Antioxidant capacity and antibacterial activity of some phyto-spices against some bacterial isolates of foods origin


Affiliation
1Department of Microbiology, Kano University of Science and Technology, Wudil
2Department of Biological Science, Kano University of Science and Technology, Wudil

*Corresponding Author: e-mail: aliyujanzaki@gmail.com
Tel: +234 (0) 8062113271

Abstract
Spices that are mostly of plant origin are used in the preparation of almost all processed food to enhance palatability, tastiness, sweetness, and its overall acceptability, without taking into consideration of its medicinal values. The study was conducted to determine the antioxidant capacity and antibacterial activity of the extracts of Allium sativum, Syzygium aromaticum, and Zingiber officinale against some bacterial isolates of foods origin including Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella dysenteriae, and Staphylococcus aureus. Bacterial isolates of food origin were collected from the laboratory unit of the Department of Microbiology, Kano University of Science and Technology, Wudil. Antioxidant capacity of the extracts used was determined using 2,2-diphenylpicrylhydrazyl (DPPH) assay while agar disc diffusion techniques were used in the determination of the antibacterial activity. Results show that extracts of the spices exhibited a strong antioxidants capacity that ranges from 89.5% to 97.5% at high concentrations of the extracts with methanol extracts being the most active. Methanolic extracts shows zone of inhibition ranging from 16.45mm to 5.26mm while that of aqueous extracts were 10.32mm to 4.32mm. Meanwhile isolates of E. coli and S. aureus were the most sensitive with 16.45 and 15.32. This study concluded that the antibacterial effect of methanolic extract of Allium sativum extract was stronger in comparison, followed by Syzygium aromaticum and Zingiber officinale against five test bacteria isolated of food origin. Therefore, this study revealed that spices produced using Allium sativum, Syzygium aromaticum and Zingiber officinale have an antibacterial property and can be used for food preservation.

Key words: Antibacterial activity, antioxidant capacity, methanolic extracts, Spices.

INTRODUCTION
Food borne microbiologic risks are a significant food safety concern since they can cause as many cases of sickness as possible each year (Stahl and Sies, 2003). Many experts and stakeholders advocate for the creation of a science- and risk-based food safety system, in which decision-makers prioritize hazards and actions based on the most up-to-date data on the distribution and reduction of risks (Caswell and Mojduszka, 2010).
A spice is plant material such as seed, fruit, root, bark, or other plant parts that are used to taste or color food. Fresh, whole dried, or pre-ground dry spices are also possible options (Addis and Sisay, 2015). Spices are usually dried. For convenience, spices can be processed into powder; because whole dried spices have the greatest shelf life, they may be purchased and kept in larger quantities, saving money per serving. Fresh spices, like ginger, are normally more delicious than their dried counterparts, but they are also more costly and have a shorter shelf life (Ahmad et al., 2020).

Spices can be found in medicine, religious rites, cosmetics, and perfumery. The antioxidant action of most herbs and spices is mostly due to phenolic chemicals, particularly flavonoids, which influence nutrition through a variety of mechanisms, including altering the absorption of other nutrients. Cumin and fresh ginger were shown to have the highest antioxidant activity in one research reported by Ninfali et al. (2007).

Spices have a distinct scent and flavor that comes from molecules called phytochemicals or secondary metabolites (Addis and Sisay, 2015). Phytochemicals are antibacterial molecules found in spices that attract beneficial organisms while repelling dangerous ones; they also function as photoprotectants and adapt to environmental changes. Spices include a wide range of phytochemicals, including isoflavones, anthocyanins, and flavonoids, among others (Dashdorj et al. 2015).

Microbial resistance has harmed the efficacy of various antimicrobial drugs, which are now used to extend the shelf life and raise the safety of food items in the food business and suppress disease-causing germs in medicine (Grass et al., 2013). As a result, new antimicrobial agents that can overcome resistance must be developed. Many spices, including clove, oregano, thyme, cinnamon, and cumin, were found to have significant antibacterial and antifungal activity against bacteria that cause food spoilage, such as Bacillus subtilis and Pseudomonas fluorescens, pathogens like Staphylococcus aureus and Vibrio parahaemolyticus, harmful fungi like Aspergillus flavus, and even antibiotic-resistant bacteria like methicillin-resistant bacteria (Ninfali et al., 2007).

Another reason for the growing interest in spices and their extracts is their availability, which has less side effects, harmfulness, and biodegradability than conventional preservatives, which can have negative health impacts if used or exposed for extended periods of time. As a result, the antioxidants capacity and antibacterial activity of some plant sources of spices against some public health isolates produced from food items were determined in this study.

MATERIALS AND METHODS
Culture Acquisition and Purification
Culture of Bacillus cereus, Escherichia coli, Salmonella Typhi, Shigella dysentriae, and Staphylococcus aureus were obtained from the microbial bank of the Department of Microbiology, Kano University of Science and Technology, Wudil. After collection isolates were subcultured to ascertain its integrity on selective media of Mannitol Salt Agar (MSA), Blood Agar, Eosine Methylene Blue (EMB), Salmonella Shigella Agar (SSA), and each organism was further identified by Gram staining and biochemical tests as described by Dua et al. (2013).

Spices Collection
The methods described by Grass et al. (2013) was adopted for spices collection and extracts preparation. In this method, spices (garlic, cloves, and ginger) were collected from Wudil market in a polyethene bags on market day (Friday) in the month of November 2020. Immediately after collection samples were transported directly to the laboratory unit of the Department of Microbiology, KUST, Wudil for analysis.
Extracts Preparation
The collected spices (garlic, ginger and clove) were cleaned, surface sterilized with methanol and dried under sterile laminar air flow chamber. The dried plant material of each plant species was grounded into fine powder to pass 100 mm sieve. About 100g of the fine powder were soaked in 200 ml of methanol and distilled water with stirring for 48h, filtered and centrifuged at 9000 rpm for 10 min and filtered again through Whatman filter paper No. 41 (Whatman 1441-125) for methanol and aqueous extract respectively. The filtrates were evaporated and dried at 40°C under reduced pressure using rotatory vacuum evaporator as described by Tiwari et al. (2009).

Sterility of Extracts
Each of the extracts was tested for growth of contaminants. This was done by making serial dilution of 1g of each extract up to 10⁶. Two micro liters (2ml) of the diluents were aseptically inoculated on Nutrient Agar plates and incubated at 37 °C for 24 hours. The plates were observed for growth. Absence of microbial growth in the extract indicated their sterility. Sterile extracts were used to test for antimicrobial efficacy according to (Friedman et al., 2004).

Determination of Antibacterial Activities
The Agar Well Diffusion method as described by Pandey and Singh (2011) was used. By this method, 0.1 ml of the respective standardized inoculums (0.5 McFarland turbidity standard = 1.0 x 10⁸ cfu/ml) of each test bacterium was spread into sterile Mueller Hinton Agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer (5.0 mm diameter) was used to make wells aseptically in the agar plates. The extracts were prepared and serially diluted in a two-fold dilution to achieve different concentrations of 200, 100, 50, and 25 mg/ml respectively for each extract. Subsequently, 1ml of each concentration of the extracts was introduced into the wells earlier bored Agar plates. The extracts were allowed to diffuse into the medium (kept for 1 hour on the bench before incubation) at 37°C for 24 hours. Ciprofloxacin was used as a positive control, while Dimethyl Sulfoxide (DMSO) was the negative control. Antibacterial activity of the extracts was determined by measurement of clear zones of inhibition produced around the wells using a transparent meter ruler. The diameter of the zones indicated the degree of susceptibility of the test bacteria.

Determination of Minimum Inhibitory Concentration (MIC)
The broth dilution method was used according to CLSI, (2013), in this method solution of 200mg/ml was prepared for the extract. One ml of nutrient broth was dispersed into four test tubes and sterilized by autoclaving at 121°C after 15min. The extract was serially diluted from the solutions of 250mg/ml of the solution to obtain varying concentrations were 200, 100, 50 and 25mg/ml. About (0.1)ml each of standard inoculums were inoculated into the various test tubes containing varying concentrations and then, a set of test tubes containing only nutrient broth were used as negative control. However, another set of test tubes containing nutrient broth and test organisms were used as positive control. All the test tubes and control were then incubated at 37 for 24h. The least concentration of the oil extract in the test tube with no turbidity was considered as the minimum inhibitory concentration (MIC) as described by Gill and Holley, (2006).

Determination of Minimum Bactericidal Concentration (MBC)
This was an offshoot of the previously determined minimum inhibitory concentration of the oil extract in the test tubes with no turbidity was taken as the Minimum Inhibitory Concentration (MIC). Subsequently, those tubes that showed no turbidity were plated out on sterile nutrient agar plate and incubated at 37°C for 24h. Absence of growth after incubation period of 48hrs was considered as the MBC (Burt, 2004).
Evaluation of Antioxidant Capacity

In order to evaluate the antioxidant potential through free radical scavenging by test samples, the change in optical density of DPPH solution radical is monitored. According to Cheung et al. (2003), the samples extract (0.2ml) was diluted with methanol and 2ml of DPPH solution (0.5mm) was added. After 30 minutes, the absorbance was measured at 517nm and the antioxidant activity percentage was calculated using the formula;

\[
AA\% = 100 - \left( \frac{Abs_{\text{sample}} - Abs_{\text{blank}}}{Abs_{\text{control}}} \times 100 \right)
\]

where, \(Abs_{\text{sample}}\) = average absorbance of the sample, \(Abs_{\text{blank}}\) = absorbance of the higher concentration sample, \(Abs_{\text{control}}\) = average absorbance of the negative control.

Statistical Analysis

The values obtained were subjected to analysis using student t-test, that is, one-sample t-test and independent sample t-test at \(p< 0.5\) significance level of probability to obtain the minimum concentration of the extract that might inactivates the pathogens using SPSS version 20.

RESULTS AND DISCUSSION

Analysis of the extract properties shows that aqueous ginger extract has the highest residue of 8.22g while the least residue (2.98g) was obtained from the methanolic extract of garlic. Aqueous and methanolic extracts of garlic were found to be dark brown and light brown, that of clove were dark yellow and light yellow, and for ginger it was deep yellow and pale yellow respectively, as shown in Table 1.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Garlic Methanol</th>
<th>Aqueous</th>
<th>Clove Methanol</th>
<th>Aqueous</th>
<th>Ginger Methanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract residue (g)</td>
<td>2.98</td>
<td>5.32</td>
<td>4.22</td>
<td>7.91</td>
<td>5.58</td>
<td>8.22</td>
</tr>
<tr>
<td>Extract Color</td>
<td>Dark brown</td>
<td>Light brown</td>
<td>Dark yellow</td>
<td>Light yellow</td>
<td>Deep yellow</td>
<td>Pale yellow</td>
</tr>
</tbody>
</table>

Key: g – Gram(s)

The bacteria in this study were chosen because of their role in food deterioration and food poisoning. *Staphylococcus aureus* is one of the most prevalent causes of food poisoning, whereas *Bacillus cereus, Escherichia coli, Salmonella Typhi*, and *Shigella dysentriae* release toxins and other metabolites that cause human gastroenteritis. All of the examined bacterial strains were suppressed by *Allium sativum* extract, which was followed by *Syzygium aromaticum* extract, and lastly by *Zingiber officinale* extract which appeared to be potentially active against four bacterial strains; *B. cereus, E. coli, S. Typhi*, and *S. dysentriae* but less efficient against *S. Typhi*.

Using the disc diffusion method, three plant species were tested for antibacterial activity against food poisoning pathogens, *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella typhi*, and *Shigella dysentriae*. Figure 1 show the results of the antibacterial activity tests on various plant extracts. The findings demonstrated that all plant extracts, to varying degrees, were potentially beneficial in reducing the growth of food poisoning bacteria. In a dosage of 10 mg/ml, *A. sativum* and
Syzygium aromaticum extracts were the most efficient at inhibiting bacteriological growth of all harmful bacteria tested using methanol and aqueous solvent respectively. Other plant extracts have varying levels of antibacterial action against bacterial types that cause food poisoning.

*Shigella dysentriae* was the most resistant strain to plant extracts, followed by *S. Typhi*, whereas *S. aureus* and *B. cereus* were the most vulnerable strains to the extracted plants, respectively. Furthermore, tests were carried out to identify their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against all the tested bacterial strains in order to assess their bacteriostatic and bactericidal characteristics. Figure 2 shows the minimum inhibitory concentration of the plant extracts, *Allium sativum* methanolic extract inhibited *S. aureus* and *E. coli* growth in a test tube containing 2.5 mg/ml concentration of the extract, similar to *S. aromaticum*.
methanolic extract that reduced the visible growth of *B. cereus* and *Salmonella Typhi*. While for the aqueous extract, *A. sativum* produced an MIC at 5.0 mg/ml, against *S. aureus* and *S. Typhi*.

![Figure 2a](image_url)  
**Figure 2a** – The Zones of Inhibition as Produced by the Methanol Extract (10mg/ml) of *Allium sativum* (Garlic), *Syzygium aromaticum* (Clove), and *Zingiber officinale* (Ginger) against some bacterial isolates of foods origin

![Figure 2b](image_url)  
**Figure 2b** – The Zones of Inhibition as Produced by the Aqueous Extract (10mg/ml) of *Allium sativum* (Garlic), *Syzygium aromaticum* (Clove), and *Zingiber officinale* (Ginger) against some bacterial isolates of foods origin

The MBC was confirmed by the lack of bacterial growth in a subsequent test tube proceeding to the MIC tubes after successful subculture. *Allium sativum* methanolic extract had bactericidal action against the pathogenic bacteria tested (*S. aureus* and *E. coli*) with an MBC of 5 mg/ml, *S. aromaticum* methanolic extract had an MBC of 10 mg/ml, with the exception of *S. dysentriae* and *Z. officinale*.
had an MBC of 10 mg/ml for *S. aureus* and *B. cereus*, and 12.5 mg/ml for *S. typhi* and *E. coli*. The MBC for *S. dysentriae* was above 15 mg/ml, since MBC was not achieved even at 15mg/ml of the aqueous extract of the tested plants extracts. The MIC and MBC values of the effective plant extracts revealed that *A. sativum* and *S. aromaticum* might be employed to control and prevent food-borne pathogens and illnesses as shown in figure 3.

**Figure 3a** – Minimum Inhibitory Concentration of the Methanol Extracts of Garlic, Clove, and Ginger against the tested bacteria.

**Figure 3b** – Minimum Inhibitory Concentration of the Aqueous Extracts of Garlic, Clove, and Ginger against the tested bacteria.
DISCUSSION

In the present study, result of the DPPH scavenging activity revealed that *A. sativum* has highest potentiality in scavenging activity compared to *S. aromaticum* and *Z. officinale*. The percentage inhibitions for DPPH assay ranged from 89.5% to 97.5%. The higher the number, the greater the hydrogen-donating ability and thus the higher the antioxidant activity of the spice extracts. Therefore, DPPH values have the highest antioxidant activities. *Allium sativum* extract had the highest DPPH inhibition and this situation could be due to high content of phenolic components such as *S.*
*Syzygium aromaticum* and *Z. officinale* content, these phenolic components with known antioxidant activity (Tural and Turhan, 2017).

Individual phenolic acids, if present in large concentrations as primary ingredients, may mediate the DPPH radical scavenging action of spices, although other compounds in modest quantities or synergy between them may also play a role (Keser et al., 2012). In methanol solution, DPPH behaves as a stable free radical that readily takes an electron or hydride radical and transforms into a stable diamagnetic molecule. DPPH radicals are converted to hydrazine by interacting with appropriate reducing agents. Also, the DPPH test assesses the extract's potential to donate hydrogen to the DPPH radical, resulting in DPPH solution bleaching (Asimi et al., 2013). Spice plants' phenolic components have been shown to contribute considerably to their ability to scavenge DPPH radicals (Kim et al., 2011). Each of the spice extracts demonstrated substantial hydrogen peroxide scavenging activity that was dependent on the concentration and did not differ significantly amongst the spices (P 0.05). Keser et al. (2012) reported that water and methanol extracts of *Allium sativa* could scavenge hydrogen peroxide in an amount-dependent way. Despite the fact that hydrogen peroxide is not extremely reactive, its removal from the cell is vital in cell defense because it can cause the cells to produce a harmful hydroxyl radical (Das et al., 2011).

The large difference in range of MIC of *A. sativum* and other sources of extracts used in this study as also observed in many studies might be owing to significant differences in extraction methods, components, and bacterial strains utilized. Variations in MIC across plant extracts may also be due to differences in chemical composition and volatile nature of compounds. *Syzygium aromaticum* extract, on the other hand, was shown to be active against *B. cereus, S. aureus,* and *E. coli* at a dosage of (10 mg/ml). Verma et al. (2012), Qader et al. (2013), and Mahboubi et al. (2015) found similar results on analysis of the same sources of spices.

However, these findings support those of Mahfuzul Hoque et al. (2007) and Pandey and Singh (2011) which reported that *Z. officinale* was less effective against most of the tested bacterial strains. This is comparable with the findings of Dua et al. (2013), who showed *Z. officinale* to be potentially active with MICs ranging from 6.25 to 12.5 mg/ml. However, a larger quantity of *Z. officinale* extract, up to 60 mg/ml, may be required to be active against food spoilage bacteria, and these findings were similar to those reported by Sheikh et al. (2010).

Several researches had examined the effectiveness of plant extracts and their active chemicals as antimicrobial agents for controlling the growth of bacteria that cause food poisoning and spoiling (Bourn and Prescott, 2002). Some researchers believe that antimicrobial components of plant extracts (terpenoid, alkaloid, and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane, causing disruption and a flux of protons towards the cell exterior, resulting in cell death, or that antimicrobial components of plant extracts (terpenoid, alkaloid, and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane, causing disruption and inhibiting enzymes required for (Burt, 2004; Gill and Holley, 2006). Other researchers linked the plant extracts' inhibitory impact to their hydrophobicity, which allows them to react with proteins in microbial cell membranes and mitochondria, disrupting their structures and modifying their permeability (Friedman et al., 2004; Tiwari et al., 2009). The findings of this study imply that plant extracts that have been shown to be potentially useful can be utilized as natural preservatives to prevent food poisoning and preserve food without the harmful effects of artificial preservatives.
CONCLUSION
The proliferation of various harmful bacterial strains can lead to food deterioration. The use of chemical preservatives in the food industry and on food products is the most common method of preventing food deterioration. Because of the negative health impacts of these chemical preservatives, there is a greater desire for potentially effective, healthful, safer, and natural food preservatives. Plant extracts such as *A. sativum* and *S. aromaticum* have been shown to be potentially active against the strains of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, and *Shigella dysentriae*. Based on the findings of this study spices extracts such as *A. sativum*, *S. aromaticum*, and *Z. officinale* can be utilized as natural alternatives in food preservation to reduce the burden of food borne poisoning and illnesses. These may be used in food formulation.

Acknowledgement
The authors wish to express their thanks to all the supporting staff of Microbiology Laboratory, Kano University of Science and Technology, Wudil for the bacterial food isolates and other pertinent support.

References


CLSI (2015). M100-S25 performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement.


