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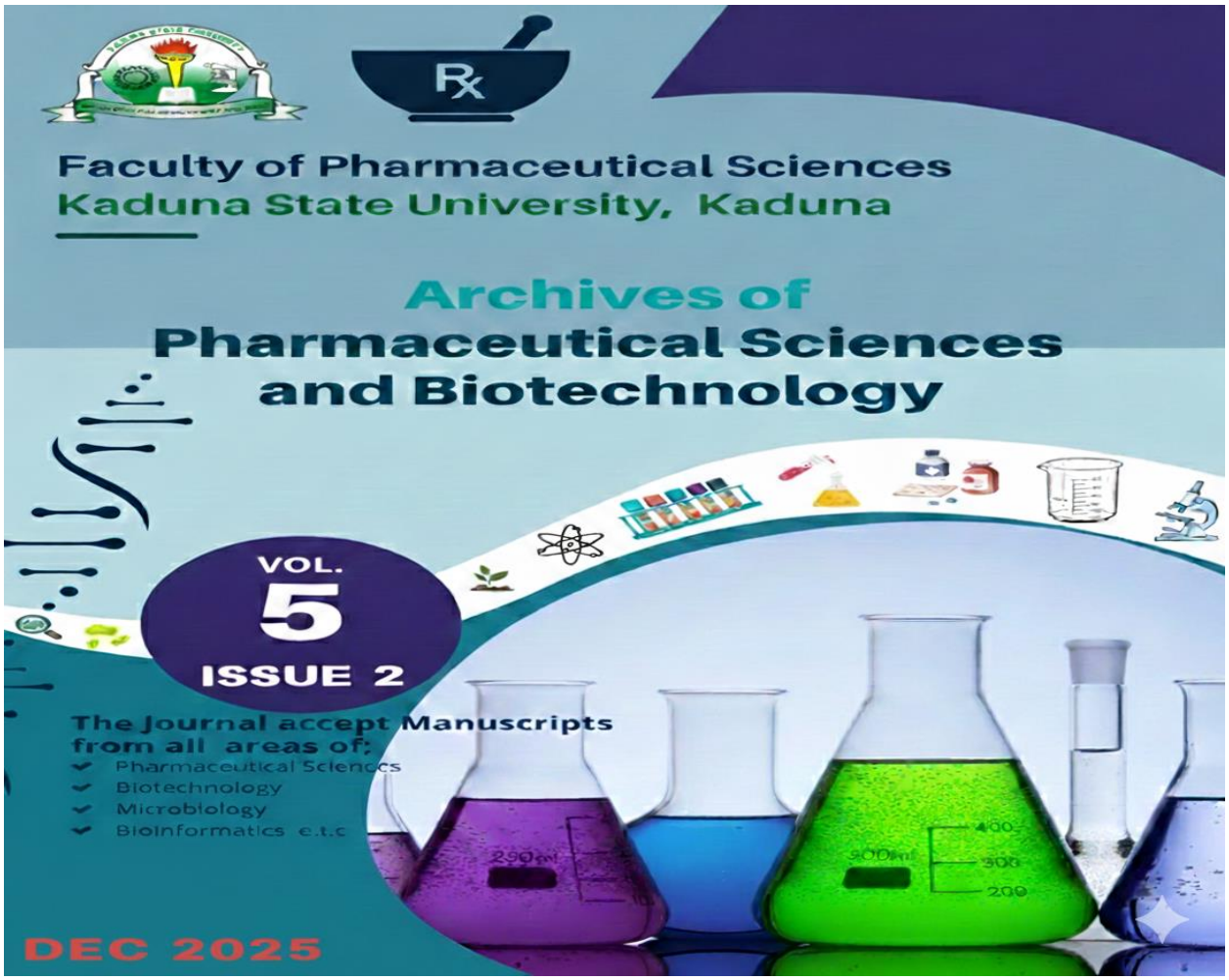

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Antibacterial Efficacy of *Eucalyptus Globulus* and *Mentha Piperita* against Multidrug-Resistant Bacteria Associated with Pneumonia in Kaduna

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ABSTRACT

Background: On the scale of global infectious disease incidence, pneumonia ranked as one of the major leading infectious diseases with a debilitating impact and high death rate among children below 5 years in Nigeria, with the highest deaths among children less than 2 years, and northern Nigeria is considered a hotspot. Since below 30% of infected children receive prompt antibiotic treatment and the growing antimicrobial resistance profile of associated bacteria, pneumonia accounts for approximately 16–19% annual deaths, resulting in an estimated 162,000 annual deaths at 18 deaths per hour in Nigeria, and is triggered by low vaccination coverage, indoor air pollution and malnutrition.

Aim: This study was designed to determine the antibacterial efficacy of *Eucalyptus globulus* and *Mentha piperita* against multidrug-resistant bacteria isolated from pneumonia patients in Kaduna, Nigeria for alternative and better treatment option.

Methods: A total of 15 multidrug-resistant (isolates with MARI ≥0.5) bacterial isolates, 3 from each bacterium (*Ps. aeruginosa*, *K. pneumoniae*, *Streptococcus pneumoniae*, *E. coli*, and *S. aureus*) from pneumonia patients attending Barau Dikko Teaching Hospital, Kaduna, were collected from our previous study on the prevalence of multidrug-resistant bacterial pneumonia in Kaduna, Nigeria. The antimicrobial activity of *Mentha piperita* and *Eucalyptus globulus* ethanolic extracts on multidrug-resistant isolates was evaluated using the Kirby-Bauer disc diffusion method, while Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays were determined using the CLSI method.

Results: Overall assessment of the susceptibility profile of the selected isolates that were 100% MDR and MARI ≥ 0.5 showed that 80% of the isolates were resistant to tetracycline, amoxicillin and chloramphenicol, 73.3% were resistant to cefotaxime, 60% were resistant to cotrimoxazole and amoxicillin-clavulanic acid and 53.3% resistant to imipenem while most of the isolates were still susceptible to ciprofloxacin (73.3%) and azithromycin (53.3%). *Mentha piperita* extract exhibited significant antimicrobial activity against all tested multidrug-resistant bacteria, with MIC values ranging from 250 mg/ml to 450 mg/ml, while *Eucalyptus globulus* extract also showed activity, but with higher MIC values. The combination of *Mentha piperita* and *Eucalyptus globulus* extracts showed enhanced antimicrobial activity with zones of inhibition higher than when tested alone, and as compared with the control.

Conclusion: The study demonstrates the potential of *Mentha piperita* and *Eucalyptus globulus* extracts as natural antimicrobial agents against pneumonia-causing bacteria. The combination of these extracts may offer a promising approach for the treatment of pneumonia.

Keywords: *Mentha piperita*, *Eucalyptus globulus*, bacteria, pneumonia, multidrug resistance

INTRODUCTION

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Pneumonia is an inflammatory disease of the lungs characterised by inflammation of the alveoli, the air sacs where oxygen and carbon dioxide are exchanged (Sattar *et al*., 2024). It is an infection that is caused by a wide variety of pathogens, mainly bacteria, viruses, or fungi and less commonly by other microorganisms (Mackenzie, 2016). The largest causative agents are bacteria, accounting for 39–60% prevalence and resulting in approximately 7 million deaths annually (Zhang *et al*., 2023). Among bacterial pathogens, the most common causative agents are *Streptococcus pneumoniae* (*S. pneumoniae*), *Haemophilus influenzae* (*H. influenzae), Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli) and Staphylococcus aureus* (*S. aureus*) (Mackenzie, 2016). Bacterial pneumonia continues to be one of the most serious public health problems due to its medical and economic burden (Cilloniz *et al*., 2013). Both community-associated pneumonia (CAP) and hospital-associated pneumonia (HAP) have been reported to increase morbidity and death in people of all ages around the world (Roquilly *et al*., 2015). Childhood mortality and adult hospitalisation due to pneumonia remain increasing in low- and middle-income countries, especially Nigeria (Selvi & Vaithilingan, 2024). Community and hospital-acquired pneumonia are the two main types of pneumonia. According to hospital-based studies in Africa, CAP is linked to approximately 15% increase in adult inpatient hospital mortality (Mulugeta *et al*., 2023). It is the most common cause of adult hospitalisation and mortality, accounting for 10% in Kenya, 11.9% in Nigeria, 17% in Ethiopia and 51,000 admissions with 10,000 deaths in Malawi each year (Bjarnason *et al*., 2018). In Spain, *S. pneumoniae* was the leading species in causing bacterial pneumonia, which accounted for 31.7% (Curran *et al*., 2008). Varying prevalence of bacterial pneumonia was reported in different parts of Ethiopia; 42.9% in southern Ethiopia and 32.1% in central Ethiopia, with *S. pneumoniae* and *K. pneumoniae* being predominant isolates, respectively (Nurahmed *et al*., 2020). Bacterial pneumonia causes complications for everyone, but individuals with weakened immune systems, children, and the elderly are at higher risk (Sampson *et al*., 2022). In the community, with a high prevalence, patients living with HIV/AIDS, especially those who have co-infection with one or more microorganisms, and aged individuals are more susceptible to infections with bacterial pneumonia (Tilahun *et al*., 2023). The study further showed that positive culture rate was slightly higher in women than in men, and higher prevalence rates of lower respiratory tract infections were observed in age groups greater than or equal to 45 years (Tilahun *et al*., 2023). Treatment of bacterial pneumonia has been associated with multidrug resistance (MDR) (resistance to at least three or more than three classes of antibiotics), extensively drug-resistant (XDR) (resistance to all antibiotic classes except one), and pan-drug resistant (PDR) (resistance to all groups of antibiotics), noting that resistance to one or more groups of antimicrobial agents may be innate or acquired. Antimicrobial resistance crisis has continued to surge due to the emergence and dissemination of antibiotic-resistant pathogens in hospitals and the environment, inappropriate drug use, overuse and consumption of drug-resistant pathogens from animal sources and crops (Giedraitiene *et al*., 2011). In China, Jinghua *et al*. (2017)

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had reported *S. aureus* isolated from a pneumonia patient to express resistance to penicillin, erythromycin, tetracycline, and clindamycin, while *S. pneumoniae* was highly resistant to erythromycin, azithromycin and clindamycin. *E. coli* was also resistant to ampicillin, gentamicin, and ciprofloxacin, while *K. pneumoniae* had the highest resistance to gentamicin and ampicillin. Similarly, resistance to cotrimoxazole was 100% among *S. aureus* and *S. pneumoniae* isolated from pneumonia patients, while *K. pneumoniae* was resistant to most of the antibiotics, showing more than 50% resistance to ceftriaxone and cefotaxime drugs respectively (Shrestha *et al*., 2013). Nigeria's analysis of pneumonia-associated bacteria among HIV/AIDS patients showed that *P. aeruginosa* isolated from pneumonia patients attending Federal Medical Centre, Ido-Ekiti, Ekiti State, were highly resistant to all antibiotics tested, including ciprofloxacin and ceftazidime, whereas *E. coli, S. aureus* and *K. pneumoniae* were resistant to commonly prescribed drugs (Ojo-Bola *et al*., 2014). Also, another study conducted by Assefa (2022) showed that Gram-negative bacteria isolated from pneumonia patients could express a high resistance profile to ampicillin, tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole. On the other hand, most of the isolates were less resistant to amikacin. Also, methicillin-resistant *S. aureus* has been isolated from a pneumonia patient sample (Ibrahim *et al*., 2024). While drugs like beta-lactams (amoxicillin, amoxicillin-clavulanate), macrolides (azithromycin and clarithromycin), tetracyclines, and, in complicated cases, fluoroquinolones (levofloxacin) are commonly used in the treatment of pneumonia (File *et al*., 2004), antibiotic resistance against these drugs is significantly problematic and is of high profile, making treatment options very skewed and burdensome. To reduce the burden and spread of multidrug resistance and effectively ensure patient therapeutic outcome, ethnomedicinal plants have sometimes been deployed in the past by locals and folk medicine to treat ailments. This concept encouraged us to evaluate the impact of the combined activity of *Eucalyptus globulus* and *Mentha piperita*, used in the treatment of pneumonia by folk medicine in Nigeria, against multidrug-resistant bacteria isolated from pneumonia. *Eucalyptus globulus* Labill. is an African medicinal plant of the Myrtaceae family, and widely grows in tropical, temperate, and subtropical regions (Sadiq*et al*., 2023). Phytochemical analysis shows that *Eucalyptus globulus* is a rich source of polyphenols, terpenoids, eucalyptol and cineole (Falahati *et al*., 2005). Further studies show that *Eucalyptus globulus* has been used for the treatment of many diseases such as influenza, dysentery and skin diseases (Shiekh *et al*., 2025). *Eucalyptus globulus* extract has many properties, such as antibacterial, anti-cancer, anti-inflammatory, painkiller, antioxidant, anti-blood proliferation, anti-malaria, anti-mold and antivirus properties (Adebola *et al*., 2001). While *Mentha piperita* (Peppermint leaves) is a hybrid herbaceous plant, originally from the crossing between spearmint and water mint (Hudz *et al*., 2023). Peppermint leaves are used worldwide, especially in Western and Middle Eastern countries, in the forms of tinctures, teas, infusions, essential oils and extracts (Hudz *et al*., 2023). Its pharmacological properties include antimicrobial, anaesthetic, anthelmintic,

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antifungal, and antioxidant activities (Li *et al*., 2017; Hudz *et al*., 2023). These properties are strongly related to the effects of the main ingredient, menthol, which has great industrial and pharmacological importance (Pathare, 2024). Due to the current public burden of pneumonia in Nigeria, knowledge on the prevalence patterns, geographical distribution, the major causes of bacterial pneumonia and antibiotic resistance among inpatients and outpatients in Barau Dikko Teaching Hospital (BDTH), Kaduna, was assessed in this study as these data may be used to devise a means to stem the emergence and subsequent spread of infections and drug resistance by the organism. More so, alternative efficacy options using ethnomedicinal plants of *Eucalyptus globulus* and *Mentha piperita*, against multidrug-resistant bacteria isolated from pneumonia in BDTH, Kaduna was evaluated.

MATERIALS AND METHODS

Materials, Reagents and Bacteriological Media

Light Microscope (wild mill Switzerland), sterile petri dishes, glass cylinders, conical flasks, test tubes, Test tube rack, Staining Tray, glass rod, Bunsen burner, face mask, hand gloves, wire loop, laboratory incubator (Baird and Tatlock ltd Essex), beakers, glass slide, sterile syringe and needle, sterile swab stick, weighing balance (W & T every limited Birmingham), micropipette (Huawei chemical Zheilang chin), Hot air oven (Baird and Tatlock ltd Essex), glass spreader, and an autoclave (Adelphi mfg Co Ltd UK).Crystal violet, iodine, Ethanol, Safranin, Normal saline, Acetic acid (Avondale Laboratory, England), Chloral hydrate (BDH Laboratory Chemicals Division, Poole, England), Dragendorff's reagent, Picric acid reagent, Mayer's reagent, Phloroglucinol, Sudan red solution, Methylene blue. Blood agar, Mueller-Hinton agar (oxoid), Mueller-Hinton broth (oxoid).

Antibiotics Disc: ciprofloxacin, imipenem, amoxicillin, cefotaxime, chloramphenicol, amoxicillin-clavulanate, azithromycin, tetracyclines.

Bacterial Collection and Antimicrobial Resistance Study

A total of 15 multidrug-resistant isolates (3 from each bacterium: *Ps. aeruginosa*, *K. pneumoniae*, *Streptococcus pneumoniae*, *E. coli*, and *S. aureus*) with MARI ≥0.5 already identified and susceptibility determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) from pneumonia patients attending Barau Dikko Teaching Hospital, Kaduna, were collected from our previous study (Igwe *et al*., 2025a) titled "Prevalence of multidrug-resistant bacterial pneumonia in Kaduna, Nigeria".

Plant Materials

Eucalyptus globulus and *Mentha piperita* were collected from the link road, Unguwan Rimi GRA, and the plant species were identified in the herbarium of the Pharmacognosy Department, in the Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna. The leaves were air-dried in shade and milled to fine particles for extraction.

Plant Extract Preparation and Determination of Percentage Yield

Using the standard Maceration method, 100 grams of dried and pounded leaves of *Eucalyptus globulus* and *Mentha piperita* were added to 1000ml each of 60% ethanol. The ethanolic extract mixtures were

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separately preserved at laboratory temperature for 48 hours and were stirred every few hours with a glass rod. The admixtures were then filtered using the suction pump and transferred into a dish, and left on water bath for evaporation. The percentage yields of the extracts were determined using the method described by Osman *et al*. (2019) below:

$$\%\text{Yield} = \frac{\text{Weight of Dried Extract}}{\text{Weight of Raw Material}} \times 100$$

Phytochemical Screening

The following phytochemical tests were carried out using standard methods:

i. **Test for Saponins (Frothing Test):** Five hundred milligrams of both extracts were dissolved in 10ml of water and shaken vigorously for 30 seconds, and allowed to stand for one hour. The occurrence of a frothing column or honeycomb-like froth of at least 1 cm in height and persisting for at least 30 minutes indicates the presence of saponins (Sofowora, 2008).

ii. **Test for Steroids/Terpenes (Lieberman/Burchard Test):** To each of the plant extracts, one ml of acetic anhydride was added to 0.5g, and then dissolved in one ml of chloroform. Furthermore, concentrated sulphuric acid was then added gently to the side of the test tube to form a lower layer at the junction of the two liquids. Formation of green/blue-green indicates the presence of steroids/cholesterol, and red-violet/pink for triterpenoids (Evans, 2002).

iii. **Test for Flavonoids (Sodium Hydroxide Test):** 0.5g of each of the extracts was dissolved in distilled water and filtered. 2ml of 10% aqueous sodium hydroxide solution was then added. The solution was observed for the presence of yellow colour. A change in colour from yellow to colourless or on the addition of dilute hydrochloric acid was used as an indication for the presence of flavonoids (Evans, 2002).

iv. **Test for Tannins (Ferric Chloride Test and Lead Acetate Test):** 0.5g of extracts was dissolved in 5ml of water and filtered. Two drops of ferric chloride solution were added to the filtrate. Appearance of blue, black or green or blue-green (condensed/catechic tannins) precipitate indicates the presence of tannins. For the lead acetate test, to 0.5g of extract, 2ml of ethanol was added, followed by two drops of lead subacetate solution. The appearance of a whitish yellow precipitate indicates the presence of tannins.

v. **Test for Alkaloids:** For each of the plants, 1 g of extract was stirred with 20ml of 1% aqueous hydrochloric acid on water bath and filtered. The filtrate was then basified with concentrated NH_4OH and extracted with 2ml of 1% HCl. The chloroform layer was then extracted with 2ml of 1% HCl. The aqueous layer was divided into four portions for the following tests: To the first portion, two drops of 1ml of freshly prepared Dragendorff's reagent were added and observed. To the second portion, two drops of 1ml Mayer's reagent

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were added and observed. To the third, two drops of 1ml Wagner's reagent were also added. The fourth portion was used as a control. Appearance of rose red to brownish, white to yellowish or cream colour and brown or reddish brown precipitates, respectively, indicates the presence of alkaloids (Evans, 2002).

Determination of Bacteria Susceptibility to Plant Extracts

The antibacterial activity of *Eucalyptus globulus* and *Mentha piperita* to multidrug-resistant isolates from pneumonia patients was determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA). The test bacteria were individually sub-cultured in a freshly prepared, sterilised Mueller-Hinton Agar by the streak plate method and incubated at 37 °C for 24 hours. Then, well-isolated overnight cultured colonies of the same morphology type were selected from the culture media. A loopful of each colony from the surface of MHA was picked with a sterile wire loop and transferred into a sterilised test tube containing 5ml sterile normal saline solution. This was then adjusted until it matches with 0.5 McFarland turbidity standard. 200µl of the standardised bacteria were then seeded into 20ml of already prepared MHA stored in a water bath at 40 °C. Using a sterile 6mm cork borer, 5 wells were bored at equal distances (15mm). The bottoms of the wells were sealed with one drop of the sterile Mueller-Hinton agar to prevent leakage of the extracts under the agar. By using a sterile pipette, five different concentrations of each of the plant extracts (1000mg/ml, 750mg/ml, 500mg/ml, 250mg/ml, and 125mg/ml) in separate plates were allowed for diffusion time of about 15 minutes before incubation at 37 °C for 24 hours. After an overnight incubation period, the diameter of the zone of inhibition was measured in millimetres using a transparent meter rule. This was done in duplicates, and the average of the two readings was taken to be the zone of growth inhibition of the test agents against the bacterial isolates, in accordance with the CLSI guideline.

Minimum Inhibitory Concentration (MIC) of the Crude Extracts against Multidrug-Resistant Pneumonia Bacteria

Minimum inhibitory concentration (MIC) was determined according to the broth microdilution method. Various concentrations ranging from 500mg/ml to 200mg/ml of each of the extracts were prepared through twofold serial dilution. A stock solution of the extract (500mg/ml) was prepared by dissolving the ethanolic extract in dimethyl sulfoxide (DMSO) and diluted to obtain concentrations of 450mg/ml, 400mg/ml, 350mg/ml, 300mg/ml, 250mg/ml, and 200mg/ml. The obtained concentrations were added to a test tube containing 1ml of Mueller-Hinton broth, with each test tube containing 200µl of the standardised microbial inoculum (adjusted to 0.5 McFarland standard). The positive control was ciprofloxacin, while the DMSO served as the negative control. The test tubes were incubated at 37 °C for 24 hours. The MIC was regarded as the lowest concentration of the extract that did not show any visible growth after 24 hours of incubation compared with the control.

Determination of Minimum Bactericidal Concentration (MBC)

The in vitro bactericidal concentration based on MIC for each of the plant extracts was

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determined as previously described with slight modifications. The MBC were determined by incorporating various concentrations of extracts (500–200 mg/ml) in Mueller-Hinton broth, that shows no growth during MIC determination. A loopful from each tube was sub-cultured onto agar plates and incubated for a further 24 hours at 37 °C. The least concentration at which no growth was observed was picked as the MBC.

RESULTS

Antibiotic Susceptibility Profile of Selected Bacterial Isolates

Overall assessment of the susceptibility profile of the isolates showed that 80% of the isolates were resistant to tetracycline, amoxicillin and chloramphenicol, 73.3% were resistant to cefotaxime, 60% were resistant to cotrimoxazole and amoxicillin-clavulanic acid, while most of the isolates were still susceptible to ciprofloxacin (73.3%) and azithromycin (53.3%). Most disturbing was that the isolates were 53.3% resistant to imipenem, a drug not commonly prescribed for children's pneumonia (Table 1).

Table 1: Antimicrobial Susceptibility Profile of Bacterial Pneumonia Isolates from Clinical Samples in Kaduna, Nigeria

S/N	Isolate Code	Bacteria isolated	AZT	C	AMC	AMX	IMP	CTX	CIP	SXT	TET	NART	MARI	CRP
1	DS2	*S. pneumoniae*	R	R	S	R	R	S	S	S	R	5	0.6	MDR
2	DS23	*S. pneumoniae*	R	R	S	R	S	R	R	R	S	6	0.7	MDR
3	SPT18	*S. pneumoniae*	S	R	R	R	S	R	R	S	R	6	0.7	MDR
4	DS41	*K. pneumoniae*	R	R	R	S	R	R	S	R	S	6	0.7	MDR
5	DS43	*K. pneumoniae*	R	R	R	S	S	S	R	R	R	6	0.7	MDR
6	DS45	*K. pneumoniae*	R	R	R	S	R	R	S	R	S	6	0.7	MDR
7	DS53	*S. aureus*	S	S	R	R	S	R	S	R	R	5	0.6	MDR
8	DS77	*S. aureus*	S	R	R	R	S	R	S	R	R	6	0.7	MDR
9	SPT90	*S. aureus*	S	S	R	R	S	R	S	R	R	5	0.6	MDR
10	SPT140	*E. coli*	S	R	S	R	R	R	S	S	R	5	0.6	MDR
11	SPT156	*E. coli*	S	R	S	R	R	R	S	R	R	6	0.7	MDR
12	SPT187	*E. coli*	R	S	R	R	R	S	R	S	R	6	0.7	MDR
13	DS202	*P. aeruginosa*	R	R	S	R	R	S	S	S	R	5	0.6	MDR
14	SPT232	*P. aeruginosa*	S	R	S	R	R	R	S	S	R	5	0.6	MDR
15	SPT250	*P. aeruginosa*	S	R	R	R	S	R	S	R	R	6	0.7	MDR
% Antimicrobial Resistance			46.7	80	60	80	53.3	73.3	26.7	60	80			
% Antimicrobial susceptibility			53.3	20	40	20	46.7	26.7	73.3	40	20			
% MARI ≥ 0.2													100	
% MDR														100

Phytochemical Screening of Ethanolic Extracts of *Mentha piperita* and *Eucalyptus globulus*

The phytochemical results of the ethanolic extract of *Mentha piperita* showed that it contains alkaloids, glycosides, sterols and flavonoids, while that of *Eucalyptus globulus* showed it

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containflavonoid, saponins, and glycosides. Alkaloid, steroid and phenol were not detected in *Eucalyptus globulus* (Table 2).

Table 2: Phytochemical Screening of *Mentha piperita* and *Eucalyptus globulus* Extract

S/N	Phytochemical Constituent	*Mentha piperita*	*Eucalyptus globulus*
1	Alkaloid	+	-
2	Phenols	-	-
3	Flavonoids	+	+
4	Tannins	-	+
5	Glycosides	+	+
6	Saponins	-	+
7	Steroid	+	-

Key: + = Present, - = absent

Evaluation of Bioactivity of *Mentha piperita* and *Eucalyptus globulus* Ethanolic Extract against Multidrug-Resistant Bacteria Isolated from Pneumonia Patients

Ethanolic extracts of *Mentha piperita* showed significant zone inhibitory activity against multidrug-resistant *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa* at 500mg/ml when compared with the standard drug (ciprofloxacin) used as a control (Table 3).

Table 3: Zone of Inhibition of Ethanolic Extract of *Mentha piperita* Leaves

S/N	Organisms	Concentration in mg/ml					Control Ciprofloxacin
		125	250	500	750	1000	
1	*K. pneumoniae*	11.5	16	22.5	23	25	25
2	*E. coli*	11.5	19	23	23.5	24	22
3	*S. aureus*	15	18	20	22	22.5	22
4	*S. pneumoniae*	13	17	20	21	22	20
5	*Ps. aeruginosa*	18	20	22	23	24	22

The minimum inhibitory concentration (MIC) of the ethanolic extract of *Mentha piperita* was 250mg/ml for *K. pneumoniae* and *S. pneumoniae*, 350mg/ml for *E. coli*, *S. aureus* and 400mg/ml for *P. aeruginosa* (Table 4).

Table 4: Minimum Inhibitory Concentration (MIC) of Ethanolic Extract of *M. piperita* Leaves

Bacteria	Ciprofloxacin	Concentration of Extract in mg/ml						
		500	450	400	350	300	250	200
K. pneumoniae	-	-	-	-	-	-	-	+
E. coli	-	-	-	-	-	+	+	+
S. aureus	-	-	-	-	-	+	+	+
S. pneumoniae	-	-	-	-	-	-	-	+
Ps. aeruginosa	-	-	-	-	+	+	+	+

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Key: + = growth, - = no growth

The minimum bactericidal concentration (MBC) of *M. piperita* at 350mg/ml was observed against *K. pneumoniae* and *S. pneumoniae*, while a higher concentration of the extract had activity at 400mg/ml against *S. aureus*, *Ps. aeruginosa* and *E. coli* (Table 5).

Table 5: Minimum Bactericidal Concentration (MBC) of Ethanolic Extract of *M. piperita* Leaves

Bacteria	Ciprofloxacin	Concentration of Extract in mg/ml				
		500	450	400	350	300
K. pneumoniae	-	-	-	-	-	+
E. coli	-	-	-	-	+	+
S. aureus	-	-	-	-	+	+
S. pneumoniae	-	-	-	-	-	+
P. aeruginosa	-	-	-	-	+	+

Key: + = growth, - = no growth

On comparing *Eucalyptus globulus* extract with standard controlled drug(ciprofloxacin), a significant zone of inhibition was observed at 750mg/ml, and activity was also observed against all the multidrug-resistant isolates tested (Table 6).

Table 6: Zones of Inhibition of Ethanolic Extract of *Eucalyptus globulus* Leaves

Bacteria	Control	Concentration of Extract in mg/ml				
	Ciprofloxacin	125	250	500	750	1000
K. pneumoniae	20	10	15	17	20	22
E. coli	21	10	10.5	13	18	20
S. aureus	20	10.5	11.5	12.5	20	20
S. pneumoniae	22	10.5	11.5	14	20	21.5
Ps. aeruginosa	22	10	14.5	17	21	22.5

Clear broths without growth were observed against all the bacteria tested at 400mg/ml, indicating its MIC value, except for *E. coli* and *S. aureus*, which had an MIC of 350mg/ml and 300mg/ml, respectively (Table 7).

Table 7: Minimum Inhibitory Concentration (MIC) of Ethanolic Extract of *Eucalyptus globulus* Leaves

Bacteria	Control	Concentration of Extract in mg/ml						
	Ciprofloxacin	500	450	400	350	300	250	200
K. pneumoniae	-	-	-	-	+	+	+	+
E. coli	-	-	-	-	-	+	+	+
S. aureus	-	-	-	-	-	-	+	+
S. pneumoniae	-	-	-	-	+	+	+	+
Ps. aeruginosa	-	-	-	-	+	+	+	+

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Keys: + = Growth, - = No growth.

Although the extract exhibited bactericidal activity against *E. coli* at 400mg/ml, and 350mg/ml for *S. aureus*, respectively. The minimum bactericidal concentration of *Eucalyptusglobulus* against all the tested multidrug-resistant bacteria isolates in Kaduna was determined to be 450 mg/ml (Table 8).

Table 8: Minimum Bactericidal Concentration (MBC) of Ethanolic Extract of *Eucalyptusglobulus* Leaves

Bacteria	Positive Control Ciprofloxacin	Concentration of Extract in mg/ml						
		500	450	400	350	300	250	200
K. pneumoniae	-	-	-	+	+	+	+	+
E. coli	-	-	-	-	+	+	+	+
S. aureus	-	-	-	-	-	+	+	+
S. pneumoniae	-	-	-	+	+	+	+	+
Ps. aeruginosa	-	-	-	+	+	+	+	+

Key: + = Growth, - = No growth

Studies of the Combined Ethanolic Extracts of *Eucalyptus globulus* and *Mentha piperita*

Table 9 shows that there was an increase in activity when the crude ethanolic extract of *Eucalyptus globulus* and *Mentha piperita* at 500mg/ml were combined, which led to an increased zone of inhibition compared to when tested alone. The result showed that the activity of the combined extract is concentration dependent (the higher the concentration of the admixture, the higher the zone of inhibition expressed), highest activity was observed at 1000mg/ml. The zone of inhibition of the combined extract was observed to be higher than that of ciprofloxacin when compared.

Table 9: Susceptibility of the Multidrug-Resistant Isolates to Combined Ethanolic Extracts of *Eucalyptus globulus* and *Mentha piperita*

Bacteria	Positive Control Ciprofloxacin	Concentration of Extract in mg/ml				
		125	250	500	750	1000
K. pneumoniae	22	-	15	20	22	24
E. coli	19	-	17	22	23	23
S. aureus	20	-	15	22	22	23
S. pneumoniae	20	-	16	21	22	22
Ps. aeruginosa	20	-	15	20	21	24

Key: - = no growth

The combined extracts had MIC activity at 250mg/ml against *S. aureus*, *E. coli* and *K. pneumoniae*. It also showed activity against *S. pneumoniae* and *Ps. aeruginosa* at MIC

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350mg/ml and 400mg/ml, respectively. In general, at 400mg/ml, the combined extracts could inhibit the growth of all the tested multidrug-resistant bacteria isolates tested (Table 10).

Table 10: Minimum Inhibitory Concentration (MIC) of Combined Ethanolic Extracts of *Eucalyptus globulus* and *Mentha piperita*

Bacteria	Positive Control Ciprofloxacin	Concentration of Extract in mg/ml						
		500	450	400	350	300	250	200
K. pneumoniae	-	-	-	-	-	-	-	+
E. coli	-	-	-	-	-	-	-	+
S. aureus	-	-	-	-	-	-	-	+
S. pneumoniae	-	-	-	-	-	+	+	+
Ps. aeruginosa	-	-	-	-	+	+	+	+

Key: + = Growth, - = No growth

Further assessment of the combined extracts showed MBC activity at 300mg/ml against *S. aureus*, *E. coli* and *K. pneumoniae*. It also showed activity against *S. pneumoniae* and *Ps. aeruginosa* at MBC of 350mg/ml. In general, at 400mg/ml, the combined extracts could cause the death of all the tested multidrug-resistant bacteria isolates (Table 11).

Table 11: Minimum Bactericidal Concentration (MBC) of Combined Ethanolic Extracts of *Eucalyptus globulus* and *Mentha piperita*

Bacteria	Positive Control Ciprofloxacin	Concentration of Extract in mg/ml						
		500	450	400	350	300	250	200
K. pneumoniae	-	-	-	-	-	-	+	+
E. coli	-	-	-	-	-	-	+	+
S. aureus	-	-	-	-	-	-	+	+
S. pneumoniae	-	-	-	-	-	+	+	+
Ps. aeruginosa	-	-	-	-	+	+	+	+

Key: + = Growth, - = No growth

DISCUSSION

Drug resistance among bacterial organisms is of public health concern, and this leads to the development of newer antibiotics from medicinal plants for pharmaceutical research (Cowan, 1999). This is why this research reports the antimicrobial activity of *Eucalyptus globulus* and *Mentha piperita* (ethanolic extract). The phytochemical analysis revealed that the ethanolic extract of *Mentha piperita* contains alkaloids, glycosides, sterols, and flavonoids, while *Eucalyptus globulus* contains flavonoids, saponins, tannins, and glycosides but lacks alkaloids, steroids, and phenols. These phytochemicals represent a repository of bioactive compounds with well-documented antimicrobial mechanisms. Alkaloids, flavonoids, saponins, and tannins collectively inhibit bacterial cell wall synthesis, disrupt membrane integrity, interfere with enzymatic activity, and bind

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to bacterial proteins, mechanisms broadly complementary to conventional antibiotics. The public health significance of these findings extends to the potential for developing affordable, locally available antimicrobial agents that can address the antibiotic resistance crisis, particularly in resource-limited settings where access to last-resort antibiotics is constrained by cost and supply chain challenges. The phytochemical content of *M. piperita* documented in this study corresponds to published findings. Ivanescu *et al*. (2021) confirmed the presence of alkaloids, flavonoids, terpenes, and glycosides as the principal bioactive classes in *M. piperita*, noting that menthol and menthone are the chief antimicrobially active compounds attributable to these phytochemical classes. Sharifi-Rad *et al*. (2013) in a phytochemical and antibacterial analysis of *Mentha piperita*reported the presence of alkaloids, flavonoids, steroids, tannins, and phenols, consistent with the current findings. For *Eucalyptus globulus*, Gonçalves *et al*. (2022) comprehensively reviewed the phytochemical profile and confirmed the presence of tannins, saponins, terpenoids, glycosides, alkaloids, and flavonoids, with flavonoids and tannins being the primary antimicrobial classes, consistent with the detection of these compounds in the present study's ethanolic extract. Future research should move beyond qualitative phytochemical screening to quantitative determination of individual bioactive compounds using advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Nuclear Magnetic Resonance (NMR) spectroscopy. Isolation and purification of individual active compounds from both plants, particularly menthol, flavonoids, and tannins, would enable structure-activity relationship studies essential for lead compound identification and pharmaceutical development. Toxicological studies (acute, subacute, and chronic) in animal models are necessary to establish safety profiles before any clinical translation.

The significant inhibitory activity of ethanolic extracts of *Mentha piperita* at 500 mg/mL against all five multidrug-resistant pneumonia-causing bacteria, with MIC values of 250 mg/mL for *K. pneumoniae* and *S. pneumoniae*, 350 mg/mL for *E. coli* and *S. aureus*, and 400 mg/mL for *P. aeruginosa*, provides compelling scientific evidence for the antimicrobial potential of peppermint extract. While the concentrations required for activity are higher than conventional antibiotics, they are achievable through standardised pharmaceutical formulation. From a public health perspective, this opens a significant avenue for developing affordable, accessible phytomedicinal products that could complement existing antibiotic therapies, particularly in low-income communities where conventional antibiotics are unavailable, unaffordable, or compromised by resistance. The antibacterial activity of *M. piperita* against clinically significant pathogens is well-supported in the literature. Shalayel *et al*. (2017), in a study of MDR clinical isolates, demonstrated strong inhibitory activity of peppermint extracts against MRSA, carbapenem-resistant *E. coli*, and *K. pneumoniae*, directly corresponding to the bacterial species tested in this study. Frunza *et al*. (2019) evaluated *M. piperita* essential oil against MDR *S. aureus*, *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, and

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Acinetobacter baumannii, confirming bactericidal activity and proposing it as a potential therapeutic option. Fraternale *et al*. (2018) demonstrated significant synergy between *M. piperita* essential oil and gentamicin against MRSA, *K. pneumoniae*, and *P. aeruginosa*, with gentamicin MIC reductions of 4- to 50-fold, suggesting that synergistic combinations with antibiotics may allow dose reduction and overcome resistance. The peppermint phytochemical study by Sharifi-Rad *et al*. (2013) confirmed antibacterial activity against *S. pneumoniae* and *K. pneumoniae* using ethanol extracts of leaves, the same extraction solvent and target organisms used in the current study. Future research should investigate the mechanism of action of *M. piperita* extract against these MDR pneumonia pathogens through time-kill kinetics, electron microscopy, and molecular docking studies targeting bacterial efflux pumps and cell wall synthesis enzymes. In vivo studies using animal infection models are essential to bridge the gap between in vitro efficacy and clinical translatability. Pharmacokinetic studies should determine the bioavailability of the active compounds when administered by inhalation, the most clinically relevant route for respiratory infections. The potential for synergy between *M. piperita* extract and ciprofloxacin, both of which were effective against the study's isolates, should be explored through checkerboard assays and FIC index calculations. Collaboration with pharmaceutical companies to develop standardised, quality-controlled phytomedicinal formulations for clinical trials is urgently needed.

Eucalyptus globulus extract demonstrated significant inhibitory activity against all five MDR bacterial isolates at 750 mg/mL, with an MIC of 400 mg/mL for most organisms and MBC at 450 mg/mL. Although the activity required higher concentrations than *M. piperita*, the broad-spectrum efficacy across both Gram-positive and Gram-negative pathogens, including the intrinsically resistant *P. aeruginosa*, is scientifically significant. Eucalyptus is widely available, economically accessible, and culturally familiar as a medicinal plant across West Africa, making it an ecologically appropriate candidate for phytomedicinal development in Nigeria. The public health relevance is amplified by the context of the BDTH findings: given that most pneumonia isolates showed high resistance to affordable antibiotics but susceptibility to expensive alternatives, eucalyptus extract could serve as a complementary or adjunctive therapy, particularly for patients who cannot afford second-line antimicrobials. The antibacterial activity of *E. globulus* against clinically significant MDR bacteria is well-documented. Mulyaningsih *et al*. (2011) demonstrated significant antimicrobial activity of *E. globulus* leaf and fruit oils against MDR bacteria, including *S. aureus* and *P. aeruginosa*, attributing the activity primarily to the 1,8-cineole (eucalyptol) component. Schuck *et al*. (2023), in a systematic review of the antibacterial properties of *E. globulus* essential oil against MRSA, concluded that it possesses bactericidal properties supported by MIC and zone-of-inhibition studies across multiple research groups. Gonçalves *et al*. (2022) documented the broad antimicrobial phytochemical repertoire of *E. globulus*, including tannins and flavonoids and noted their proven activity against respiratory and skin pathogens. Ita *et al*. (2016) in a study conducted in Ethiopia confirmed antimicrobial activity of aqueous *E. globulus*

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leaf extracts against *S. aureus*, *E. coli*, and other pathogens, supporting the African ethnomedicinal use of eucalyptus for respiratory conditions. To accelerate the therapeutic development of *E. globulus* extracts, future studies should identify and isolate the principal antimicrobial component(s) likely to be 1,8-cineole, eucalyptol, or macrocarpal compounds using bioassay-guided fractionation. Dose-optimisation studies are needed to determine whether formulation improvements (e.g., nano-encapsulation, nebulisation for inhalation) can achieve effective concentrations at the site of pulmonary infection with reduced systemic doses. Toxicological and hepatotoxicity profiling in animal models are essential prerequisite for any clinical development. The integration of ethnobotanical knowledge of eucalyptus use in Northern Nigerian traditional medicine with scientific validation of its antimicrobial potential represents a promising pathway for evidence-based phytomedicine development.

The most clinically significant finding of this study is the synergistic activity observed when the ethanolic extracts of *M. piperita* and *E. globulus* were combined. The combined extract at 500 mg/mL produced zones of inhibition greater than either extract alone and comparable to or exceeding ciprofloxacin, the positive control. The MIC of the combined extract against *S. aureus*, *E. coli*, and *K. pneumoniae* was 250 mg/mL lower than either extract individually. At 400 mg/mL, the combined extract inhibited all five MDR bacterial species, and at 400 mg/mL MBC, it achieved a bactericidal effect against all tested organisms. The concentration-dependent nature of this synergy and its ability to suppress *P. aeruginosa*, the most resistant isolate in this study, at achievable concentrations, is particularly significant from a therapeutic perspective. This combination opens the possibility of formulating a polyherbal antimicrobial product with a broad-spectrum MDR activity profile, which could have tremendous public health value in the context of sub-Saharan Africa's antibiotic resistance crisis. Synergistic antimicrobial combinations involving plant extracts are increasingly documented in the scientific literature. Fraternale *et al*. (2018) demonstrated that *M. piperita* essential oil acts synergistically with gentamicin, producing FIC indices of 0.07–0.30 across multiple MDR pathogens, representing dramatic MIC reductions. These synergistic effects are mediated by complementary mechanisms: terpene alcohols (from peppermint) increase membrane permeability, facilitating entry of phenolic compounds (from eucalyptus) that inhibit intracellular enzymes. Mulyaningsih *et al*. (2011) explicitly studied combinations of *E. globulus* oil with antibiotics and other essential oils against MDR bacteria, demonstrating marked synergy. The concentration-dependent activity of the combined extract mirrors the dose-response behaviour reported by Frunza *et al*. (2019) for *M. piperita* essential oil. The superior activity of the combined extract over ciprofloxacin, a reserve 'Watch' antibiotic in this study, echoes findings reported by multiple African phytomedicine research groups who have documented plant extract activity exceeding that of standard antibiotics against MDR clinical isolates. The synergistic activity of the combined *M. piperita* and *E. globulus* extract represents a promising lead for pharmaceutical development and merits immediate follow-up research.

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CONCLUSION AND RECOMMENDATIONS

From the results obtained, it can be concluded that both the ethanolic leaf extracts of *Eucalyptus globulus and Mentha piperita* have antibacterial activity against the multidrug-resistant strains, which are attributed to pneumonia and can be used in the development of newer agents to reduce the rate of drug resistance. Therefore, the following are recommended: checkerboard microdilution assays to calculate fractional inhibitory concentration (FIC) indices and formally classify the interaction as synergistic, additive, or indifferent; time-kill kinetic studies to characterise the bactericidal dynamics; in vivo pharmacological studies in rodent pneumonia models to assess therapeutic efficacy, pharmacokinetics, and safety; phytochemical profiling of the combined extract by GC-MS and HPLC to identify which compound classes are responsible for the enhanced activity; pre-clinical toxicology testing and eventual progression to Phase I/II clinical trials in a Nigerian tertiary hospital setting are recommended. Regulatory frameworks for phytomedicines in Nigeria, under the National Agency for Food and Drug Administration and Control (NAFDAC), should be engaged early in the development pathway to facilitate eventual approval and commercialisation of a safe, effective, locally produced antimicrobial product.

REFERENCES

1. Adebola, O.O., Olaifa, G.C., Adebola, P.O., & Akinyele, A.J. (2001). Phytochemical and antibacterial studies of Eucalyptus globulus. Journal of Medical Plants Research, 5(2), 182–190.
2. Adewemimo, A., Kalter, H.D., Perin, J., *et al*. (2017). Direct estimates of cause-specific mortality fractions and rates of under-five deaths in the Northern and Southern regions of Nigeria by verbal autopsy interview. PLOS ONE, 12(5), e0178129. https://doi.org/10.1371/journal.pone.0178129
3. Assefa, M. (2022). Multi-drug resistant gram-negative bacterial pneumonia: aetiology, risk factors, and drug resistance patterns. Pneumonia, 14(1), 4. https://doi.org/10.1186/s41479-022-00096-z
4. Bjarnason, A., Westin, J., Lindh, M., Andersson, L.-M., Kristinsson, K.G., Löve, A., Baldursson, O., & Gottfredsson, M. (2018). Incidence, aetiology, and outcomes of community-acquired pneumonia: a population-based study. Open Forum Infectious Diseases, 5(2), ofy010. https://doi.org/10.1093/ofid/ofy010
5. Cilloniz, C., Ewig, S., Polverino, E., Marcos, M.A., Esquinas, C., Gabarrus, A., Mensa, J., & Torres, A. (2013). Microbial aetiology of community-acquired pneumonia and its relation to severity. Thorax, 66(4), 340–346. https://doi.org/10.1136/thx.2010.143982
6. Cowan, M.M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582. https://doi.org/10.1128/cmr.12.4.564

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 1 - 20 Submitted 8/7/2025 Accepted 17/12/2025
APSBJournal

7. Curran, A., Doménech-Puigcerver, S., Clemente, M., Cabellos, C., Fernández-Viladrich, P., Verdaguer, R., & Ribera, E. (2008). Bacterial pneumonia in HIV-infected patients: use of the pneumonia severity index and influence of current antiretroviral therapy. HIV Medicine, 9(8), 609–615. https://doi.org/10.1111/j.1468-1293.2008.00608.x
8. Evans, W.C. (2002). Trease and Evans' Pharmacognosy (15th ed.). Saunders/Elsevier.
9. Falahati, M., Tabrizi, N.O., & Jahaniani, F. (2005). Anti-dermatophyte activities of Eucalyptus camaldulensis in comparison with Griseofulvin. Iranian Journal of Pharmacology & Therapeutics, 4(2), 80–83.
10. File, T.M. Jr., & Niederman, M.S. (2004). Antimicrobial therapy of community-acquired pneumonia. Infectious Disease Clinics of North America, 18(4), 993–1016. https://doi.org/10.1016/j.idc.2004.07.011
11. Fraternale, A., Schiavano, G.F., Taffi, S., Brundu, S., Balestra, E., Perno, C.F., Clementi, M., Brandi, G., & Magnani, M. (2018). Elucidation of the synergistic action of Mentha piperita essential oil with common antimicrobials. PLoS ONE, 13(8), e0201237. https://doi.org/10.1371/journal.pone.0201237
12. Frunza, M., Brezuica, R., Corbu, V.M., Chitigoi, N., Soica, C.M., Borcan, F., Dănilă, G., & Dumitrascu, V. (2019). Evaluation of essential oil obtained from Mentha × piperita L. against multidrug-resistant strains. Microorganisms, 7(9), 312. https://doi.org/10.3390/microorganisms7090312
13. Gera, R., Singh, V., Mujwar, S., & Bhardwaj, P. (2013). Antibiotic resistance pattern of bacterial isolates causing pneumonia at a tertiary care hospital. International Journal of Pharmaceutical Sciences and Research, 4(8), 3164–3169.
14. Giedraitienė, A., Vitkauskienė, A., Naginienė, R., & Pavilonis, A. (2011). Antibiotic resistance mechanisms of clinically important bacteria. Medicina (Kaunas), 47(3), 137–146.
15. Gonçalves, A.C., Rodrigues, M.J., Neves, B., Cruz, M.T., Lopes, V., Duarte, M.F., & Custódio, L. (2022). Phytochemical properties and diverse beneficial roles of Eucalyptus globulus Labill.: A review. Horticulturae, 7(11), 450. https://doi.org/10.3390/horticulturae7110450
16. Hudz, N., Kobylinska, L., Pokajewicz, K., Horčinová Sedláčková, V., Fedin, R., Voloshyn, M., Myskiv, I., Brindza, J., Wieczorek, P.P., & Lipok, J. (2023). Mentha piperita: Essential oil and extracts, their biological activities, and perspectives on the development of new medicinal and cosmetic products. Molecules, 28(21), 7444. https://doi.org/10.3390/molecules28217444
17. Ibrahim, R.A., Wang, S.H., Gebreyes, W.A., Mediavilla, J.R., Hundie, G.B., Mekuria, Z., Ambachew, R., Teklu, D.S.,

Kreiswirth, B., Beyene, D., & Berhe, N. (2024). Antimicrobial resistance profile of Staphylococcus aureus isolated from patients, healthcare workers, and the environment in a tertiary hospital in Addis Ababa, Ethiopia. PLoS ONE, 19(8), e0308615. https://doi.org/10.1371/journal.pone.0308615

18. Ita, S.O., Joseph, E., & Ubi, M.E. (2016). Phytochemical and antimicrobial potentials of leaves extract of Eucalyptus globulus from Maichew, Tigray, Ethiopia. MedCrave Online Journal of Cell Science & Report, 3(2). https://doi.org/10.15406/mojcsr.2016.03.00068
19. Ivanescu, B., Miron, A., & Corciova, A. (2021). Sesquiterpene lactones from the Artemisia genus: biological activities and methods of analysis. Journal of Analytical Methods in Chemistry, 2021, 6687172. https://doi.org/10.1155/2021/6687172
20. Jinghua, W., Xiaolan, Y., Shan, S., Yan, S., & Li, Q. (2017). Bacterial distribution and drug resistance in lower respiratory tract infections in pediatric patients in China. Experimental and Therapeutic Medicine, 13(5), 2372–2376. https://doi.org/10.3892/etm.2017.4228
21. King, C., Siddle, M., Adams, O., Ahmar, S., Ahmed, T., Bakare, A.A., Bakare, D., Burgess, R.A., Colbourn, T., McCollum, E.D., Olowookere, T., Salako, J., Uchendu, O., Graham, H.R., Falade, A.G.; INSPIRING Consortium. (2022). Prevalence of pneumonia and malnutrition among children in Jigawa state, Nigeria: a community-based clinical screening study. BMJ Paediatrics Open, 6(1), e001640. https://doi.org/10.1136/bmjpo-2022-001640
22. Li, Y., Liu, Y., Ma, A., Bao, Y., Wang, M., & Sun, Z. (2017). In vitro antiviral, anti-inflammatory, and antioxidant activities of the ethanol extract of Mentha piperita L. Food Science and Biotechnology, 26(6), 1675–1683. https://doi.org/10.1007/s10068-017-0217-9
23. Mackenzie, G. (2016). The definition and classification of pneumonia. Pneumonia, 8, 14. https://doi.org/10.1186/s41479-016-0012-z
24. Mulugeta, B., Tesfahun, E., Mamo, T., Mohammed, E., Endewent, E., & Ayele, M. (2023). Determinants of community-acquired pneumonia among adult patients attending Debre Berhan University Hakim Gizaw Hospital, Northeast Ethiopia: a case-control study. International Journal of General Medicine, 16, 5271–5279. https://doi.org/10.2147/IJGM.S434954
25. Mulyaningsih, S., Sporer, F., Reichling, J., & Wink, M. (2011). Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. Pharmaceutical Biology, 49(9), 893–899. https://doi.org/10.3109/13880209.2011.553625

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 1 - 20 Submitted 8/7/2025 Accepted 17/12/2025
APSBJournal

26. Nurahmed, A.M., Haile, G., Awulachew, M.T., Shibiru, T., & Teshome, M. (2020). Bacterial pathogens and antibiotic susceptibility profiles in community-acquired pneumonia: a cross-sectional study at Jimma University Medical Centre. BMC Infectious Diseases, 20, 576. https://doi.org/10.1186/s12879-020-05307-0
27. Ojo-Bola, O., Fayemi, O., & Sule-Odu, A. (2014). Antibiotic resistance pattern of pneumonia-associated bacteria among HIV patients at Federal Medical Centre Ido-Ekiti, Nigeria. International Research Journal of Pharmacy, 5(6), 479–483.
28. Osman, A., Abadi, R.S.M., & Ayoub, S.M.H. (2019). The effect of extraction method and solvents on the yield and antioxidant activity of certain Sudanese medicinal plant extracts. The Journal of Phytopharmacology, 8(5), 248–252.
29. Pathare, A.D. (2024). An overview of the herbal plant: peppermint. International Journal of Pharmaceutical Research and Applications, 9(4), 840–845.
30. Pharmaceutical Society of Nigeria – Partnership for Advocacy in Children and Family Health (PSN-PACFAH). (2022). 20% of Kaduna children die before their 5th birthday. https://drpcngr.org/20-of-kaduna-children-die-before-their-5th-birthday-psn-pacfah/
31. Roquilly, A., Marret, E., Abraham, E., & Asehnoune, K. (2015). Pneumonia prevention to decrease mortality in intensive care units: a systematic review and meta-analysis. Clinical Infectious Diseases, 60(1), 64–75. https://doi.org/10.1093/cid/ciu747
32. Sadiq, M.U., Shah, A., Haleem, A., Shah, S.M., & Shah, I. (2023). Eucalyptus globulus mediated green synthesis of environmentally benign metal-based nanostructures: a review. Nanomaterials, 13(13), 2019. https://doi.org/10.3390/nano13132019
33. Sampson, U.Z., Onuh, P.E., Jibril, D.A., Osuchukwu, E.C., & Alhassan, A.I. (2022). Distribution and antibiogram profile of bacteria associated with pneumonia among hospitalised patients in Kano State, Nigeria. Tropical Journal of Natural Product Research, 6(2), 243–247. https://doi.org/10.26538/tjnpr/v6i2.14
34. Sattar, S.B.A., Nguyen, A.D., & Sharma, S. (2024). Bacterial pneumonia. StatPearls [Internet]. National Centre for Biotechnology Information. https://www.ncbi.nlm.nih.gov/books/NBK513321/
35. Schuck, D.C., Ribeiro, E.L., Casali, M., Daré, R.G., Soares, A.A., Marques, J.A., & Bento, C.S. (2023). Antibacterial properties of Eucalyptus globulus essential oil against MRSA: a systematic review. International Journal of Molecular Sciences, 24(7), 6212. https://doi.org/10.3390/ijms24076212
36. Selvi, M., & Vaithilingan, S. (2024). Childhood pneumonia in low- and middle-income countries: a systematic review of prevalence, risk factors, and healthcare-seeking

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 5 Issue 2 December, 2025 ISSN: 2971_611X
Page 1 - 20 Submitted 8/7/2025 Accepted 17/12/2025
APSBJournal

behaviours. Cureus, 16(4), e57636. https://doi.org/10.7759/cureus.57636

37. Shalayel, M.H.F., Al-Mazrooa, S.A., Al-Hazmi, M.H., Al-Ansari, A.H., Al-Shahrani, A.A., & Al-Assiri, A. (2017). Antibacterial activity of peppermint (Mentha piperita) extracts against some selected pathogenic bacteria. Pakistan Journal of Medical and Health Sciences, 11(2), 816–819.
38. Sharifi-Rad, M., Nazaruk, J., Polito, L., Morais-Braga, M.F.B., Rocha, J.E., Coutinho, H.D.M., Sharifi-Rad, R., & Sharifi-Rad, J. (2013). Antibacterial activity and phytochemical analysis of Mentha piperita L. (peppermint), an important multipurpose medicinal plant. American Journal of Plant Sciences, 4(4), 685–690. https://doi.org/10.4236/ajps.2013.44A085
39. Shiekh, R.A.E., Atwa, A.M., Elgindy, A.M., Mustafa, A.M., Senna, M.M., Alkabbani, M.A., & Ibrahim, K.M. (2025). Therapeutic applications of eucalyptus essential oils. Inflammopharmacology, 33(1), 163–182. https://doi.org/10.1007/s10787-024-01588-8
40. Shrestha, P., Bhattarai, N., & Marhatta, M.N. (2013). Bacteriological profile and antibiogram of lower respiratory tract infections in a tertiary care hospital. Journal of the Institute of Medicine Nepal, 35(3), 22–26.
41. Sofowora, A. (2008). Medicinal plants and traditional medicine in Africa (3rd ed.). Spectrum Books.
42. Tilahun, M., Gebretsadik, D., Seid, A., Gedefie, A., Belete, M.A., Tesfaye, M., Kebede, E., & Shibabaw, A. (2023). Bacteriology of community-acquired pneumonia, antimicrobial susceptibility pattern and associated risk factors among HIV patients, Northeast Ethiopia: cross-sectional study. SAGE Open Medicine, 11, 20503121221145569. https://doi.org/10.1177/20503121221145569
43. Zhang, C., Fu, X., Liu, Y., Zhao, H., & Wang, G. (2023). Burden of infectious diseases and bacterial antimicrobial resistance in China: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet Regional Health – Western Pacific, 43, 100972. https://doi.org/10.1016/j.lanwpc.2023.100972