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ANTIBACTERIAL ACTIVITY OF THE EXTRACTS OF *Piper guineense* (Schumach), and *Zingiber officinale* (Roscoe) AND HONEY ON SELECTED PNEUMONIC BACTERIA IN NIGERIA

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ABSTRACT: Aim and Objective: Pneumonia is still a serious disease in most developing countries. Although, pneumonia can be treated with antibiotics but the issue of bacterial resistance to antibiotics leads to treatment failure. Therefore, there is the need to seek for alternative therapy of this infection. In this study, different extracts of *Piper guineense* (Seed), rhizome of *Zingiber officinale* and honey used to treat different ailments in folk medicine were examined for possible antibacterial activity on commonly encountered pneumonic bacteria in Ondo State, Southwest, Nigeria. The seeds of *Piper guineense* (African Black Pepper) and rhizome of *Zingiber officinale* (Ginger) were separately extracted using ethanol, N-hexane and hot water according to standard methods. Susceptibility of selected pneumonic bacteria to the extracts and honey singly and in combinations was determined using agar diffusion assay. The MIC, the MBC and the phytochemical profile of the extracts were carried out using standard methods. The *in-vitro* antibacterial assay of the different extracts of seed of *Piper guineense*, rhizome of *Zingiber offiniale* and honey shows that they exerted growth inhibitory activity on the test pneumonic bacteria with zone diameter ranging from 6.25 to 21.25mm which was superior to the one mediated by control antibiotics (amoxicillin, perfloxacilin, ciprofloxacin and chloramphenicol) used with zone diameter ranging from 1.25 to 16.0mm. Phytochemicals such as saponin, tannin, flavonoid, terpenoid, alkaloid and cardiac glycosides were detected in the extracts and honey. The findings showed that the different extracts of seeds of *Piper guineense*, rhizome of *Zingiber officinale* and honey exerted growth inhibitory activity that was superior to that of the antibiotics used against the test pneumonic bacteria in the *in-vitro* assay. Therefore the extracts has novel antibacterial properties that could be harnessed as alternatives for treating bacterial pneumonia.

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Keywords: *Piper guineense*, *Zingiber officinale*, honey, Pneumonic Bacteria, antibacterial activities

INTRODUCTION

Pneumonia is an inflammatory condition of the lung primarily affecting small air sac known as alveoli (Leach, 2009; McLuckie, 2009). Symptoms of pneumonia include productive or dry cough, chest pain, fever and difficulty breathing (Ashby and Turkington, 2007). Although, it can be treated with antibiotics. However, most of these antibiotics are becoming less effective because of the alarming rate of development of resistance to antibiotics by many bacterial species thereby leading to treatment failure (Mene 'ndez and Torres, 2007; Odokor and Addo; 2011; Thenmozhi *et al.*, 2014).

Over the years, plant extracts and plant-derived medicines have been reported to have an immense impact on the overall health and wellbeing of man (Anyanwu and Nwosu, 2014). For example, *Piper guineense*, has been reported to have antibacterial, antioxidant and anti-inflammatory properties (Balogun et al., 2016). *Zingiber officinale* another plant has been reported for treating upper respiratory tract infections, cough, and bronchitis (Shukla and Singh, 2007) while honey has been reported as an effective natural cure for certain infections, such as respiratory diseases and for the healing of skin burns and wounds (Basualdo et al., 2007; Bizerra, 2012; Boukraa, 2013; John-Isa et al., 2022).

Therefore in this study, the extract that is made from seeds of *Piper guineense* (Schumach), the extract from the rhizome of *Zingiber officinale* (Roscoe) and honey singly and in combinations were examined for possible antibacterial activity on common bacteria causing pneumonia in Ondo State, Southwest, Nigeria in

searching for alternatives to antibiotics to treat bacterial pneumonia.

MATERIALS AND METHODS

Sample collection

Seeds of *Piper guineense* and rhizome of *Zingiber officinale* (Ginger) were purchased at the Oja Oba market, Akure. The plants were used to prepare a voucher specimen (NO. FHI.113939) which was deposited at Forest Herbarium, Ibadan, Oyo State, Nigeria. The honey used was harvested from a bee farm at Ile-Ife, Osun State, Nigeria. The Pneumonic bacteria used were isolated from volunteered patients attending the chest clinic of selected Government hospitals in Ondo State. Ninety-five percent analytical grade of Ethanol and N-hexane were used for the extraction. Antibiotics disc (Maxicare Medical Laboratory) and 0.45 µm Millipore membrane filter were purchased from Megababs Scientific Concept, Akure, Nigeria.

Study design/ period/ place

The study was carried out at the Department of Microbiology, Federal University of Technology, Akure between the period of May and August, 2023. Extracts prepared from the seeds of *P.guineense*, extracts prepared from the rhizome of *Z. officinale* and honey were singly and in combinations evaluated for growth inhibitory activity on selected pneumonic bacteria in *in vitro* assays. The pneumonic bacteria were isolated from the sputum of pneumonic patients using standard microbiological methods.

Standardization of bacterial inoculum

The standardization of the bacterial isolates was carried out by diluting a 6 hours old broth cultures of the isolates in test tubes and compared with a 0.5 McFarland standard. The bacterial suspension was adjusted to a density equivalent of approximately 10^8 CFU/ml (Idowu et al., 2020)

Extraction of plant components

The seeds of *Piper guineense* and rhizome of *Zingiber officinale* were seperately pulverized using electric grinder after sun-drying. The resulting powder was separately parked and stored in sterile labelled containers at room temperature (30 ± 2^0C) until extraction. For extraction, three types of extracting solvents namely: ethanol, N-hexane and hot water were used. Extraction was carried out as described by Ogundare (2006) with slight modification. One hundred gram (100 g) of the powdered seeds of *P. guineense* and rhizome of *Z. officinale* were separately soaked in 1000 ml each of the three types of solvents used in three different containers for three days with regular agitation. The extracts from each extractant was filtered by passing through muslin cloth and then Whatman No1. filter paper to obtain crude extract. The ethanol and N-hexane filtrates were evaporated in a rotary evaporator to remove the solvent used while the aqueous filtrate was evaporated at 40^0C in water bath until dried extract samples were obtained according to Babajide at al. (2023). All the extracts were sterilized using 0.45 µm Millipore membrane filter. The extracts sterility was confirmed by method of Sule and Agbabiaka (2008). The concentrated extract (3000 mg) was dissolved in 10ml of distilled water at room temperature (30 ± 2^0C) to make 300 mg/ml, sterilized using membrane filter of 4.5 µm Millipore size. This serves as stock extract. The prepared extract solution were refrigerated at 4^0C until use.

Determination of phytochemicals in the extracts of *Piper guineense* and *Zingiber officinale* and Honey

Quantitative and qualitative screenings were carried out on the prepared plants extracts and honey using the method of Daniel et al. (2020).

In-vitro assessment of antibacterial activity of the prepared extracts of *Piper guineense* and *Zingiber officinale* and Honey on the isolated pneumonic bacteria

Susceptibility of the bacterial isolates to the prepared plant extracts and honey was determined using the agar diffusion method according to Prescott et al. (2008).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC was determined using the method of Babajide et al. (2023) with slight modification while the MBC of the extract was determined by plating 0.5ml of the samples from all the tubes with no visible growth on Mueller Hilton Agar and incubated at 37˚C for 18hrs. Any of the plates on which there was no growth was taken as the MBC of the extract.

Data analysis

All data generated were subjected to statistical analysis using SPSS 17 version and the data obtained were analyzed by one-way analysis of variance (ANOVA). Means were compared by Duncan's new multiple range test and considered statistically significant at $p \leq 0.05$

Results

Percentage recovery of the extracts of *Piper giuneense* and *Zingiber officinale*

The hot water extraction resulted in the highest percentage recovery of the extracts of *P. giuneense* (67.24%) and *Z. officinale* (56.52%) while the N-hexane extraction had the least percentage recovery (Table 1).

Growth inhibitory activity of the different extracts of *Piper guineense, Zingiber officinale* and Honey on bacterial species isolated from pneumonic patients

Both the ethanolic seed extract of *P. guineense* and ethanolic rhizome extract of *Z. officinale* and the honey used in this study exerted growth inhibitory activity against all the test pneumonic bacteria with zone diameter which ranged from 7.50 to 21.25 mm on *Streptococcus pneumoniae*, 5.25 to 20.75 mm on *Staphylococcus aureus* and 5.50 to 13.25 mm on *Klebsiella pneumoniae*. The highest growth inhibition however was exerted by the combination of the ethanolic seed extract of *P. guineense*, ethanolic rhizome extract of *Z. officinale* and honey (ESRHO) (Table 2).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the combination of the extracts of *Piper giuneense, Zingiber officinale* and Honey

The MICs of ESRHO (ethanolic seed extract of *P. guineense* + ethanolic rhizome extract of *Z. officinale* + honey) of the prepared extracts and honey on the bacterial species isolated from pneumonic patients are 75 mg/ml, 150 mg/ml and 150 mg/ml for *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae* respectively while the MBC was 150 mg/ml, 300 mg/ml, 300 mg/ml of the extracts for *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae* respectively (Table 3).

Phytochemical profile of the extracts and honey

Phytochemical profile in the extracts revealed the presence of Saponin, Tannin, Terpenoid, Flavonoid, Alkaloid and Cardiac glycosides (Table 4). The quantitative profile showed the amount of bioactive components that are present in the prepared extracts and honey used in this study (Table 5). The highest value of flavonoids was observed in the ethanolic rhizome extract of *Z. officinale* and the least in honey.

Table 1: Percentage recovery of the extracts of *Piper giuneense* and *Zingiber officinale*

Solvent	Original weight/Input (g)	Extracted Weight/Output (g)	Percentage Recovery (%)
A	100	5.51	22.00
B	100	5.86	11.72
C	100	33.62	67.24
D	100	6.04	12.08
E	100	2.48	4.98
F	100	28.26	56.52

KEY: A= Ethanolic seed extract of *Piper giuneense*
B= N-hexane seed extract of *Piper giuneense*
C= Hot water seed extract of *Piper giuneense*
D= Ethanolic rhizome extract of *Zingiber officinale*
E= N-hexane rhizome extract of *Zingiber officinale*
F= Hot water rhizome extract of *Zingiber officinale*

Table 2: Growth inhibitory activity of different extracts of *Piper guineense, Zingiber officinale* and Honey on bacterial species isolated from Pneumonic Patient patients

Treatment @ 300mg/ml	**Diameter zone of inhibition (mm) of Pneumonic bacterial isolates**		
	***Streptococcus pneumoniae*(n=4)**	***Staphylococcus aureus* (n=4)**	***Klebsiella pnuemoniae*(n=4)**
A	13.25±2.50^{cdef}	10.50±1.29^{cdef}	7.75±3.304^{bcd}
B	11.25±6.70^{cd}	9.00±3.56^{cdef}	8.50±2.65^{bcd}
C	7.50±2.89 ^{abc}	6.25±2.06^{bc}	6.50±3.42^{bc}
D	14.50±4.04^{cdef}	8.50±3.00^{cde}	5.50±2.65^{abc}
E	19.75±6 .^{ef}	10.00±3.83^{cdef}	8.00±4.24^{bcd}
F	10.25±3.50 ^{bcd}	8.50±2.89^{cde}	9.50±4.51^{cd}
G	15.25±5.12^{cdef}	9.75±2.06cdef	9.25±3.50^{bcd}
H	15.00±4.62^{cdef}	7.00±1.15^{cd}	6.75±2.75^{bcd}
I	10.00±8.04^{bcd}	6.25±1.71^{bc}	7.50±2.65^{bcd}
J	15.00±5.77^{cdef}	12.50±7.05^{def}	10.00±4.24^{cd}

K	20.75±7.411^{f}	14.50±4.65^{f}	7.75±4.57^{bcd}
L	16.00±4.55def	5.25±2.63^{abc}	8.50±3.51^{bcd}
M	15.50±4.36^{cdef}	10. 00±2.16^{cdef}	11.00±3.56^{cd}
N	**21.25±4.00^{f}**	**20.75±6.65^{g}**	**13.25±2.22^{d}**
O	13.50±4.43^{cdef}	9.75±2.63^{cdef}	10.00±3.16^{cd}
P	15.50±4.80^{cdef}	13.00±4.97^{ef}	6.75±2.75^{bcd}
Q	15.25±3.77^{cdef}	8.75±2.22^{cdef}	6.50±2.89^{bc}
R	13.75±3.30^{cdef}	9.50±2.38^{cdef}	9.00±3.65^{bcd}
S	12.25±4.03^{cde}	8.25±2.22^{cde}	11.50±3.70^{cd}
PERFLOXACILIN	16.00±.00^{def}	14.00±0.00^{ef}	0.00±0.00^{a}
AMOXACILIN	3.25±.50^{ab}	1.25±0.50^{ab}	0.00±0.00^{a}
CIPROFLOXACIN	0.00±.00^{a}	0.00±0.00^{a}	10.00±4.08^{cd}
CHLORAMPHENICOL	0.00±.00^{a}	0.00±0.00^{a}	3.00±1.15^{ab}
Sterile distilled water	0.00±.0000^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Values are represented as Mean± SD of duplicates, values in the same row carrying same superscript are not different significantly and (P <0.05) according to new Duncan's multiple range test.

KEY:
A= Ethanolic seed extract of *Piper giuneense* + Honey, B= Ethanolic seed extract of *Piper giuneense* alone, C= Honey alone, D= Hot water seed extract of *Piper giuneense* + Honey, E= Hot water seed extract of *Piper giuneense* alone, F= N-hexane seed extract of *Piper giuneense* + Honey, G= N-hexane seed extract of *Piper giuneense* alone, H= Ethanolic rhizome extract of *Zingiber officinale* + Honey, I= Ethanolic rhizome extract of *Zingiber officinale* alone, J= Hot water rhizome extract of *Zingiber officinale* + Honey, K= Hot water rhizome extract of *Zingiber officinale* alone, L= N-hexane rhizome extract of *Zingiber officinale* + Honey, M= N-hexane rhizome extract of *Zingiber officinale* alone, N= Ethanolic seed extract of *Piper giuneense* + Honey alone + Ethanolic rhizome extract of *Zingiber officinale*, O= Ethanolic seed extract of *Piper giuneense* + Ethanolic rhizome extract of *Zingiber officinale*, P = Hot water seed extract of *Piper giuneense* + Honey + Ethanolic rhizome extract of *Zingiber officinale*, Q= Hot water seed extract of *Piper giuneense* + N-hexane rhizome extract of *Zingiber officinale* + Honey, R= N-hexane seed extract of *Piper giuneense* + Honey + N-hexane rhizome extract of *Zingiber officinale*, S= N-hexane seed extract of *Piper giuneense* + N-hexane rhizome extract of *Zingiber officinale*,, Perfloxacilin, Amoxacilin , Ciprofloxacin, Chloramphenicol, Sterile distilled water

Table 3: Minimum inhibitory concentration (MIC) (mg/ml) and minimum bactericidal concentration (MBC) (mg/ml) of ethanolic extract of *Piper giuneense and Zingiber officinale* in honey

BACTERIAL ISOLATES	MIC (mg/ml)	MBC (mg/ml)
Streptococcus pneumoniae	75	150
Staphylococcus aureus	150	300
Klebsiella pneumoniae	150	300

Table 4: Qualitative phytochemical profile of *Piper guineense* / *Zingiber officinale* and honey

Active component	A	B	C	D	E	F	G
Saponin	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+
Phlobatannin	-	-	-	-	-	-	-
Flavonoid	+	+	+	+	+	+	+
Steroid	-	-	-	-	-	-	-
Terpenoid	+	+	+	+	+	+	+
Alkaloid	-	-	+	+	-	+	-
Anthraquinone	-	-	-	-	-	-	-
Cardiac glycosides							
Legal test	+	+	+	+	+	+	+
Keller kiliani test	+	+	+	+	+	+	+
Salkwoski test	+	+	+	+	+	+	+
Lieberman test	-	-	-	-	-	-	-

KEY: A= Ethanolic seed extract of *Piper giuneense*, B= Ethanolic rhizome extract of *Zingiber officinale*, C= Hot water seed extract of *Piper giuneense*, D= Hot water rhizome extract of *Zingiber officinale*, E= N-hexane seed extract of *Piper giuneense*, F= N-hexane rhizome extract of *Zingiber officinale*, G= Honey

Table 5: Quantitative phytochemical profile of *Piper guineense* / *Zingiber officinale* and honey

Extract	**Saponin mg/g**	**Tannin mg/g**	**Flavonoid mg/g**	**Phenol mg/g**	**Terpenoid mg/g**	**Glycosides mg/g**	**Alkaloid %**
A	263.4546 ± 0.25717^{e}	6.9656± 0.00765^{g}	0.3192± 0.00544^{c}	12.4094 ± 0.04270^{e}	8.4840± 0.03761^{a}	24.1479± 0.04547^{a}	0.0000± 0.00000^{a}
B	1.6364± 0.25713^{a}	2.8122± 0.00765^{c}	0.8500± 0.00544^{e}	14.1908 ± 0.04270^{f}	9.4681± 0.03761^{c}	25.3376± 0.04547^{c}	0.0000± 0.00000^{a}
C	6.5455± 0.25713^{c}	5.1430± 0.00765^{f}	0.3077± 0.00544^{c}	8.3333± 0.04270^{c}	26.7819± 0.03761^{e}	46.2701± 0.04547^{e}	15.7500± 15.7500^{c}
D	7.8182± 0.25713^{d}	3.4341± 0.00765^{d}	0.6654± 0.00544^{d}	14.3720 ± 0.04270^{g}	26.2234± 0.03761^{d}	45.5949± 0.04547^{d}	11.0000± 0.110000^{b}
E	2.9091± 0.25713^{b}	1.7847± 0.00765^{a}	.1423± 0.00544^{b}	1.5097± 0.04270^{b}	8.5372± 0.03761^{a}	24.2122± 0.04547^{a}	0.0000± 0.00000^{a}
F	2.5455± 0.25713^{b}	2.6445± 0.00765^{b}	0.3154± 0.00544^{c}	8.6051± 0.04270^{d}	9.4149± 0.03761^{c}	25.2733± 0.04547^{c}	16.1090±0.16.1 090^{d}
G	1.4545± 0.25713^{a}	3.7424± 0.00765^{e}	0.0154± 0.00544^{a}	1.1775± 0.04270^{a}	9.2021± 0.03761^{b}	25.0161± 0.04547^{b}	0.0000±0.00^{a}

Values are represented as Mean± SD of duplicates, values in the same row carrying same superscript are not different significantly (P <0.05) according to new Duncan's multiple range test.

KEY: A= Ethanolic seed extract of *Piper giunense*
B= Ethanolic rhizome extract of *Zingiber officinale*
C= Hot water seed extract of *Piper giunense*
D= Hot water rhizome extract of *Zingiber officinale*
E= N-hexane seed extract of *Piper giunense*
F= N-hexane rhizome extract of *Zingiber officinale*
G= Honey

Values are represented as Mean± SD of duplicates, values in the same row carrying same superscript are not different significantly (P <0.05) according to new Duncan's multiple range test.

Discussion

Pneumonia is an inflammatory ailment of the pulmonary system that primarily impacts diminutive air sacs recognized as alveoli (Leach, 2009; McLuckie, 2009). It can be caused by bacteria, fungi and viruses. Although, it can be treated with antibiotics. The issue of bacterial resistance to antibiotics leads to treatment failure (Mene 'ndez and Torres, 2007; Odokor and Addo; 2011; Thenmozhi et al., 2014). Therefore in this study, extracts of two plants used in folk medicine to treat respiratory diseases, that is seeds of *Piper guineense* (Schumach) and rhizome of *Zingiber officinale* (Roscoe) and honey were examined for possible antibacterial activity on commonly encountered pneumonic bacteria in Ondo State, Southwest, Nigeria in *in-vitro* assays.

In this study, the combination of the ethanolic seed extract of *Piper guineense* and ethanolic rhizome extract of *Zingiber officinale* and honey exerted the highest growth inhibitory activity on all the test pneumonic bacteria which ranged from 13.25 to 21.25 mm in zone diameter. This inhibition was greater than that of the N-hexane, hot water extracts and the control antibiotics on the organisms. This is in agreement with the report of Dash et al. (2011) and Mbaey-Nwaoha and Onwuka (2014) that the ethanolic extracts of C*entella asiatica* had higher antimicrobial activities than n-hexane and water extracts in their respective studies. The combination of the extracts in honey in this study has broad-spectrum activity against all the isolated bacteria. This could be as a result of secondary metabolites that are present in the plant extracts and the honey. Phytochemicals such as saponin, tannin, flavonoid, terpenoid, alkanoid and cardiac glycosides were detected in the plants and the honey. It has been reported that these phytochemicals are toxic to microbial cells by exerting both bacteriostatic and bactericidal effects on microorganisms (Okigbo and Igwe, 2007). Each of these bioactive compounds has antibacterial effect on the bacterial species. For instance, Sen et al., (1998) reported that saponins bind with cholesterol inside cell leading to the formation of saponin-cholesterol complex which results in lysing of the cells. It can disturb also the permeability of bacterial cells by binding to the outer membrane (Winter, 1994 and Arabski et al., 2009). Tannins are multidentate ligands which may bind to proteins, mainly by hydrophobic interactions and hydrogen bonds and as a result of this, the inhibition of bacteria metabolism is achieved (Theisen et al., 2014). Also, Biharee et al. (2020) and Donadio et al. (2021) reported that flavonoids can suppress nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism. It also reduces adhesion and biofilm formation, porin on the cell membrane, membrane permeability, and pathogenicity, all of which are crucial for bacterial growth. Furthermore, Terpenoid has been reported to possess ability to act as adjuvants for antimicrobials (Langeveld et al., 2014; Barbieri et al., 2017).

Conclusion:

The combination of the ethanolic extract of the seeds of *Piper giuneense*, the ethanolic extract of the rhizome of *Zingiber officinale* and honey is very effective in inhibiting the growth of the isolated pneumonic bacteria than conventional antibiotics. Therefore the ethanolic extract has novel antibacterial properties that could be harnessed as alternatives for treating bacterial pneumonia.

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