In Vitro Antibacterial Activity of Different Extracts of Zingiber Officinale against Bacterial Isolates Responsible for Food Spoilage

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Abstract

Ginger (Zingiber officinale) is a medicinal plant that has been widely used all over the world in food as spices. The study was aimed to investigate the phytochemical constituents and antibacterial activity of ginger extracts against some pathogenic bacteria responsible for food spoilage. The aqueous, ethanol and n-hexane extracts from ginger were prepared, screened for phytochemical analysis and tested for antibacterial activity against 6 pathogenic bacteria (Klebsiella pneumoneae, Salmonella typhi, Shigella spp, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus). The result of phytochemical screening of the extracts showed that the Ginger extracts contain Alkaloid, Anthraquinone, saponin, phenol, Flavonoid, terpenoid and glycoside, steroid and reducing sugar, while resin was absent. Statistical analysis of the result showed that ethanol extract demonstrated highest antibacterial activity with average zone of inhibition of 13.77 ± 2.16 mm among the isolates, followed by aqueous extracts (11.67 ± 1.54 mm) while least average zone of inhibition was recorded by n-hexane extract (9.64 ± 1.22 mm. Based on the susceptibility of the organisms to the extracts, E. coli was found to be the highest susceptible organisms with average zone of inhibition of 13.6 ± 1.23 mm, followed Shigella (13.3 ± 1.63 mm), Salmonella typhi (12.7 ± 2.01 mm), S. aureus (12.5 ± 1.62 mm), Pseudomonas (10.8 ± 1.08mm) while least average zone of inhibition is shown by Klebsiella (9.2 ± 1.66 mm). There is no significant different 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 ginger and also used as dietary supplement for food preservation.

Keywords: Ginger, Extract, Pathogenic bacteria, Antibacterial activity, Phytochemicals

Introduction

Natural products are a major source of new natural drugs and their use as an alternative medicine for treatment of various diseases has been increased in the last few decades [1]. In comparison to the formulated drugs, the herbs and spices have fewer side effects. They are also inexpensive, show better patient tolerance and are readily available for low socioeconomic population [2]. In recent years, in view of their beneficial effects, use of spices or herbs is gradually increasing not only in developing countries but also in developed countries [3]. The antimicrobial activity of spices is due to specific phytochemicals or essential oils [4]. The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, and types of microorganism, composition of the food, pH value and temperature of the environment [5].

Ginger (Zingiber officinale) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases [6]. Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections [7]. Ginger belongs to Zingiberaceae family [8]. The Zingiberaceous plants have strong aromatic and medicinal properties and characterized by their tuberous or non-tuberous rhizomes [9]. Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. The plant is reported to have antibacterial, anti-oxidant, antiprotozoal, anti-fungal, anti-emetic, anti-rhinoiral, anti-inflammatory, anti-insecticidal activity [10]. Reported pharmacological activities of ginger include antipyretic, analgesic, ant tissues in addition to hypersensitive effects [11]. The ginger plant has a long history of cultivation known to originate in China and then spread to India, South East Asia, West Africa and the Caribbean [12,13]. Ginger
contains up to 3% of an essential oil that causes the fragrance of the spice [14].

Medically ginger is used as a stimulant and carminative, and is used frequently for drypepsia and colic [14]. It has a saliagogue action, stimulating the production of saliva. It also used to disguise the taste of medicines. Ginger promotes the release of bile from the gall bladder [15,16]. Ginger may also decrease joint pain from arthritis, may have blood thinning and cholesterol lowering properties and may be useful for the treatment of heart diseases and lungs diseases [14-17]. The characteristic odour and flavour of ginger root is caused by a mixture of gingerone, shogaoles and gingerols, volatile oils that make up about 1-3% of the weight fresh ginger. The gingerols increase the motility of the gastrointestinal tract and have analgesic, sedative and antibacterial properties [14]. Ginger has been found effective by multiple studies for treating nausea caused by seasickness, morning sickness and chemotherapy [18]. Ginger has been reported to be effective for the treatment of inflammation, rheumatism, cold, heat cramps, and diabetes [19,20]. The objective of the present study was to investigate the phytochemical constituents and antibacterial activity of ginger extracts against some pathogenic bacteria responsible for food spoilage.

**Materials And Methods**

**Sample collection and identification**

Ginger (Zingiber officinale) rhizomes were used in this study and were purchased from Rimi market in Kano city, Nigeria. Identification and authentication of the plant material was done at compounding laboratory in the Department of Pharmaceutical Technology, School of Technology Kano with the following voucher number SOT/PCT/01/085. Voucher specimen has been deposited there for future reference.

**Bacterial strains**

Six different bacterial strains responsible for food spoilage including Klebsiella pneumoniae, Salmonella typhi, Shigella spp, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus were obtained from Laboratory of Science Lab Technology, School of Technology Kano. All bacterial organisms were isolated from spoiled fruits and diagnosed to the species level by using different available procedures including Gram's stain, cultural characterization and Biochemical tests including; Indole, Methyl red, Vougues Proskaeur, Catalase, Citrate utilization and coagulase tests. The strains were maintained on Nutrient agar slants.

**Preparation of extracts**

Three types of extracts (aqueous, ethanolic and n-hexane extracts) from ginger were prepared separately. The fresh ginger rhizomes were washed and air dried for fourteen days. After drying, the ginger slices were ground to fine powder using sterile pestle and mortar. 20 g powder of each garlic and ginger was soaked in 200 ml of distilled water, ethanol and n-hexane separately. The flakes were kept at room temperature for 3 days with intermittent shaking. The mixture was filtered using Whatman filter paper. The ethanol and n-hexane extracts were evaporated at 50°C using rotary evaporator while the aqueous extract was evaporated at 80°C in water bath. All dried extract samples were dissolved in 10% DMSO separately to the final concentration of 200 mg/ml as a stock concentration. The extract solutions were stored at 4°C.

**Phytochemical screening**

Phytochemical screening was done to ascertain the presence of bioactive component present in the Ginger. Presence of Alkaloid, saponin, Glycoside, Tannin, flavonoid, resin, steroid, terpenoid, Anthraquinones, Protein and amino acid e.t.c were determined using procedure described by Sofowora [21].

**Test for alkaloid**

(Wagner’s test) To 0.1 ml of the extract in a test tube, 3 drops of Wagner’s reagent (iodine in Potassium iodide) were added. Formation of brown precipitate was observed. This showed presence of Alkaloids

**Test for flavonoids**

(Lead acetate test) Extracts were treated with few drops of lead acetate solution. The formation of yellow precipitate indicated the presence of flavonoids.

**Test for glycoside**

Ten (10) ml of 50% Tetraoxosulphate (vi) acid was added to 1ml of the extract in a separate test tube and the mixture was heated gently for 15 minutes followed by addition of 10ml of Fehling solution. A brick red precipitate indicated the presence of Glycoside.

**Test for reducing sugar**

(Fehling's test) To 1ml of the extract in a separate test tube, 2ml of distilled was added followed by addition of Fehling solution (A + B) and the mixture was warmed at 40°C. The appearance of brick red precipitation, at the bottom of the test tube, indicates the presence of reducing sugar.

**Test for saponins**

(Foam test): Half gram (0.5g) of the powdered sample was dispensed in a test tube and 5ml of distilled water was added and shake vigorously. Persistent froth (foam) that lasted for about 10 minutes indicated the presence of saponin.

**Test for steroid**

To 2ml of the sample, 2ml of acetic acid was added and the solution was kept in ice for cooling. 2ml of concentrated Tetraoxosulphate (vi) acid was added carefully. Color changes, from violet to blue/bluish green indicated the presence of steroids.

**Test for tannin**

(Gelatin test): To 2ml of the extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicated the presence of tannins.

**Test for phenol**

(Ferric chloride test) Extracts were treated with 4 drops of ferric chloride solution. The formation of bluish black color indicated the presence of phenols.

**Test for terpenoid**

(Salwoki test) About 5ml of the extract was added with 2ml
of chloroform and 3ml of concentrated Tetraoxosulphate (vi) acid. Reddish brown colour of interface, indicate the presence of Terpenoids.

**Test for anthraquinones**

About 2ml of the extract was added into a test tube and 5ml of Benzene was added and shaken, then 5ml of the 10% Ammonia solution was also added followed by shaking. The formation of pink color in the lower phase showed positive test and presence of Anthraquinones.

**Antimicrobial assay**

The antimicrobial assay of Ginger extracts was performed by disc diffusion method as described by Kirby-Bauer [22]. All the experiments were performed under sterile conditions. The Mueller Hinton agar plates were inoculated separately with the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by E. coli (17.2 mm) at 10 µg /ml. The zone of inhibition of the control (Ciprofloxacin 10 µg) ranges from to 19-22 mm

**Statistical Analysis**

The data of average zone of inhibition produced by the isolates against the antibiotics used was analyzed using One-Way ANOVARs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at p<0.05.

**Results**

**Phytochemical screening**

The phytochemical constituent of aqueous, ethanol and n-hexane extracts of Ginger is presented in Table 1. The result showed that the aqueous, ethanol and n-hexane Ginger extracts contain the following Phytochemicals; Alkaloid, Anthraquinone, saponin, phenol, Flavonoid, terpenoid and glycose, steroid and reducing sugar, while resin was absent. The ethanol extract has the highest number of phytochemical components.

**Antibacterial activity of ginger aqueous extract**

The antibacterial activity of aqueous ginger extract is presented in Table 2. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by E. coli (17.2 mm) at 10 µg /ml. The zone of inhibition of the control (Ciprofloxacin 10 µg) ranges from to 19-22 mm

**Antibacterial activity of ginger ethanol extract**

The antibacterial activity of ethanol ginger extract is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by Shigella spp (17.7 mm) at 10 µg /ml. The zone of inhibition of the control (Ciprofloxacin 10 µg) ranges from to 19-22 mm

**Antibacterial activity of ginger n-hexane extract**

The antibacterial activity of n-hexane ginger extract is presented in Table 4. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by E. coli (14.7 mm) at 10 µg /ml. The zone of inhibition of the control (Ciprofloxacin 10 µg) ranges from to 19-22 mm

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>n-hexane extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Resin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

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

| Table 1: Phytochemical constituents of aqueous, ethanol and n-hexane extracts of Ginger

<table>
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<tr>
<th>Isolates</th>
<th>Concentration (µg /ml)</th>
<th>Zone of inhibition (mm)</th>
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<tr>
<td>Klebsiella pneumoniae</td>
<td>0.00 10.80</td>
<td>11.40 ± 0.20</td>
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<tr>
<td>Salmonella typhi</td>
<td>0.12 10.80</td>
<td>12.50 ± 0.17</td>
</tr>
<tr>
<td>Shigella sp</td>
<td>0.12 10.70</td>
<td>13.20 ± 0.20</td>
</tr>
<tr>
<td>Pseudomonas aerogenosina</td>
<td>0.17 10.40</td>
<td>14.40 ± 0.30</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.20 10.50</td>
<td>15.60 ± 0.17</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.21 10.40</td>
<td>16.10 ± 0.20</td>
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</tbody>
</table>

| Table 2: Antibacterial activity of Ginger aqueous extract

<table>
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| Table 3: Antibacterial activity of Ginger ethanol extract

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<td>15.60 ± 0.17</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.21 10.40</td>
<td>16.10 ± 0.20</td>
</tr>
</tbody>
</table>

| Table 4: Antibacterial activity of Ginger n-hexane extract
inhibition of the control (Ciprofloxacin 10 µg) ranges from to 19-22mm

Discussion

In the present study antibacterial effect of ginger extracts was evaluated by disc diffusion method. The phytochemical constituent of aqueous, ethanol and n-hexane extracts of Ginger is presented in Table 1. The result showed that the aqueous, ethanol and n-hexane Ginger extracts contain the following Phytochemicals; Alkaloid, Anthraquinone, Flavonoid, terpenoid and glycoside, steroid and reducing sugar, while saponin, resin and phenol were absent. The ethanol extract has the highest number of phytochemical components compared to water and n-hexane. This is due to better solubility of the bioactive constituents in ethanol than in water and n-hexane.

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, antipsomadnic, antihyperglycemic, antiinflammatory and immunomodulatory properties [23]. Flavonoids are also present in all the extracts as a potent water-soluble antioxidant and free radical scavenger which prevent oxidative cell damage and also have strong anticancer activity [24,25]. It also helps in managing diabetes induced oxidative stress. According to this study, Alkaloid is present in all the extracts. Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anaesthetics and Central Nervous Stimulants [26,27]. Alkaloids are known to play some metabolic roles and control development in living system [28]. It also interferes with cell division, hence the presence of alkaloids in ginger, clove, onion, garlic and black pepper could account for their use as antimicrobial agents. Aboaba, et al. [29] had reported that the antimicrobial properties of substances are desirable tools in food spoilage and food safety. This suggests that the ginger extracts which have been confirmed to contain alkaloids may also be useful as preservatives in food. Steroids are importance in pharmacy as they possess compounds like sex hormones and can be used for drug production [30]. Tannin and saponin were present in all the extracts except aqueous extract. Saponins protect against hypercholesterolemia and antibiotics properties [31]. In addition, it has been documented that saponins have antitumor, antioxidant and antimutagenic activities and can lower the risk of human cancers by inhibiting the growth of cancer cells [32,33]. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins [34].

The results of this study indicated that different extracts of ginger have broad spectrum antibacterial activity with variable degree of sensitivity against the tested bacterial species responsible for food spoilage. The antibacterial activity of ginger extracts could be attributed to the chemical properties of ginger as mentioned above. Statistical analysis of the result showed that ethanol extract demonstrated highest antibacterial activity with average zone of inhibition of 13.77 ± 2.16 mm among the isolates. This could be attributed to higher number of phytochemicals recorded. Aqueous extracts exerted antibacterial activity against the tested isolates with average zone of inhibition of 11.67 ± 1.54 mm while least average zone of inhibition was recorded by n-hexane extract with 9.64 ± 1.22 mm. The result of this study was in conformity with that of Onyeagba, et al. [35] who found the effect of ethanol extract of ginger against Bacillus spp. and Staphylococcus aureus. They also found the antimicrobial activity of the ethanol extract of ginger; lime and garlic against broad range of bacteria including Bacillus spp., Staphylococcus aureus, Escherichia coli, and Salmonella spp. The results of antibacterial activity of ginger extracts in this study shows similarity with study conducted by Gull, et al. [36] in which the result of their study showed the potent antimicrobial activity of the ginger extract against all the tested bacterial food pathogens namely Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholerae, Klebsiella spp. and Salmonella spp. The extract of ginger showed highest zone of inhibition (11.67 ± 1.53mm) against Salmonella spp. and lowest zone of inhibition (8.0 ± 1.73mm) against Escherichia coli. Ginger extract also showed lower zone of inhibition (8.67 ± 2.52mm) against Staphylococcus aureus compared to the Gram-negative bacteria. Based on the susceptibility of the organisms to the extracts, E. coli was found to be the highest susceptible organisms with average zone of inhibition of 13.6 ± 1.23 mm, followed Shigella [13.3 ± 1.63 mm], Salmonella typhi [12.7 ± 2.01 mm], S. aureus [12.5 ± 1.82 mm], Pseudomonas [10.8 ± 1.08mm] while least average zone of inhibition is shown by Klebsiella [9.2 ± 1.66 mm].

Conclusion

In conclusion, this study has shown that ginger extracts possess medicinal properties, antibacterial activity and that the inhibition of bacterial growth was dose dependent. The results of the present study show that ginger ethanol extracts are more effective against all tested bacterial strains than ginger aqueous and n-hexane extracts. E. coli and Shigella were also more susceptible to the ginger 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. The results of present study have provided the justification for therapeutic potential of ginger and also used as dietary supplement for food preservation.

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