

Full Length Research Paper

Antibacterial potential of extracts of the roots of *Zingiber officinale* against bacterial strains commonly associated with nosocomial infections

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Zingiber officinale, commonly known as ginger, has been used as a medicinal plant for decades for medical and culinary purposes. This study aimed to determine the antimicrobial potential of ginger root extracts using the Kirby-Bayer agar diffusion method to compare the zones of inhibition of the extracts to those of synthetic antibiotics against five clinical bacterial pathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*). Ginger extracts showed more antimicrobial activity against the five test organisms compared to the synthetic antibiotics. The least resistant bacterium was *S. aureus* while the remaining four bacterial strains were strongly resistant to most of the antibiotics. Antimicrobial activity of ginger root extracts at various concentrations revealed that *E. coli* had the lowest concentration (1.2 mg/ml) in 20 mg/ml while the highest concentration (9.1 mg/ml) was observed for *S. aureus* in 75 mg/ml. Phytochemical screening of the ginger root extracts revealed the presence of all the tested secondary metabolites (saponins, tannin, flavonoids, glycoside, terpenoids and alkaloids). A number of phytochemicals present in ginger were identified to be possibly responsible for the antibacterial activity of ginger roots. These could be used independently or in combination with synthetic antibiotics to create more efficient antibiotics. Findings of this study can contribute to on-going research towards identifying alternative treatment of nosocomial infections that eliminate the use of antibiotics. With these findings, scientists could be employed in the health sector to create antibiotics that are not resisted by pathogens that cause infections in health care facilities.

Key words: Antibacterial, ginger, phytochemical, zone of inhibition.

INTRODUCTION

Antibiotics are antimicrobial medicines that are used to treat and prevent bacterial infections. When used properly, antibiotics can save lives by fighting these infections. However, the misuse and abuse of these

drugs has led to an increase in drug resistance which makes it difficult to successfully treat bacterial infections and control microbial pathogenicity. The misuse of antibiotics over the years has caused the development of

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Figure 1. Ginger roots used in this study.

multi drug resistance (MDR) in pathogenic bacteria. This is where the demand for natural products such as ginger comes in as alternative medical treatment for these pathogenic bacteria. Antibiotics are classified based on their modes of action such as cell wall inhibition which prevent cell wall generation, cell membrane inhibition which causes disorganization of the cell membrane and nucleic acid synthesis inhibition which block the action of DNA gyrase and topoisomerase IV, therefore inhibiting DNA replication (Verma et al., 2008).

The use of antibiotics when it is not appropriate promotes antibiotic resistance. According to the Centre for Disease Control and Prevention (2013), one-third to one-half of antibiotics use in humans is unnecessary or inappropriate. Resistance to antibiotics results in serious illness, longer recovery from disease, more-frequent or longer hospitalization, more doctor visits, and more-expensive treatments.

All natural antibiotics are plant derivatives or other natural substances that show strong antimicrobial properties and as such may be recommended by medical professionals for use as first line treatments to combat viral, bacterial, fungal, or parasitic infections. These natural alternatives work very effectively in helping patients get healthy all the while avoiding the negative side effects of conventional antibiotics. Conventional antibiotics are static; they are bound to have limitations which allow bacteria to develop mechanisms that resist their effect. On the other hand, natural plant based antibiotics such as ginger are not static and as such are able to come up with new ways of eliminating bacteria.

The use of ginger as a naturally obtained antimicrobial agent has great implications. The continuing occurrence of superbugs that are resistant to the most lethal of synthesised antibiotics is becoming a global concern. Research shows that more than 3 million people die

annually from bacteria related infections (Pang et al., 1995).

Previous studies have shown the medicinal benefits of ginger extracts which warrants the need for it to be further investigated. As Gizanna et al. (2005) report, ginger has been applied to remedy ailments such as muscle conditions and arthritis. The antibacterial potential of ginger extracts is well documented in its ability to control different diseases as well as disease prevention (Rahmani et al., 2014). Ginger has been shown to have potential against some clinical isolates (Omoya and Akharaiyi, 2011) as well as some food borne bacteria (Islam et al., 2014). More studies on the antibacterial activity of ginger extracts against nosocomial pathogens are necessary in order to elucidate their antibiotic potential. This study aimed to assess resistance patterns of bacterial strains that commonly cause nosocomial infections against compounds extracted from ginger roots in order to determine the antimicrobial potential of the ginger root extracts.

MATERIALS AND METHODS

Extracts isolation and preparation

The variety of roots used in this study was Chinese ginger roots which were purchased at Spar Grocery Store in Palapye, Botswana (Figure 1). The roots were about a month's old at the time of purchase. The ginger roots (weighing around 3 kg) were thoroughly washed with distilled water, peeled and chopped into small equal fragments of about 1 cm³. After drying, fragments were incubated in an oven at 40°C for 24 h. They were then ground into fine powder. About 10 g of the ginger powder was dissolved in 100 ml of 96% methanol. The same amount of powder was dissolved in 100 ml of distilled water which was used as a negative control. The mixtures were sonicated to break down the cell walls of the ginger roots mechanically thereby releasing their content without degrading the

Table 1. Inhibitory zones of different antibiotics on 5 different bacterial isolates.

Antibiotic	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Enterobacter aerogenes</i>	<i>Pseudomonas aeruginosa</i>
Ampicillin	R	R	R	R	R
Amoxicillin	R	R	R	R	R
Gentamycin	I	R	I	R	R
Augmentin	S	R	I	R	R
Tetracycline	R	R	R	R	R
Clindamycin	S	R	R	R	R
Erythromycin	I	R	R	R	R
Ciprofloxacin	R	R	R	R	I
Nalidixic acid	I	R	I	R	R

R=Resistant, I=intermediate, and S=Susceptible.

Table 2. Zones of inhibition (mm) of 96% methanol and distilled water extracts of ginger roots on five bacterial isolates.

Bacterial Isolate	Inhibition zone in 96% methanol (mm)	Inhibition zone in distilled water (mm)
<i>Staphylococcus aureus</i>	17 ± 1.53	0
<i>Klebsiella pneumoniae</i>	15 ± 1.37	0
<i>Escherichia coli</i>	20 ± 1.85	0
<i>Enterobacter aerogenes</i>	17 ± 1.34	0
<i>Pseudomonas aeruginosa</i>	21 ± 1.27	0

cells. After sonication, the mixtures were filtered with a Whatman No. 1 filter paper and the filtrates were placed in the hood for 24 h to allow evaporation to occur.

Antibiotic susceptibility testing

Five clinical isolates (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*) were obtained from the Botswana International University of Science and Technology (BIUST) microbiology laboratory. Disk diffusion method (Collins et al., 1995) was used to determine the susceptibility of isolates to antibiotics. A 0.5 McFarland standard was used. Twenty four hour-old cultures were spread on prepared Mueller-Hinton Agar (MHA) plates and allowed to diffuse. Synthetic antibiotics were carefully placed on to the media with sterilized forceps. The plates were then inverted and placed in an incubator for 24 h. This allowed the determination of the presence or absence of a zone of inhibition. Isolates with the highest resistance to most of the antibiotics were selected to test against ginger root extracts.

Antibiotic sensitivity testing

The following antibiotics were used in this study; ampicillin, amoxicillin, ciprofloxacin, clindamycin, erythromycin, gentamycin, augmentin, tetracycline and nalidixic acid. Antibiotic sensitivity testing was done as described by Pierce-Hendry and Dennis (2010). Filter paper discs (7 mm in diameter) were prepared and sterilised in an autoclave. They were then placed into the ginger extracts to soak up the content. Using sterile cotton swabs, the cultures were aseptically swabbed onto sterile MHA plates. Using ethanol dipped and flamed forceps, the antibiotic discs were placed over the seeded MHA plates and sufficiently separated from each

other to avoid overlapping of the inhibition zones. The plates were incubated at 37°C for 24 h and the diameter of the inhibition zones was measured. Antimicrobial activity of ginger extracts on bacterial pathogens was tested at different concentrations of 20, 50 and 75 mg/ml. For all experiments, there were three technical repeats as well as three biological repeats. Results of these were averaged.

Phytochemical tests

Phytochemical screening of the ginger root extracts was performed as described by Harborne (1998) to determine the presence of the following bioactive compounds; saponins, tannins, terpenoids, glycosides, flavonoids and alkaloids which all give ginger its antimicrobial properties.

Statistical analysis

Data was analysed and expressed as mean ± standard deviation. All tests were carried out in triplicate to improve accuracy.

RESULTS

The sensitivity of the five bacterial isolates was tested against different synthetic antibiotics. Augmentin and clindamycin both showed high antimicrobial activity against *S. aureus*. *S. aureus* was the least resistant of the five bacterial strains. The other four bacteria showed high resistance against most of the antibiotics showing that most antibiotics had low antimicrobial activity against them (Table 1).

Large zones of inhibition by methanol extracts were

Table 3. Inhibition concentrations of ginger root extracts against bacterial pathogens at different concentrations.

Multi-Drug Resistant Bacterial strain	20 mg/ml	50 mg/ml	75 mg/ml	Gentamicin	Distilled Water
<i>Staphylococcus aureus</i>	2.5	5.2	9.1	10	0
<i>Klebsiella pneumoniae</i>	2.2	4.1	7.2	12	0
<i>Escherichia coli</i>	1.2	3.2	5.7	13	0
<i>Enterobacter aerogenes</i>	1.3	2.4	4.2	9	0
<i>Pseudomonas aeruginosa</i>	1.5	2.7	4.8	13	0

Table 4. Phytochemical constituents of ginger extracts.

Secondary metabolite	Distilled water extract			96% Methanol extract		
Saponins	-	-	-	+	+	
Tannins	-	-	-	+	+	
Flavonoids	-	-	-	+	+	
Glycosides	-	-	-	+	+	+
Terpenoids	-	-	-	+	+	+
Alkaloids	-	-	-			+

+ = present at low concentration, ++ = present at high concentration, +++ = present at very high concentration, - = absent.

observed while distilled water extracts showed no inhibition zones. The highest inhibition zone was observed for *P. aeruginosa* while *K. pneumoniae* had the lowest zone of inhibition (Table 2). Table 3 shows the antimicrobial activity of the ginger root extracts tested at different concentrations. Gentamicin was used as a positive control while distilled water was used as a negative control. Inhibition concentrations ranged from 9 to 13 mg/ml for the control gentamicin. The highest recorded concentration was 9.1 mg/ml for *S. aureus* in 75 mg/ml while *E. coli* recorded the lowest inhibition concentration of 1.2 mg/ml in 20 mg/ml (Table 3).

Phytochemical screening showed that no bioactive compounds were present in distilled water extracts while all of the tested compounds were present in 96% methanol extracts with terpenoids and glycosides being present at higher concentrations than the other compounds (Table 4). Alkaloids were present at lower levels than any other compound.

DISCUSSION

This study demonstrated that methanol extracts contained compounds that showed antimicrobial properties against both the Gram positive and Gram negative isolates. Ginger extracts were shown in this study to comprise secondary metabolites such as flavonoids, alkaloids, terpenoids, tannins and saponins. This has been proved by other studies in the past (Batista et al., 1994; Barre et al., 1997). The phytochemicals tested for were those that are linked with the antimicrobial

activity of the ginger roots because they serve as a defence mechanism against invasion by microorganisms (Bensky et al., 1993). Flavonoids that are present in ginger extract serve as hydroxylated phenolic substances that help plants respond to microbial infections. Saponins on the other hand have antimicrobial activity because they are able to leak some proteins and enzymes from the plant cell (Zablotowicz et al., 1996). As explained by Shimada et al. (2006), the antimicrobial property of tannins comes from their ability to bind to proline rich proteins and interfere with protein synthesis. Terpenoids, which were present in the methanol extract, interact with compounds such as vitamin A to give plants direct protection from biotic and abiotic stresses (Bensky et al., 1993).

There are some advantages associated with the use of natural sources of antibiotics such as reduced side effects, tolerance by patients, easy accessibility and the fact that most of these plant sources are renewable resources found in nature (Vermani, 2002). However, toxic effects of the ginger root extracts were not tested in this study. As such, their exact effectiveness can only be determined after toxicity tests have been done on eukaryotic cells to determine whether or not these extracts are safe for human consumption. Alternatively, extracts such as soybean oil and other oils from edible plants may be used for extraction. Studies have been conducted on the extraction of bioactive compounds present in ginger using solvents such as ethanol and methanol (Bailey-Shaw et al., 2008). Various extraction methods such as reflux, sonication and steam distillation have also been employed in ginger extraction. Because

of time limitations, the sonication method in which sound waves are applied to agitate the cellular particles of the plant was employed in this study. A frequency of >20 kHz used for extraction has high overall efficiency and high yields of the product. Thermal stability and rapid extraction time prevents the bioactive compounds from degrading (Balladin et al., 1997).

Pathogens such as *E. aerogenes* and *P. aeruginosa* used in this study have proven over the years to be resistant to many antibiotics due to the permeability barriers in their outer membrane made up of lipopolysaccharides. This allows them to colonize the surface of biofilms making them resistant to antibiotics. The resistance of these pathogens could however be reduced by antibiotic inhibitors found in plants such as ginger (Kim et al., 1995). It is possible that the maturity of the plant, the solvent used for extraction and the extraction method used to attain the secondary metabolites affected the variation of the antimicrobial activity against the test microbes.

According to Fujii (1994), morphological differences between Gram positive and negative bacteria influence the effects of the antimicrobial agents. Gram negative bacteria have influx pumps that do not allow intercellular build-up of antibacterial agents (Farzaneh and Carvalho, 2015). Therefore, there is a need to develop new antibiotics that can overcome or suppress these pumps to recover the potency of antibiotics. The use of medicinal herbs such as ginger and other medicinal plants could help in combating this problem.

According to Burt (2004), the mechanisms of action of plant based drugs and their antimicrobial activity is based on the following, the antibiotic's ability to breakdown the cytotic membrane of the organism; interaction with proteins in the membrane; disintegration of the outer membrane to release LPS; destabilizing forces that leak ions into the cell; cell content coagulation and synthesis of enzymes. Complementary studies could be carried out to determine the pharmacokinetics of plant extracts, their purity as well as quantification of the bioactive compounds. Further studies could help in strengthening the potential of novel medicinal plants as cost effective agents against bacterial infections. The issue of safety of the use of herbal medicines is crucial in the public health sector because of the enormous number of people that are likely to consume the product.

It is evident from this study that ginger has antimicrobial activity against both Gram positive and negative bacteria. It is then reasonable to recommend that ginger and any other edible plant that has been proven to have antimicrobial properties be included in diet for benefits such as reducing the chances of developing bacterial infections which might help prevent frequent abuse of antibiotics. This could also help in reducing costs of treatment and development of new adverse drugs while preventing recurrent infections. It is however of paramount importance to determine the toxicity of the

bioactive compounds found in every plant that is being explored for drug development by testing their side effects and pharmaceutical properties. We could not determine the *in vivo* side effects of the paper disks but for further research it is important that plants used for traditional medical practices be accompanied with thorough knowledge of the plant's bioactive compounds as well as efficacies that are backed up by scientific evidence. Following this study, toxicity studies should be carried out to check the effect of the ginger abstracts on human beings. This is to check if they are safe or not safe for use in humans as potential treatment against nosocomial infections. In conclusion, ginger roots extracts have the potential to be used to make non-synthetic antibiotics against nosocomial infections to reduce reliance on synthetic antibiotics which promote multi-drug antibiotic resistance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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