

Antibacterial and Phytochemical Analysis of Methanolic Extract of *Zingiber Officinale* (GINGER) on Some Bacterial Pathogens

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Abstract

Original Research Article

Ginger (*Zingiber officinale*) is a rhizome or root of the plant which is mostly used as a spice or a folk medicine. The study was aimed at determining the antibacterial property, phytochemical screening as well as the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of methanolic extract of dried ginger powder, using the agar well diffusion method against seven clinical bacterial isolates: *K. pneumonia*, *S. pyogenes*, *S. typhi*, *S. paratyphi A*, *S. paratyphi B*, *S. paratyphi C* and *S. pneumonia*. The antibacterial screening of the extract showed a moderate zones of inhibition in all the tested organisms at different concentration (80mg/ml, 160mg/ml and 200mg/ml) except *K. pneumonia* which was resistance to all the concentration that was used. However, higher diameter of zone of inhibition was recorded at high concentration (200mg/ml) with zones of inhibition ranging from 15.00±1.00mm in *S. pyogenes*, and 9.00±0.00 mm in *S. pneumoniae*. While lower zones of inhibition were recorded at 80 and 160mg/ml in all the organisms from 6.50±0.50mm in *S. paratyphi A* to 13.00±0.00mm in *S. pyogenes*. The Qualitative phytochemical screening of the methanolic extract, revealed the presence of nine different phytochemicals which include alkaloid, flavonoids, saponins, cardiac glycosides, anthraquinones, sterols, phlobatanins and terpenes, whereas the Quantitative phytochemical estimation of the extract revealed an appreciable amount of phenols, flavonoids, tannins, alkaloids and saponins at a concentration of 1371.405±206.551, 564.223±35.556, 386.925±5.058, 273.540±0.857 and 104.325±1.042 respectively. The results of this study revealed that the bacterial isolate used in the study exhibited different sensitivities towards methanolic extract of ginger. Thus, ginger which normally forms part of ingredients in food could provide protection against some infections caused by these pathogenic bacteria.

Keywords: *Zingiber officinale*, Antibacterial property, pathogens and phytochemical screening.

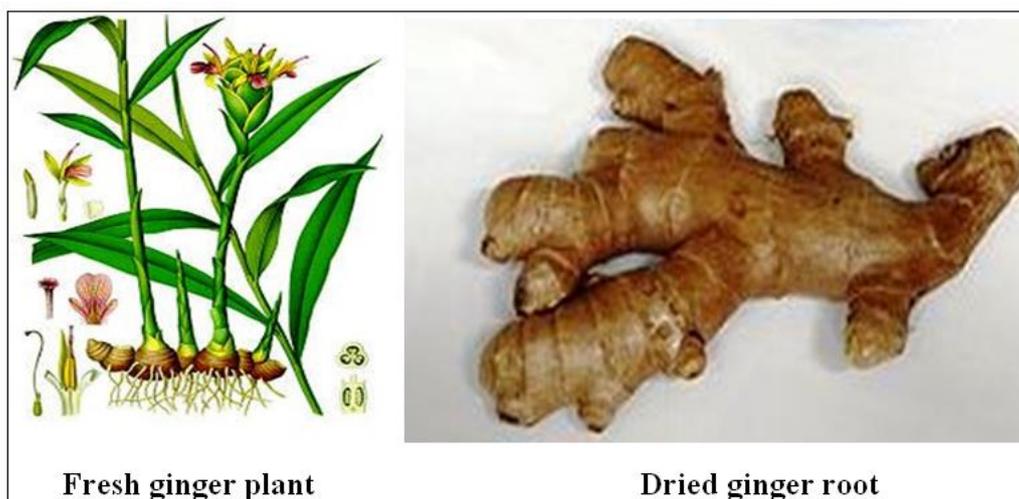
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INTRODUCTION

Medicinal plants are becoming popular in various fields of medicine, pharmaceuticals, nutraceuticals, cosmetics and food supplements. For centuries, plant parts and derivatives such as leaves, roots, stems etc. have been used for medicinal purposes. Different medicinal plants are used traditionally in Africa and Asian for medicinal purposes [1]. However, increase in antibiotic-resistant strains of pathogenic microorganisms has necessitated the use, development and growth of traditional medicine. Investigation for antimicrobial and other bioactive properties are receiving significant attention by scientist all over the world as more traditional plants are constantly been

studied in order to combat the antibiotic resistance challenge [2]. This has consequence leading to the invention of reasonable numbers of effective antibiotics against these resistances bacterial. 1998 WHO report estimated that over 80% of people in developing countries use and rely on traditional medicine [3].

Ginger (*Zingiber officinale*) is a flowering plant, which belongs to *Zingiberaceae* family [4]. Whose rhizome, root or simply ginger, is widely used as a spice or a folk medicine. The *Zingiberaceae* plants are known to have tuberous or non-tuberous rhizomes with strong medicinal and aromatic properties [5].



Fresh ginger plant

Dried ginger root

Source: (Ugwu *et al.*, 2011) [6]

Ginger (*Zingiber officinale*) has been used widely for the treatment of different ailments including arthritis, cramps, rheumatism, sprains and sore throats, pains, constipation, indigestion, vomiting as well as hypertension, dementia, muscular aches, fever and infectious diseases [7]. Ginger has direct anti-microbial activity and it has been used for the treatment of various bacterial infections and diseases [8].

MATERIALS AND METHODS

Sample Collection and Identification

Fresh corn of *Zingiber officinale* was obtained from Zungeru Central Market, Niger State Nigeria and was identified in the Department of Biological Sciences of Federal University of Technology Minna.

Processing of Plant Material

The corn of *Zingiber officinale* samples obtained were chopped and dried at room temperature of about (29-32°C) in microbiology laboratory for two weeks and it was processed into powder by pounding them using laboratory mortar and pestle.

Extraction Procedure

Two hundred grams (200g) of the powdered sample was weighed with a laboratory chemical balance and poured into a round bottom flask containing 500ml of methanol where its active component was extracted by reflux extractor method at 60°C for 2 hours according to the method described by Ugwu *et al.*, [6] and Kabiru *et al.*, [3]. The extract was filtered using muslin cloth and the filtrate was slowly evaporated to dryness using the steam bath. The crude extract was weighed and yield preserved in the freezer.

Test Organisms

The test organism (7 bacteria isolates) that was used for this study, were all obtained from the microbiology unit of General Hospital laboratory Minna, Niger state Nigeria as pure and subsequently confirmed

in the laboratory. The isolates include; *K. pneumonia*, *S. pyogenes*, *S. typhi*, *S. paratyphi A*, *S. paratyphi B*, *S. paratyphi C* and *S. pneumonia*. They were maintained by sub-culturing them on nutrient agar slant and stored at 4°C.

Phytochemical Analysis

Qualitative Phytochemical Estimation of the Crude Extract

Qualitative phytochemical analysis was conducted on the methanolic extract of *Zingiber officinale* using the method described by [9]. To determine the secondary metabolites such as alkaloid, flavonoids, saponins, cardiac glycosides, anthraquinones, sterols, phlobatanins that may be present in the extract.

Quantitative Phytochemical Estimation of the Crude Extract

The quantitative phytochemical screening of the extract for Total Alkaloids, Total Saponins, Total Flavonoid, Total Tannin and Total Phenol was carried out following standard procedures according to the method described by Singleton *et al.*, [10]; Oloyede [11]; Emmanuel *et al.*, [12]. At the Centre for Genetic Engineering and Biotechnology Laboratory, Federal University of Technology Minna, Niger State.

Statistical Analysis

The data are presented as mean \pm S.E.M. All the data were analyzed by one-way ANOVA and differences between the means were assessed with Duncan Multiple comparison test. Differences were considered significant at $p < 0.05$. All analyses were carried out using Statistical Package for Social Science (SPSS) version 20 (USA). For the same treatment, values affected by the same superscripts letter (a-d) are not significantly different).

Antimicrobial Susceptibility Test Standardization of Inoculums

One loopful of the test isolates were sub cultured at 37°C for 18hour in a nutrient broth culture thereafter suspended in sterile nutrient broth. It was standardized by gradually adding normal saline to compare its turbidity to McFarland standard 0.5 which is equivalent to density of 1.0×10^6 CFU/ml according to Clinical Laboratory Standards Institute.

Preparation of Extract Concentrations

Three different concentrations (80mg/ml, 160mg/ml and 240mg/ml) were obtained by dissolving 80mg, 160mg and 240mg of the extract in 1 ml of dimethyl sulfur oxide (DMSO) in different test tubes.

Susceptibility Testing of Plant Extracts

Mueller Hinton agar was prepared, sterilized and then inoculated with the standardized test organisms using the spread plate method and a sterile cork borer of diameter 6mm was used to bore equidistant wells onto the agar plates. The 80, 160 and 200mg/ml of the extract were transferred separately into the wells and the standard drug (Chloramphenicol) was used as the positive control while DMSO served as the negative control. The plates were incubated at 37°C for 24 hours and the average zones of inhibition were then measured to the nearest millimeter using ruler [13].

Test for Minimum Inhibitory Concentration (MIC)

One ml of the inhibited extract solution of the treatments of 240mg/ml were added to 1ml of Mueller Hinton Broth (MHB) and subsequently transferred. 1ml from the first test tube to the next, for up to the eighth test tube to obtain concentrations 240, 160, 80, 40, 20, 10, 5, and 2.5 mg/ml. Then 1ml of 24 h old culture of test bacterial organisms (1.0×10^6 cell/ml) was inoculated into each test tube and mixed thoroughly. The test tubes

were then incubated at 37°C for 24 h. The tube with the lowest dilution with no detectable growth was considered as the MIC. The same procedure was repeated using two test tubes, the first been the Positive control test tube which contains the MHB and the test organisms without the extract and the second been a negative control which contain MHB and 1 ml of the extract without the test organisms were also incubated alongside. The lowest concentration of the extract that did not show any visible turbidity (bacteria growth) was regarded as minimum inhibitory concentration MIC tubes [14].

Test for Minimum Bactericidal Concentration (MBC)

The test tubes that showed no visible turbidity (growth) in the MIC assay after incubation of the batch of the test tubes were sub cultured on freshly prepared nutrient agar plates and then incubated at 37°C for 24 hours. The minimum bactericidal concentration was considered as the concentration of the extract that did not show any growth on agar plate after incubation.

RESULTS AND DISCUSSION

RESULT

The qualitative Phytochemical screening of methanolic extract of ginger showed the presence of flavanoids, alkaloid, glycosides, saponins, tannin, phenols, cardiac glycosides, anthraquinones, steroids, phlobatannins and terpenes. The Quantitative phytochemical estimation of the extract which revealed an appreciable amount of phenols, flavonoids, tannins, alkaloids and saponins at a concentration of 1371.405 ± 206.551 , 564.223 ± 35.556 , 386.925 ± 5.058 , 273.540 ± 0.857 and 104.325 ± 1.042 respectively as showed on Table-1 below:

Table-1: Result of Qualitative phytochemical analysis of methanolic extract of *Z. officinale*

Phytochemicals	Qualitative analysis	Quantitative analysis
Alkaloids	+	273.540 ± 0.857
Flavonoids	+	564.223 ± 35.556
Tannins	+	386.925 ± 5.058
Saponins	+	104.325 ± 1.042
Phenols	+	1371.405 ± 206.5
Cardiac glycosides	+	ND
Anthraquinones	+	ND
Steroids	+	ND
Phlobatannins	+	ND
Terpenes	+	ND

Values are expressed in Mean \pm Standard Error of Mean

Key: + = present, ND= Not Determined

Table-2: Antimicrobial Activity of the Methanol Extract of *Z. officinale* on the selected bacteria isolates

Organisms	80 mg/ml	160 mg/ml	240 mg/ml	Chloramphenicol (30µg/ml)
<i>K. pneumonia</i>	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	16.67±0.57 ^a
<i>S. pyogenes</i>	12.00±2.00 ^c	13.00±0.00 ^c	15.00±1.00 ^c	17.67±0.57 ^a
<i>S. typhi</i>	10.00±1.00 ^{bc}	11.50±0.50 ^{bc}	14.50±0.50 ^c	14.33±1.00 ^a
<i>S. paratyphi A</i>	11.00±1.00 ^c	12.00±2.00 ^{bc}	14.50±0.50 ^c	16.33±1.15 ^a
<i>S. paratyphi B</i>	6.50±0.50 ^b	13.00±0.00 ^c	13.50±0.50 ^c	21.00±1.00 ^c
<i>S. paratyphi C</i>	8.50±1.50 ^{bc}	9.50±0.50 ^b	10.00±1.00 ^b	14.67±1.53 ^a
<i>S. pneumonia</i>	8.50±0.50 ^{bc}	9.00±0.00 ^b	9.00±0.00 ^b	16.67±0.57 ^a

Values are expressed in Mean ± Standard Error of Mean

a, b, c, means in the same row; values with the same superscript on the same row have no significance difference at p<0.05

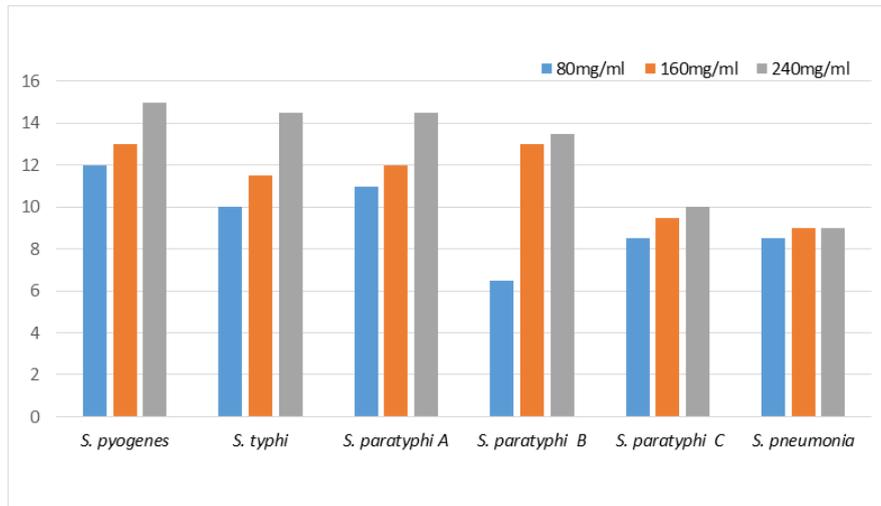


Fig-1: showing the average zone of inhibition of the methanolic extract of *Z. officinale* against the selected test organisms at different concentration

Table-3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Z. officinale*

Organisms	MIC(mg/ml)	MBC(mg/ml)
<i>S. pyogenes</i>	10	20
<i>S. typhi</i>	40	80
<i>S. paratyphi A</i>	10	40
<i>S. paratyphi B</i>	20	80
<i>S. paratyphi C</i>	40	160
<i>S. pneumonia</i>	20	160

DISCUSSION

The search for medical plants, herbs and spices for development of new therapeutic agents has been known over the years. Likewise, the mutation and resistance of disease causing agents to therapeutic medicines have also lead to continual search for better ways to treat, cure, manage and prevent health problems. In this study, the agar well diffusion method was used to evaluate the antibacterial potency of the plant extract and the result showed moderate zones of inhibition against all the tested organisms at different concentration (80, 160 and 200mg/ml) except in *K. pneumonia* with no inhibition in any of the concentration used. However, the highest diameter of the zone of inhibition was recorded at concentration 240mg/ml, with zones ranging from

15.00±1.00mm in *S. pyogenes* to 9.00±0.00mm in *S. pneumonia* as showed in Table-2. This agrees with the findings of Igwoezikpe *et al.*, [15] that the extract of *Zingiber officinale* have antibacterial potency against some pathogenic bacterial and could be used for the treatment of infections.

From Figure-1, the average zone of inhibition of the plant extract increased with increased in the concentration of the extract. This could be as a result of the increased in the concentration of the active component of the extract [16].

The qualitative phytochemical screening of the methanol extract of *Z. officinale* revealed the presence of

phytochemicals which include flavonoids, alkaloid, cardiac glycosides, saponins, anthraquinones, phlobatanins, sterols and terpenes (Table-1) which agrees with the findings of Ali *et al.*, [7]. The presence of these constituents no doubt may be responsible for the plant to be medicinal in nature owing to the fact that most of the phytochemical constituent present are reported to be used for the treatment of one ailment or the other as reported by Kenner and Requena [16]. Quantitative phytochemical estimation of the extract revealed an appreciable amount of phenols, flavonoids, tannins, alkaloids and saponins at a concentration of 1371.405 ± 206.551 , 564.223 ± 35.556 , 386.925 ± 5.058 , 273.540 ± 0.857 and 104.325 ± 1.042 respectively. The presences of these phytochemicals could be responsible for the efficacy of this plant in the treatment of infections or diseases [4].

Alkaloid is a natural product which contains heterocyclic nitrogen atoms with basic properties that are naturally produced by large number of organisms including animals, plants, bacteria and fungi [17]. They play important role in the protection of plant against pathogenic microorganisms, insects and herbivores. They have also been known for many pharmacological activities including antihypertensive, antiarrhythmic, antimalarial and anticancer activity. Therefore, high amount of alkaloid in this plant is an indication of its various medicinal values.

Phenol and phenolic compounds are the most widely distributed phytochemicals in the plant kingdom. They are large and complex group of chemical constituents found in plants [18]. Phenols are plant secondary metabolites that play important role in defense against infections. They possess several characters that are beneficial to humans including antioxidant properties which are important in protecting the body against free radical mediated disease [19]. Phenol has also been known to increase secretion of bile and reduces blood cholesterol level. They also have antibacterial activity against some strains of bacteria [20].

Saponins have been known to be antimicrobial. They inhibit the growth of mold and help to protect plants against insect attack hence, they are considered as part of plants defense systems. Saponin mixtures present in plants possess diverse biological effects when consumed by animals. They have been found to significantly affect growth, feed intake and reproduction in animals [21].

Furthermore, the result of the MIC value ranged from 6.25mg/ml to 50mg/ml and that of the MBC ranged from 25mg/ml to 200mg/ml. The low MIC and MBC values is an indication that the plant exert good antibacterial potency against the test organisms especially *S. pyogenes* which had the highest zones of

inhibition with lowest MIC and MBC values of 6.25 and 25mg/ml respectively.

CONCLUSION

The result of the study shows that the methanolic extract of *Zingiber officinale* has antibacterial activity against *S. pyogenes*, *S. typhi*, *S. paratyphi A*, *S. paratyphi B*, *S. paratyphi C* and *S. pneumonia* and could be used for the treatment of infections causes by these bacteria. The result of the study has also provided justification for therapeutic potentiality of this plant and different bacterial species exhibited different sensitivities towards the methanolic extract of ginger. However, the toxicity of the plant and its compatibility with the mammalian system is essential in order to enhance its exploitation in this regard in the treatment of diseases caused by resistant or multi drug resistant strains of bacteria.

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