Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2023, Vol. 39, No.(6):
Pg. 1479-1494

Selected Coastal Plants as Potential Treatment for *Pneumonia* Disease: Determination of Their Phytochemicals and Antibacterial Activity Against Some *Pneumonia* Bacteria

WAHDINI HANIFAH[1]*, YOSIE ANDRIANI[1]*, NOR ATIKAH MOHAMED ZIN[1], DINI RYANDINI[2], FADZILLAH ADIBAH ABDUL MAJID[1], BEGINER SUBHAN[4], and TENGKU SIFZIZUL TENGKU MUHAMMAD[1]

[1]Institute of Climate Adaptation and Marine Biotechnology, Universiti Malaysia Terengganu, Mengabang Telipot 21030, Kuala Nerus, Terengganu, Malaysia.
[2]Microbiology Department, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Central Java, Indonesia.
[4]Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor, Bogor, West Java, Indonesia.
*Corresponding author E-mail: yosie.hs@umt.edu.my

http://dx.doi.org/10.13005/ojc/390608

(Received: October 02, 2023; Accepted: December 11, 2023)

ABSTRACT

Pneumonia is a respiratory infection caused by microorganisms including bacteria. Current treatment with antibiotics leads to bacterial resistance. An alternative treatment involves utilizing coastal plants. In this study, five parts of eleven coastal plants underwent phytochemicals screening and investigated for their antibacterial activity against five pneumonia bacteria. Cold extraction was performed using hexane and methanol, successively. Qualitative phytochemicals screening and antibacterial testing were done using several reagents and agar well diffusion method, respectively. The results revealed that almost all hexane and methanolic fractions from coastal plants showed antibacterial activity, except Vitex rotundifolia leaves. The highest activity was shown by hexane fraction of Rhodomyrtus tomentosa leaves. Among the methanolic fractions, Syzigium grande twigs exhibited the highest antibacterial property. Phytochemical screening revealed the presence of flavonoids in all active fractions, potentially correlating with their antibacterial activity. In summary, some selected coastal plants have the potential to act as anti-pneumonia bacteria agents.

Keywords: Extraction, Methanol, Hexane, Phytochemicals, Coastal plants, Anti-pneumonia bacteria.

INTRODUCTION

A type of disease that spreads worldwide is respiratory diseases, including asthma[1–3], influenza[4], tuberculosis[3], lung cancer[2,3], chronic obstructive pulmonary disease (COPD)[1–3], pulmonary fibrosis[5], pneumonia[6]. Over the past 150 years, respiratory diseases have remained a significant cause for disability and mortality[3]. According to Centers for Disease Control and Prevention[7], respiratory

diseases were among the top ten causes of death in 2020. The development of respiratory diseases is caused by various factors, including smoking, exposure to air pollution, and infection by microorganisms.

One of infectious disease agents is bacteria, and some studies have reported that bacteria have caused 10%-30% of all infectious diseases in human, leading to millions of deaths every year[8–11]. Several bacterial species commonly identified as causative agents of respiratory tract infections that have contributed to global mortality and morbidity rates include *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus cohnii*, *Pseudomonas aeruginosa*, and *Escherichia coli*[12–14]. Pneumonia is more frequently caused by *Gram-negative* bacteria than *Gram-positive* bacteria[15]. These bacteria can cause several types of pneumonia, such as Community Acquired Pneumonia (CAP) and Hospital Acquired Pneumonia (HAP), based on the place where the infection occurs. CAP is commonly caused by bacteria like *K. pneumoniae*[16], *S. pneumoniae*[17], and *E. coli*[18], while HAP is caused by *P. aeruginosa*[15].

Current drugs used to treat the infection include antibiotics. However, existing antibiotics have been less effective due to the increasing bacterial resistance to these antimicrobial substances[19]. The rise in antibiotic resistance among pathogenic microbial agents, along with antibiotics' adverse effects on the human body, has prompted researchers worldwide to explore and discover new alternative drugs to address this issue. One approach involves investigating natural products as potential alternative treatments. Previous studies have reported that medicinal plants have been used to treat several respiratory diseases like pneumonia, cough, cold, bronchitis, and asthma, and these plants include *Glycyrrhiza glabra* (liquorice), *Hyssopus officinalis* (mint)[20], *Magnifera indica* (mango)[21], *Psidium guajava* (guava)[22], *Allium sativum* (garlic)[23], *Allium cepa* (onion)[24], *Zingiber officinale* (ginger) and *Eucalyptus globuluus* (blue gum eucalyptus)[25]. Also, the World Health Organization (WHO) reported that herbal medicines are used as traditional medicine in about 88% of all countries. In addition, natural products are used in over 40% of pharmaceutical formulations[26] and herbal medicine is used for basic health care in around 70-95% populations of developing countries[27].

Coastal plants have been known as the natural product that produces bioactive compounds and acts in several biological activities. For instance, the extract of *Avicennia marina*, which is rich in total phenol and flavonoid, has been shown to possess antibacterial properties against *P. aeruginosa*[28]. Apart from mangrove trees, other flowering coastal plants may be found along the shore in the coastal areas and above the high tide line. In addition, coastal plants has been used traditionally as a medicinal treatment in the community, such as *Xylocarpus granatum* being used to treat dyspnea by the Indonesian community[29], the root of *Acanthus licifolius* to treat asthma and cough, and *Aegiceras corcniculatum* to treat asthma[30,31]. Coastal plants hold potential as an alternative approach to combat respiratory infections caused by pathogenic bacteria. However, compared to terrestrial plants, research on the antibacterial activity of coastal plants remains limited.

Based on the research gap identified in the literature, this study investigated the potency of antibacterial properties from several coastal plants to determine their suitability as an alternative treatment for pneumonia caused by bacterial infections. The study was conducted using several locally available coastal plants (Fig. 1), which were extracted using hexane and methanol successively, then subjected to phytochemical screening and antibacterial tests using agar well diffusion. As reported by Andriani *et al.,* 2023[32] *S. alba, P. tectorius, P. pongamia*, and *H. tiliaceus* were active against *K. pneumoniae*. However, they have not been tested against other pneumonia-causing bacteria. Therefore, the antibacterial activities of these three coastal plants need to be evaluated against other pneumonia-causing bacteria. The outcomes of this study will provide new knowledge about which coastal plants have high potential as an alternative treatment against pneumonia bacteria.

Fig. 1. Selected coastal plants in this study

MATERIALS AND METHODS

Sample Collection and Preparations

The selected coastal plants were collected from various areas within the state of Terengganu, Malaysia, including Kuala Nerus district (Universiti Malaysia Terengganu, Tanjung Gelam Beach, and Tok Jembal) shown in Fig. 2, and Marang district (Bukit Kor) shown in Fig. 3. Several parts of the plant were used, such as leaves, twigs, seeds, rinds, and flowers. The description of the coastal plants (scientific and local names), parts of the plants used, and sampling locations are shown in Table 1. The images of the coastal plants are shown in Fig. 1. The weight of all parts of the plants was measured before the drying process. The samples were dried using a freeze dryer and ground into powder using a grinder or blender.

Table 1: Selected Coastal Plants with their local names, part of the plants used, and sampling area location

Plant Species	Local Names	Part Used	Location
Canavalia rosea	Beach bean/Coastal Jack-bean	Leaves, Twigs, Seeds, Rinds	Tanjung Gelam Beach Area
Hibiscus tiliaceus	Sea hibiscus/coast cottonwood	Leaves	UMT Area
Ipomoea pescaprae	Beach morning glory/tapak kuda	Leaves, Twigs	UMT Area
Melastoma malabathricum	Senduduk	Leaves, Twigs, Flowers	Tok Jembal Area
Pandanus tectorius	Mengkuang Laut/Screw Pine	Leaves	Tanjung Gelam Beach
Pongamia pinnata	Indian beech/pongame oiltree	Leaves, Twigs, Seeds, Rinds	UMT Area
Rhodomyrtus tomentosa	Kemunting	Leaves	Bukit Kor
Syzigium grande	Sea apple/Jambu Laut	Leaves, Twigs	Tanjung Gelam Beach Area
Sonneratia alba	Mangrove apple/perepat	Leaves, Twigs	UMT Area
Terminalia catappa	Sea almond/Indian almond	Leaves	UMT Area
Vitex rotundifolia	Round leaved chaste tree	Leaves, Twigs	Tanjung Gelam Beach Area

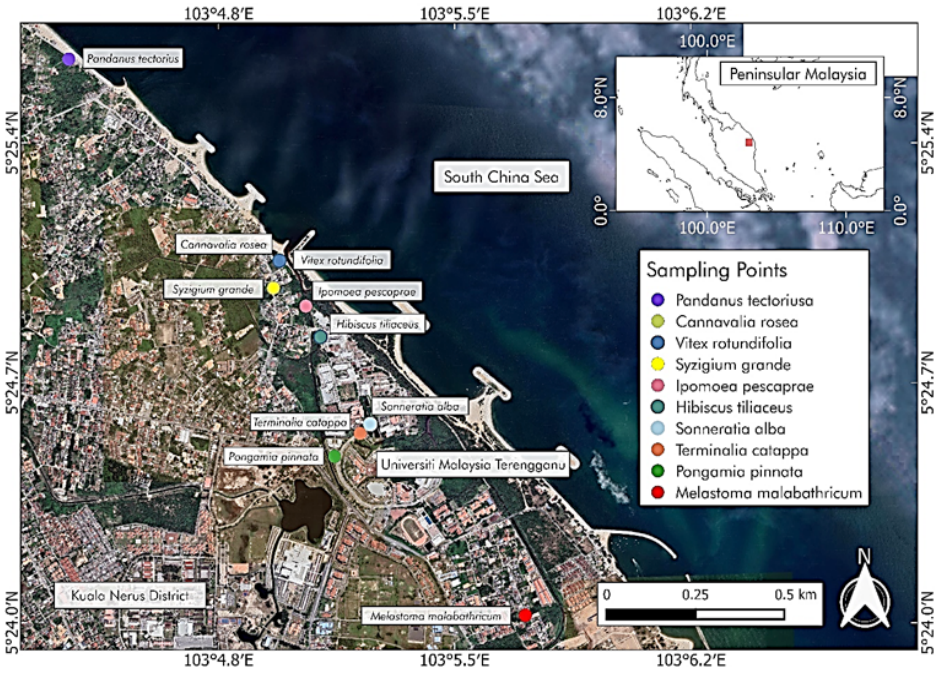

Fig. 2. Sampling Site at Kuala Nerus District

Fig. 3. Sampling site at Marang District

Extraction of Plant Samples

The powder samples were extracted using hexane and methanol successively to produce hexane and methanol fractions. The filtrate was filtered using Whatman paper No. 1 and then evaporated using a rotary evaporator at 50-60°C, 40 rpm until the solvent evaporated. The yield obtained was stored in a cold room and used to screen for antibacterial activity against selected pathogenic bacteria.

Phytochemical Screening

The fraction samples were proceeded to phytochemical constituent testing. Phytochemicals of active fractions were tested using different chemical tests to detect different phytoconstituents using a standard procedure[33–35]. The tests were performed for phenols and tannins (Ferric chloride test), flavonoids (Alkaline reagent test), alkaloids, terpenoids (Salkowski's test), steroids (Liebermann-Burchard test), saponins (Frothing test, glycosides (Keller-kiliani test), and quinones.

Antibacterial assay

The antibacterial assay of the hexane and methanolic fractions of several coastal plants was tested against five human pathogenic bacteria using the agar well diffusion method[36]. Two *Gram-positive* bacteria were represented by *Staphylococcus aureus* and *Staphylococcus cohnii.* Meanwhile, *Gram-negative* bacteria were represented by *Klebsiella pneumoniae, Escherichia coli*, and *Pseudomonas aeruginosa*. The pneumonia bacteria were sub-cultured to Nutrient Agar (NA) medium and incubated for 24 hours at 37°C.

Modified agar well diffusion method

The method used in this research was based on the modified agar well diffusion method developed by Magaldi *et al.,* (2004)[36]. Mueller Hinton Agar (MHA) medium was used for antibacterial susceptibility testing. The cultured bacteria were then diluted in sterilized dH_2O until it obtained an optical density (OD) of 0.5 MacFarland (1.0x108 CFU/mL). All fractions were tested with selected bacteria. The bacteria cultures were then swabbed on Mueller Hinton Agar (MHA) medium using a sterilized cotton swab and left for 5 min for drying. 10 mg/mL of fractions was diluted in 1 mL DMSO in a sterilized 1,5 mL Eppendorf tube. The well of agar medium was made using sterilized forceps with a diameter of 6 mm. 10 mg/mL of fractions were loaded into each well and incubated at 37°C for 24 hours. The inhibition zone formed was measured in millimetre. The resulting inhibition zone (IZ) was categorized as listed on Table 2[37–39]. The active fractions were then proceeded to the minimum inhibitory testing.

Table 2: Inhibition zone categorization

Inhibition zone (mm)	Symbol	Interpretation/categorization
0 mm	-	No activity
<10 mm	+	Weak activity
10–14.9 mm	++	Good activity
≥15 mm	+++	Strong activity

Minimum inhibitory concentration

Test of minimum inhibitory concentration was only done to the fractions that were active to form the inhibition zone against pneumonia bacteria. Two-fold serial dilution was done to obtain several concentrations. The concentrations used in this research were 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.625 mg/mL. The positive control was 0.01 mg of antibiotic gentamycin and the negative control was DMSO. The agar plate medium was divided into different parts, and each sample was loaded into each well. The plate was incubated for 24 h at 37°C. After 24 h the inhibition zone was measured to see the lowest concentration that could inhibit the bacteria.

Statistical analysis

The statistical analysis of the triplicate data for the inhibition zone diameter values and minimum inhibitory concentration (MIC) values was performed using the Windows version 26 of the Statistical Packages for Social Sciences (SPSS) software. Each experimental value was presented as the mean and standard deviation (SD). To assess the significance of differences and interactions between variables, a one-way analysis of variance (ANOVA) was conducted, followed by the Post hoc Duncan test. The corresponding p-values of the test were compared to determine statistical significance. In this study, p-values<0.05 were considered significant.

RESULTS AND DISCUSSION

Phytochemical Screening of Fractions of Selected Coastal Plants

The qualitative phytochemical analysis of fractions from selected coastal plants revealed different phytochemical constituents, including quinone, saponin, steroid, glycoside, alkaloid, flavonoid, terpenoid, phenol and tannin, as listed in Table 3. The majority of the fractions contained bioactive compounds including flavonoid, steroid, glycoside, alkaloid, and terpenoid. These bioactive compounds have been associated with antibacterial properties. However, it is important to note that the presence of phytochemicals can be influenced by several factors, such as genetic variations, environmental conditions, time of harvest, and geographical factors[40].

Table 3: Phytochemical screening of selected coastal plants' fractions

Plant Sample	Parts of plant	Types of fractions	Code	Phytochemicals Constituents							
				Quinone	Saponin	Phenol & Tannin	Flavonoid	Alkaloid	Glycoside	Terpenoid	Steroid
Canavalia rosea	Leaf	Hexane	CRLH	-	+	-	+	-	-	-	-
	Twig	Methanol	CRLM	-	+	-	+	+	+	-	+
	Twig	Hexane	CRTH	-	-	-	-	-	-	-	+
	Twig	Methanol	CRTM	-	+	-	+	+	+	-	+
	Seed	Hexane	CRSH	-	-	-	+	-	-	-	+
	Seed	Methanol	CRSM	-	-	+	+	+	+	+	+
	Rinds	Hexane	CRRH	-	-	-	+	-	-	-	+
	Rinds	Methanol	CRRM	-	+	-	+	+	+	+	+
Hibiscus tiliaceus	Leaf	Methanol	HTLM	-	+	+	+	-	+	-	+
	Leaf	Hexane	HTLH	-	-	-	-	-	-	-	+
Ipomoea pescaprae	Twig	Methanol	TKTM	-	+	-	+	+	+	-	+
	Twig	Hexane	TKTH	-	-	-	+	-	-	+	+
	Leaf	Hexane	TKLH	-	-	-	+	-	-	-	+
	Leaf	Methanol	TKLM	-	-	+	+	+	+	+	+
Melastoma malabathricum	Leaf	Hexane	MMLH	-	-	-	-	-	-	-	+
	Leaf	Methanol	MMLM	+	+	+	+	+	+	+	+
	Flower	Methanol	MMFM	+	+	+	+	-	+	+	-
	Flower	Hexane	MMFH	-	-	-	+	-	+	+	-
	Twig	Methanol	MMTM	-	+	+	+	+	+	+	+
	Twig	Hexane	MMTH	-	-	-	+	-	-	-	+
Pandanus tectorius	Leaf	Hexane	PTLH	-	-	-	+	-	-	-	+
	Leaf	Methanol	PTLM	-	+	-	+	-	+	-	+
Pongamia pinnata	Leaf	Methanol	PPLM	-	+	-	+	-	+	-	+
	Twig	Methanol	PPTM	-	+	-	+	-	-	-	+
	Twig	Hexane	PPTH	-	-	-	+	-	-	-	+
Pongamia pinnata	Leaf	Hexane	PPLH	-	-	-	-	-	-	-	+
	Seed	Hexane	PPSH	-	-	-	+	-	+	-	-
	Seed	Hexane	PPSH OIL	-	-	-	+	-	+	-	-
	Seed	Methanol	PPSM	-	+	-	+	+	+	-	+
	Rinds	Hexane	PPRH	-	-	-	+	-	+	-	+
	Rinds	Methanol	PPRM	-	-	-	+	+	+	+	+
Rhodomyrtus tomentosa	Leaf	Hexane	RTLH	-	-	-	+	+	-	-	-
	Leaf	Methanol	RTLM	-	+	+	+	+	+	+	+
Sonneratia alba	Twig	Hexane	SATH	-	-	-	+	-	-	-	+
	Leaf	Hexane	SALH	-	-	-	-	-	-	-	+
	Leaf	Methanol	SALM	-	+	+	+	+	-	-	-
	Twig	Methanol	SATM	+	+	+	+	+	+	-	-
Syzigium grande	Leaf	Hexane	SGLH	+	-	-	+	-	-	-	+
	Leaf	Methanol	SGLM	-	+	+	+	+	+	+	+
	Twig	Hexane	SGTH	-	-	-	+	-	-	-	+
	Twig	Methanol	SGTM	+	+	+	-	+	+	+	+
Terminalia catappa	Leaf	Methanol	TCLM	-	+	+	+	-	+	+	+
	Leaf	Hexane	TCLH	-	-	-	-	-	-	-	+
Vitex rotundifolia	Leaf	Hexane	VRLH	-	-	-	+	-	-	+	+
	Leaf	Methanol	VRLM	-	+	-	+	+	+	+	+
	Twig	Hexane	VRTH	-	-	-	-	-	-	+	+
	Twig	Methanol	VRTM	-	-	+	+	+	+	+	+

*(-) absence of phytochemicals group compound, (+) presence of phytochemicals group compound

Antibacterial activity of Fractions of Selected Coastal plants

The antibacterial activity was tested using agar well diffusion method with pre-screening of all fractions at a concentration of 10 mg/mL against *Gram-negative* bacteria (*K. pneumoniae, E. coli*, and *P. aeruginosa*) and *Gram-positive* bacteria (*S. aureus* and *S. cohnii*). In this study, a total of 47 fractions were obtained from different plant species, parts, and extract types. These fractions consisted of

24 hexane fractions and 23 methanolic fractions. The results of the antibacterial activity for the methanolic and hexane fractions are presented in Table 4 and Table 5, respectively. Based on the comparison, methanolic fractions of *T. catappa, R. tomentosa*, *M. malabatahricum*, and *S. grande* had a broader spectrum of action as they inhibited the growth of all pneumonia bacteria with an inhibition zone range of 10-19.50 mm. However, seven hexane fractions and three methanolic fractions showed no activity against all pneumonia bacteria. Thus, the methanolic fractions showed better antibacterial activity than hexane fractions against *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. cohnii*.

Methanol is known to be effective in extracting more bioactive compounds compared to other organic solvents[41]. The better antibacterial activity of methanolic fractions in this study is in line with that in previous research by Mahmud (2018)[42], as well as Martin and Kinyanjui (2014)[43]. In addition, the methanol fraction attracted polar compounds, while the hexane fraction attracted non-polar compounds. Flavonoids can be extracted from both polar and non-polar fractions. According to Table 3, more of methanolic fractions exhibited flavonoid compounds compared to the hexane fractions, thereby correlating with its antibacterial properties. According to Al Mamari (2022)[44], the flavonoid group compound present in methanolic fractions acts as a main bioactive group compound that correlates with an antibacterial activity. The study by Majdanik *et al.,*[45] found that flavonoid acted as the main bioactive compound, possessed antibacterial properties, and is potentially effective against a wide array of microorganisms because of its ability to complex with extracellular, soluble proteins, and bacterial cell.

The highest inhibition zones from methanolic fractions against several tested bacteria are as shown in Table 4. Based on the result, the highest inhibition zones came from the following tests: *S. grande's* twigss against *P. aeruginosa* (19.33 mm), *M. malabathricum's* leaves against *P. aeruginosa* (18.50 mm), *T. catappa's* leaves against *P. aeruginosa* (17.50 mm), and *S. alba's* leaves against *P. aeruginosa* (17.00 mm) (Fig. 4a-d), but these inhibition zones were lower than the zone formed by gentamicin. Notably, the leaf fractions demonstrated the highest antibacterial activity compared to other plant parts and were most effective against *P. aeruginosa*. The study by Courtney and Cock[46] investigated extracts from different parts of *Terminalia spp*. It was found that the leaf extracts were more potent in inhibiting the bacteria than fruit, bark or seed extracts. According to Borges *et al.,*[47], the higher antibacterial potential of a fraction is associated with its high polarity, which enables the extraction of all the phenolic compounds that are supposed to have antibacterial activity. Different types of plants and different parts of the plants have varying concentrations of their compounds, which will lead to different inhibitory effects. Noumedem *et al.,*[48] stated that the variability of antimicrobial activity between plant extracts might be due to the presence of different compounds in each plant.

Meanwhile, the highest inhibition zones from hexane fractions were *R. tomentosa's* leaves against *Gram-positive* bacteria *S. cohnii* (22.67 mm) and *S. aureus* (21.67 mm), as listed in Table 5 and Fig. 4c-d. These inhibition zone values were comparable to the positive control (Gentamicin) values against *S. cohnii* (31.00 mm) and *S. aureus* (21.00 mm), as shown in Table 6. These results correlated with the result of phytochemical screening in Table 3, which showed this fraction contained flavonoid and alkaloid group compounds that possess antibacterial properties. The study by Idris *et al.,* investigated the Total Flavonoid Contents (TFC) of hexane fractions of *R. tomentosa*, and it showed the presence of flavonoids with less amount than in methanolic fractions[49]. Besides, the hexane fraction also contained alkaloids, which could also contribute to its antibacterial properties. According to Markham *et al.,*[50] and Khan *et al.,*[51], alkaloids can interact with the bacterial cytoplasmic membrane, DNA assimilation and prevent the efflux pump.

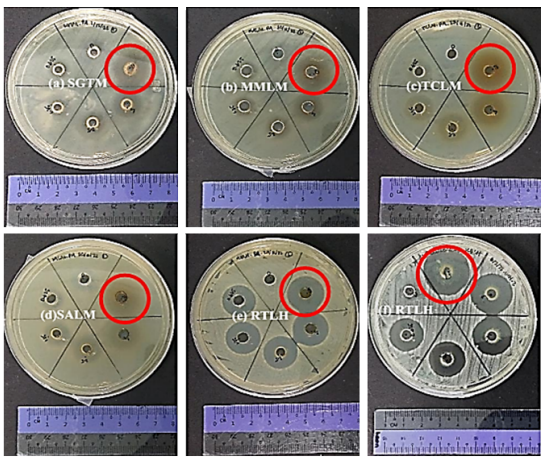

Fig. 4. The highest inhibition zone of methanol and hexane fractions of selected coastal plants. a-d are against *P. aeruginosa*; c is against *S. aureus*; d is against *S. cohnii*

Table 4: Antibacterial activity of methanol fractions against *Pneumonia* bacteria

Name of Sample	Code	Part	Inhibition Zone of Fractions Against Bacteria in Diameter (mm)				
			Gram-negative Bacteria			*Gram-positive* Bacteria	
			K. pneumoniae	*E. coli*	*P. aeruginosa*	*S. cohnii*	*S. aureus*
Canavalia rosea	CRLM	Twig	-	-	9.50 ± 0.71^{+}	-	-
	CRTM	Twig	-	-	9.00 ± 0.00^{+}	-	-
	CRSM	Seed	-	-	9.67 ± 0.24^{+}	-	-
	CRRM	Rind	-	-	11.17 ± 0.24^{++}	-	9.83 ± 0.62^{+}
Hibiscus tiliaceus	HTLM	Leaf	-	-	10.33 ± 0.24^{++}	-	-
Ipomoea pescaprae	TKLM	Leaf	-	-	7.00 ± 0.00^{+}	-	-
	TKTM	Twig	9.50 ± 0.41^{+}	-	12.17 ± 0.85^{++}	10.83 ± 0.24^{++}	11.92 ± 0.92^{++}
Melastoma malabathricum	MMLM	Leaf	11.83 ± 0.85^{++}	11.00 ± 0.71^{++}	18.50 ± 1.47^{+++}	15.67 ± 0.47^{+++}	13.50 ± 0.41^{++}
	MMFM	Flower	11.58 ± 1.01^{++}	12.67 ± 0.94^{++}	14.50 ± 0.41^{++}	14.00 ± 0.00^{++}	11.67 ± 0.94^{++}
	MMTM	Twig	10.50 ± 0.41^{++}	12.33 ± 1.25^{++}	13.33 ± 0.85^{++}	-	11.50 ± 1.22^{++}
Pandanus tectorius	PTLM	Leaf	-	-	-	-	-
Pongamia pinnata	PPLM	Leaf	-	-	10.50 ± 0.71^{++}	-	-
	PPTM	Twig	-	-	11.92 ± 0.72^{++}	9.83 ± 0.24^{+}	-
	PPSM	Seed	-	-	-	-	10.83 ± 0.24^{++}
	PPRM	Rind	9.83 ± 0.62^{+}	-	-	-	11.33 ± 1.25^{++}
Rhodomyrtus tomentosa	RTLM	Leaf	12.00 ± 1.63^{++}	13.33 ± 0.24^{++}	15.25 ± 0.74^{+++}	12.50 ± 0.41^{++}	12.42 ± 2.16^{++}
Sonneratia alba	SALM	Leaf	11.75 ± 0.74^{++}	15.83 ± 1.03^{+++}	17.00 ± 0.71^{+++}	15.33 ± 0.47^{+++}	-
	SATM	Twig	12.50 ± 0.00^{++}	13.67 ± 2.49^{++}	14.50 ± 0.41^{++}	15.67 ± 0.47^{+++}	-
Syzigium grande	SGLM	Leaf	12.33 ± 0.47^{++}	12.67 ± 0.47^{++}	16.00 ± 0.41^{+++}	13.17 ± 0.47^{++}	12.67 ± 0.47^{++}
	SGTM	Twig	11.67 ± 1.25^{++}	15.00 ± 0.41^{+++}	19.33 ± 0.94^{+++}	13.00 ± 0.00^{++}	14.83 ± 0.62^{++}
Terminalia catappa	TCLM	Leaf	13.92 ± 0,66^{++}	16.83 ± 2.01^{+++}	17.50 ± 1.22^{+++}	16.17 ± 0.62^{+++}	16.50 ± 0.74^{+++}
Vitex rotundifolia	VRLM	Leaf	-	-	-	-	-
	VRTM	Twig	-	-	-	-	-

The values describe mean ± standard deviation, - = No inhibition zone, + = weak activity (<10 mm), ++ = good activity (10-14.9 mm), +++ = strong activity (≥ 15mm)

Table 5: Antibacterial activity of hexane fractions against pneumonia bacteria

Name of Sample	Code	Part	Inhibition Zone of Fractions Against Bacteria in Diameter (mm)				
			Gram-negative Bacteria			*Gram-positive* Bacteria	
			K. pneumoniae	*E. coli*	*P. aeruginosa*	*S. cohnii*	*S. aureus*
Canavalia rosea	CRLH	Leaf	-	-	-	-	10.00 ± 0.00^{++}
	CRTH	Twig	-	-	-	11.67 ± 0.47^{++}	16.00 ± 0.82^{+++}
	CRSH	Seed	-	-	-	-	8.67 ± 0.24^{+}
	CRRH	Rind	-	-	-	11.83 ± 0.24^{++}	16.33 ± 0.47^{+++}
Hibiscus tiliaceus	HTLH	Leaf	-	-	-	11.42 ± 0.42^{++}	-
Ipomoea pescaprae	TKTH	Twig	-	-	-	-	-
	TKLH	Leaf	-	-	-	-	-
Melastoma malabathricum	MMLH	Leaf	-	-	-	-	-
	MMTH	Twig	-	-	-	-	-
	MMFH	Flower	-	-	-	9.83 ± 0.62^{+}	13.50 ± 0.71^{++}
Pandanus tectorius	PTLH	Leaf	9.50 ± 0.41^{+}	-	-	13.33 ± 0.47^{++}	15.17 ± 0.24^{+++}
Pongamia pinnata	PPLH	Leaf	-	-	-	-	9.67 ± 0.47^{+}
	PPTH	Twig	-	-	-	13.33 ± 0.94^{++}	17.17 ± 0.62^{+++}
	PPSH OIL	Seed	-	-	-	-	9.83 ± 0.24^{+}
	PPSH	Seed	-	-	-	-	10.67 ± 0.62^{++}
	PPRH	Rind	11.25 ± 0.61^{++}	-	11.83 ± 1.03^{++}	12.67 ± 0.24^{++}	15.00 ± 0.82^{+++}
Rhodomyrtus tomentosa	RTLH	Leaf	-	-	-	22.67 ± 0.94^{+++}	21.67 ± 1.25^{+++}
Sonneratia alba	SATH	Twig	-	-	-	-	-
	SALH	Leaf	-	-	-	-	-
Syzigium grande	SGLH	Leaf	12.33 ± 1.70^{++}	-	-	11.67 ± 0.47^{++}	12.42 ± 0.31^{++}
	SGTH	Twig	-	-	-	12.33 ± 0.85^{++}	15.33 ± 0.47^{+++}
Terminalia catappa	TCLH	Leaf	-	-	-	-	14.67 ± 0.94^{++}
Vitex rotundifolia	VRLH	Leaf	-	-	-	-	-
	VRTH	Twig	-	-	-	-	9.67 ± 0.47^{+}

The values describe mean ± standard deviation, - = No inhibition zone, + = weak activity (<10 mm), ++ = good activity (10-14.9 mm), +++ = strong activity (≥ 15mm)

Based on the Duncan test ($p<0.05$), the RTLH fraction with a concentration of 10 mg/mL showed the highest activity against *S. aureus*, followed by the RTLH fraction with a concentration of 10 mg/mL against S. cohnii. In addition, compared to the study by Sinulingga *et al.,*[52] whose reported the 600 mg/mL of hexane fraction had an IZ value of 20.13 mm against *S. aureus*, the current study has a better result due to the lower concentration of fraction (10 mg/mL) producing a higher IZ value (21.67 mm) against *S. aureus*. Kamarudin *et al.,* stated that *R. tomentosa* contained antibacterial candidate compounds, which are Rhodomentones A and B compounds extracted using n-hexane, ethyl acetate, and 95% ethanol[53]. However, another study done by Mordmuang *et al.,* reported that the MIC value of the ethanolic extract of *R. tomentosa* against *S. aureus* is 16 µg/mL, and the MIC value of the compound rhodomyrtone is 0.5 µg/mL[54].

The results of the post hoc Duncan test ($p<0.05$) indicated the level of interaction among various variables, including coastal plant species, type and concentration of fractions, and bacteria species. The most effective combination was the hexane fraction of *R. tomentosa* leaves at concentrations of 10 mg/mL and 5 mg/mL against *S. aureus* and *S. cohnii*, respectively. This was followed by the methanolic fraction of *S. grande's* twigs at a concentration of 10 mg/mL against *P. aeruginosa*. The third position was occupied by the methanolic fraction of *M. melastoma's* leaves at a concentration of 10 mg/mL against *P. aeruginosa*, and the methanolic fraction of *T. catappa's* leaves at a concentration of 10 mg/mL against *P. aeruginosa*.

Previous studies suggested that the observed effects may be attributed to the presence of the same active substances in different fractions but at varying minimum concentrations[55-51]. At lower concentrations, the bioactivity might no longer be detectable or the ability of the fraction to inhibit bacterial growth may decrease. Therefore, the high concentration of one or more active substances in a fraction may explain its efficiency in inhibiting microbial growth. Additionally, the susceptibility of each bacterial species also influences the antibacterial activity of the fractions.

The current study used Dimethyl sulfoxide (DMSO) as the negative control and antibiotic gentamicin as the positive control. According to the results presented in Table 6, the negative control did not exhibit any inhibition zones. In contrast, the positive control demonstrated inhibition zone values of 23 mm against *K. pneumoniae*, 18 mm against *E. coli*, 22.5 mm against *P. aeruginosa*, 31 mm against *S. cohnii*, and 22 mm against *S. aureus*. The results align with the literature stating that gentamicin is one of the antibiotics that is effective against several bacterial infections, widely against *Gram-negative*, also against *Gram-positive* bacteria, including *Staphylococci strains* and beta-haemolytic group *Streptococci*. It has also been shown to inhibit 90% of pathogen Enterobacteriaceae, so this can be a perfect model as a comparison to inhibition zone of several pneumonia bacteria[56,57].

Table 6: Antibacterial activity of DMSO and antibiotic gentamicin against pneumonia bacteria

Bacteria species	Inhibition Zone in Diameter (mm)	
	Positive Control Gentamicin	Negative Control DMSO
Klebsiella pneumoniae	$23.00 \pm 0.00^{+++}$	-
Escherichia coli	$18 \pm 0.00^{+++}$	-
Pseudomonas aeruginosa	$22.50 \pm 0.00^{+++}$	-
Staphylococcus cohnii	$31.00 \pm 0.00^{+++}$	-
Staphylococcus aureus	$22.00 \pm 0.00^{+++}$	-

The values describe mean ± standard deviation, - = No inhibition zone, + = weak activity (<10 mm), ++ = good activity (10-14.9 mm), +++ = strong activity (≥ 15mm).

Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) was determined in order to investigate the concentration limit at which the fractions can inhibit bacterial growth[58]). It is important to note that the MIC is distinct from the Minimum Bactericidal Concentration (MBC), which represents the concentration at which microbial death occurs. However, when the MIC value is closer to the MBC, it indicates a more bactericidal effect of the fractions[59]. In this study, the MIC test was done for the active fractions, which showed the inhibition zone in the pre-screening against pneumonia bacteria. To determine the MIC, a two-fold dilution of the fractions was carried out, resulting in a series of concentrations ranging from the highest to the lowest (10, 5, 2.5, 1.25, and 0.625 mg/mL)[59]. The result showed that every active fraction has a different MIC, as evidenced by the zone of inhibition formed (Table 7).

Table 7: MIC of fractions of selected coastal plants against pneumonia bacteria

Name of Sample	Part Used	Types of Fractions	Code	MIC (mg/mL) of selected fractions against bacteria*				
				K. pneumoniae(d)	*E. coli*(e)	*P. aeruginosa*(c)	*S. aureus*(a)	*S. cohnii*(b)
Canavalia rosea	Leaves	Hexane	CRLH (v)				5^{+}	
	Twigs		CRTH (n)				$0{,}625^{+}$	$0{,}625^{+}$
	Seeds		CRSH (v)				5^{+}	
	Rinds		CRRH (n)				$0{,}625^{+}$	$1{,}25^{+}$
Hibiscus tiliaceus	Leaves		HTLH (s)					$1{,}25^{+}$
Melastoma malabathricum	Flowers		MMFH (o)				$1{,}25^{+}$	$2{,}5^{+}$
Pandanus tectorius	Leaves		PTLH (l)	5^{+}			$0{,}625^{+}$	$0{,}625^{+}$
Pongamia pinnata	Twigs		PPTH (m)				$0{,}625^{++}$	$0{,}625^{+}$
	Leaves		PPLH (u)				$2{,}5^{+}$	
	Seeds		PPSH (r)				$0{,}625^{+}$	
	Seeds Oil		PPSHO (u)				$2{,}5^{+}$	
	Rinds		PPRH (g)	$0{,}625^{+}$		$0{,}625^{+}$	$0{,}625^{+}$	$1{,}25^{+}$
Rhodomyrtus tomentosa	Leaves		RTLH (h)				$0{,}625^{+++}$	$0{,}625^{++}$
Syzigium grande	Leaves		SGLH (k)	$0{,}625^{+}$			$0{,}625^{+}$	$1{,}25^{+}$
	Twigs		SGTH (n)				$0{,}625^{+}$	$0{,}625^{+}$
Terminalia catappa	Leaves		TCLH (r)				$1{,}25^{+}$	
Vitex rotundifolia	Twigs		VRTH (u)				$2{,}5^{+}$	
Canavalia rosea	Leaves	Methanol	CRLM (v)			5^{+}		
	Twigs		CRTM (v)			5^{+}		
	Seeds		CRSM (u)			$2{,}5^{+}$		
	Rinds		CRRM (r)			$2{,}5^{+}$	5^{+}	
Hibiscus tiliaceus	Leaves		HTLM (s)			$1{,}25^{+}$		
Ipomoea pescaprae	Twigs		IPTM (j)	$0{,}625^{+}$		$1{,}25^{+}$	$0{,}625^{+}$	$2{,}5^{+}$
	Leaves		IPLM (w)			10^{+}		
Melastoma malabathricum	Leaves	Methanol	MMLM (b)	0,625+	0,625+	0,625+	0,625+	0,625+
	Flowers		MMFM (d)	0,625+	0,625+	0,625++	0,625+	1,25+
	Twigs		MMTM (i)	0,625+	2,5+	0,625+	1,25+	
Pongamia pinnata	Leaves		PPLM (u)			2,5+		
	Twigs		PPTM (p)			1,25+		5+
	Seeds		PPSM (r)				0,625+	
	Rinds		PPRM (q)	2,5+			2,5+	
Rhodomyrtus tomentosa	Leaves		RTLM (e)	0,625+	0,625+	0,625+	1,25+	1,25+
Sonneratia alba	Leaves		SALM (e)	0,625+	0,625++	0,625++		0,625+
	Twigs		SATM (f)	0,625+	0,625+	0,625+		0,625+
Syzigium grande	Leaves		SGLM (d)	0,625+	1,25+	0,625++	1,25+	0,625+
	Twigs		SGTM (c)	1,25+	0,625+	0,625++	1,25+	1,25+
Terminalia catappa	Leaves		TCLM (a)	0,625++	0,625++	0,625++	0,625++	0,625+

*The values in (mg/mL) represent the minimum concentration of fraction to inhibit the growth of pneumonia bacteria listed in the table, the categories of the IZ value at that minimum concentration were described by + = weak activity (<10 mm), ++ = good activity (10-14.9 mm), +++ = strong activity (≥ 15mm). The different letters assigned in the table indicate significant differences among subsets of the inhibition zone means, resulting from the interaction between variables, as determined by the Duncan test ($p \leq 0.05$).

Based on Table 7, all fractions have different minimum inhibitory concentrations ranging from 5 mg/mL to 0.625 mg/mL. The highest result of MIC was 5 mg/mL for CRLH, CRSH, and CRRM against *S. aureus*, PTLH against *K. pneumoniae*, CRLM and CRTM against *P. aeruginosa*, and PPTM against *S. cohnii*. Table 7 also revealed that the majority of all parts from *M. malabathricum* (leaves, twigs, and flowers) extracted using methanol were able to inhibit all pneumonia bacteria at a minimum concentration of 0.625 mg/mL. However, its flower fraction exhibited inhibition against *S. cohnii* at a minimum concentration of 1.25 mg/mL. Its twig fraction inhibited *E. coli* at a minimum concentration of 2.5 mg/mL, inhibited *S. aureus* at a minimum concentration of 1.25 mg/mL, and showed no inhibition against *S. cohnii*. Various studies have also investigated the antibacterial activity of *M. melastoma's* leaves, but studies on other parts such as its twigs and flower are still lacking. The studies by Alwash *et al.,*[60] and Diris *et al.,*[61] revealed that methanol leave extract of this plant can inhibit

S. aureus, E. coli, and *P. aeruginosa* at varying concentrations. Purwanto[62] tested the methanol fraction of its leaves against *E. coli* and found that the minimum concentration of 1 mg/mL can inhibit the *E. coli*; this MIC result is higher than our MIC result, which is 0.625 mg/mL.

Meanwhile, for the hexane fraction of M. Malabtahricum, only its flower fraction was active, with MIC of 1.25 mg/mL against *S. aureus* and MIC of 2.5 mg/mL against *S. cohnii*. The study by Ropisah[63] stated that methanol and hexane fractions of *M. malabathricum's* leaves could inhibit *E. coli*. The study by Aslam *et al.,*[64] revealed that flowers and leaves of M. malabathrichum contain kaempferol, which includes flavonoid group compound and acts as an antibacterial agent.

Methanolic and hexane fractions of *S. grande* (leaves and twigs) have antibacterial activity with various minimum concentrations ranging from 1.25 to 0.625 mg/mL against different pneumonia bacteria. Producing a result similar to this study, the study by Ong *et al.,*[65] found that ethanolic fractions from leaves and stems of *S. Grande* were effective against *E. coli*, *S. aureus*, and *P. aeruginosa*. In addition, another study was done by sarvesan *et al.,*[66], who investigated the antibacterial activity of leaf essential oil of *S. grande* with a concentration range from 25 mg/mL to 5 mg/mL against pneumonia bacteria.

Moreover, the methanolic fraction from *T. catappa's* leaves can inhibit the growth of all pneumonia bacteria with the lowest concentration 0.625 mg/mL. The previous study by Mbengui *et al.,*[67] showed that methanolic extracts of *T. catappa's* barks also inhibited all those bacteria used in this study. Courtney and Cock[46], revealed that methanolic fraction of *T. catappa's* leaves can inhibit *K. pneumoniae* with MIC 2.85 mg/mL. However, the study by Balala *et al.,*[68], showed that the ethanolic of *T. catappa's* leaves could inhibit *S. aureus* with the concentration of the extract from 5mg/mL to 10 mg/mL.

Furthermore, Fig. 5 displays a graph depicting the inhibition zones (mm) of the 20 active plant fractions at the lowest concentration (0.625 mg/mL) tested against pneumonia bacteria. Based on Fig. 5, all fractions tested at the MIC value of 0.625 mg/mL demonstrated the ability to inhibit pneumonia-causing bacteria. Among them, the methanolic fraction derived from *T. catappa's* leaves (TCLM) exhibited the broadest spectrum of activity against the five pneumonia bacteria, resulting in the highest inhibition zone values: 11.17 mm against *E. coli*, 10.50 mm against *P. aeruginosa*, 10.25 mm against *S. aureus*, 10.17 mm against *K. pneumoniae*, and 9.67 mm against *S. cohnii*. However, the highest inhibition zone values observed at the minimum concentration were found in the hexane fraction of *R. tomentosa's* leaves against *S. aureus* (16.75 mm) and *S. cohnii* (14.17 mm).

Figure 5 further illustrates that among all the tested fractions at the minimum inhibitory concentration (0.625 mg/mL), *S. aureus* was the bacterium most effectively inhibited. In addition, Table 7 indicates that all concentrations of the plant fractions exhibited antibacterial activity against *S. aureus*. This bacterium proved to be the most susceptible species among all the *Pneumonia* bacteria tested, as indicated by the letter "a" in the Duncan test results with a p-value <0.05. *S. aureus* belongs to the category of *Gram-positive* bacteria, which are generally more susceptible compared to *Gram-negative* bacteria. These findings align with previous studies that have reported *Gram-negative* bacteria to be more resistant than *Gram-positive* bacteria[69,70].

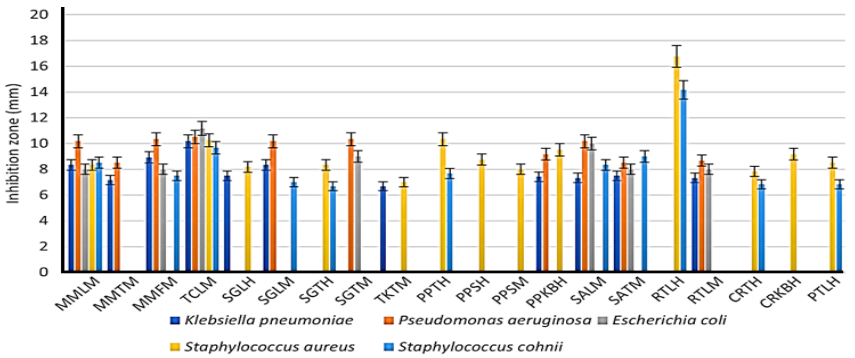

Fig. 5. Graphic of Inhibition zone (mm) of active plant fraction with the minimum inhibitory concentration (MIC) 0,625 mg/mL. y-axis is the inhibition zone (mm); x-axis are the fraction samples

CONCLUSION

The present study involving selected coastal plants revealed the presence of various phytochemicals such as flavonoids, alkaloids, terpenoids, phenols, tannins, quinones, saponins, and glycosides. The methanolic and hexane fractions of the plants demonstrated antibacterial effects, with methanolic fractions exhibiting a broader spectrum of action by inhibiting the growth of all pneumonia bacteria compared to hexane fractions. However, the hexane fraction of *R. tomentosa* showed the highest inhibition zone against *S. aureus* (21.67±1.25) and *S. cohnii* (22.67±0.94) with MIC 0.625 mg/mL. CRTH, CRRH, PTLH, PPTH, PPSH, PPRH, RTLH, SGLH, SGTH, IPTM, MMLM, MMTM, MMFM, PPSM, RTLM, SALM, SATM, SGLM, SGTM, and TCLM fractions were able to inhibit *Pneumonia* bacteria at a minimum concentration of 0.625 mg/mL. Among the tested bacteria, *S. aureus* was found to be the most susceptible to the inhibitory effects of the fractions. The presence of phytochemical compounds, particularly flavonoids, in the fractions may account for their antibacterial activity. This study provides valuable information on the potential use of herbal medicine derived from coastal plants for the prevention and treatment of bacterial *Pneumonia.* Further research is warranted to isolate and purify the active compounds responsible for the observed antibacterial activity.

ACKNOWLEDGEMENT

The authors would like to extend acknowledgement to Universiti Malaysia Terengganu (UMT), Terengganu, Malaysia for funding (International Partnership Research Grant), with vot number: 55319 and reference number: UMT/PPPI/2-2/2/23 (64).

Conflict of interest

There are no conflicts of interest in this study.

REFERENCES

1. Woodruff, P. G.; Van, D. B. M.; Boucher, R. C.; Brightling, C.; Burchard, E. G.; Christenson, S. A.; Han, M. K.; Holtzman, M. J.; Kraft, M.; Lynch, D. A.; Martinez, F. D.; Reddel, H. K.; Sin, D. D.; Washko, G. R.; Wenzel, S. E.; Punturieri, A.; Freemer, M. M.; Wise, R. A. American Thoracic Society/National Heart, Lung, and Blood Institute Asthma-Chronic Obstructive Pulmonary Disease Overlap Workshop Report. *Am. J. Respir. Crit. Care Med.*, **2017**, *196*(3), 375–381. https://doi.org/10.1164/rccm.201705-0973WS.
2. Espada-Sánchez, M.; Sáenz de Santa María, R.; Martín-Astorga, M. del C.; Lebrón-Martín, C.; Delgado, M. J.; Eguiluz-Gracia, I.; Rondón, C.; Mayorga, C.; Torres, M. J.; Aranda, C. J.; Cañas, J. A. Diagnosis and Treatment in Asthma and Allergic Rhinitis: Past, Present, and Future. *Appl. Sci.*, **2023**, *13*(3). https://doi.org/10.3390/app13031273.
3. Geddes, D. *The History of Respiratory Disease Management. Medicine (Baltimore).*, **2016**, *44*(6), 393–397.
4. Macias, A. E.; McElhaney, J. E.; Chaves, S. S.; Nealon, J.; Nunes, M. C.; Samson, S. I.; Seet, B. T.; Weinke, T.; Yu, H. The Disease Burden of Influenza beyond Respiratory Illness. *Vaccine.,* **2021**, *39*, A6–A14. https://doi.org/10.1016/j.vaccine.2020.09.048.
5. Krishna, R.; Chapman, K.; Ullah, S. Idiopathic Pulmonary Fibrosis; StatPearls Publishing, Treasure Island (FL)., **2023**.
6. Torres, A.; Cilloniz, C.; Niederman, M. S.; Menéndez, R.; Chalmers, J. D.; Wunderink, R. G.; van der Poll, T. Pneumonia. *Nat. Rev. Dis. Prim.*, **2021**, *7*(1). https://doi.org/10.1038/s41572-021-00259-0.
7. CDC. Deaths and Mortality. CDC/National Center for Health Statistics. https://www.cdc.gov/nchs/fastats/deaths.htm.
8. Srikacha, N.; Ratananikom, K. Antibacterial Activity of Plant Extracts in Different Solvents against Pathogenic Bacteria: An *in Vitro Experiment. J. Acute Dis.*, **2020**, *9*(5), 223. https://doi.org/10.4103/2221-6189.291288.
9. Cleaveland, S.; Laurenson, M. K.; Taylor, L. H. Diseases of Humans and Their Domestic Mammals: Pathogen Characteristics, Host Range and the Risk of Emergence. *Philos. Trans. R. Soc. B Biol. Sci.*, **2001**, *356* (1411), 991–999. https://doi.org/10.1098/rstb.2001.0889.
10. Taylor, L. H.; Latham, S. M.; Woolhouse, M. E. *J. Risk Factors for Human Disease Emergence. Philos. Trans. R. Soc. B Biol. Sci.*, **2001**, *356*(1411), 983–989. https://doi.org/10.1098/rstb.2001.0888.

11. Woolhouse, M. E.; Gowtage-Sequeria, S. Host Range and Emerging and Reemerging Pathogens; **2005**. www.cdc.gov/eid.
12. Huang, Y.; Jiao, Y.; Zhang, J.; Xu, J.; Cheng, Q.; Li, Y.; Liang, S.; Li, H.; Gong, J.; Zhu, Y.; Song, L.; Rong, Z.; Liu, B.; Jie, Z.; Sun, S.; Li, P.; Wang, G.; Qu, J. Microbial Etiology and Prognostic Factors of Ventilator-Associated Pneumonia: A Multicenter Retrospective Study in Shanghai. *Clin. Infect. Dis.*, **2018**, *67*(suppl_2), S146–S152. https://doi.org/10.1093/cid/ciy686.
13. Ruiz-Bastián, M.; Falces-Romero, I.; Ramos-Ramos, J. C.; de Pablos, M.; García-Rodríguez, J. Bacterial Co-Infections in COVID-19 Pneumonia in a Tertiary Care Hospital: Surfing the First Wave. *Diagn. Microbiol. Infect. Dis.*, **2021**, *101*(3). https://doi.org/10.1016/j.diagmicrobio.2021.115477.
14. Scott, H.; Zahra, A.; Fernandes, R.; Fries, B. C.; Thode, H. C.; Singer, A. J. Bacterial Infections and Death among Patients with Covid-19 versus Non Covid-19 Patients with Pneumonia. *Am. J. Emerg. Med.*, **2022**, *51*, 1–5. https://doi.org/10.1016/j.ajem.2021.09.040.
15. Poovieng, J.; Sakboonyarat, B.; Nasomsong, W. Bacterial Etiology and Mortality Rate in Community-Acquired Pneumonia, Healthcare-Associated Pneumonia and Hospital-Acquired Pneumonia in Thai University Hospital. *Sci. Rep.*, **2022**, *12*(1). https://doi.org/10.1038/s41598-022-12904-z.
16. Ko, W.-C.; Paterson, D. L.; Sagnimeni, A. J.; Hansen, D. S.; Gottberg, A.; Mohapatra, S.; Jose, #; Casellas, M.; Goossens, H.; Mulazimoglu, L.; Trenholme, G.; Klugman, K. P.; Mccormack, J. G.; Yu, V. L. Community-Acquired Klebsiella Pneumoniae Bacteremia: Global Differences in Clinical Patterns., **2002**, 8.
17. Cilloniz, C.; Martin-Loeches, I.; Garcia-Vidal, C.; Jose, A. S.; Torres, A. Microbial Etiology of Pneumonia: Epidemiology, Diagnosis and Resistance Patterns. *Int. J. Mol. Sci.*, **2016**, *17* (12). https://doi.org/10.3390/ijms17122120.
18. John, T. M.; Deshpande, A.; Brizendine, K.; Yu, P. C.; Rothberg, M. B. Epidemiology and Outcomes of Community-Acquired *Escherichia coli Pneumonia*. *Open Forum Infect. Dis.*, **2022**, *9*(1). https://doi.org/10.1093/ofid/ofab597.
19. Fair, R. J.; Tor, Y. Antibiotics and Bacterial Resistance in the 21st Century. *Perspect. Medicin. Chem.*, **2014**, *6*, 25–64. https://doi.org/10.4137/PMC.S14459.
20. Shinwari, Z. K.; Khan, I.; Naz, S.; Hussain, A. Assessment of Antibacterial Activity of Three Plants Used in Pakistan to Cure Respiratory Diseases. *African J. Biotechnol.*, **2009**, *8*(24), 7082–7086.
21. Haider, M.; Zhong, L. Ethno-Medicinal Uses of Plants from District Bahawalpur, Pakistan. *Curr Res J Biol Sci.,* **2014**, *6*, 183–190.
22. Ishtiaq, M.; Mahmood, A.; Maqbool, M. No Title Indigenous Knowledge of Medicinal Plants from Sudhanoti District (AJK), *Pakistan. J. Ethnopharmacol.,* **2015**, *168*, 201–207.
23. Sher, H.; Hussain, F. Ethnobotanical Evaluation of Some Plant Resources in Northern Part of *Pakistan. African J. Biotechnol.*, **2009**, *8*(17), 4066–4076.
24. Mahmood, A.; Mahmood, A.; Naveed, I.; Memon, M, M.; Bux, H.; Majeed, M, Y.; Mujtaba, G.; Mumtaz, M, S. Indigenous Medicinal Knowledge of Common Plants Used by Local People of Hattian Bala District, Azad Jammu and Kashmir (AJK), *Pakistan. J. Med. Plants.,* **2011**, *5*, 5517–5521.
25. Pranskuniene, Z.; Balciunaite, R.; Simaitiene, Z.; Bernatoniene, J. Herbal Medicine Uses for Respiratory System Disorders and Possible Trends in New Herbal Medicinal Recipes during COVID-19 in Pasvalys District , Lithuania. *Int. J. Environ. Res. Public Heal. Public Heal.*, **2022**, *19*(8905), 1–13.
26. WHO. Catalysing Ancient Wisdom and Modern Science for The Health of People and The Planet. World Health Organization. https://www.who.int/initiatives/who-global-centre-for-traditional-medicine (accessed **2023**-01-21).
27. Singh, P.; Shukla, R.; Kumar, A.; Prakash, B.; Singh, S.; Dubey, N, K. Efect of Citrus Reticulata and *Cymbopogon Citratus* Essential Oils on Aspergillus Favus Growth and Afatoxin Production on Asparagus Racemosus. *Mycopathologia.,* **2010**, *170*(3), 195–202.
28. Ravikumar, S.; Syed Ali, M.; Ramu, A.; Ferosekhan, M. Antibacterial Activity of

Chosen Mangrove Plants Against Bacterial Specified Pathogens. *World Appl. Sci. J.*, **2011**, *14*(8), 1198–1202.
29. Prabowo, Y.; Irawan, H.; Dosen, A. P.; Kelautan, I.; Umrah, F. Extraction of Secondary Metabolites Compound in Mangrove Xylocarpus Granatum Leaves with Different Solvents; **2014**.
30. Genilar, L. A.; Kurniawaty, E.; Mokhtar, R. A. M.; Audah, K. A. Mangroves and Their Medicinal Benefit: *A Mini Review. Ann. Rom. Soc. Cell Biol.*, **2021**, *25*(4), 695–709.
31. Vinoth, R.; Kumaravel, S.; Ranganathan, R. Therapeutic and Traditional Uses of Mangrove Plants. *J. Drug Deliv. Ther.*, **2019**, *9*(4-s), 849–854. https://doi.org/10.22270/jddt.v9i4-s.3457.
32. Andriani, Y.; Hanifah, W.; Kholieqoh, A.; Abdul Majid, F.; Hermansyah, H.; Amir, H.; Muhammad, T. T. Antibacterial Activity of Hexane and Methanol Fractions of Some Selected Plants against *Klebsiella Pneumoniae*. *J. Adv. Pharm. Technol. Res.* **2023**, *14*(3), 220. https://doi.org/10.4103/JAPTR.JAPTR_183_23.
33. Harborne, J. B. Methods of Plant Analysis. In Phytochemical Methods.; Springer: Dordrecht., **1984**.
34. Sofowara, A. A Medicinal Plants and Traditional Medicine in Africa; Spectrum Books Ltd: Ibadam-Nigeria, **1993**.
35. Trease, G. E.; Evans, W. C. Pharmacognosy, 11th ed.; Bailliere Tindall: London, **1989**.
36. Magaldi, S.; Mata-Essayag, S.; Hartung De Capriles, C.; Perez, C.; Colella, M. T.; Olaizola, C.; Ontiveros, Y. Well Diffusion for Antifungal Susceptibility Testing. *Int. J. Infect. Dis.*, **2004**, *8*(1), 39–45. https://doi.org/10.1016/j.ijid.2003.03.002.
37. Andriani, Y.; Wahid, E.; Mohamad, H.; Yosie, A.; Effendy, M. A. W.; Sifzizul, T. M. T.; Habsah, M. Antibacterial, Radical-Scavenging Activities and Cytotoxicity Properties of Phaleria Macrocarpa (Scheff.) Boerl. Leaves in HepG2 Cell Lines. *IJPSR.,* **2011**, *2*(7), 1700–1706.
38. Ahmad, R.; Ali, A. M.; Israf, D. A.; Ismail, N. H.; Shaari, K.; Lajis, N. H. Antioxidant, Radical-Scavenging, Anti-Inflammatory, Cytotoxic and Antibacterial Activities of Methanolic Extracts of Some Hedyotis Species. *Life Sci.*, **2005**, *76* (17), 1953–1964. https://doi.org/10.1016/j.lfs.2004.08.039.
39. Mohamad, H.; Rashid, Z. M.; Shaari, K.; Latip, J.; Lajis, M. N. H.; Ali, A. M. Antibacterial and DPPH Free Radical-Scavenging Activities of Methanolic Extracts of Aaptos Sp. (Marine Sponges). *Pertanika J. Trop. Agric. Sci.*, **2009**, *32*(1), 43–50.
40. Ganjewala, D.; Gupta, A. K. Study on Phytochemical Composition, Antibacterial and Antioxidant Properties of Different Parts of Alstonia Scholaris Linn., **2013**, *3*(2), 379–384.
41. Shafodino, F. S.; Lusilao, J. M.; Mwapagha, L. M. Phytochemical Characterization and Antimicrobial Activity of Nigella Sativa Seeds. *PLoS One* **2022**, *17*(8 August). https://doi.org/10.1371/journal.pone.0272457.
42. Mahmud, M.S.-A.-. Antimicrobial Activity of Methanol, n-Hexane and Dichloromethane Extract of Portulaca Oleracea. *Adv. Pharmacol. Clin. Trials.,* **2018**, *3*(1), 1–4. https://doi.org/10.23880/apct-16000122.
43. Martin, W.; Kinyanjui, J. C. Antimicrobial Activity of Solvent Extracts from the Leaves of Tarchonanthus Camphoratus (Asteraceae). ~ 123 ~ *J. Pharmacogn. Phytochem.*, **2014**, *3*(1), 123–127.
44. Al Mamari, Hamad, H. Biochemistry. In Organic Chemistry; Intech Open: New York, **2022**, 452. https://doi.org/10.5772/intechopen.94825.
45. Miklasińska-Majdanik, M.; Kępa, M.; Wojtyczka, R. D.; Idzik, D.; Wąsik, T. J. Phenolic Compounds Diminish Antibiotic Resistance of Staphylococcus Aureus Clinical Strains. *Int. J. Environ. Res. Public Health.,* **2018**, *15*(10). https://doi.org/10.3390/ijerph15102321.
46. Courtney, R.; Cock, I. E. Comparison of the Antibacterial Activity of Australian Terminalia Spp. Extracts against Klebsiella Pneumoniae: A Potential Treatment for Ankylosing Spondylitis. *Inflammopharmacology.*, **2022**, *30*(1), 207–223. https://doi.org/10.1007/s10787-021-00914-8.
47. Borges, A.; José, H.; Homem, V.; Simões, M. Comparison of Techniques and Solvents on the Antimicrobial and Antioxidant Potential of Extracts from Acacia Dealbata and Olea Europaea. *Antibiotics.,* **2020**, *9*(2). https://doi.org/10.3390/antibiotics9020048.

48. Noumedem, J. A. K.; Mihasan, M.; Lacmata, S. T.; Stefan, M.; Kuiate, J. R.; Kuete, V. Antibacterial Activities of the Methanol Extracts of Ten Cameroonian Vegetables against *Gram-negative* Multidrug-Resistant Bacteria., **2013**.
49. Idris, M.; Sukandar, E. R.; Purnomo, A. S.; Martak, F.; Fatmawati, S. Antidiabetic, Cytotoxic and Antioxidant Activities of Rhodomyrtus Tomentosa Leaf Extracts. *RSC Adv.*, **2022**, *12*(39), 25697–25710. https://doi.org/10.1039/d2ra03944c.
50. Markham, P. N.; Westhaus, E.; Klyachko, K.; Johnson, M. E. *Multiple Novel Inhibitors of the NorA Multidrug Transporter of Staphylococcus Aureus.*, **1999**, *43*(10), 2404–2408.
51. Khan, I. A.; Mirza, Z. M.; Kumar, A.; Verma, V.; Qazi, G. N. Piperine, a *Phytochemical Potentiator of Ciprofloxacin against Staphylococcus aureus.*, **2006**, *50*(2), 810–812. https://doi.org/10.1128/AAC.50.2.810.
52. Sinulingga, S. E.; Hasibuan, P. A. Z.; Suryanto, D. Antibacterial Activity of Karamunting (Rhodomyrtus Tomentosa (Aiton) Hassk) Leaf Extract and Fractions. *Asian J. Pharm. Clin. Res.*, **2018**, *11*(3), 163–165. https://doi.org/10.22159/ajpcr.2018.v11i3.23505.
53. Kamarudin, E.; Zainol, H.; Anuar, T. S.; Hussain, R. Rhodomyrtus Tomentosa Leaves Phytochemistry and Biological Activities: An Update Review. *Malaysian J. Med. Heal. Sci.*, **2021**, *17*, 334–343.
54. Mordmuang, A.; Brouillette, E.; Voravuthikunchai, S. P.; Malouin, F. Evaluation of a Rhodomyrtus Tomentosa Ethanolic Extract for Its Therapeutic Potential on *Staphylococcus aureus* Infections Using in Vitro and in Vivo Models of Mastitis. *Vet. Res.*, **2019**, *50*(1), 1–11. https://doi.org/10.1186/s13567-019-0664-9.
55. Nair, R.; Chanda, S. Antimicrobial Activity of Terminalia Catappa, Manilkara Zapota and Piper Betel Leaf Extract. *Indian J. Pharm. Sci.*, **2008**, *70*(3), 390–393.
56. Akhtar, B.; Muhammad, F.; Aslam, B.; Kashif, M.; Sharif, A. International Journal of Biological Macromolecules Pharmacokinetic pro Fi Le of Chitosan Modi Fi Ed Poly Lactic Co-Glycolic Acid Biodegradable Nanoparticles Following Oral Delivery of Gentamicin in Rabbits. *Int. J. Biol. Macromol.*, **2020**, *164*, 1493–1500. https://doi.org/10.1016/j.ijbiomac. 2020.07.206.
57. Altucci, P.; Sapio, U.; Esposito, E. Gentamicin: Antibacterial Activity *In vitro* and Clinical Studies. *Chemotherapia (Basel).*, **1965**, *10* (66), 312–320.
58. Henrique, M.; Melo, F.; Mirian, I.; Raimunda, M.; Silva, C.; Monteiro-neto, V.; Aliança, A. Antimicrobial Potential of Streptomyces Ansochromogenes (PB 3) Isolated From a Plant Native to the Amazon Against *Pseudomonas Aeruginosa.*, **2020**, *11* (October), 1–11. https://doi.org/10.3389/fmicb.2020.574693.
59. Rekha, S. R.; Kulandhaivel, M.; Hridhya, K. V. Antibacterial Efficacy and Minimum Inhibitory Concentrations of Medicinal Plants against Wound Pathogens. *Biomed. Pharmacol. J.*, **2018**, *11*(1), 237–246. https://doi.org/10.13005/bpj/1368.
60. Alwash, M. S. A.; Ibrahim, N.; Yaacob, W. A.; Din, L. Bin. Antibacterial, Antioxidant and Cytotoxicity Properties of Traditionally Used Melastoma *Malabathricum* Linn Leaves. *Adv. J. Food Sci. Technol.*, **2014**, *6*(1), 6–12. https://doi.org/10.19026/ajfst.6.3022.
61. Diris, M. N.; Basri, A. M.; Metali, F.; Ahmad, N.; Taha, H. Phytochemicals and Antimicrobial Activities of *Melastoma malabathricum* and *Melastoma Beccarianum* Leaf Crude Extracts. *Res. J. Phytochem.*, **2016**, *11*(1), 35–41. https://doi.org/10.3923/rjphyto.2017.35.41.
62. Purwanto, S. Uji Aktivitas Antibakteri Fraksi Aktif Ekstrak Daun Senggani (*Melastoma malabathricum* L) Terhadap *Escherichia coli*. *J. Keperawatan Sriwij.*, **2015**, *2*(2), 84–92.
63. Ropisah, M.; Wan Nur Aqilah, W. M. S.; Nurul Haziqah, Y.; Alsya Haneeza, M. S.; Mohd Syafid, A.; Sheikh Ahmad Izaddin, S. M. G.; Shanthi, A. Phytochemical Analysis and Biological Activities of Melastoma Malabathricum and Dissochaeta Gracilis. *ASM Sci. J.*, **2020**, *13*(Specialissue6), 1–6.
64. Aslam, M.; Ahmad, M. S.; Ahmad, M. A. An Up-to-Date Review on Phytochemical Constituents and Pharmacological Activities of *Melastoma Malabathricum. Int. J. Pharm. Pharm. Sci.*, **2017**, *8*(5), 76–91.

65. Ong, B.; Chua, K.; Norulaiman, Y.; Nuziah, H.; Azah, Nor, M. Phytochemical Screening and Anti-Food-Borne Bacterial Properties of Syzigium Grande Ethanolic Extract. In Proceedings of The Conference On Forestry and Forest Products Research 2013; Rahim, S., Lim, H., Farhana, Huda, M., Mahmudin, S., Eds.; Forest Research Institute Malaysia: Kuala Lumpur, **2013**, 302–307.
66. Sarvesan, R.; Eganathan, P.; Saranya, J.; Sujanapal, P. Chemical Composition and Antimicrobial activity of Leaf *Essential oil* of Syzygium Grande (Wight) Walp. *J. Essent. oil-Bearing Plants.,* **2015**, *18*(3), 642–646. https://doi.org/10.1080/0972060X.2014.958572.
67. Mbengui, R.; Guessennd, N.; M'boh, G.; Golly, J.; Okou, C.; Nguessan, J.; Dosso, M.; Djaman, J. Phytochemical Screening and Study of Comparative Antibacterial Activity of Aqueous and Alcoholic Extracts of the Leaves and Barks of Terminalia Catappa on Multiresistant Strains. *J. Appl. Biosci.*, **2013**, *66*(0), 5040. https://doi.org/10.4314/jab.v66i0.95000.
68. Balala, L.; Francis, E.; Salares, O.; Balala, L. M. Phytochemical Screening and Antimicrobial Activity of Terminalia Catappa L. Leaf Extract Against Potential Pathogens of Animals. *J. Sci. Eng. Technol.*, **2018**, *6*(November), 15–26.
69. Mujeeb, F.; Bajpai, P.; Pathak, N. Phytochemical Evaluation, Antimicrobial Activity, and Determination of Bioactive Components from Leaves of Aegle Marmelos. *Biomed Res. Int.,* **2014**, *2014*. https://doi.org/10.1155/2014/497606.
70. Chanda, S.; Rakholiya, K.; Nair, R. Antimicrobial Activity of *Terminalia Catappa* L. Leaf Extracts against Some Clinically Important Pathogenic Microbial Strains. *Chin. Med.*, **2011**, *02*(04), 171–177. https://doi.org/10.4236/cm.2011.24027.