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Antibacterial property of commonly used spices in Abuja, Nigeria

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ABSTRACT— Spices are additives to improve the flavor, taste, and colour of food. Spices are also known to extend shelf life by inhibiting growth or decreasing food borne pathogens. The study is aimed to evaluate seven spices for antibacterial properties on some bacteria. The local and botanic names of the seven spices are Ehuru (*Monodora myristica*), Uziza (*Piper guineese*), Turmeric (*Curcuma longa*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Cayenne pepper (*Capsicum annum*), cinnamon (*Cinnamonum cassie*) were purchased in the market in Abuja, Nigeria and identified in Herbarium Unit of National Institute for Pharmaceutical Research and Development, these spices were dried and pulverized into powder. These powders were extracted with 70% methanol into crude extracts. The crude extracts were screened for antibacterial property against *Salmonella typhi*, *Escherichia coli*, *Staph. aureus and Bacillus subtilis* at varied concentration of 80,40, 20 and 10 mg/mL using the agar. *Curcuma longa* and *Piper guineese* inhibited the growth of test bacteria at the concentration of 10 mg/mL while *Cinnamonum cassie* and Capsicum *annuum* inhibited the growth of three of the test bacteria. *Zingiber officnale* crude extract was found to be resistance against to *E. coli* and *Salmonella typhi*, while Allium *sativum* extract was also exhibit resistance to *Sal. typhi* and E. coli growth. *Monodora myristica* crude extract was found to have not inhibitory potential on the test bacteria. The concentration of 10 mg/mL of each of the extract was found that *C. lunga* had the highest zone of inhibitions against the test bacteria. The study revealed the antibacterial potentials of these spices on opportunistic and spoilage microorganisms and can therefore be used in food preparations as medicinal additives.

KEYWORDS: Spices, methanolic extraction, spice crude, agar diffusion antibacterial activity, test bacteria, growth inhibition

1. INTRODUCTION

Spices have been applicable as food additives. They improve the flavor, taste and color of food, as well as extend the shelf life of food by inhibiting the growth or decrease the food borne pathogens. Plants have been known as natural antimicrobials for long and have been applied for the preservation of food and other consumable products. Spices such as cinnamon, garlic, ginger, and mint, among many others can substitute the health remedies. Most of spices show antimicrobial activity against bacteria, yeasts, and molds. The biological activity of spices based on the phenolic compounds, so can be effectively applied as food preservatives. Spices can be classified according to their antimicrobial activities into three categories; the first classified as strong (cinnamon, clove, mustard), the second as medium (all spices, sage, bay leaf, caraway, coriander, cumin, rosemary, thyme, oregano), and the third as weak (black pepper, red pepper, ginger) [35]. Although there has been a massive development in technology and medicine in recent years, many countries however, still practice folk medicine in the treatment of diseases and infections as well as relief of pains using natural plant products such as herbs and spices. Aside addition of color, taste and flavor, spices have been reported to confer health benefits to its consumers such as antioxidant, antidiabetic, anti-inflammation, antifungal, and antibacterial among many other benefits [9]. The antibacterial activities of M. myristica have been reported by various studies including [2], [3], [27], [10], [4], [34] among many others using varying

solvent during the extraction process. Antibacterial activities of Zingiber officinale has been evaluated and reported by scientist across the globe including but not limited to reports of [15], [5], [22], [26], [33], [31], [6], [1]. Allium sativum have been known to possess antibacterial activities against both Gram positive and negative bacterial due to the presence of potent and active compound known as allicin [11]. [20] evaluated effects of solvent used in the extraction of A. sativum. In their reports, some solvents exhibited antibacterial activities while others did not at the same concentration against the same microorganism. Study by [36] also reported similar pattern of antibacterial activities exhibited by methanolic extract of A. sativum against all the test microorganisms. Study of [38] pointed out effect of climate and regions on the antimicrobial activities of A. sativum. In their results, antimicrobial activities of A. sativum from China were different from those from Turkey, thus, explaining the varying reports documented so far.

The antimicrobial activities of C. annuum against Gram positive bacteria were also reported by [17], [18], [21], [12]. There were studies conducted by [37], [40], [30], [32] among many others have reported antibacterial activities of C. cassie against both Gram positive and negative bacteria. The antibacterial activity of C. lunga have been reported by [7], [14], [24], [16] against Gram positive and negative bacteria. The antibacterial activity of Piper guineense was reported by [28] against S. aureus and E. coli with susceptibility higher in E. coli. This study is aimed at the determining the antibacterial property of Monodora myristica, Zingiber officinale, Allium sativum, Capsicum annuum, Cinnamonum cassie, Piper guineense and Curcuma longa against of selected Gram positive and negative bacteria.

2. MATERIALS AND METHODS

2.1 Collection of Spices

Ehuru (Monodora myristica), Uziza (Piper guineese) was purchase at Karmo market Abuja, while Turmeric (Curcuma longa), Garlic (Allium sativum), Ginger (Zingiber officinale), Cayenne pepper (Capsicum annum), cinnamon (Cinnamonum cassie) was purchased at Garki market Abuja. These spices were identified in the Herbarium Unit of National Institute for Pharmaceutical Research and Development (NIPRD) by taxonomist.

2.2 Extractions of the spices

Each of the spice plant materials were dried at 250C for 3 day and grind into powder. The pulverized materials were sieved in muslin and 250 g was added into 70% methanol for 24 hours. The supernatant was dried at 380C to obtained crude extract.

2.3 Test microorganisms

Salmonella typhi, Escherichia coli, Staphylococcus aureus and Bacillus subtilis were obtained from Department of Microbiology and Biotechnology of National Institute for Pharmaceutical Research and Development. These test microorganisms were authenticated using selective media and biochemical tests.

2.4 Antimicrobial susceptibility study

Agar diffusion technique was used (NCLS, 2003). One gram each of spice crude was dissolved in 1.0mL of dimethysuphloxide (DMSO) and added to 5.0mL sterile distilled water. A concentration of 80 mg/mL was made and further 1:2 dilutions to obtained 40, 20, 10 mg/ml. Muller Hinton agar Petri dishes were prepared and each inoculated with specific test bacteria and allowed to dry. Five wells were bored on seeded Muller Hinton agar with 6mm cork borer. The base covered with molten agar to avoid flow at the base. 100µl of the diluted crude was dispensed on the labeled wells. This procedure was repeated for each of the test bacteria. All plates were incubated at 370C. The zones of inhibition of each plate were observed and measured with

Vernier clapper and records recorded accordingly.

2.5 Statistical analysis

All data obtained was statistically analyzed using the one-way ANOVA. The data was expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

Table 1 depicts the antimicrobial activity of the methanol extract of M. myristica against S. typhi, Escherichia coli. Staphylococcus aureus and B. subtilis. M. myristica was found to be active against B. subtilis only at all concentration (80, 40, 20 and 10 mg/mL). M. myristica had no activity against S. typhi, E. coli, and S. aureus, S. typhi, E. coli and S. aureus.

Table 1: The antimicrobial activity of methanolic extract of M. myristica against test bacteria

	Zone of Inhibition (mm)				
Isolate	**80** (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**C** (50 µg/mL)
S. typhi	-	-	-	-	18.0 ± 0.0
E. coli	-	-	-	-	19.0 ± 0.0
S. aureus	-	-	-	-	12.0 ± 0.0
B. subtilis	11.5 ± 0.7	10.5 ± 0.7	10.0 ± 0.0	10.0 ± 1.4	12.0 ± 0.0

Key: - = No activity, S. typhi = Salmonella typhi, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis.

Table 2 shows the antimicrobial activity of Zingiber officinale against S. typhi, E. coli, Staph aureus and B. subtilis. Z. officinale had no activity against S. typhi and E. coli without zone of inhibition. Z. officinale had against Staph aureus and B. subtilis at 80, 40 and 20 mg/mL concentrations.

Table 2 Antimicrobial activities of methanol extract of Zingiber officinale

Isolate	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**C** (50 µg/mL)
S. typhi	-	-	-	-	15.0 ± 0.0
E. coli	14.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	17.0 ± 0.0
S. aureus	21.0 ± 1.4	17.0 ± 1.4	15.0 ± 0.0	13.0 ± 1.4	26.0 ± 1.4
B. subtilis	-	-	-	-	24.0 ± 0.0

Key: - = No activity, S. typhi = Salmonella typhi, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis.

Table 3 represents results of antibacterial activities of Allium sativum methanol extract. A. sativum was found to be active against S. aureus and E. coli across all concentration with the highest (21.0 ± 1.4 mm) zone of inhibition exhibited against S. aureus at 80 mg/mL and the lowest (11.0 ± 0.0 mm) against E. coli at three different concentration (40, 20 and 10 mg/ml).

Table 3: Antimicrobial activity of Allium sativum against test bacteria

Isolate	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**C** (50 mg/mL)
S. typhi	-	-	-	-	17.5 ± 0.7
E. coli	-	-	-	-	19.0 ± 0.0
S. aureus	11.0 ± 1.4	5.5 ± 3.5	5.5 ± 0.7	-	20.0 ± 0.0
B. subtilis	9.3 ± 5.7	5.5 ± 3.5	6.0 ± 0.0	-	19.0 ± 0.0

Key: - = No activity, S. typhi = Salmonella typhi, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis.

Capsicum annuum exhibited antimicrobial activities against all the test microorganisms with zones of inhibition measured to be ≥ 11.0 ± 0.0 mm. The highest zone (15.0 ± 0.0 mm) of inhibition was recorded against B. subtilis at concentration of 80 mg/mL while the lowest (11.0 ± 0.0 mm) was recorded against all the test microorganisms but at varying concentration as depicted in Table 4.

Table 4. Antimicrobial activities of spices of methanol extract Capsicum annuum

Isolates	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**C** (50 µg/mL)
S. typhi	12.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	12.0 ± 0.0
E. coli	12.0 ± 0.0	12.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	18.0 ± 0.0
S. aureus	13.0 ± 0.0	12.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	28.0 ± 0.0
B. subtilis	15.0 ± 0.0	11.0 ± 0.0	-	-	25.0 ± 0.0

Key: - = No activity, S. typhi = Salmonella typhi, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis.

Antimicrobial activities of methanol extract of C. cassie was highest at concentration of 80 mg/mL and 40 mg/mL against B. subtilis with a diameter of18.0 ± 0.0 mm and 15.0 ± 0.0 mm respectively followed by S. typhi (14.5 ± 3.5 mm) and S. aureus (14.5 ± 0.7 mm). The lowest (9.0 ± 0.0 mm) zones of inhibition were however recorded against S. typhi at concentration of 40 mg/ml. S. aureus was resistant to other concentrations (i.e., 40, 20 and 10 mg/mL) aside 80 mg/ml as shown in Table 5.

Table 5. Antimicrobial activities of spices of methanol extract of Cinnamonum cassie

Isolates	Zone of Inhibition (mm)				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**C** (50 µg/mL)
S. typhi	14.5 ± 3.5	10.5 ± 0.7	9.0 ± 0.0	9.0 ± 0.0	12.0 ± 0.0
E. coli	11.5 ± 0.7	10.5 ± 0.4	10.5 ± 0.7	9.5 ± 0.7	17.0 ± 0.0
S. aureus	14.5 ± 0.7	-	-	-	19.0 ± 0.0
B. subtilis	18.0 ± 0.0	15.0 ± 0.0	12.0 ± 0.0	11.0 ± 0.0	19.0 ± 0.0

Key: - = No activity, S. typhi = Salmonella typhi, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis.

Table 6 indicates the antimicrobial activity of methanol extract of Piper guineense against test bacteria. P. guineense was active against all the test microorganisms. The highest zone (11.0 ± 0.0 mm) of inhibition was recorded against S. typhi followed by 10.5 ± 0.7 mm against E. coli both at 80 mg/mL. P. guineense lowest zones (8.0 ± 0.0 and 8.5 ± 0.7 mm) of inhibition were recorded against S. aureus and B. subtilis at 40 mg/mL and 10 mg/mL respectively.

Table 6: Antibacterial activity of Piper guineense against test bacteria

Isolates	**Zone of Inhibition (mm)**				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**C** (50 mg/mL)
S. typhi	14.0 ± 0.0	12.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	17.0 ± 0.0
E. coli	12.0 ± 0.0	12.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	19.0 ± 0.0
S. aureus	14.5 ± 0.4	13.5 ± 0.7	12.5 ± 0.7	11.0 ± 0.0	29.0 ± 0.0
B. subtilis	14.0 ± 1.4	14 ± 2.8	11.0 ± 0.0	10.0 ± 0.0	23.0 ± 0.0

Key: - = No activity, S. typhi = Salmonella typhi, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis.

Antimicrobial activities of methanol extract of C. longa shown on Table 7. Curcuma longa had activity against S. typhi, E. coli, Staph aureus and B. subtilis. evidence by zone of inhibition recorded across varying concentrations. The highest zone (11.0 ± 0.0 mm) of inhibition was obtained against S. typhi followed by E. coli (10.5 ± 0.7 mm) while the lowest (8.0 ± 0.0 mm) zone of inhibition was recorded against S. aureus.

Table 7 Antimicrobial activities of methanol extract Curcuma longa

Isolate	**Zone of Inhibition (mm)**				
	80 (mg/mL)	**40** (mg/mL)	**20** (mg/mL)	**10** (mg/mL)	**C** (50 mg/mL)
S. typhi	11.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	9.5 ± 0.7	15.5 ±
E. coli	10.5 ± 0.7	10.0 ± 0.0	9.5 ± 0.7	9.5 ± 0.7	20.5 ±
S. aureus	8.5 ± 0.7	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	27.5 ±
B. subtilis	9.5 ± 0.7	9.5 ± 0.7	9.0 ± 0.0	8.5 ± 0.7	21.5 ±

Key: - = No activity, S. typhi = Salmonella typhi, E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis.

3.1 Discussion

In this study, the antimicrobial activities of methanol extract of M. myristica was evaluated and found to be effective against only Gram-positive B. subtilis across all concentrations. This result is similar to the one obtained by [2] where only B. subtilis among the Gram- positive bacteria was susceptible to ethanol extract of M. myristica at the highest concentration of 33 mg/mL used. In this study, S. aureus and E. coli were resistant to methanol extract of M. myristica, which is similar to the result obtained by [2]. However, [27] reported slightly dissimilar result. In their study, E. coli was susceptible to both soluble and insoluble ethylacetate

fractions of M. myristica with 30 mm and 21 mm zone of inhibition respectively. This variation could be as a result of different solvent used or the fact that [27] used fractions against crude extract used in this study. Gram negative bacteria were shown to be resistant to crude extracts of M. myristica in this study, which is similar to results of [4] as significant zones of inhibition were recorded at high concentrations (150 and 100 mg/mL) whereas concentrations less than 100 mg/mL showed little or no inhibition against the Gram-negative bacteria tested. In this study, E. coli and S. typhi demonstrated resistance against Z. officinale across all concentrations with zones of inhibition less than 12 mm obtained from B. subtilis and S. aureus test microorganisms. [26] however, reported Z. officinale to be active against E. coli with 15.00 ± 3.54 mm and 13.00 ± 2.66 mm zones of inhibition obtained at 100% and 50% fresh Z. officinale respectively. This activity could be associated with method of processing and state (dried or fresh) of the rhizomes before extraction as the dried rhizomes of Z. officinale was used in this study. However, [26] evaluated antibacterial activities for both fresh and dried rhizomes of Z. officinale and activities were recorded with 15.00 ± 3.54 mm for the fresh and 14.50 ± 6.08 mm for the dried Z. officinale. E. coli 82MR and S. typhi ATCC 19430 demonstrated susceptibility in the studies carried out by [22] with zones of inhibition 13.66 ± 1.54 and 9.66 ± 0.57 mm respectively. These activities recorded against E. coli 82MR and S. typhi ATCC 19430 could be attributed to the hydro-distillation extraction process used.

In this study, A. sativum was active against both Gram positive (S. aureus) and negative (E. coli) bacteria with zone of inhibition of 21.0 ± 1.4 mm and 14.0 ± 0.0 mm respectively. Antibacterial activities of A. sativum were also reported against S. aureus and E. coli by [15]. Although B. subtilis was resistant to methanol extract of A. sativum at all concentration in this study was however not the case as reported by [15]. In their study, A. sativum exhibited antibacterial activities across all the concentration used (25, 50, 100 and 200 µg/mL) with zones of inhibition ranging from 11.00 ± 0.32 to 16.55 ± 0.25 mm. [20] evaluated effects of solvent used in the extraction of A. sativum. In their reports, some solvents exhibited antibacterial activities while others did not at the same concentration against the same microorganism. Thus, solvents to a great extent plays important role in the antimicrobial properties of A. sativum. From their results, methanol extract of A. sativum was active against all the test microorganisms in their study and the activities were concentration dependent. The higher the concentration the bigger the zone of inhibition recorded, likewise the smaller the zones recorded with lower concentration. Study by [36] also reported similar pattern of antibacterial activities exhibited by methanolic extract of A. sativum against all the test microorganisms. C. annuum was found to be more active against B. subtilis, S. aureus, E. coli and S. typhi with zones of inhibition ranging from 11.0±0.0 to 15.0±0 mm. This finding agreed with results of [17], [37], [40], [30], which reported C. annuum to be active against the test isolates in this study. However, results of this study did not correspond with that of [12], [32], which reported low zones (≤ 9.7 ± 0.26 mm) of inhibition against S. aureus and E. coli when challenged with ethanolic and aqueous extract of C. annuum. [18] also reported resistance among Gram negative bacteria to C. annuum. [21] reported that selective antibacterial ability in C. annuum is due to the presence of dihydrocapsaicin that has effect on mainly the cell wall containing peptidoglycan. Piper guineense in this study showed antibacterial activities against all the test microorganisms with zones of inhibition between 8.0 ± 0.0 to 11.0 ± 0.0 mm with S. typhi and E. coli being the most susceptible. The ethanol and aqueous extracts reported by [28] all had antimicrobial activities against S. aureus and E. coli which is similar to the result obtained in this study. C. lunga had varied zones of inhibition with different concentration when challenged with B. subtilis, E. coli, Staph. aureus and Sal. typhi which was similarly to reports of [7], [14], [24] reported higher zones of inhibition against Staph. aureus and E. coli ranging 21.3 to 26 mm. Though, [16] reported lower zones of inhibition for E. coli and Staph. aureus at 100 mg/mL.

3.2 Conclusion

The seven (7) spices evaluated for antibacterial properties were all active against one or more of the test microorganisms at varying concentration. Difference in antimicrobial activities within the spices could be associated to different phytochemicals presents in each individual spice since they all came from different genera. The varying activities of individual spice against the test microorganisms are often related to the bacterial cell wall. These spices justify inclusion in foods to enhance its elongation and preservation against food borne pathogens.

4. References

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