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Bioactive Compound Profiling and In-vitro Antimicrobial Study of Ginger (*Zingiber officinale* Roscoe) Extract against Pneumococcal Bacteria[#]

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ABSTRACT

High morbidity and mortality rate associated with pneumococcal infection globally is of major concern most especially among infant. This burden is equally worsened by multidrug resistance strains due to indiscriminate consumption of antibiotics. Hence, need for constant search for cheap and effective bioactive compounds as alternative antimicrobials for the treatment of pneumococcal infection. Bioactive compound profiling and *in-vitro* antibacterial activity of ginger methanol extract against two predominant pneumococcal agents; *Streptococcus pneumonia* and *Haemophilus influenza* were investigated. Gas Chromatography Mass Spectroscopy (GC-MS) was used for the identification and quantification of bioactive compounds in the ginger methanol extract. The antibacterial activity and Minimum Inhibitory Concentration (MIC) of the extract was determined using Agar well diffusion. Twenty-seven (27) matched bioactive compounds were detected in the sample. Zingerone (17.70%), α-zingiberene (13.30%), (6)-shogaol (10.84%), α-Farnesene (6.26%), β-Funebrene (5.61%), 6-gingerol (5.18%), α-curcumene (4.15%) were the major compounds present. All other identified compounds had less than 4% composition by peak area each. The antibacterial activity of the ginger crude methanol extract against *S. pneumonia* and *H. influenza* were 2.33 mm and 9.33 mm. MIC of the extract against the isolates was 10%. In conclusion ginger crude methanol extract contain an array of bioactive compounds and the extract exhibited antibacterial activity against predominant pneumococcal agents. Ginger extract can be harnessed for the production of new antimicrobials to combat pneumococcal infection.

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Introduction

Pneumococcal infection is known for high morbidity and mortality worldwide, especially among children below 5 years (Walker et al., 2013), the burden of which is worsened by antibiotic resistance as a result of prominent antibiotic consumption (Buckley et al., 2019; Lewnard and Lo, 2020). It causes upper respiratory infections such as otitis media, sinusitis and severe invasive pneumococcal diseases (Bogaert et al., 2004). The incidence of IPD in developed countries is estimated to be 8-34 cases per 100,000 inhabitants with geographical variance (WHO, 2008), the mortality rate of which is between 10% and 30% (Bravo, 2009). Organisms such as *Streptococcus pneumonia* and *Haemophilus influenzae*, among others, have been widely reported as predominant causal organisms. Humans are the main targets of pneumococcal diseases, colonization of which may be influenced by multiple factors. Though not entirely clear, there is no doubt that the local host immune response plays an important regulatory role in the trafficking of pathogens in the nasopharynx (Song et al., 2013). Furthermore, there has been huge demands for natural immunity boosters like spices and herbs, with regard to the recent Covid-19 global pandemic.

Plant use in disease treatment is as old, but still complementary medicine, especially in developing countries where access to standard health services are almost non-existent (El-Shemy et al., 2007; Sevindik et al., 2017; Akgül et al., 2022), the limitation of which is absence of scientific evaluation. However, recent investigations have been to ascertain the compounds

responsible for such medicinal effects. Spices are important medicinal agents of natural sources (Kına et al., 2021; Krupodorova et al., 2022). Globally, they are used to enhance both flavour and aroma of foods, but, usage in preserving food quality and treatment of infections or diseases have been widely recognized (Panpatil et al., 2013), thus indicating their antimicrobial properties, due to the presence of certain naturally derived bioactive components (Maharjan et al., 2019; Uysal et al., 2021; Mohammed et al., 2022). Being organic and natural, they are widely accepted than synthetic additives and as such, their safety not questioned when compared (Sanusi et al., 2017).

Ginger (*Zingiber officinale* Roscoe), one of the most widely known perennial herbs, is derived from the underground stems or rhizomes of the plant (Sharifi-Rad et al., 2017). It is widely used as a spice and flavoring agent around the world and also medicinally (Ogodo and Abia, 2013; Sharifi-Rad et al., 2017). Additionally, *Zingiberaceae* species have been reported to have oils and alkaloids that possess antimicrobial, anti-parasitic, immune booster, antioxidant and other important biological properties. Now extensively employed in the management of different infections, ginger rhizome can be in the form of fresh paste, ginger tea (flavoring), dried powder or preserved slices (El-Ghorab et al., 2010) and also incorporated into many commercial products. It is most especially, a go-to product for sore throat, cough and some other upper respiratory tract infections, so, in vitro antimicrobial activity against two predominant pneumococcal agents, *S pneumonia* and *H influenza* was aimed at. Furthermore, analysis of inherent bioactive compounds using GC-MS would give an insight into the numerous bioactive compounds responsible for some of the earlier mentioned medicinal properties.

Figure 1. Ginger rhizome

Materials and Methods

Sample collection

Fresh ginger roots were purchased from a local produce market in Ibadan, Oyo State, Nigeria. They were identified and authenticated at the Department of Botany Herbarium, University of Ibadan Nigeria, labeled and transported to the laboratory for immediate microbiological analysis.

H influenzae and *S pneumonia* were obtained from the culture collection center of the University College Hospital, Ibadan, Nigeria.

Sample preparation

The rhizomes were washed to remove soil, peeled and washed again in clean water. Samples were surface sterilized with 70% ethanol and allowed to evaporate, after which they were subsequently rinsed in sterile distilled water. Samples were dried in the oven and further pulverized into powder using a laboratory blender. 200 g of powdered ginger were extracted using 500 mL methanol for three days after which the extract was concentrated using a rotary evaporator and kept at 4^{o}C. 1g of the extract was dissolved, in 5 mL of Dimethylsulfoxide (DMSO) to make a 20 % (200mg/mL) solution.

Antimicrobial sensitivity test

Agar well diffusion method was used (Baur et al., 1966). Test cultures were maintained on sterile nutrient broth for 18 hrs. They were further diluted out to 0.5 McFarland standard of approximately 1.5 x 10^8 CFU/mL. Cultures (0.1 mL) were aseptically inoculated on sterile Mueller-Hinton Agar (LabM, UK) plates and spread evenly. Wells were made, using a sterilized cork-borer (6 mm), and 0.1 mL of the extract was introduced into respective wells on the plates. Incubation was done in an upright position at 37^{o} C for 24 hours and diameter of inhibition zones were measured (mm).

Qualitative phyto-compound screening

Screening was carried out to evaluate the presence of alkaloids, tannins, flavonoids, saponins, glycosides, phlobotannins, terpenoids and steroids according to the method of Akroum et al. (2017)

Analysis of bioactive compounds

Identification and quantification of organic compounds in the extract was done using the Gas chromatography Mass Spectroscopy (GC-MS) method of Gavamukulya et al. (2015). Analysis spectra was determined by matching with reference spectra (National Institute of Standards and Technology, NIST14.Lib).

Results and Discussion

The agar well diffusion test indicated that ginger extract inhibited the growth of both test organisms, as presented in Table 1. Zones of inhibition for *H. influenzae* ranged between 9 and 11mm, with a mean value of 9.33mm, while those for *S. pneumonia* was between 1 and 4mm, with a mean value of 2.33mm. This is indicative of the fact that *H. influenza* was more sensitive to the ginger extract. Similar report of ginger, having low activity against S. pneumonia was made by Horne et al. (2001). The antimicrobial activity of ginger extract, concentrates and essential oil, as reported, showed promising inhibitory effect against some pathogens such as *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa* (Albaridi and Yehia, 2021; Sanusi et al., 2019; Riaz et al., 2015; Harmalkar and Desai, 2011), *Escherichia coli, Candida albicans* and *Aspergillus niger* (Kumar et al., 2016). Such effect can be attributed to its constituent monoterpenes and sesquiterpenes, as they are capable of altering the permeability and fluidity of the plasma membrane of microorganisms (Lopez et al., 2017).

Table 1. Antimicrobial activity of ginger extract against *H. influenzae and S. pneumonia*

	Test Isolates/ Diameter zone of inhibition (mm)	
	H. influenzae	*S. pneumoniae*
Zone 1	9	4
Zone 2	11	2
Zone 3	8	1
Mean value	9.33	2.33
Augmentin (30µg)	11.0	0

Key: Zone 1-3= Replicates

Table 2. Minimum Inhibitory Concentration of ginger extract against *H. influenza and S. pneumonia*

Extract concentration (%)	Test isolate/Optical Density @ 540nm	
	H. influenza (1.701 initial conc.)	*S. pneumonia (*1.567 initial conc.)
10	1.654 ± 0.01^{a}	1.539 ± 0.00^{a}
20	1.506 ± 0.00^{b}	1.517 ± 0.00^{b}
30	1.476 ± 0.00^{c}	1.478 ± 0.00^{c}
40	1.469 ± 0.00^{c}	1.471 ± 0.00^{d}

Values are mean ± standard deviation. Values reported with same superscript in the same column indicated no significant difference at 5% probability

Table 3. Qualitative phytochemical profile of methanol extract of ginger

Phytochemical compounds	Qualitative profile
Alkaloids	++
Tannins	+
Glycosides	+
Saponin	++
Steroids	-
Flavanoids	+
Terpenoids	+
Pholobotannins	-

Key: ++ Abundantly present; +Fairly present; -Absent

Table 4. Matched Bioactive compounds generated in the methanol extract of ginger by GC-MS Peak Report TIC

Peak Number	Retention time (minutes)	% Composition by Area	Matched Compound IUPAC Name	Chemical Formula
1	1.704	0.30	Silanediol, dimethyl-	C_2H_8OCSi
2	3.779	2.94	Eucalyptol	$C_{10}H_{18}O$
3	5.585	0.85	Isoborneol	$C_{10}H_{18}O$
4	6.114	0.39	Decanal	$C_{10}H_{20}O$
5	6.587	2.65	2,6-Octadienal, 3,7-dimethyl-, (Z)	$C_{10}H_{16}O$
6	6.753	2.88	β-Myrcene	$C_{10}H_{16}$
7	6.986	3.36	2,6-Octadienal, 3,7-dimethyl-, (E)	$C_{10}H_{16}O$
8	7.022	2.61	Citral	$C_{10}H_{16}O$
9	8.470	2.99	Geranyl isobutyrate	$C_{14}H_{24}O_2$
10	9.783	4.15	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$
11	9.923	13.30	1,3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-, [S-(R*,S*)]	$C_{15}H_{24}$
12	10.001	1.47	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	$C_{15}H_{24}$
13	10.058	6.26	α-Farnesene	$C_{15}H_{24}$
14	10.276	5.61	(3R,3aR,7R,8aS)-3,8,8-Trimethyl-6- methyleneoctahydro-1H-3a,7-methanoazulene	$C_{15}H_{24}$
15	10.613	1.55	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) -, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	$C_{15}H_{24}$
16	10.727	1.12	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	$C_{15}H_{24}$
17	11.339	1.23	β-Bisabolene	$C_{15}H_{24}$
18	11.775	17.70	2-Butanone, 4-(4-hydroxy-3-methoxy phenyl)-	$C_{11}H_{14}O_3$
19	12.180	0.74	7-epi-cis-sesquisabinene hydrate1	$C_{15}H_{26}O$
20	4.935	1.18	n-Hexadecanoic acid	$C_{16}H_{32}O$
21	17.405	3.94	(E)-1-(4-Hydroxy-3-methoxyphenyl)d ec-3-en-5-one	$C_{17}H_{24}O_3$
22	17.447	1.36	3-Decanone, 1-(4-hydroxy-3-methoxy phenyl)-	$C_{17}H_{26}O_3$
23	18.002	10.84	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	$C_{17}H_{24}O_3$
24	18.339	0.92	1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione	$C_{17}H_{24}O_4$
25	18.910	5.18	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one	$C_{17}H_{26}O_4$
26	20.160	2.50	1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one	$C_{17}H_{24}O_3$
27	23.631	2.07	1-(4-Hydroxy-3-methoxyphenyl)tetradec-4-en-3-one	$C_{17}H_{24}O_3$

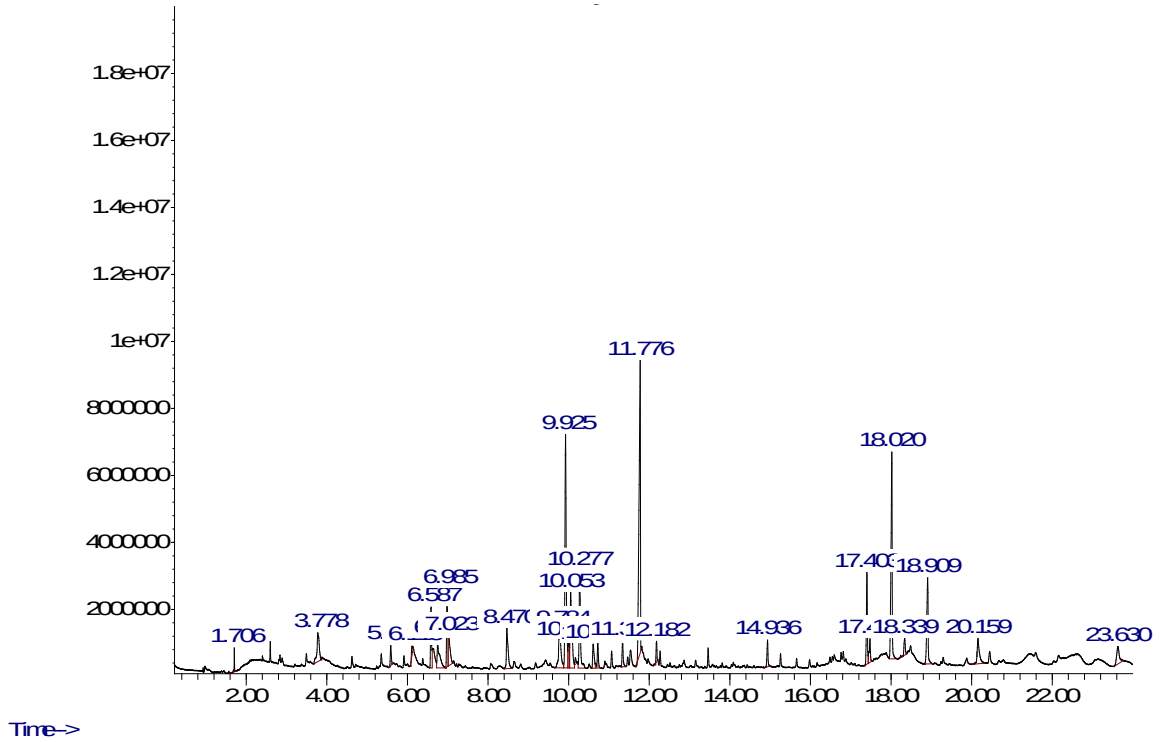

Figure 2. Total Ion chromatogram of methanol extract of ginger

However, variations in antibacterial activity reported for different studies have been linked to differences in genetics, soil mineral availability, environmental as well as climatic factors (Sanusi et al., 2019), cultivation type, vegetative stage (Ghasemzadeh et al., 2016) and even extraction method (Mesomo et al., 2013). The antibiotics (augmentin) used as the control inhibited the growth of *H. influenza*, with 11mm inhibition zone while *S. pneumonia* was resistant. These findings revealed that The ginger extract was more effective in inhibiting the growth of *S. pneumonia* than the synthetic antibiotic. There was significant reduction in growth concentration with increasing extract concentrations, however, the minimum concentration that inhibited the growth of both organisms was 10% (v/v), having the most reduced growth concentrations of 1.654 ± 0.01 and 1.539 ± 0.00 at 540nm for *H. influenza and S. pneumonia*, respectively (Table 2).

The results obtained from this study represented an important step towards the effective characterization of the metabolite compounds spectrum from ginger, using GC-MS analysis. Preliminary qualitative phytochemical analysis revealed the plant to be rich in compounds such as alkaloids and saponins. Tannins, glycosides, flavonoids and terpenoids were fairly present while steroids and pholobotannins were absent, as indicated in table 3. In accordance, Riaz et al. (2015) also reported the absence of steroids and traces of pholobotannins, Shamsuddeen et al. (2009), however reported the detection of steroids in both aqueous and ethanol extract of ginger. Antimicrobial properties of compounds like phlobotannins, saponins, alkaloids, flavonoids, tannins, steroids and glycoside have been previously reported (Suleiman et al., 2019), thus the inhibitory effect observed in the current study.

The total ion chromatogram (TIC) showed the GC-MS profile of the compounds identified (Figure 2). Over 60 volatile and non-volatile bioactive compounds have been reported to be present in ginger (Bilal et al., 2015). Findings from this study revealed twenty-seven (27) matched bioactive compounds in methanol extract of ginger, the highest percentages being at peak 18, 2-Butanone, 4-(4-hydroxy-3-methoxy phenyl)- (peak area 17.70%; zingerone), peak 11, 3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-, [S-(R*,S*)] (peak area 13.30%; α-zingiberene), peak 23, 1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one (peak area 10.84%; (6)-shogaol), peak 13, α-Farnesene (peak area 6.26%), peak 14, (3R, 3aR, 7R, 8aS) -3, 8, 8-Trimethyl-6-methyleneoctahydro-1H-3a,7-methanoazulene (peak area 5.61%; β-Funebrene), peak 25, 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one (peak area 5.18%; 6-gingerol), and peak 10, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (peak area 4.15%; α-curcumene). These compounds constituted more than 50% while all other identified compounds had less than 4% composition by peak area each (Table 4). Gunasena et al. (2022), in a study on the volatile compounds in ginger essential oil, identified monoterpene compounds like Geranial (17.88%), 1,8-Cineole (14.96%), Neral (13.99%), Camphene (7.53%), and α-Farnesene (3.51%) as the major compounds, however, sesquiterpenes, similar to those obtained in this study (α-Zingiberene, α-Farnesene and Curcumene) were also identified. These compounds, according to Zhang et al. (2017) and Abdullahi et al. (2020), contributed to significant antimicrobial activity.

2-Butanone, 4-(4-hydroxy-3-methoxy phenyl)-, a pungent component of ginger has potent anti-inflammatory, antidiabetic, antilipolytic, antidiarrhoeic, antispasmodic, antimicrobial as well as property of enhancing growth and immune stimulation. Contrary to the findings of this study, it was reported to be absent in fresh ginger but cooking or heating transforms gingerol to zingerone (Bilal et al., 2015). Antimicrobial activity has also been linked to the richness of ginger bioactive molecules like zingerone and 1-(4-Hydroxy-3-

methoxyphenyl) dec-4-en-3-one (Rigane et al., 2018). α-zingiberene has been established to be a major ginger constituent (Sharifi-Rad et al., 2017; Abdullahi et al., 2020; Snuossi et al., 2016) which has shown significant antimicrobial activity with some Gram-positive and Gram negative bacteria (Andrade et al., 2012).

Irrespective of the fact that some of these matched compounds are in low concentrations, they can have an effect on the overall efficiency of the antimicrobial activity through synergistic interaction with the other constituents (Giles et al., 2010). However, major components mostly account for more than 50% of total composition, but their proportions are not usually related to activity. This is indicative of the fact that compounds with small proportions are as important for pharmacological action as the major ones (Galindo et al., 2010).

Conclusion

Methanol extract of *Zingiber officinale* showed antimicrobial activity against *Streptococcus pneumonia* and *Haemophilus influenza*, with 2.33mm and 9.33mm inhibition zones, respectively. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, glycosides, flavonoids and terpenoids while steroids and pholobotannins were absent. The GC-MS analysis of compounds in the ginger methanol extract showed twenty-seven (27) matched bioactive compounds, among which Zingerone, α-zingiberene, (6)-shogaol, α-Farnesene, β-Funebrene, 6-gingerol and α-curcumene were the major components. As such, ginger extract can be harnessed for the production of new antimicrobials to combat pneumococcal infection.

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