test tube to form the second diluent (10⁻²) and the process was continued to the sixth diluent (10⁻⁶). For counts of different microbial types, inocula were aseptically collected from different diluent for total bacterial count, 0.1ml inoculum was collected from fourth diluent of each sample (10⁻⁴) while inocula for fungi count were collected from the second diluent of each sample (10⁻²). Similarly, inocula from the second diluents (10⁻²) were also used for the selective culture and count of *staphylococcus* and *salmonella/shigella* count. For each culture, 0.1ml of the inoculum was aseptically transferred into the surface of fresh sterile appropriate agar in plate with the aid of a glass spreader; the inoculum was spread evenly over the surface of the medium in plate (spread plate technique). Such inoculated plate as sealed, labeled and incubated as follows: Nutrient agar plate for total bacterial count were incubated at 37°C for 24 to 48 hours while Sabouraud dextrose agar plate (for fungal count) were incubated at temperature 28 to 32°C for 2 to 5 days. The mannitol salt agar plate (for *staphylococcus* count) was incubated at 37°C as well as the *salmonella shigella* agar culture plates and for 24 to 42 hours. On establishment of growth, the number of colonies in each

culture plates was counted with the aid of a colony counter and the formula below was used to calculate the number of colony forming units of the organism per gramme of the sample:

$$\text{TVC (cfu/g)} = 1/V \times N \times D$$

Where:

TVC = total viable count expressed as colony forming unit per gramme (cfu/g)

V = volume of inoculum cultured

N = number of colonies counted in each case

D = dilution factor

Antimicrobial Test

Tests were conducted with the water and ethanol extract of the seasonings to show their respective ability to inhibit the growth of the pathogen isolates. The disc diffusion test (Cheesbrough, 2000) [6] was used. The antimicrobial test discs were prepared with absorbance paper (Whatman No. 1) cut in circles of 5mm with the aid of an office paper perforator. The cut discs were boiled in distilled water for an hour (to remove possible residual preservative), drained dry and sterilized by autoclaving. To test for the activity of the extract against the isolates, the isolates in question was inoculated onto a sterile agar plate, then the prepared disc

was dipped into the concentrated extract and allowed to absorb. Carefully, with the aid of a flame pair of forceps, the extract bearing disc was transferred to the inoculated plate. Three extract discs were used for each plate and they were placed at about the same distance from one another and not less than 1cm from the edge of the petri dish. A separate plate was inoculated with each of the test isolates and a standard antibiotic was used in place of the extract disc. Also the inoculated plates with their respective disc were incubated as described earlier. They were observed daily for growth and for the existence of clear zones around the extract disc as a show of inhibition. Where there were inhibition zones, the diameter of such zone were measured with the aid of a transparent ruler to record the extent of activity of the test extract against the organisms.

Statistical Analysis

All data obtained from the analysis of the seasonings samples were subjected to Statistical Analysis System (SAS, 2000). Software package Analysis of Variance (ANOVA) was done and means separation using Fischer least significant difference (LSD) to determine significant difference at 5% probability.

Results and Discussion

Table 1: Anti-Nutrient Properties

Sample	Phytate	HCN	Phenol	Flavonoid	Alkaloid	Tannin
Benny	0.12 ^a ±0.01	2.48 ^b ±0.03	0.29 ^d ±0.00	0.21 ^c ±0.02	0.05 ^a ±0.01	0.11 ^a ±0.00
Curry	0.25 ^d ±0.00	0.56 ^a ±0.08	0.18 ^c ±0.00	0.29 ^d ±0.03	0.05 ^{bc} ±0.01	0.33 ^c ±0.00
Thyme	0.13 ^b ±0.00	1.27 ^a ±0.64	0.36 ^e ±0.00	0.33 ^e ±0.02	0.07 ^c ±0.01	0.36 ^d ±0.00
K.glory	0.12 ^a ±0.00	7.97 ^a ±0.57	0.04 ^a ±0.00	0.11 ^b ±0.01	0.04 ^{ab} ±0.00	0.13 ^b ±0.00
Onga	0.12 ^c ±0.05	2.56 ^c ±2.91	0.18 ^b ±0.14	0.22 ^a ±0.08	0.06 ^a ±0.02	0.21 ^a ±0.12

Values show means of triplicate analysis ± Standard deviation.

Figures with different superscripts in the same column are significantly different (p<0.05)

Table 1 shows the anti-nutrients composition in the regular seasonings. The result shows variations of significant different (p<0.05) in the values of the different anti-nutrients in the different seasonings. Phytate content of the seasonings varied from 0.12%±0.00 to 0.25%±0.00. Phytate was highest in curry (0.25%) and least in kitchen glory (0.12±0.00). Phytate compete with dietary-minerals and render them insoluble and as such non-absorbance during digestion. The value of hydrogen cyanide in the seasonings is very low with a range of 0.56mg/kg±0.08 to 7.97mg/kg. It was highest in kitchen glory and least in curry powder. Hydrogen cyanide is toxic and can be dangerous at high concentrations. However, the range of these toxic substances in the test seasonings was found to be far lower than the critical level of 50.0mg/kg reported by (Onwuka, 2005) [16].

Phenol in the seasoning was in the concentration range of 0.04%±0.00 to 0.36%±0.00. Although phenol is considered to be an anti-nutrient, it has some health benefits in that it possesses antioxidant activity which can protect the body against harmful oxidative processes. Also, phenol has strong antimicrobial activity and forms the main components of disinfectants and antiseptics.

Alkaloids content of the seasonings was in the range of 0.04%±0.01 to 0.06%±0.01. It was highest in thyme

seasoning (0.07%) and least in kitchen glory seasoning (0.04%). According to Harbone, 1973, Alkaloids are toxic when ingested and can cause widespread changes in body physiology. Notwithstanding, Ezeocha *et al*, 2013 observed that alkaloids are now recognized more on the basis of their pharmacological activity rather than toxicity. The flavonoid content of the seasonings was in the range of 0.11 to 0.33%. Thyme had the highest flavonoid content while kitchen glory had the least flavonoid, like phenols, have health benefit courtesy of their antioxidant properties. Value for tannins was least (0.11%±0.00) in Benny seasoning and at highest (0.36%±0.00) in thyme. Tannins are regarded as such anti-nutrient due to their ability to coagulate proteins thereby interfering with protein bioavailability as nutrient (Kim *et al*, 2011).

Generally, therefore it was observed that there were significant differences (p<0.05) between the anti-nutrient content of the different seasonings. Some of these anti-nutrients are known to possess toxicity (e.g. HCN, alkaloids etc) and are therefore recorded as toxic substances in the seasonings. The variation in the levels of the toxic anti-nutrients in the seasonings was attributed to possible effects of processing during production. However, the results of the anti-nutrients were all low compared to what is obtained in most leafy vegetables.

Table 2: Toxic element composition of Regular Seasoning (Mg/100g)

Sample	Fe	Zn	Cu	Mn	Pb
Benny	1.89 ^d ±0.023	2.23 ^a ±0.023	0.68 ^b ±0.439	0.10 ^{ab} ±0.439	0.02 ^a ±0.000
Curry	1.75 ^b ±0.023	12.6 ^d ±0.0630	0.29 ^a ±0.000	0.29 ^b ±0.000	0.03 ^a ±0.046
Thyme	1.92 ^a ±0.000	9.85 ^c ±0.023	0.22 ^a ±0.006	0.22 ^{ab} ±0.006	0.03 ^a ±0.046
K.glory	1.83 ^c ±0.031	10.1 ^c ±0.115	0.12 ^a ±0.010	0.12 ^{ab} ±0.010	0.00 ^a ±0.000
Onga	0.99 ^a ±0.046	6.45 ^b ±0.064	0.07 ^a ±0.006	0.67 ^a ±0.006	0.00 ^a ±0.000

Values show means of triplicate analysis ± standard deviation.

Figures with different superscripts in the same column are significantly different (p<0.05)

Table 2 show the toxic heavy metal content of the regular seasonings. Lead was not detected in two of the seasonings, Onga and Kitchen glory but was in the range of 0.02mg/100g to 0.03mg/100g in the other seasonings. The lead content of the seasonings were all below the minimal acceptable level of 0.05mg/100g (WHO, 2000).^[19] Notwithstanding, the concentration of lead, the toxic metal has a cumulative build up effect when ingested over time. The concentration of iron in the seasonings was in the range of 0.99mg/100g to 1.92mg/100g. Thyme seasoning had the highest iron concentration while Onga seasoning had the least. Dietary iron is essential because of the role of the

element in building blood as well as in oxygen transport (Abbaspour *et al*, 2014)^[1].

The level of iron in seasonings implies that the seasonings may play complementary roles as sources of dietary iron. The values for zinc content of the seasonings was between 2.23mg/100g and 12.6mg/100g while that of Copper and Manganese were between 0.07mg/100g and 0.69mg/100g and from 0.01mg/100g to 0.67mg/100g respectively. Copper, Zinc and Manganese are required in small amount for good functioning of the body. While observing the possible benefit of having dietary mineral, Zinc, Iron, Copper etc in the seasonings, the need for caution was noted as the minerals have long time effects on the body.

Table 3: The Microbiota of Regular Food Seasonings

Sample	Bacterial (cfu/g x 10 ³)	Fungal (cfu/g x 10 ³)
Benny	17.0 ^c ±1.73	1.67 ^b ±0.58
Curry	9.67 ^a ±1.15	0.00 ^a ±0.00
Thyme	8.67 ^a ±1.15	1.33 ^b ±0.58
Kitchen glory	14.7 ^b ±2.31	2.00 ^b ±0.00
Onga	15.0 ^b ±1.00	1.67 ^b ±0.58

Values shows means of triplicate analysis ± standard deviation.

Figures with different superscript in the same column are significantly difference (p<0.05)

The microbiota of the seasonings is shown in table 3 above. The result shows significant differences (p<0.05) in mean microbial loads of the different seasonings. The bacterial load ranged from 8.67x10³cfu/g ±1.15 to 17.0 x 10³cfu/g ±1.73 while the mean fungal load was in the range of 0.00 to 2.00 x 10³cfu/g ±0.00. Benny seasoning had the highest bacterial load while thyme had the least bacterial load. The highest fungal load was recorded at kitchen glory seasonings while the least which is non-detection of fungi was observed in curry seasoning.

The variation in the microbial load was attributed to possible varied levels of inhibition of microbial proliferation in the seasonings which may also be due to their varied

phytochemical constituents. Varied levels of phytochemicals with established antimicrobial activity were recorded in the seasonings including phenols, alkaloids, tannins, HCN etc (Table 1). Secondly, the low microbial load of the seasonings could be due to processing techniques during production which may be tailored toward discouraging microbial growth.

The presence of the microbes observed in this research work was considered not to be of serious concern since all the seasonings are used in cooked foods and the heat of cooking destroys most bacteria except the spores in some cases such as *Bacillus species* (Ververidis *et al*, 2007)^[18].

Table 4: Antimicrobial Activities of Regular food Seasonings

Sample	<i>Staphylococcus</i>	<i>Bacillus</i>	<i>Pseudomonas</i>	<i>Yeast</i>	<i>Aspergillus</i>
Benny	7.67 ^b ±0.58	9.33 ^{ab} ±0.58	9.60 ^b ±0.58	7.67 ^{ab} ±0.58	8.33 ^b ±0.58
Curry	10.7 ^c ±0.58	11.0 ^b ±1.73	12.7 ^c ±0.58	10.3 ^c ±1.15	10.3 ^c ±0.58
Thyme	11.3 ^c ±0.58	13.7 ^c ±0.58	13.3 ^c ±0.58	8.00 ^b ±1.00	11.7 ^c ±0.58
K.glory	7.67 ^b ±0.58	8.33 ^a ±0.58	9.33 ^b ±0.58	6.67 ^{ab} ±0.58	6.33 ^a ±0.58
Onga	0.00 ^a ±0.00	8.00 ^a ±1.00	6.67 ^a ±0.58	6.33 ^a ±0.58	6.33 ^a ±0.58

Values show means of triplicate analysis ± standard deviation.

Figures with different superscripts in the same column are significantly different (p<0.05)

Table 4 shows the antimicrobial activity of the seasonings. The result shows variation of significant differences (p<0.05) in the diameters of the inhibition zones created by extracts of the seasonings. There were also variations between levels of resistance susceptibility of the different test organisms to the different test seasonings. The variations were of significant differences (p<0.05).

All the seasonings were potent against the organisms but to varying extents. Against *Staphylococcus aureus*, Onga was the least active and did not inhibit the organisms while the potent ones had diameters of inhibition zones ranging from 7.67mm to 11.3mm. Kitchen glory seasoning was the least while thyme had the highest value. Similarly, thyme extract was the most potent against *Bacillus* (subtiles) with

inhibition zones of $13.7\text{mm} \pm 0.58$ while Onga had the least of $8.00\text{mm} \pm 1.00$. *Pseudomonias aureginiosa* had the least resistance against thyme having been inhibited to the time of 13.3mm diameter where as its resistance to Onga was very high and show inhibition of first 6.67mm diameter.

On a general scale, the yeast and the mould *aspergillus* were less inhibited than the bacteria. The inhibition zone created by the different seasonings against yeast was in the range of 6.33mm to 10.3mm while that of *Aspergillus niger* was between 6.33mm and 11.7mm. The variation in the potency of the seasonings against the different microbes was perhaps due to the different phytochemicals (and their concentrations) in the seasonings as well as possible effects of processing on them. Also, the resistance of the different microbes determines their levels of inhibition by antibiotics (Cushine, 2005) [7].

It is recommended, based on findings in this work that the consumption of these regular seasonings should not be discouraged. However, they should be used for cooked foods such that heat will act on the anti-nutrients and microorganisms to improve the safety level. The use of the seasonings in non-heat processed foods should be discouraged.

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