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Original Research Article

Determination of Susceptibility of Foodborne Pathogens Isolated among Children to the Extracts of Commonly used Spices

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Abstract: Foodborne diseases are illnesses resulting from the consumption of food contaminated with pathogenic microorganisms, their toxins, or harmful chemicals. The study was aimed to investigate the phytochemical constituents and antibacterial efficacy of some spices (*Zingiber officinale*, *Allium sativa*, *Piper nigrum* and *Syzygium aromaticum*) extracts against some food borne pathogens (*Klebsiella* spp, *Salmonella* spp, *Shigella* spp, and *Escherichia coli*). Ethanol extracts from the spices were prepared, screened for phytochemical constituents using conventional methods and tested for antibacterial activity using agar well diffusion methods. Phytochemical screening of the extracts showed that the extracts contain Alkaloid, Anthraquinone, saponin, tannin, phenol, steroid, flavonoid and terpenoid. Alkaloid was found to be the most abundant constituent. The result of Antibacterial activity of the extract showed that the extract demonstrated activity against the isolates. Based on the susceptibility of the organisms to the extracts, E. coli was found to be the highest susceptible organisms, followed Salmonella typhi while least activity was shown by Klebsiella. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts range from 3.125 to 50 mg/ml. There is no significant difference on the susceptibility of the organisms against the extracts at $p < 0.05$. The results of present study have provided the justification for therapeutic potential of the extracts and also its use as dietary supplement for food flavouring and preservation.

Keywords: Antibacterial Activity, Extract, Pathogenic Bacteria, Phytochemical.

INTRODUCTION

Foodborne diseases are illnesses resulting from the consumption of food contaminated with pathogenic microorganisms, their toxins, or harmful chemicals. The World Health Organization (WHO) estimates that more than 200 diseases are transmitted through contaminated food, with diarrheal diseases accounting for the majority of cases. Globally, unsafe food causes hundreds of millions of illnesses annually, with children under five years bearing a disproportionately high burden of severe disease and death (WHO, 2025). The economic consequences are equally substantial due to healthcare costs, reduced productivity, and long-term health complications (Ali *et al*., 2018b).

Among children, foodborne diarrhea is primarily caused by bacterial pathogens such as

Escherichia coli (especially enterotoxigenic and enteropathogenic strains), *Salmonella* species, *Shigella* species, *Campylobacter jejuni*, *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens*. Viral agents such as rotavirus and norovirus and protozoan parasites such as *Giardia lamblia* and *Cryptosporidium* species also contribute significantly to childhood diarrhe (Pundir *et al*., 2010). These pathogens are commonly transmitted through contaminated food, drinking water, improperly cooked meals, poor food handling practices, and inadequate personal hygiene. Recent global evidence indicates that contaminated food remains an important source of diarrheal diseases among children, particularly in resource-limited settings (Nas *et al*., 2018).

Foodborne bacterial infections usually manifest with symptoms such as watery or bloody diarrhea, vomiting, nausea, abdominal cramps, dehydration, fever, electrolyte imbalance, and weight loss. Severe infections may lead to complications including septicemia, hemolytic uremic syndrome, kidney failure, neurological disorders, growth retardation, and death if not promptly diagnosed and treated. Persistent diarrhea also contributes significantly to childhood malnutrition, impaired cognitive development, weakened immunity, and poor educational outcomes (Ali *et al*., 2018c).

The growing challenge of antimicrobial resistance has stimulated interest in identifying alternative antimicrobial agents from natural sources. Medicinal plants and culinary spices have received considerable scientific attention because they contain numerous bioactive phytochemicals with antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties (Balogun *et al*., 2016). Commonly used spices such as garlic (*Allium sativum*), ginger (*Zingiber officinale*), cloves (*Syzygium aromaticum*), black pepper (*Piper nigrum*), cinnamon (*Cinnamomum verum*), and chili pepper (*Capsicum* species) contain phytochemicals including allicin, gingerol, curcumin, eugenol, cinnamaldehyde, thymol, carvacrol, flavonoids, tannins, alkaloids, phenolic compounds, and terpenoids. These compounds exhibit broad-spectrum antimicrobial activity against numerous foodborne bacterial pathogens through mechanisms such as disruption of bacterial cell membranes, inhibition of protein synthesis, interference with nucleic acid replication, suppression of biofilm formation, and inhibition of bacterial enzyme activity (Van Vuuren and Viljoen, 2011).

Although spices have been traditionally used for centuries as food preservatives and medicinal agents, scientific evaluation of their effectiveness against locally isolated foodborne pathogens remains limited in many parts of Nigeria. Determining the susceptibility of bacterial isolates obtained from diarrheic children to extracts of commonly consumed spices may identify inexpensive, readily available, and culturally acceptable alternatives or complementary therapies for managing foodborne bacterial infections. Furthermore, such studies may contribute to the discovery of novel antimicrobial compounds capable of combating multidrug-resistant bacteria.

Study Area

The study sites include Sheikh Muhammad Jidda specialist Hospital, Muhammad Abdullahi Wase Teaching Hospital and Murtala Muhammad Specialist Hospital all in Kano Metropolis. Kano State is located in the North-western Nigeria, it is coordinated at latitude 11^0 30’ N and longitude 8^0 30’ E (Wikipedia, 2021). It shares borders with Kaduna State to the South-West, Bauchi State to the South-East, Jigawa State to the East and Katsina State to the North. It has a total area of 20,131km^2 (7,777sqm) and projected population of 17.5 million as of 2026 (WPR, 2026).

Collection and Identification of Plant Materials

Potential medicinal plants traditionally being used for the treatment of diarrhea infection were used for the study. Accordingly, four spices namely ginger, clove, black pepper and garlic claimed to be traditionally used against diarrhea infections were obtained. Identification and authentication of the plant materials was conducted at Herbarium unit in the Department of Plant Science Bayero University Kano. Voucher specimen was deposited there for future reference. The samples were washed with water to remove dust and rinsed with distilled water. Samples were air dried for two-weeks and pulverized into powder form using sterile mortar and pestle in the laboratory as described by Ali *et al*., (2017). The powdered samples were bagged in a black polythene bag and store in air tight container for further use.

Extraction of Plant Materials

Ethanol was used as solvent in the extraction process. About 100 g of the fine powder of the plant's parts were subjected to Soxhlet extraction by using ethanol for 48 hours. The extract was evaporated and dried at 40 °C under reduced pressure using rotary vacuum evaporator. The extract yields were weighed, stored in dark air tight container at 4°C (Kumar, 2020).

Phytochemical Screening

Preliminary Phytochemical Screening

Phytochemical screening of the plant materials was conducted using the method adopted by Tiwari *et al*., (2011). Wagner's test for determination of alkaloid, Ferric chloride test for phenol, gelatin test foe tannin, lead acetate test for flavonoid, foam test for saponin, acetic acid test for steroid, Salkowski test for terpenoid detection, Fehling's test for glycoside and Benzene test for determination of Anthraquinones.

Quantitative Phytochemical Analysis

Various methods will be employed in determining the number of bioactive components (phytochemicals) present in the plant materials. Terpenoids, steroids, and tannins will be determined using Spectrophotometric method while phenol will be determined using Folin Ciocalteu procedure. The alkaloids, flavonoids, and the content of saponins will be evaluated using analytical method (Ali *et al*., 2018a)

Test Isolates

Six different bacterial isolates namely *Klebsiella* spp, *Salmonella* spp, *Shigella* spp, and *Escherichia coli* were isolated from the stool samples of children diagnosed with foodborne illness the study hospitals. All bacterial organisms were identified using different available procedures including Gram's stain, cultural characterization and Biochemical tests including; Indole, Methyl red, Voges Proskauer, Catalase, Citrate utilization and coagulase tests. The isolates were maintained on Nutrient agar slants before use.

Antibacterial Activity of the Extracts

The agar well diffusion method was used to determine the antibacterial activity of the spices extracts as described by Ali *et al*., (2017). A 0.1 ml volume of the different standardized organisms (0.5 McFarland) were introduced onto the surface of freshly prepared Mueller Hinton agar in a sterile Petri dish and allowed to set and then labeled. A 6 mm sterile Cork borer was used to punch holes (i.e. 5 wells) in the inoculated agar and then removed. Four of the wells were filled with different concentrations of the extract which were labeled accordingly; 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml, while the 5th well contained the control, 5 mg/mL of Ciprofloxacin (Oxoid UK) as positive control in this research. These were left on the bench for 1 hour to enable proper diffusion of the extracts and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along straight line. The experiment was conducted in triplicate and average value were evaluated (Ali *et al*., 2017).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined using the micro-dilution method as described by Clinical and Laboratory Standards Institute (CLSI, 2010). Serial two-fold dilutions of all the extracts were prepared with sterile saline in a 96-well microtiter plate, obtaining a concentration range from 100 to 6.25 mg/mL (100, 50, 25, 12.5 and 6.25 mg/mL). It was followed by addition of 5 µL of the isolates suspension which were added to the wells containing the dilutions. Each dose was assayed in triplicate. Un-inoculated wells containing sterile saline were used as controls. After incubation for 24 hours at 37°C, the samples were observed. MIC was recorded as the lowest concentration of each plant extract that inhibited the bacterial growth as detected by the absence of visual turbidity. To estimate the MBC, an aliquot of each well that did not show microbial growth in the prior tests were swabbed on the entire surface of Mueller Hinton Agar plates and then incubated for 24 hours at 37°C. The lowest concentration that prevented the bacterial growth was registered as MBC (Ali *et al*., 2017).

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the extracts used were analyzed using one way Analysis of Variance (ANOVA) with the aid of statistical program SPSS (Statistical package for Social Sciences) version 21.0. Significance level for the differences for both the tests will be set at $p<0.05$.

RESULT

Qualitative phytochemical Constituents

The phytochemical constituents of aqueous of rhizome of *Zingiber officinale*, bulb of *Allium sativa*, seeds of *Piper nigrum* and buds of *Syzygium aromaticum* are tabulated in table 1. The result showed the present of diverse range of phytochemicals such as alkaloid, flavonoid, glycoside, reducing sugar, saponin, steroid, phenol, anthraquinone and tannin.

Table 1: The phytochemical constituents of the Spices

Phytochemicals	*Z. officinale*	*A. sativa*	*S. aromaticum*	*P. nigrum*
Alkaloids	+	+	+	+
Flavonoid	+	+	+	+
Glycosides	+	+	-	-
Reducing sugar	+	+	-	-
Saponin	+	+	+	+
Steroids	+	+	+	+
Phenols	+	+	+	+
Terpenoid	+	+	+	+
Anthraquinones	+	+	+	+
Tannin	+	+	-	+

Key: + = Presence of phytochemical, - = absent of phytochemical

Quantitative phytochemical Constituents

The quantitative phytochemical constituents of aqueous of rhizome of *Zingiber officinale*, bulb of *Allium sativa*, seeds of *Piper nigrum* and buds of *Syzygium aromaticum* are tabulated in table 3. Alkaloids were found to be the most abundant phytochemical in all the spices.

Table 2: Phytochemical content of the Spices

Phytochemicals	*Z. officinale*	*A. cepa*	*S. aromaticum*	*P. nigrum*
Alkaloids	12.15±0.04	11.30±0.09	9.60±0.12	13.10±0.11
Flavonoid	4.50±0.02	4.00±0.01	3.00±0.09	5.25±0.10
Glycosides	0.08±0.01	1.80±0.02	0.00±0.00	0.00±0.00
Reducing sugar	0.10±0.01	3.62±0.05	0.00±0.00	0.00±0.00
Saponin	1.20±0.01	5.30±0.03	3.70±0.00	0.06±0.00
Steroids	0.07±0.00	0.05±0.00	1.80±0.04	1.27±0.01
Phenols	0.10±0.01	0.08±0.01	0.10±0.01	0.70±0.01
Terpenoid	0.15±0.00	0.09±0.01	1.70±0.01	0.17±0.01
Anthraquinones	1.20±0.01	1.30±0.01	1.20±0.03	0.90±0.01
Tannin	3.70±0.04	4.50±0.02	0.00±0.00	2.20±0.03

Antibacterial Activity of the Extracts

The antibacterial activity of ethanol extract is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates, types of spices and concentration of the extracts.

Table 3: Antibacterial Activity of the Extracts against the Isolates

Concentration (mg/ml)/Zone of Inhibition (mm)							
Isolates	**Extract**	**25**	**50**	**75**	**100**	**Control**	**p-value**
Klebsiella	*Z. officinale*	09.30±0.00	10.70±0.00	12.20±0.00	14.30±0.00	21	0.07054
	A. sativa	10.28±0.20	12.64±0.12	13.62±0.17	14.54±0.17		
	S. aromaticum	10.8±0.20	11.4±0.12	13.2±0.17	15.4±0.17		
	P. nigrum	10.80±0.20	11.40±0.12	13.20±0.17	15.40±0.17		
Salmonella	*Z. officinale*	09.60±0.12	12.10±0.17	12.70±0.26	13.70±0.26	22	0.14871
	A. sativa	12.50±0.12	13.90±0.17	15.40±0.25	17.20±0.20		

Concentration (mg/ml)/Zone of Inhibition (mm)							
Isolates	**Extract**	**25**	**50**	**75**	**100**	**Control**	**p-value**
	S. aromaticum	12.80±0.12	13.50±0.17	14.40±0.25	16.20±0.20		
	P. nigrum	12.80±0.12	13.50±0.17	14.40±0.25	16.20±0.20		
Shigella spp	*Z. officinale*	09.40±0.00	11.70±0.00	12.20±0.19	14.30±0.21	21	0.09372
	A. sativa	10.70±0.32	12.80±0.25	15.10±0.32	18.80±0.37		
	S. aromaticum	11.7±0.32	12.8±0.25	15.1±0.32	19.7±0.37		
	P. nigrum	11.70±0.32	12.80±0.25	15.10±0.32	18.60±0.37		
E. coli	*Z. officinale*	10.30±0.12	11.30±0.17	12.60±0.20	13.30±0.11	22	0.17281
	A. sativa	12.30±0.32	13.90±0.20	15.10±0.12	16.80±0.32		
	S. aromaticum	13.30±0.32	14.20±0.20	15.80±0.12	16.90±0.32		
	P. nigrum	13.3±00.32	14.20±0.20	15.80±0.12	16.90±0.32		

MIC and MBC of the Extracts

Minimum inhibitory concentration and MBC of ethanol extracts of the spices are represented in Table 4. The result showed dilutions of various concentrations of the extracts can inhibit and/or kill the isolates.

Table 4: MIC and MBC of the Extracts against the Isolates

Isolates	Extract	MIC (mg/ml)	MBC (mg/ml)
Klebsiella spp	*Z. officinale*	12.5	50
	A. sativa	6.25	25
	S. aromaticum	6.25	25
	S. aromaticum	6.25	25
Salmonella spp	*Z. officinale*	12.5	50
	A. sativa	6.25	25
	S. aromaticum	6.25	25
	P. nigrum	6.25	25
Shigella spp	*Z. officinale*	12.5	50
	A. sativa	6.25	25
	S. aromaticum	6.25	50
	P. nigrum	6.25	25
Escherichia coli	*Z. officinale*	12.5	50
	A. sativa	3.125	12.5
	S. aromaticum	3.125	12.5
	P. nigrum	6.25	25

DISCUSSION

The results of the present study suggested that several phytochemicals were present in the spices (*Zingiber officinale*, *Allium sativa*, *Piper nigrum* and *Syzygium aromaticum*) extract. The presence of the phytochemicals can be correlated with the fact that solvent extracts showed antibacterial activity against the bacterial isolates. Phytochemicals give plants their colour, flavor, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites (Ali *et al*., 2018a; Nas *et al*., 2018; Ali *et al*., 2018b). The phytochemicals alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin and tannin were present in all the spices extracts according to this study. The results are in accordance with the findings of other authors who have studied these plant (Ali *et al*., 2018a; Nas *et al*., 2018; Ali *et al*., 2018b).

According to this study, Alkaloid is present in the extracts. Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anaesthetics and Central Nervous Stimulants (Ali *et al*., 2018c). Alkaloids are known to play some metabolic roles and control development in living system. It also interferes with cell division, hence the presence of alkaloids in clove could account for their use as antimicrobial agents. Ali *et al*., (2018b) had reported that the antimicrobial properties of spices are desirable tools in food spoilage and food safety. This suggests that the spices extracts which have been confirmed to contain alkaloids may also be useful as preservatives in food. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties (Nas *et al*., 2018). Flavonoids are also present in the extract as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity. It also helps in managing diabetes induced oxidative stress. Steroids are importance in pharmacy as they possess compounds like sex hormones and can be used for drug production (Inas *et al*., 2017). Tannin and saponin were present in the extract. Saponins protect against hypercholesterolemia and antibiotics properties (Ali *et al*., 2018a). In addition, it has been found that saponins have antitumor, antioxidant and anti-mutagenic activities and can lower the risk of human cancers by inhibiting the growth of cancer cells (Nas *et al*., 2018). The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins (Ali *et al*., 2018d).

The results of antibacterial activity of the spices extracts against foodborne pathogens shows that the extracts were effective against the tested isolates. *E. coli* and *Salmonella* were also more susceptible to the extracts in comparison with the *Shigella* and *Klebsiella* respectively. The result of antimicrobial activity of spices in this study was inconformity with the study conducted by many researchers (Ibrahim *et al*., 2010; Aneja *et al*., 2010; Akrayi and Abdulrahman, 2013; Ali *et al*., 2018b; Ali *et al*., 2018c). The antibacterial activities of the spice's extracts are expected due to the presence of compounds such as alkaloid, flavonoids and tannin. The results obtained in this study corroborate with the report of Khan and Siddiqui (2007) who evaluated the antibacterial potential of some spices which showed strongest antibacterial activity against some bacterial isolates. The result of MIC and MBC of the extracts showed that dilutions of various concentrations of the spices can inhibit and/or kill the isolates.

Antimicrobial susceptibility testing of bacterial isolates against extracts in this study provides valuable information regarding their therapeutic potential. Significant inhibition of bacterial growth by garlic, ginger, cloves, and black pepper would suggest that these plants contain bioactive compounds capable of suppressing pathogenic bacteria. Phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, and essential oils present in these spices have been shown to damage bacterial cell walls, alter membrane permeability, inhibit enzyme activity, interfere with DNA replication, and reduce biofilm formation (Farid *et al*., 2023). Differences in susceptibility among bacterial isolates may be explained by variations in bacterial cell wall structure, production of resistance enzymes, efflux pumps, biofilm formation, and the phytochemical composition of each spice extract (Suleiman *et al*., 2023). Gram-positive bacteria often show greater susceptibility than Gram-negative bacteria because the outer membrane of Gram-negative organisms acts as an additional barrier that limits penetration of many antimicrobial compounds (Grace, 2023).

Due to antimicrobial activity of the spices in this study, the findings would support their traditional medicinal use and suggest that they could serve as complementary antimicrobial agents alongside conventional antibiotics. However, crude plant extracts generally contain mixtures of active and inactive compounds whose concentrations vary depending on plant species, geographical location, harvesting season, extraction solvent, and extraction technique.

CONCLUSION

In conclusion, this study revealed that *Z. officinale A. sativa, S. Aromaticum* and *S. aromaticum* extracts possess medicinal properties and antibacterial activity that inhibits bacterial growth. The results of the present study show that extracts of the spices are effective against all tested bacterial isolates. *E. coli and Salmonella* were also more susceptible to the extracts while *Klebsiella* was the least susceptible. The antibacterial activities of the extracts are expected perhaps due to the present of bioactive compounds like Alkaloid, Terpenoid, Saponin, Tannin, flavonoids and Anthraquinones which were dissolved in the solvents. The results of present study have provided the justification for therapeutic potential of the spices and also used as dietary supplement for food preservation.

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