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Antibacterial Potential of Ginger (*Zingiber officinale*) Extract against Bacterial Isolates of Pulmonary Infections

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ABSTRACT

There has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Therefore, this study aims at the determination of antibacterial potential of ginger extracts against bacteria isolates of pulmonary infections. The ginger rhizome, used in the study was obtained at the botanical garden of the Department of Pharmacognosy Delta State University Abraka where extraction processes were also carried out using methanol, and sterile distilled water. Standard bacteriological culture method was used for isolation of bacteria from the pulmonary infections. Three isolates of *Streptococcus pneumonia* (5.66%), six strains of *Klebsiella pneumonia* (11.32%), sixteen strains of *Staphylococcus aureus* (30.18%), ten isolates of *Escherichia coli* (18.86%), ten strains of *Pseudomonas aeruginosa* (18.86%) and eight isolates of *Proteus mirabilis* (15.09%) were tested against extracts of ginger using agar diffusion plate method. The result revealed a significant effect of methanol extracts of the ginger in all the isolates tested, but there were some resistant strains observed in the conventional antibiotics; while the sensitivity pattern of crude extract of ginger were less effective compared to methanol extract. The present work indicates that natural rhizome (ginger) has considerable antibacterial activities against bacterial isolates of pulmonary infections.

Keywords: Anti-bacterial, ginger, pulmonary, infections, Nigeria

INTRODUCTION

The medicinal value of ginger is due to synergistic functions of the chemical composition and active constituents which make it to be widely used in the management of disease condition (Shoji *et al*., 1982). The major constituents in *zingiber officinale* are the pungent vanilloids, 6-gingerol and 6-paradol, in addition to two other phenolic compounds, shogoals and zingerone in addition to 6-gongerol (Furman*et al.*, 2000). High amounts of iron (54-62mg/100g) and calcium (1.0%-1.5%) are found in ginger rhizomes (Uma Pradeep *et al.*, 1993). Ginger rhizomes contains both volatile oils and non volatile pungent compounds which can be extracted with solvents such acetone or alcohol (Gorindarajan,1992). Ginger is known to prevent motion sickness, elevate low blood pressure, lower blood cholesterol and prevent cancer in animals (Langner*et al*., 1998). Previous study has shown that ginger was active against such organisms as Plasmodium spp.,*Shigella dysenteries, Staphylococcus aureus, Pseudomonas aureginosa, Candida albican, Eschirichia coli, Klebsiella pneumonia, Streptococcus spp.* and *Salmonella spp* (Chinese researcher, 2001).The pulmonary infection is describedasacute infections involving the nose,par nasal, sinuses, pharynx, larynx, trachea and bronchi.Theinfection occurs more frequently during the cold winter months (Cooper, 2001).

Resident bacteria of the pulmonary such as *Streptococcus pyogenes*, *Haemophilus influenza*, and *Streptococcus pneumonia* are the most common causes of bacterial infections (Slack, 2002). The normal flora competes, with pathogenic organisms for potential attachment site, and often produces substances (toxins/acids) which are bactericidal (Chamberlin, 2002). This study is set to determine the effectiveness ofginger as an alternative antimicrobial agent in the treatment of pulmonary infections.

MATERIALS AND METHODS

Apparatus

Ginger, chemical beam balance, hot air oven, methanol, normal saline, distilled water, cotton wool, soxhlet apparatus, blending machine, retort stand, conical flask, test tubes, Bunsen burner, sterile universal containers and Petri dishes. All the equipment, glasswares, reagents and consumables were products of CamLab: quality lab product and scientific laboratory supplies (UK).

Collection of Test Organisms

Fifty- three bacterial isolates which comprises of three strains of *Streptococcus pneumonia*, six strains of *klebsiella pneumonia*, sixteen strains of *Staphylococcus aureus*,ten isolates of

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Escherichia coli, ten isolates of *Pseudomonas aeruginonas*, eight strains of *Proteus mirabilis* and three control organisms used namely *Staphylococcus aureus*(NCTC) 6571, *Pseudomonas aeruginonas*(NCTC) 10662, *Escherichia coli* (NCTC) 10416 were collected from the Department of Microbiology, Faculty of Science, Delta State University, Abraka.

Collection and Authentication of Ginger
Fresh ginger rhizomes were obtained from the botanical garden of the Department of Pharmacognosy, Delta State University Abraka Southern Nigeria. The identification and confirmation of the ginger was carried out in thePharmacognosy Laboratory of Delta State University by a pharmacist who specialized in Pharmacognosy.

Solvents for Extraction
The solvents chosen for the ginger extraction were methanol, normal saline and distilled water for crude method extraction.

Preparation of Ginger for Extraction
The ginger was rinsed with sterile distilled water to remove dirty materials on the rhizomes. These were then crushed into a smaller piece using a clean sterile mortar and pestle.

Methanol extraction
Ginger was crushed using a clean sterile mortal and pestles. Crushed ginger of 100 g was accurately weighed out into an extraction tumbler and covered with cotton wool. Soxhlet extraction using methanol was performed.

Distilled water extraction (crude method)
100g of the crushed ginger was put into a glass basin;100mls of the sterile distilled water was added. It was covered and allowed to soak for 72 hours. The ginger was blended using the blending machine and filtered through a bed of sterile cotton wool. The filtrate was then sterilized using a membrane filter of 0.45µm pure size, pressure being applied using a syringe. Then 25ml was measured out from the extract and pour into a sterile universal container.

Sterility test of the extract
Exactly, 2mls of each extracts was added to 8mls of peptone water and incubated at 37^{o}C for 24 hours. It was subculture on MacConkey agar, blood agar, and chocolate agar plates, incubated at 37^{o} C again for 24 hours and plates were observed for growth; but no growth was found.

Preparation of test organisms
Microbial cultures were prepared from nutrients agar slopes by inoculating pure cultures of each organism into 3 ml sterile peptone water and each of the bijou bottles were incubated for 24 hours at 37^{o}C.

Sub-culturing of the test organisms
The test organisms were sub-cultured on nutrient agar slopes which were prepared using the manufacturer's specification of 28 g to 100 ml of water. A stock solution was prepared and sterilized using the autoclave at a temperature of 121°C for 15 minutes. The solution was then allowed to cool and then poured into sterile bijou bottles, slanted and allowed to solidify. The test organisms were streaked on the agar using a flamed wire loop and incubated at 37°C for 24 hours.

Identification of Isolates
All the bacteria isolates were identified and confirmed based on morphological, physiological and biochemical test (Cheesbrough, 2000; Cowan, and Steel, 1974; Cruikshank *et al.*, 1975).

Antibacterial sensitivity testing using agar diffusion plate method
Twenty millilitres each of prepared Muller Hinton agar was dispensed into Mac Cartney bottle and sterilized by autoclaving for 15 minutes at 121^{o}C. The agar was cooled to 45^{o}C before pouring into Petri dishes and allowed to set. Then, 1 in 10 dilution of the overnight broth culture of the bacteria was prepared in order to obtain confluent colonies and then flooded on dried plates of Muller Hinton agar and left on the working bench for about 5 minutes to diffuse. Excess diluted broth culture on agar surface was discarded into discard jar and placed on the working bench undisturbed and a sterile cork bore No4 was used to make wells on the agar. Each well was filled with different dilution and concentration of each of ginger extract (Neat, 1/2, 1/4, 1/8,1/16, 1/32 and 1/64 control.
The plates were then incubated at 37^{o}C and examined for zones of inhibition after 24 hours.
Sensitivity: It was taken as a zone of inhibition of 12 mm diameter or more than that of the control organisms (Baker *et al*., 1980).

RESULTS
In table 1:the entire control organisms, showed the sensitive of up to 1/8 dilution of the methanol extract while three isolates, of *Streptococcus pneumonia* were sensitive up to 1/4 dilution also the *Klebsiella*

pneumonia. None of the isolates shows sensitivity up to 1/16 dilution. E. coli (3), *Pseudomonas aeruginosa* (3) *Proteus mirabilis* (2), *Staphylococcus aureus* (4) and *Klebsiella* (2), were inhibited at 1/16 dilution. Few strains such as *Klebsiella pneumonia* (1), *Staphylococcus aureus* (3), *Escherichia coli* (2), *Pseudomonas aeruginosa*(1), *Proteus mirabilis* (1) were inhibited up to 1/32 dilution. Table 2: this shows the sensitivity pattern of isolates to crude extract of ginger. All the control organisms shows the sensitivity up to 1/8 dilution of ginger extract variation occur from1/2dilution while *Klebsiella pneumonia* (5), *Staphylococcus aureus*(13), *Echerichia coli* (7), *Pseudomonas aeruginosa (4) and Proteus mirabilis*(4), shows sensitive to 1/2dilution while all the three isolates of *Streptococcus pneumonia* were sensitive. Out of the three isolates of *Streptococcus pneumonaie*, (2) were sensitive todilution (1), to 1/8 dilution but none to 1/16 dilution.

Three isolates of *Klesiella pneumonia*, *Staphylococcus aureus* (10), *Escherichia coli* (8), *Psuedomonasaeruginosa*(3) *and Proteus mirabilis* (3) were inhibited by 1/4 dilution. However, their proportion of sensitivity reduces with reduction in the concentration of ginger extracts. No organism shows sensitive up to 1/64 dilution. But *Staphylococcus aureus*(1), *Escherichia coli* (1) showed sensitivity up to 1/32 dilution while other isolates, were resistant to 1/32 dilution of ginger extracts. Table 3: shows original sensitivity pattern of the organisms to conventional antibiotics. Majority of the isolates were found to be sensitive to Gentamycin. Ciprofloxacin, Recophin, Ceftazidine, Ofloxacin while Chloraphenicol was found to be less effective. As only 3 out of 16 isolates of *Staphylococcus aureus* were sensitive to Chlorophenicol while others were resistant to it.

Table 1: Sensitivity pattern of susceptible organisms to methanol extract of ginger

Control/test organisms	No. of Isolates	Neat		1:2		1:4		1:8		1:16		1:32		1:64	
Control		S	R	S	R	S	R	S	R	S	R	S	R	S	R
Staphylococcus aureusNCTC 6571	1	1	0	1	0	1	0	1	0	1	0	01		01	
Pseudomonas aeruginosaNCTC 10662	1	1	0	1	0	1	0	1	0	01		01		01	
*Escherichia coli*NCTC 104996	1	1	0	1	0	1	0	1	0	01		01		01	
Test															
Strept. Pneumonia	3	3	0	3	0	3	0	2	1	0	3	0	3	0	3
Klebsiella pneumonia	6	6	0	6	0	6	0	3	3	2	4	1	5	0	6
Staphylococcus aureus	16	16	0	10	6	8	8	6	0	4	12	3	13	0	16
Escherichia coli	10	10	0	82		64		4	6	3	7	28		0	10
Pseudomonas aeruginonas	10	10	0	7	3	6	4	4	6	3	7	1	9	0	10
Protesu mirabilis	8	8	0	7	1	6	2	4	4	2	6	1	7	0	8

S- Sensitive; R- Resistance

Table 2: Sensitivity Pattern of Susceptible Organisms to Distilled Water Extract of Ginger.

Species	No of Isolates	Neat		1:2		1:4		1:8		1:16		1:32		1:64	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
*Staphylococcus aureus*NCTC 6571	1	1	0	1	0	1	0	1	0	1	0	0	1	0	1
*Pseudomonas aeruginosa*NCTC 10662	1	1	0	1	0	1	0	1	0	0	1	0	1	01	
*Escherichia coli*NCTC 10496	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1
Streptococcus pneumonia	3	3	0	3	0	3	0	1	2	0	3	0	3	0	3
Klebsiella pneumonia	6	6	0	6	0	6	1	4	2	3	3	2	4	0	6
Staphylococcus aureus	16	16	0	16	0	14	2	13	3	10	6	9	7	0	16
Escherichia coli	10	10	0	8	2	7	3	4	6	3	7	2	8	0	10
Pseudomonas aeruginosa	10	10	0	5	10	3	7	2	8	1	9	0	10	0	10
Proteus mirabilis	8	8	0	5	3	4	4	3	5	2	6	1	7	0	8

S – Sensitive R – Resistance

Table 3: Sensitivity Pattern of Susceptible Organisms to Conventional Antibiotics

Species	No of Isolates	GEN		C.F		CRO		CHL		OFL		OFL		CAZ	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
*Staphylococcus aureus*NCTC 6571	1	1	0	1	0	1	0	1	0	1	0	0	1	0	1
*Pseudomonas aeruginosa*NCTC 10662	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1
*Escherichia coli*NCTC 10496	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1
Streptococcus pneumoniae	3	3	0	3	0	3	0	0	3	3	0	3	0	0	3
Klebsiella pneumonia	6	6	0	5	1	4	2	0	6	4	2	5	1	0	6
Staphylococcus aureus	16	16	0	16	0	16	0	3	13	16	0	16	0	0	16
Escherichia coli	10	10	0	8	2	3	7	0	10	7	3	2 8		0	10
Pseudomonas aeruginosa	10	3	7	8	2	5	5	0	10	8	2	6	4	0	10
Proteus mirabilis	8	5	3	80		80		08		80		80		0	8

S – Sensitivity R – Resistance

DISCUSSION

In this study, all the test organisms were susceptible to ginger extracts (methanol, and distilled water) but at different concentrations which could be due to solvent action of each extract. The susceptibility to methanol extracts more than distilled water could be as a result of methanol's ability to interfere with the bacteria cell. Despite this fact, ginger has proven itself to possess antibacterial properties against most aetiological agents of pulmonary infections as earlier reported by Datta*et al*.(1997). This finding confirmed the work of Chinese researchers (2001) who reported ginger as chemotherapeutic agents against organisms *Plasmodium spp. Shigelladysenteria, Staphylpcoccusaureus, Pseudomonas aeruginosa, Candida albican, Escherichia coli, Klebsiella pneumonia, Streptococcus spp.* although, our study did not include some of the organisms reported.

It was observed that antimicrobial activity of ginger decreases with increase in dilutions. Most of the isolates were sensitive to at least 1/8 dilution. Therefore the antimicrobial activity of concentrated ginger extracts were better than the diluted ones, but this could lead to adverse effect i.e. toxicity when taken in large amounts. It has earlier been stated that the presence of bioactive compounds in ginger could lead to inhibition of blood platelet aggregation and caused increased in bleeding due to inhibition of prostaglanding, leukotrienes, and thromoboxane biosynthesis (Argento*et al*., 2000).

In this study, it was noted that ginger has a broad spectrum in antibacterial properties, against both gram positive and gram negative bacteria. Findings have shown that methanolic extract of ginger injected subcutaneously (100gm/kg) in dogs infected with Dirofilariaimmitis reduced microfilarial concentrations in theblood by 98% (Datta*et al*., 1997) which was not determined in this study. However, it further confirms the efficacy of ginger as potential antimicrobial agent.

CONCLUSION

In the present study, ginger has demonstrated effective antimicrobial activities against*Streptococcus pneumonaie, Klebsiella pneumonia, Staphylpcoccusaureus, Escherichia coli, Proteus mirabilis.* This suggests the usefulness of ginger as an alternative to conventional chemotherapeutic agents.

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Conflict of Interest: The authors declare no conflict of interest

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