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Bactericidal Activity of Extracts and Major Fractions of Endophyte Metabolites of Fungus Cladosporium Velox against Pneumonia and Diarrhea Pathogens

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The resistance of bacteria to drugs is a problem in the treatment of diarrhea and pneumonia. A new source of antibiotic with high sensitivity and potential to develop as new drugs is required. This study was conducted to determine the fungus of Cladosporium and test the crude extract and the major fraction on the bacteria that cause pneumoniae and the diarrhea. Firstly, the fungus was determined its species with molecular identification. The method for bacterial sensitivity tests was carried out by disc diffusion; MIC and MBC analysis using liquid dilution; and the data were statistically analyzed using One Way ANOVA. The species was identification as *Cladosporium velox* shows the inhibition ranging from 29.9-38.84 mm; all MBC/MIC values <2 indicate the extract can kill all the bacteria tested. These results indicate that the antibacterial metabolites of C. velox are bactericidal and have the potential to be developed as candidates for anti-pneumonia and anti-diarrheal drugs.

Key words: Bactericidal, *Cladosporium velox*, diarrhea, pneumonia.

1. INTRODUCTION

Pneumonia is the main cause of death due to infection in children under five, which kills around 2,400 children every day (WHO, 2017). This data is supported by a report from UNICEF which states that of the 5.6 million deaths under five, nearly 16% are caused by infectious pneumonia. In tropical areas such as Indonesia, these bacteria have an indication of an infection rate in humans of 15% -30%. The main cause of pneumonia is infection by the bacteria *Klebsiella pneumoniae* and *Streptococcus pneumonia*. In particular, *S. pneumoniae* causes 70-90% of pneumonia in children under five and more than 40% in adults (EUCAST, 2018; Valencia et al., 2016).

Diarrheal disease is often shown with more stool consistency symptoms than usual, followed by a more frequent frequency of the day. Bacteria that cause diarrhea include *E. coli* and *Salmonella*, which are bacterial contaminants in food and beverages. Approximately 1.7 billion cases of diarrhea each year occur in children with a mortality rate of around 525,000 in children under five (Halim et al., 2017).

Pneumonia caused by bacteria, viruses, or fungi that would make inflammation in the lung parenchyma, starting from the alveoli to the bronchi or bronchioles which causes the exchange of oxygen and carbon dioxide disrupted (WHO, 2017). This disease could spread from individuals which would show symptoms in pathological stage marked by the alveoli containing a mixture of inflammatory exudate, bacteria, and white blood cells (Walker and Whittlesea, 2012).

Diarrhea and pneumonia are still a big challenge in health because several types of microorganisms that cause these diseases are starting resistant to antibiotics such as ciprofloxacin (Puspitarini et al., 2019). The high mortality rate caused by diarrhea and pneumonia and the urge to find another antibiotics alternative to find the effective anti-diarrheal and anti-pneumonic. Several studies on anti-diarrhea and anti-pneumonia obtained compounds from various sources, namely: medicinal plant *Combretum dolichopetalum* (Nwuke, 2020), lemon fruit extract, endophytic fungi *Fusarium* and *Colletrichum* (Ekawati and Darmanto, 2015). Several studies have shown that looking for antibacterial extracts for diarrhea and pneumonia by utilizing microorganisms because it is more efficient and easier to propagate with an appropriate procedure.

Many studies that have reported the scope of interest in the study of new drugs of various antibiotic resistance by bacterial pneumonia and diarrhea require new drug alternatives sourced from metabolic extracts of endophyte microorganisms with high antibacterial activity. Studies on the antibacterial activity of *Cladosporium* spp. showed potential against the following bacteria: *E. coli* (Hoepfner et al., 2012; Hulikere M & Joshi, 2017; Khan et al., 2016; Mahadevamurthy et al., 2016; Singh et al., 1994; Sugijanto & Dorra, 2016), *S. aureus (Khiralla et al., 2016)*, *S. epidermis, B. subtilis (Hulikere M & Joshi, 2017)*, *B. megaterium, B. stearothermophilus (Hoepfner et al., 2012)*. Crude extract of *C. oxysporum (Hartanti et al., 2016)* showed antibacterial sensitivity against *E. coli* ATCC 8739 and *S. aureus* ATCC 6538 (Hartanti et al., 2016; Valencia et al., 2016). Studies on antibacterial pneumonia such as *K. pneumoniae* and *S. pneumoniae*

have been reported using metabolites produced by *Penicillium sp (Rossiana et al., 2017)*; *Thymbra spicata (Deshmukh et al., 2015; Hartanti et al., 2016)*; *Alternaria sp (Deshmukh et al., 2015)*. Based on this information, although diarrhea-causing bacteria have been tested with metabolites from *Cladopsorium* spp, this study is the first to test the sensitivity of pneumonia bacteria using extracts from *Cladosporium (Talha, 2020)*.

On previous research, shown the active compounds from the fermented extract of *Cladosporium* sp (ZHAO & TALHA, 2021). Which is an endophytic microfungi Sargassum cineureum has been carried out by Examinati, (2018). The crude extract has high cytotoxic properties with the IC50 value category being very active as an anticancer against breast cancer cell lines MCF-7, HeLa, and DU-145 cell lines (Wulandari et al., 2018). The purification results of the metabolite compounds from the genus *Cladosporium* indicated the presence of major extracts which were thought to be cladosporin derivatives belonging to the polyketide group. The cladosporin have a broad spectrum of sensitivity as antibacterial (Silber et al., 2014).

To complete the classification of the genus *Cladosorium* and evaluate its antimicrobial potential, the aim of this study was to determine the genus species and evaluate the sensitivity of the main extracts and fungal fractions to bacteria that cause diarrhea and pneumonia.

2. RESEARCH METHODOLOGY

Isolate *Cladosporium* sp. EN-S01, is a collection from the Microbiology Laboratory of the Department of Biology, Padjadjaran University, Indonesia. The standard bacteria tested in this study were: *Klebsiella pneumoniae* ATCC 700603, *Streptococcus pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, and *Salmonella typhimurium* ATCC 49416 which is a collection of Rumah Sakit Pendidikan (RSP), Faculty of Medicine, Padjadjaran University, Indonesia. Growth media for bacteria and fungi used: Müler Hinton Agar (MHA) powder [OXOID], Müler Hinton Broth (MHB), PDA (Potato Dextrose Agar), PDB (Potato Dextrose Broth), and yeast extract [OXOID].

2.1 Molecular Identification and Phylogenetic Analysis

The fungus culture was grown on Potato Dextrose Agar media (PDA) for 1 week and then grown on Potato Dextrose Broth media (PDB) for 3 days. Fungal biomass was extracted by DNA by using nucleon reagent PHYTOpure (Amersan LIFE SCIENCE). PCR amplification of ITS using primary ITS 4: 5'-TCC TCC GCT TCT TGA TAT GC-3' and primary ITS 5: 5'-GGA AAA AGT AAC AAG G-3' 5. PCR purification was carried out by the PEG 6 precipitation method of continued sorting. The sequencing results were re-purified by the ethanol purification method. The nitrogen base sequence was recognized by an automated DNA sequencer (ABO PRISM 3130 Genetic Analyzer Applied Bio system). The trimming and assembling process was carried out using the Chromas Pro program then using BLAST with genomic data from the DDBJ/DNA Bank of Japan Data (http://blast.ddbj.nig.ac.ip) or NCBI/National Center for Biotechnology Information (http://ncbi.nlm.nih.gov/BLAST/) to determine the most molecularly homologous species.

The evolution analysis was concluded by using the neighbord joining tree method. Tree #1 of 6 parsimony trees (length = 231) is shown. A consistency index of 0.982684 (0.958333), a retention index of 0.956044 (0.956044), and a composite index of 0.939489 (0.916209) for all parsimony-informative sites and sites (in brackets). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with level 0 searching where the initial tree was obtained by adding random sequences (10 replications) (Yang & Talha, 2021). This analysis involves 10 nucleotide sequences. The codon position entered is 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data are eliminated. There are a total of 305 positions in the final dataset. Evolutionary analysis carried out in MEGA7 (Singh et al., 1994).

3. Fermentation, Extraction and Isolation of Secondary Metabolites

Fermentation to produce metabolites was carried out with reference to (Wulandari et al., 2018). The suspension of fungal culture was added to the physiological solution as much as 90 mL. Potato Dextroxe Yeast (PDY) liquid media was used as a fermentation medium which added a starter suspension and incubated in a shaker (150 rpm) for 4 x 24 hours.

The crude extract and ethyl acetate fraction were prepared based on the method from Examinati et al. (2018) by macerating the broth from the culture using ethyl acetate (1: 1) solvent. The filtrate was concentrated using a vacuum rotary evaporator at a temperature of 20-40ºC.

The fractionation of the metabolites was carried out by open column chromatography of silica gel G60. The major fraction was then analyzed using thin layer chromatography of GF254 silica plates and compound detection using 10% sulfuric acid staining in ethanol which was then heated with a UV strain detector lamp at λ 254 nm and 365 nm. The simplest staining is used as the target fraction which is then purified again by column chromatography technique to obtain the purity of the fraction. All extracts were stored in glass flasks, stored in the refrigerator until used.

3.1 Preparation of the Inoculum and Conditions for Microbial Growth

The pathogen bacterial strains were maintained on agar slant at 4°C and subcultured on fresh agar plates and on growth medium 24 hours before the antibacterial test. Activation of the pathogen bacteria was carried out on Mueller Hinton Agar (MHA), while for the determination of MIC and MBC the inoculum was prepared on Mueller Hinton Broth (MHB) medium and nutrient agar (Hi-Media) respectively.

3.2 Antibacterial Activity Test

The crude extract samples and column fractions were tested for their antibacterial activity by the disc diffusion method. 17,18 Samples were assayed at 100 mg/mL, 80 mg/mL, 60 mg/mL, 40 mg/mL, and 10 mg/mL. Zone of inhibition was compared with streptomycin (10 µg) and cefotaxime (30 µg) for antibiotic pneumoniae, whereas control antibiotics for diarrhea used cefotriaxone (30 µg). The effect of the solvent as a negative control using 2% DMSO solvent.

MIC and MBC crude extracts and column fractions were assessed using the broth microdilution method. Bacterial strains were inoculated into Mueller Hinton (MH) broth and allowed to grow overnight in an incubator for 24 hours with a stable temperature of 37°C, bacterial growth was compared to a standard of 0.5 McFarland. The crude extract and column fraction were added by dilution into each eagle tube containing MHB media. The bacteriostatic effect was determined by the MIC value observed when the turbidity in the liquid medium at each sample concentration could inhibit the growth of the standard test bacteria. MIC value was determined as the lowest growth inhibiting extract concentration (CLSI. 2012). The experiment was replicated three times.

The bactericidal effect is determined by MBC which can kill 99.9% of standard test bacteria. All tubes at the MIC stage were previously confirmed by sub-culture to the solid surface of the MHA medium using the swab method. The test was carried out with duplicates. The lowest growth-inducing concentration after subculture was taken as the minimum bactericidal concentration (MBC) value. All MIC tests were repeated in triplicate.

3.3 Statistical Analysis

Data were analyzed using the statistical package (SPSS, version 12.0). Experimental results are expressed as mean ± Standard Deviation (SD). Group comparisons were carried out by One Way ANOVA followed by the Post Hoc Waller-Duncan test.

4. RESULT AND DISCUSSION

4.1 Species Determination

In this study, isolates of *Cladosporium sp.* which isolated as endophyte microfungi from macroalgae *Sargassum cineureum* were determined its species based on the closest similarity by nucleotide analysis (Figure 1).

The results obtained indicate that the taxon fungus *Cladosporium* sp. The closest to homology with the DNA sequence obtained was *Cladosporium velox* strain cbs 119417 from MATERIAL TYPE with 92% homology. *C. velox* has not been widely studied, but other isolates that have been found have been found in hypersaline areas and in plant material (Zalar et al., 2007); In addition, the potential for insecticides against *Spodoptera litura* by partially purified fractions obtained from *C. velox* as an endophyte was isolated from *Tinospora cordifolia* (Singh et al., 1994).

The phylogenetic tree with the neighbor-joining tree method formed 3 in-group groups and 1 outgroup group. The in-group group is divided into one large clade, namely clade I and two smaller clades, namely clad II and clad III. Class I consists of *Penicillium digitatum*, P. *vanluykii*, *Cladosporium velox*, C. *tenuissimum*, and C. *cladosporioides*. Clad II consists of *Cladosporium colocasiae* and C. *oxysporum*, Clad III consists of *Cladosporium herbarum* and C. *bruhnei.* The outgroup group in clade IV is *Cladosporium sphaerospermum* which is needed in the construction of phylogenetic trees to provide character polarization or characteristics, namely apomorphic and plesiomorphic characters. *C. velox* is closely related to P. *digitatum* and P. *vanluykii*.

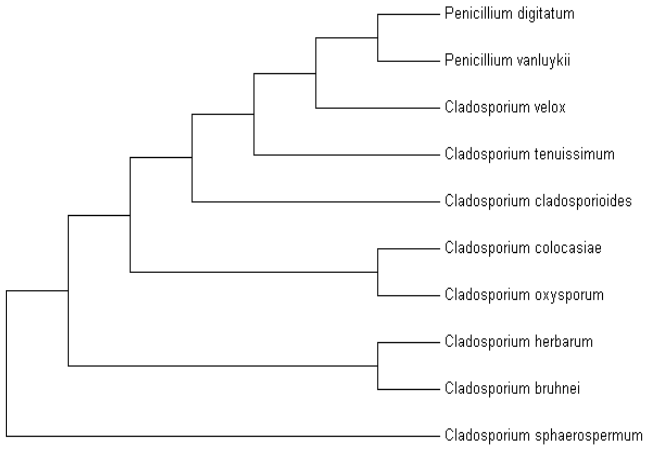

Figure 1. A neighbor-joining tree based on the nucleotide sequence of Cladosporium and other similar species selected from GenBank

4.2 Antibacterial Activity Test

The inhibition zone of the mean diameter of crude extract samples and the fraction of the antibacterial activity column of *C. velox* tested to detect good antibacterial activity against pneumoniae and diarrhea bacteria tested is tabulated in Table 1. The zone of mean inhibition of crude extract antibacterial activity and its fraction against concentration. Based on the mean value of the inhibition zone, all concentration variations ranged from 19.72 – 38.84 mm, so the crude extract and fraction column showed antimicrobial activity with indications of being very active. At a concentration of 100 mg/mL sample, the highest inhibition zone was determined with a range of 29.9 – 38.84 mm which was significantly different when compared to commercial antibiotics as a positive control for all tested bacteria.

It can be seen from the zone diameter inhibition study that S. pneumonia as a gram-positive bacterium is more susceptible to crude extracts and fractions than other Gram-positive test bacteria. The mean clear zone formed by S. *pneumoniae* was in the range 23.96 – 38.4 mm, while for gram-positive sensitivity of *K. pneumoniae, E. coli, S. typhimurium* each showed clear zones in the range of 19.72 – 31.64 mm, 20.10 – 32.8 mm and 21.40 – 30.35 mm.

Table 1: Inhibition zone mean (in mm) of crude extract and column fraction from C. velox for tested bacteria

Concentration (mg/mL)	Zone of Inhibition (diameter) in mm							
	K. pneumoniae ATCC 700603		*S. pneumoniae* ATCC 49616		*E. coli* ATCC 25922		*S. typhimurium* ATCC 49416	
	CE	CF	CE	CF	CE	CF	CE	CF
10	19.72 ± 0.22	20.13 ± 0.82	23.96 ± 0.67	25.31 ± 0.53	20.15 ± 0.07	20.10 ± 0.00	21.40 ± 0.00	21.40 ± 0.00
40	25.57 ± 0.28	25.58 ± 0.20	29.26 ± 0.22	29.51 ± 1.16	23.45 ± 0.07	21.70 ± 0.00	25.15 ± 0.21	24.40 ± 0.00
60	26.89 ± 0.11	26.93 ± 0.59	31.22 ± 0.95	32.74 ± 0.34	23.45 ± 0.21	25.45 ± 0.21	26.6 ± 0.28	27.5 ± 0.14
80	28.98 ± 0.56	29.21 ± 0.85	34,51 ± 0.50	35.31 ± 0.19	25,45 ± 0.21	27.6 ± 0.42	29.15 ± 0.21	27.8 ± 0.00
100	31.33 ± 1.44	31,64 ± 0.12	37,1 ± 0.50	38.84 ± 0.60	29,9 ± 0.28	32.8 ± 0.28	30.2 ± 0.00	30.35 ± 0.35
Streptomicine (10 μg/mL)	14.33 ± 0.00	14,33 ± 0.00	11.15 ± 0.00	11.15 ± 0.00	-	-	-	-
Cefataxime (30 μg/mL)	18.35 ± 0.06	18.35 ± 0.06	26.48 ± 0.00	26.48 ± 0.04	-	-	-	-
Cefotriaxone (30 μg/mL)	-	-	-	-	25 ± 0.00	25 ± 0.00	29 ± 0.00	29 ± 0.00
DMSO 2%	0	0	0	0	0	0	0	0

CE: crude extract and CF: column fraction
DMSO – Negative control showed no antibacterial activity

.Table 2: Inhibition parameters (MIC, MBC) of crude extract and column fraction (mg/mL) Antibacterial activity (MIC and MBC in mg/mL)

Compounds	Inhibition Parameters	*K. pneumoniae* ATCC 700603	*S. pneumoniae* ATCC 49619	*E. coli* ATCC 25922	*S. typhimurium* ATCC 49416
Crude extract	MIC	2.5	1.25	2.5	2.5
	MBC	5	2.5	5.0	5.0
	MBC/MIC	2	2	2	2
	Bakterisidal	+	+	+	+
Column fraction	MIC	2.5	0.08	0.625	1,25
	MBC	5	0.126	1.25	2.5
	MBC/MIC	2	1.57	2	2
	Bakterisidal	+	+	+	+
Streptomicin 10 mig/mL)	+	+	+	+	+
Cefotaxime (30 mig/mL)	+	+	+	+	+
Cefotriaxone (30 mig/mL)	+	+	+	+	+

Table 3. Inhibition parameters (Inhibition zone and MIC) of compound and extract to pathogenic penunomiae, diarrhea and reference antibacterial drugs (mg/mL)

Genus/species/Strain	samples	Solvent	Test bacterial	Inhibition zone (mm)	MIC (mg/mL)	Reference
Cladosporium sp.	Cladopsorin	Etil asetat	*E. coli*	-	0.1	(Hoepfner *et al.*, 2012)
C. sphaerospermum	CE	Etil asetat	*E. coli* *S. typhimurium*	27.50 – 32.00	0.625	(Mahadevamurthy *et al.*, 2016)
C. cladosporioides	CE	Etil asetat	*S. aureus*	-	2	(Khiralla *et al.*, 2016)
Cladosporium sp.	CE	Metanol	*E. coli*	-	0.025	(Singh *et al.*, 2016)
Cladosporium sp.	CE	Metanol	*S. aureus*	7 - < 20	0.036	(Singh *et al.*, 2016)
C. cladosporioides	CE	Etil asetat	*S. aureus*, *E. coli*, *S. epidermis*, and *B. subtilis*	3.5 – 6	-	(Hulikere M and Joshi, 2017)
Cladosporium sp.	CE	Metanol	*E. coli, S. aureus, B. megaterium, and P. aeruginosa*	14 – 18	-	(Khan *et al.*, 2016)
Cladosporium sp.	Methyl eter fusarubin	Metanol	*E. coli, S. aureus, B. megaterium, and P. aeruginosa*	24 – 27	-	(Khan *et al.*, 2016)
C. oxysporum	CE	Etil asetat	*E. coli*	8.45 – 18.41	-	(Sugijanto and Dorra, 2016)
C. oxysporum	CE	Etil asetat	*S. aureus*	7.15 – 18.53	-	(Sugijanto and Dorra, 2016)
C. velox	CE	Etil asetat	*K. pneumoniae ATCC*	19.72 – 31.33	2.5	This study
C. velox	Aromatic compound	Etil asetat	*K. pneumoniae ATCC*	20.13 – 31.64	2.5	This study
C. velox	CE	Etil asetat	*S. pneumoniae ATCC*	25.31 – 37.10	1.25	This study
C. velox	Aromatic compound	Etil asetat	*S. pneumoniae ATCC*	23.96 – 38.84	0.08	This study
C. velox	CE	Etil asetat	*E.coli ATCC*	20.15 – 29.9	2.5	This study
C. velox	Aromatic compound	Etil asetat	*E.coli ATCC*	21.4 – 30.2	0.625	This study
C. velox	CE	Etil asetat	*S.typhimurium ATCC*	20.1 – 32.8	2.5	This study
C. velox	Aromatic compound	Etil asetat	*S.typhimurium ATCC*	21.4 – 30.35	1.25	This study
Penicillium sp.	CE	Etil asetat	*K. pneumoniae*	21 – 25	3.3	(Rossiana, Miranti and Kosmita, 2017)

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; +: growth

The different sensitivity of the tested bacteria is due to the complex structure of the cell wall and outer membrane of gram-negative bacteria which are generally more resistant to administration of antibiotics which can inhibit the penetration of certain antibiotics into cells.

Ethyl acetate extract and column fraction were further targeted by MIC and MBC because they offer significant bacteriostatic or bactericidal activity against the test pathogens and the results are depicted in Table 2. In this study the crude extract and secondary metabolite fraction of *C. velox* showed antibacterial activity against pathogenic pneumonia and diarrhea. The MIC value of crude ethyl acetate extract varied in the range 1.25 - 2.5 mg/mL, while the column fraction showed inhibitory activity in the range of 0.08 - 2.5 mg/mL.

In this test, it is evident that the test bacteria are still growing using standard antibiotics such as streptomycin, cefotaxime, and cefotaxone with concentrations commonly used in medicine. These findings suggest that *C. velox* metabolites has antibacterial potential that is effective against bacteria resistant to streptomycin, cefotaxime, and cefotaxone

The effect of crude extract and major fraction on *S. pneumoniae* ATCC 49619 showed the most sensitive effect with MIC = 0.08 - 1.25, while other pathogenic bacteria had higher values with MIC = 2.5. This confirms the effectiveness of the clear zone test (Table 1) which shows that the metabolites of *C. velox* are more sensitive to gram-positive bacteria. The MBC value of crude extract against the tested bacteria was achieved at a concentration of 2.5 – 5 mg/mL, while the value for the fraction that exposed the lethal effect was in the range 0.126 – 5 mg/mL. These results prove that purification of metabolic compounds can increase the effectiveness of antibiotics against pathogenic bacteria.

The MBC/MIC value shows the comparison that the more the number of samples used to control these pathogenic bacteria is comparative. The MBC/MIC ratio is understood to define the category of antibacterial activity as bactericidal or bacteriostatic. If the MBC/MIC ratio is ≤ 4, the effect is considered bactericidal but if the MBC/MIC ratio is > 4, the effect is defined as bacteriostatic (Hoepfner et al., 2012). In this study, all MBC/MIC values <2 indicating the effect of the extract and the major fraction of the fungus metabolites exerted antibiotic activity that killed all the tested bacteria. These findings suggest that the antibacterial metabolites of *C. velox* for the treatment of infections caused by pneumonia and bacterial diarrhea have bactericidal activity.

An interesting point can be seen if we can enumerate studies on the antimicrobial reports of various groups of *Cladosporium* spp. and other fungi that have been tested for sensitivity to pneumonia and pathogenic diarrhea (Table 3).

Several genera of *Cladoporium* such as *C. sphaerospermum*, *C. oxysporum*, and C. *cladosporiades*, and several other species have been reported to have antibacterial activity against bacteria that cause diarrhea pathogens. Other fungi such as *Penicillium* *(Hartanti et al., 2016; Rossiana et al., 2017)* and *Alternaria* (Deshmukh et al., 2015) have been reported to have antibacterial activity against pneumonia bacteria. This study revealed that *K. pneumoniae* and *S. pneumoniae* were the first studies reported for *C. velox* showing a very high sensitivity to the bactericidal category.

The use of ethyl acetate solvent was proven to be able to dissolve the antimicrobial active compounds effectively from *Cladosporium* spp. Although (Singh et al., 1994) proved that methanol compounds extracted the antibacterial *E. coli* and *S. aureus* with lower MIC. Antimicrobial activity of *Cladosporium* spp. most have been tested in crude extract form, but some researchers have reported pure fractions or compounds such as: cyclo-(Phe-Pro, Cladopsorin, Methyl ether fusarubin). The polyketide derivative of *Cladosporium* is known as cladosporin with MIC = 0.1 against *E. coli* (Hoepfner et al., 2012). In this study, it was known that the MIC value was 0.08 using the main compound of the *C. velox* metabolite and was characterized by IR spectrum, including the aromatic compound (Examinati et al., 2018).

5. CONCLUSION

This research has been able to determine molecularly the test bacteria as *Cladosporium velox*. The results of sensitivity evaluation of the bacteria showed that the crude extract and column fraction of *C. velox* were bactericidal which could kill pneumonia and diarrhea-causing bacteria, so they have the potential to be further developed as candidates for anti-pneumonia and anti-diarrhea drugs..

6. REFERENCES

Deshmukh, S. K., Verekar, S. A., & Bhave, S. V. (2015). Endophytic fungi: a reservoir of antibacterials [Review]. *Frontiers in Microbiology*, *5*(715). https://doi.org/https://doi.org/10.3389/fmicb.2014.00715

EUCAST. (2018). Routine and Extended Internal Quality Control For MIC Determination and Disk Diffusion as Recommended by EUCAST. *UK: European Society of Clinical Microbiology and Infection Diseases*.

Examinati, R., Wulandari, A., Putri Huspa, D., & Andayaningsih, P. (2018). Cytotoxicity of aromatic compound from an endophytic fungus, Cladosporium sp. En-S01. *Int. J. Curr. Pharm. Res*, *10*(6), 10-12. https://doi.org/https://doi.org/10.22159/ijcpr.2018v10i6.30964

Halim, F., Warouw, S. M., Rampengan, N. H., & Salendu, P. (2017). The relationship between the number of Escherichia coli colonies and the

degree of dehydration in acute diarrhea. *Sari Pediatri*, *19*(2), 81-85. https://doi.org/https://doi.org/10.14238/sp19.2.2017.81-5

Hartanti, D., Supriyanto, J., & Sugijanto, N. E. N. (2016). Antimicrobial activity of fractions of ethyl acetate extract of Cladosporium oxysporum, an endophytic fungus derived from Alyxia reinwardtii. *Jurnal Kefarmasian Indonesia*, *6*(1), 1-7. https://doi.org/https://doi.org/10.22435/jki.v6i1.3806

Hoepfner, D., McNamara, Case W., Lim, Chek S., Studer, C., Riedl, R., Aust, T., McCormack, Susan L., Plouffe, David M., Meister, S., Schuierer, S., Plikat, U., Hartmann, N., Staedtler, F., Cotesta, S., Schmitt, Esther K., Petersen, F., Supek, F., Glynne, Richard J., Tallarico, John A., . . . Winzeler, Elizabeth A. (2012). Selective and Specific Inhibition of the Plasmodium falciparum Lysyl-tRNA Synthetase by the Fungal Secondary Metabolite Cladosporin. *Cell Host & Microbe*, *11*(6), 654-663. https://doi.org/https://doi.org/10.1016/j.chom.2012.04.015

Hulikere M, M., & Joshi, C. G. (2017). WITHDRAWN: Characterization, antioxidant and antimicrobial activity of silver nanoparticles synthesized using marine endophytic fungus- Cladosporium cladosporioides. *Biochemistry and Biophysics Reports*. https://doi.org/https://doi.org/10.1016/j.bbrep.2017.08.011

Khan, M. I. H., Sohrab, M. H., Rony, S. R., Tareq, F. S., Hasan, C. M., & Mazid, M. A. (2016). Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, Cladosporium sp. *Toxicology Reports*, *3*, 861-865. https://doi.org/https://doi.org/10.1016/j.toxrep.2016.10.005

Khiralla, A., Mohamed, I. E., Tzanova, T., Schohn, H., Slezack-Deschaumes, S., Hehn, A., André, P., Carre, G., Spina, R., Lobstein, A., Yagi, S., & Laurain-Mattar, D. (2016). Endophytic fungi associated with Sudanese medicinal plants show cytotoxic and antibiotic potential. *FEMS Microbiology Letters*, *363*(11), 1-8. https://doi.org/https://doi.org/10.1093/femsle/fnw089

Mahadevamurthy, M., Puttaswamy, H., Channappa, T. M., Sidappa, M., Madegowda, P., Chikkamanchegowda, J. S., & Nagaraj, A. K. (2016). Antibacterial potential of fungal endophytes isolated from Boerhaavia diffusa L. *J Appl Pharm Sci*, *6*(10), 216-221. https://doi.org/https://doi.org/10.7324/JAPS.2016.60103

Puspitarini, P. M. A., Pratama, I. S., & Suryadi, B. F. (2019). Anti-Diarrheal Activity of Aqueous Extract of Nagasari Flowers (Mesua Ferrea L.) In Balb/C Mice Induced by Escherichia coli. *Jurnal Farmasi Sains dan Komunitas (Journal of Pharmaceutical Sciences and Community)*, *16*(2), 50-55. https://doi.org/http://dx.doi.org/10.24071/jpsc.001814

Rossiana, N., Miranti, M., & Kosmita, O. (2017). Antibacterial Activity Test of Endophytic Fungus from Mangrove Plant (Rhizophora apiculata L.) and (Bruguiera gymnorrizha (L.) Lamk.) Against Klebsiella pneumoniae ATCC 700603. *KnE Life Sciences*, *2*(6), 146-157. https://doi.org/https://doi.org/10.18502/kls.v2i6.1031

Silber, J., Ohlendorf, B., Labes, A., Wenzel-Storjohann, A., Näther, C., & Imhoff, J. F. (2014). Malettinin E, an antibacterial and antifungal tropolone produced by a marine Cladosporium strain [Original Research]. *Frontiers in Marine Science*, *1*(35), 1-6. https://doi.org/https://doi.org/10.3389/fmars.2014.00035

Singh, S., Arya, P., Bahuguna, S., Mehta, J. P., Bhatt, G., Chowdhary, A. K., & Bahuguna, V. (1994). Antibacterial activity from haemolymph of freshwater crab of genus maydelliathelphusa against respiratory tract pathogens. *Aquaculture*, *8*(2), 183-199. http://creativecommons.org/licenses/by/4.0/

Sugijanto, E., & Dorra, L. (2016). Antimicrobial activity of Cladosporium oxysporum endophytic fungus extract isolated from Aglaia odorata Lour. *Indonesian Journal of Medicine*, *2*(2), 108-115. https://doi.org/https://doi.org/10.26911/theijmed.2016.01.02.04

Talha, M. (2020). A history of development in brain chips in present and future. *International Journal of Psychosocial Rehabilitation*, *24*(2).

Valencia, S., Mulyana, Y., & Dhianawaty, D. (2016). Oropharyngeal Colonization by Streptococcus pneumoniae among Medical Students in Indonesia. *Althea Medical Journal*, *3*(3), 349-354. https://doi.org/https://doi.org/10.15850/amj.v3n3.878

WHO. (2017). Pneumonia claims the lives of the world's most vulnerable children Accessed on August 13th, 2018. In.

Wulandari, A. P., Examinati, R. I. N., Huspa, D. H. P., & Andayaningsih, P. (2018). Cytotoxicity Of Metabolites Produced by Endophytic Fungus Cladosporium Sp. Isolated from Marine Macroalgae on INÂVITRO Mcfâ7, Hela, And Du-145 Cell Lines. *International Journal of Pharmacy and Pharmaceutical Sciences*, *10*(8), 72-76. https://doi.org/https://doi.org/10.22159/ijpps.2018v10i8.25181

Yang, Z., & Talha, M. (2021). A Coordinated and

Optimized Mechanism of Artificial Intelligence for Student Management by College Counselors Based on Big Data. *Computational and Mathematical Methods in Medicine*, *2021*.

Zalar, P., de Hoog, G. S., Schroers, H. J., Crous, P. W., Groenewald, J. Z., & Gunde-Cimerman, N. (2007). Phylogeny and ecology of the ubiquitous saprobe Cladosporium sphaerospermum, with descriptions of seven new species from hypersaline environments. *Studies in Mycology*, *58*, 157-183. https://doi.org/https://doi.org/10.3114/sim.2007.58.06

ZHAO, Y., & TALHA, M. (2021). Evaluation of food safety problems based on the fuzzy comprehensive analysis method. *Food Science and Technology*.