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Antibacterial Activity of Ginger (*Zingiber officinale*) Against Isolated Bacteria from the Respiratory Tract Infections

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ABSTRACT
This study was designed to investigate the bacteria found in association with respiratory tract infections (RTI) and the susceptibility of such bacteria to aqueous and methanolic extracts of *Zingiber officinale* (ginger). Bacteriological studies were carried out on sputum from 110 patients, made up of 57 males and 53 females, aged 24-80 years, attending Chest Clinic at Ekiti State University Teaching Hospital Ado-Ekiti in the year 2013. Seventeen bacteria species namely *Luteococcus sanguinuis, Corynebacterium accolens, Vibrio fluvialis, Serratia ficaria, Enterobacter aerogenes, Staphylococus aureus, Pasteurella pneumotropica, Aeromonas caviae, Vibrio mimicus, Vibrio parahaemolyticus, Pleisomonas shigelloides, Pseudomonas aeruginosa, Citrobacter youngae, Chromobacterium violaceum, Luteococcus pertonei, Actinomyces radicidentis and Klebsiella pneumoniae* were isolated from the sputum specimens. *Klebsiella pneumoniae, Corynebacterium accolens, Aeromonas caviae* and *Luteococcus sanguinis* were commonly isolated from both sexes. *Enterobacter aerogenes, Vibrio parahaemolyticus, Luteococcus peritonei, Citrobacter youngae, Pleisomonas shigelloides and Serratia ficaria* were isolated mainly from male patients while *Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio fluvialis, Vibrio mimicus, Citrobacter youngae and Chromobacterium violaceum* were isolated from female patients. The bacteria showed a high degree of resistance to the antibiotics used. All the Gram positive bacteria were resistant to cloxacillin, augmentin and tetracycline, with varied resistance to erythromycin (85%), streptomycin (68.75%) and cotrimoxazole (75%). The gram negative bacterial isolates were all resistant to augmentin, tetracyclin and amoxicillin, but were all susceptible to ofloxacin. All the bacteria tested were susceptible to both aqueous and the methanol extracts of ginger, but with higher susceptibility of bacteria to methanolic extract than the water extract. The phytochemical analysis of the ginger extracts indicated that the methanolic extract possessed phenolics, saponin, tannin and flavonoids, but no glycoside detected. On the other hand only cardiac glycoside, out of the 5 phytochemicals, was detected from the aqueous extract of ginger.
Key words: Antimicrobial Activity, Ginger, Respiratory Tract Infection

INTRODUCTION

Respiratory tract infections (RTIs) are caused by the invasion of the respiratory tract by infectious microorganisms such as bacteria and virus. An infection of this type is further classified as an upper respiratory tract infection (URI or URTI) or a lower respiratory tract infection (LRI or LRTI). Lower respiratory infections, such as pneumonia, tend to be far more serious conditions than upper respiratory infections, such as the common cold. Typical infections of the upper respiratory tract include tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media, certain types of influenza, and the common cold. Symptoms of URIs can include cough, sore throat, runny nose, nasal congestion, headache, low grade fever, facial pressure and sneezing (Eccles, *et al.,* 2007).

The lower respiratory tract consists of the trachea (wind pipe), bronchial tubes, the bronchioles, and the lungs. Lower respiratory tract infections are generally more serious than upper respiratory infections. LRIs are the leading cause of death among all infectious diseases (Beaglehole, *et al.,* 2004). The two most common LRIs are bronchitis and pneumonia (Antibiotic Expert Group, 2006). Lower respiratory tract infection, while often used as a synonym for pneumonia, can also be applied to other types of infection including lung abscess and acute bronchitis. Symptoms of LRI include shortness of breath, weakness, high fever, coughing and fatigue. However in 2002 they were still the leading cause of deaths among all infectious diseases, and they accounted for 3.9 million deaths worldwide and 6.9% of all deaths that year (Beaglehole *et al*., 2004).

According to Guthrie (2001), *Haemophilus influenzae* and *Moraxella catarrhalis* are of increasing importance in both community acquired pneumonia (CAP) and acute exacerbation of chronic bronchitis (AECB) while the importance of *Streptococcus pneumoniae* is declining. It has also become apparent the importance of atypical pathogens such as *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae* and *Legionella pneumophila*, in CAP (Guthrie, 2001)

Interest in plants with antimicrobial properties has been revived as a result of microbial resistance to commonly prescribed drugs. This resistance could be attributed to indiscriminate use of commercial drugs or not taking an antibiotic prescription according to the instruction, for example, not taking all the prescription in the treatment of infectious diseases (Aliero and Afolayan, 2006). It is important to use appropriate antibiotic selection based on the infecting organism to avoid the evolving nature of these infectious agents that could result in the emerging resistance to conventional antimicrobials (Guthrie, 2001). The high cost of conventional drugs, particularly in resource limited communities has led to the increased use of plants as an alternative for treatment of infectious diseases. Plant extracts with phytochemicals of good antimicrobial properties are of great significance in treatment of infectious diseases. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of novel antibiotic prototypes (Riaz *et al.*, 2015).

Ginger has been found effective in the treatment of a wide range of human ill-health conditions such as cataract, heart disease, migraines, struck amenorrhea, athlete's foot, bursitis, chronic fatigue, cold, flu, coughs, depression, dizziness, fever, erectile difficulties, kidney stones, Reynard's disease and viral infection (Peggy, 2006). Research on rats suggested that ginger may be useful for treating diabetes (Ody, 1997).

This study was designed to investigate the bacteria found in association with respiratory tract infections and the susceptibility of such bacteria to the aqueous and methanolic extracts of ginger.

MATERIALS AND METHODS

Study area and population

This investigation was carried out on patients visiting the Ekiti State University Teaching Hospital, Ado, Ekiti State, Nigeria over a period of five months (Feb- June, 2013.). Sputum was obtained from 110 patients into sample bottles. Information was obtained from each patient as regards, the symptomatic effect as in incessant sneezing or coughing, or very dry or wet cough or nasal discharge and antimicrobial therapy, sex and age. The sputum samples were transported in cold environment to the Microbiology Laboratory of Afe Babalola University, Ado-Ekiti, where bacteriological investigations were carried out.

Inoculation, isolation, characterization and identification of isolates.

The sputum samples were thoroughly mixed, a loopfull streaked with the aid of inoculating wire loop on nutrient, macConkey, blood and chocolate blood agars. District colonies obtained were subcultured severally until pure cultures were obtained. All isolates were then characterised using standard microbiological and biochemical tests as described by Barrow and Feltham [1993], and Cheesbrough [2005]. Bacterial isolates were identified with the help of online Gideon Informatics [1999-2014] with reference to Barrow and Feltham [1993] and Garrity *et al.*[2005].

Antibiotic susceptibility test

Antibiotic susceptibility tests on the bacterial isolates were carried out using standard antibiotics discs. Even spreads of theisolates were made on Mueller-Hinton agar plates using sterile swab sticks followed by aseptic placement of the antibiotic discs. The plates are then inverted and incubated for 24hrs after which observations were taken (Clinical and Laboratory Standards Institute, 2013). Antibiotics used are Augmentin (30µg), Ofloxacin (5µg), Gentamicin (10µg), Nalidixic acid (30µg), Nitrofurantoin (200µg), Amoxycillin (25µg), Tetracycline (25µg) for gram negative isolates, while Augmentin (30µg), Cotrimoxazole (25µg), Cloxacillin (5µg), Erythromycin (5µg), Gentamicin (10µg), Streptomycin (10µg), Tetracycline (10µg) and Chloramphenicol (10µg) were used for gram positive isolates.

Preparation of ginger extracts

Ginger (*Zingiber officinale*) used in this present study was purchased from the local market in Ado-Ekiti, Nigeria. Aqueous and methanol extracts from ginger were prepared separately. The fresh ginger rhizomes were washed, peeled, sliced and air dried for 21 days. After drying, ginger was ground to fine powder using electric blender. Ten gram each of ginger powder was soaked in 100ml of distilled water and 100ml of methanol

separately. The flasks were incubated at room temperature for 72 hours. The crude extracts were centrifuged at 3000rpm for 10 minutes at 28±2°C, and the supernatant filtered with filter paper. The extracts were concentrated using a rotary evaporator.

Microbial Susceptibility by Ginger extracts using Agar-well diffusion

The antimicrobial assay of ginger extract was performed by deep-well agar diffusion method using Mueller Hinton agar. The Mueller Hinton agar were inoculated separately with 10^7CFU of each test bacterial culture and evenly spread on entire surface of each plate. The agar was carefully punched using a cork-borer (5mm diameter) and the extracts of different concentrations were dispensed into the wells agar wells seeded with bacterial culture. The plates were left at ambient temperature for 15 minutes and then incubated at 37°C for 18-24 hours and observed for zone of inhibition. The diameter of inhibition zones was measured in millimetres.

Phytochemical analysis of ginger extract

Tests for the presence of the following plant secondary metabolites: alkaloids, saponins, tannins, flavonoids, steroids and cardiac glycosides were carried out on the aqueous and methanolic extracts of the rhizome of *Zingiber officinale* as described by Sofowora (2008).

Test for alkaloids: A volume 2ml of the extract was added to 1ml of aqueous hydrochloric acid. A few drops of saturated picric acid solution were added. A creamish precipitate obtained indicated the presence of alkaloid.

Test for tannins: To 2ml of the plant extract was dissolved in 10ml of distilled water and filtered. 2 ml of the extract was added to 2ml $FeCl_3$. If a blue-black precipitate was obtained, this indicated the presence of tannins.

Test for saponins (Frothing test): From the extract, 0.5ml was added to 5ml distilled water. Frothing presence indicated presence of saponnins.

Test for flavonoids: About 2ml of plant extract was weighed in a test tube and dissolved diluted NaOH and diluted HCl were added. The presence of yellow solution that colourless indicates the presence of flavonoids.

Test for steroids: 10ml of plant extract was dissolved in 10ml chloroform and filtered. 2ml of the filtrate was added to 2ml acetic anhydride and Conc H_2SO_4. Blue-green ring obtained the presence of terpenoids.

Test for Cardiac glycosides: 2ml of extract was added to 1ml glacial acetic acid, 1ml $FeCl_3$ and 1ml Concentrated H_2SO_4. Absence of green-blue colour indicated that cardiac glycosides were absent.

RESULTS

Bacteriological studieds were carried out on sputum from 110 patients made up of 57 males and 53 females, age 24-80 years. Seventeen bacteria species namely, *Luteococcus Sanguinuis, Corynebacterium accolens, Vibrio fluvialis, Serratia ficaria, Enterobacter aerogens, Staphylococus aureus, Pasteurella pneumotropica, Aeromonas caviae, Vibrio mimicus, Pleisomonas shigelloides, Pseudomonas aeruginosa, Citrobacter youngae, Chromobacterium violaceum, Luteococcus pertonei, Actinomyces radicidentis, V. Parahaemolyticus and Klebsiella pneumonia,* were isolated from the sputum specimens (Figure 1).

Distribution of bacteria along gender is presented in Figure 2. *K. pneumoniae, C. accolens, A. caviae* and *L. sanguinis* were commonly isolated from both sexes. *E. aerogenes, V. parahaemolyticus, L. peritonei, C. youngae P. shigelloides and* S. ficaria were isolated mainly from male patients while *S. aureus, P. aeruginosa, V. fluvialis, V. mimicus, C. youngae and C. violaceum* were isolated female patients only. No significant differences obtained in the distribution of the bacterial isolates along sex and age ($p>0.05$).

The bacteria showed a high degree of resistance to the antibiotics used. All the Gram positive bacteria were resistant to cloxacillin, augmentin and tetracycline, with varied resistance to erythromycin (85%), streptomycin (68.75%) and cotrimoxazole (75%), Table 1. The gram negative bacterial isolates were all resistant to augmentin, tetracyclin and amoxicillin, but were all susceptible to ofloxacin (Table 2). All the bacteria tested were susceptible to both aqueous and the methanol extracts of ginger, but with higher susceptibility of bacteria to methanolic extract than the aqueous extract (Table 3). The phytochemical analysis of the ginger extracts indicated that the methanolic extract possessed phenolics, saponin, tannin and flavonoids, but no glycoside detected. On the other hand only cardiac glycoside, out of the 5 phytochemicals, was detected from the aqueous extract of ginger (Table 4).

DISCUSSION

Respiratory tract infections (RTIs) continue to be a major cause of morbidity and mortality worldwide (Akoachere, 2002). Hence the need for continuous investigation of the causative agents, as well as, looking for therapeutic measures is very germane. Attention has been focused in recent studies in exploring natural

phytochemicals found in plants to ameliorate the health of humans and animals that are continuously mutilated by ever emerging drug resistant microorganisms (Gereard, 2011)

The study revealed a variety of bacteria that could play a significant role in epidemiology of respiratory diseases in Nigeria. A total of 17 bacteria species namely *Klebsiella pneumoniae, Luteococcus sanguinis, Corynebacterium accolens, Vibrio Fluvialis, Serratia ficaria, Enterobacter aerogenes, Staphylococus aureus, Pasteurella pneumotropica, Aeromonas caviae, Vibrio mimicus, Vibrio parahaemolyticus, Pleisomonas shigelloides, Pseudomonas aeruginosa, Citrobacter youngae, Chromobacterium violaceum, Luteococcus peritonei* and *Actinomyces radicidentis* were isolated from the sputum specimens. *Klebsiella pneumoniae* has the highest frequency of isolation. Some of the organisms have been reported in earlier studies in Nigeria (Taura *et al.*, 2013) and other parts of Africa (Regasa, 2014).

The bacteria showed a high degree of resistance to the antibiotics tested. The Gram positive bacteria were all resistant to cloxacillin, augmentin and tetracycline, but with varied resistance to erythromycin (85%), streptomycin (68.75%) and cotrimoxazole (75%). The gram negative isolates were wholly resistant to augmentin, tetracycline and amoxicillin, but totally susceptible to ofloxacin. The tested bacterial isolates were susceptible to both aqueous and the methanolic extract of ginger used. These findings correspond to earlier reports by Nascimento, *et al.* (2000) and Peggy, (2006).

With the high susceptibility of bacterial isolates to ginger extracts obtained from this study, it is evident that methanolic and aqueous extracts of ginger can serve as suitable antimicrobial chemotherapeutics for the treatment of some respiratory tract infections. Future studies should be devoted to the understanding of the various mechanisms of actions of the phytochemical active ingredients that are found in ginger.

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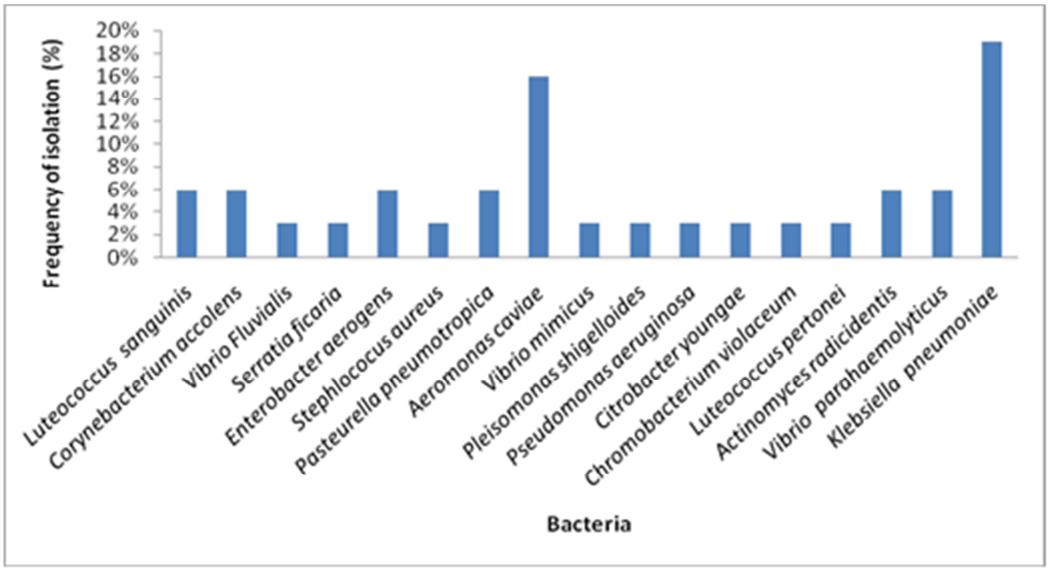

Figure 1: Frequency of bacteria isolated from sputum

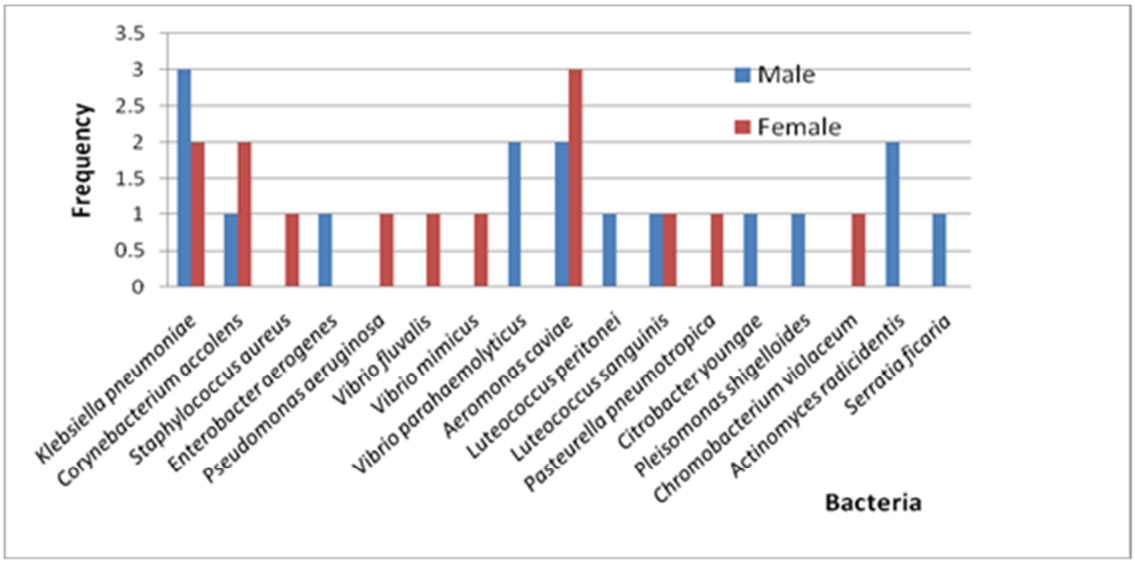

Figure 2: Distribution of frequency of bacteria isolated from sputum along sex

Table 1: Antibiotic susceptibility of gram positive bacterial isolates

Identified Isolates	GEN (10µg)	ERY (5µg)	CXC (5µg)	CHL (10µg)	STR (10ug)	AUG (30µg)	COT (25µg)	TET (10µg)
Luteococcus sanguinis	I	R	R	S	R	R	S	R
Corynebacterium accolens	S	R	R	R	R	R	R	R
Staphylococcus aureus	I	R	R	R	I	R	R	R
Corynebacterium accolens	S	R	R	R	R	R	R	R
Luteococcus sanguinis	S	I	R	S	R	R	S	R
Luteococcus peritonei	R	R	R	R	R	R	R	R
Actinomyces radicidentis	I	I	R	S	S	R	R	R
Actinomyces radicidentis	S	I	R	S	S	R	R	R
Susceptibility (%)	68.75	18.75	R	50	31.25	0	25	0
Resistance (%)	31.25	81.25	100	50	68.75	100	75	100

Key: S- Susceptible R- Resistant I- Intermediate.

Table 2: Antibiotic susceptibility of gram negative bacterial isolates

Bacterial Isolates	AUG (30µg)	TET (25µg)	AMX (25µg)	COT (25µg)	NIT (200µg)	NAL (30µg)	GEN (10µg)	OFL (5µg)
Vibrio fluvialis	R	R	R	R	R	R	R	S
Vibrio mimicus	R	S	R	S	S	R	S	S
Serratia ficaria	R	R	R	R	R	R	S	S
Pseudomonas aeruginosa	R	R	R	R	S	S	S	S
Citrobacter youngae	R	R	R	R	R	S	S	S
Plesiomonas shigelloides	R	R	R	S	R	S	S	S
Chromobaterium shigelloides	R	R	R	S	S	S	S	S
Enterobacter aerogenes I	R	R	R	R	S	S	S	S
Enterobacter aerogenes II	R	R	R	R	S	S	I	S
Pasteurella pneumotropica I	R	R	R	I	R	S	I	S
Pasteurella pneumotropica II	R	R	R	R	S	S	S	S
Aeromonas caviae I	R	R	R	R	S	S	S	S
Aeromonas caviae II	R	R	R	R	S	S	I	S
Aeromonas caviae II	R	R	R	R	S	S	I	S
Aeromonas caviae III	R	R	R	R	S	R	S	S
Aeromonas caviae IV	R	R	R	R	S	S	I	S
Vibrio parahaemolyticus I	R	R	R	S	S	S	S	S
Vibrio parahaemolyticus II	R	S	R	S	S	S	I	S
Klebsiella pneumonia I	R	I	R	R	S	R	S	S
K. pneumonia II	R	S	R	S	S	S	R	S
K. pneumonia III	R	I	R	R	S	R	S	S
K. pneumonia IV	R	S	R	S	S	S	R	S
K. pneumonia V	R	I	R	R	S	R	S	S
K. pneumonia VI	R	S	R	S	S	S	R	S
Susceptibility (%)	0	0	0	35.42	79.2	70.83	70.83	100
Resistance (%)	100	100	100	64.58	20.8	29.17	29.17	0

Table3: Antibacterial susceotibility of ginger extracts on the bacterial isolates

Bacterial Isolates	METHANOLIC EXTRACT				AQUEOUS EXTRACT			
	400mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml
Luteococcus sanguinis	7	5	3	0	5	3	0	0
Corynebacterium accolens	9	5	3	0	9	5	0	0
Serratia ficaria	7	10	0	0	5	0	0	0
Pasteurella pneumotropica	7	8	0	0	3	0	0	0
Aeromonas caviae	7	5	3	0	11	7	5	3
Pasteurella pneumotropica	9	5	3	0	9	5	0	0
Pleisomonas shigelloides	7	5	0	0	5	3	0	0
Vibrio parahaemolyticus	7	5	0	0	5	0	0	0
V. parahaemolyticus	9	5	0	0	5	3	0	0
Klebsiella pneumoniae	13	9	5	3	7	5	3	0
K. pneumoniae	13	9	5	3	9	5	3	0
K. pneumoniae	13	11	9	3	9	5	3	0
K. pneumoniae	13	5	5	3	7	5	3	0
K. pneumoniae	13	9	7	5	7	5	3	0
K. pneumonia ATCC 13883	18	15	10	7	15	10	8	5
Staphylococcus aureus (ATCC 25923)	16	15	12	9	13	11	10	8

Values in millimetre

Table 4: Qualitative phytochemical analysis of ginger extract

Phytochemicals	Aqueous Extract	Methanolic Extract
Phenolic	-	+
Saponin	-	+
Tannins	-	+
Flavonoids	-	+
Glycosides	+	-