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Effect of light exposure on secondary metabolites production of an endophytic fungus *Arthrinium rasikravindrae* and its antioxidant and anticancer activities

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Abstract. *Eltivitasari A, Rahmawati, Gemantari BM, Romadhonsyah F, Nurrochmad A, Wahyuono S, Astuti P. 2021. Effect of light exposure on secondary metabolites production of an endophytic fungus* Arthrinium rasikravindrae *and its antioxidant and anticancer activities. Biodiversitas 22: 3156-3163.* Endophytic microorganisms are one of the promising sources in producing bioactive compounds, to be developed for new drug candidates. They are found to have the ability to generate the same compounds as their host plant. Metabolite producing capacity of the endophytes is known to be affected by light exposure during fermentation process. This study focused on an endophytic fungus *Arthrinium rasikravindrae* isolated from *Coleus amboinicus* stem to reveal out its metabolite profiles due to light exposure as well as its bioactivity consequences. *A. rasikravindrae* was cultured on potato dextrose broth medium for 14 days and fermented in dark and exposed to natural light. Metabolite profiling was performed using TLC and GC-MS analysis. The activities were observed using DPPH assay for antioxidant and MTT assay for cytotoxicity potential. The results showed that *A. rasikravindrae* ethyl acetate extract produced during dark and exposed to light fermentation conditions contained different compounds but there was some which showed similarity with their host plant. Methyl octadec-9-enoate was found in all fermentation conditions as well as in *C. amboinicus* stem extract. Besides methyl octadec-9-enoate, methyl palmitate was also found present in both *A. rasikravindrae* extract fermented exposed to light and its host plant. The antioxidant activity of extract generated from dark fermentation condition was better as compared to that exposed to light with IC_{50} value of 66.36±0.53 vs 556.92±34.37 µg/mL. However, cytotoxic activity screening against several cancer cell lines exhibited opposing results in which extract from light-exposed fermentation resulted in better cytotoxic activity (IC_{50} value of 291.40 ± 2.34 µg/mL on WiDr, 336.80 ± 5.05 µg/mL on T47D, and 404.73 ± 3.46 µg/mL on Hela cell lines). Extract obtained from dark fermentation condition showed IC_{50} value of more than 500 µg/mL in all tested cancer cell lines. Preliminary examination on cytotoxic activity against WiDR cells suggested that the extract from light-exposed fermentation might induce cell death through mechanisms involving cell cycle arrest.

Keywords: Antioxidant, *Arthrinium rasikravindrae*, cytotoxic, endophytic fungus, light, metabolites profiles

Abbreviations: DPPH: 2,2-diphenyl-1-picrylhydrazyl; GC-MS: Gas Chromatography-Mass Spectrometry; MTT: 3-(4,5-dimethyl-thiazol-2-yl)-2,5-di-phenyltetrazolium bromide); TLC: Thin Layer Chromatography; PDA: Potato Dextrose Agar; PDB :Potato Dextrose Broth; RPMI: *Roswell Park Memorial Institute*; DMEM: *Dulbecco's Modified Eagle Medium*; DMSO: Dimethyl Sulfoxide

INTRODUCTION

Coleus amboinicus is a member of the family of Lamiaceae, known to contain many clinically useful bioactive compounds such as flavonoids, alkaloids, tannins, triterpenoids and saponins (Damanik et al. 2017; Aisyah et al. 2020). This medicinal plant in Indonesia is called torbangun and has been traditionally used by Bataknese as a stimulant for breast milk (a lactagogue) (Damanik 2009). *C. amboinicus* leaves were described to have pharmacological properties such as antimicrobial, antimutagenic, antitumorigenic, antiepileptic, urolithiasis, radioprotective, and neuropharmacological activities (Bhatt et al. 2013). It was reported that ethyl acetate extract from *C. amboinicus* leaves had antioxidant and cytotoxic activity against HeLa, T47D and MCF7 cells (Hasibuan et al. 2019). It was also reported that the stem extract of *Plectranthus amboinicus* was rich in antioxidants and exhibiting anti-platelet aggregation, antibacterial activity, as well as antiproliferative effects on Caco-2, HCT-15, and MCF-7 cancer cells (Bhatt et al. 2013). Major phenolics compounds reported to be found in stem extracts of *C. amboinicus* are rosmarinic acid, caffeic acid, rutin, gallic acid, quercetin, and p-coumaric acid (Bhatt et al. 2013). In addition, thymol, carvacrol, 1,8-cineole, p-cymene, spathulenol and terpinen-4-ol were shown to be the main constituents of *C. amboinicus* leaf essential oil (Arumugam et al. 2016). Some of these components (carvacrol, thymol and 1,8-cineole) were functioned as antioxidant and also used for cancer therapy (Pinheiro et al. 2015; Quiroga et al.

2015; Mahran et al. 2019). It has been observed that compounds having antioxidant properties are capable of preventing degenerative diseases (Sinha and Dabla 2015; SA Rifki et al. 2019). Additionally, some reports are available which revealed that antioxidant properties are related to the mechanism to induce apoptosis, one of the strategies in killing cancer cells (Salganik 2001; Rzepczynska et al. 2011).

Endophytic fungi are microorganisms that colonize inside the plant tissues such as roots, stems, leaves, flowers, or seeds without causing adverse effects on the host plant (Yadav 2018). Endophytes are useful for maintaining plant resistance to abiotic stresses such as increasing drought tolerance, plant disturbances during high and low temperatures, low pH environmental conditions, high salinity, and heavy metals pressure in the soil (Jalgaonwala et al. 2011; Sushma et al. 2021). Endophytes produced secondary metabolites that inhibit the growth of pathogenic bacteria, fungi and protozoa in humans, animals and plants (Martinez-Klimova et al. 2017). It has been reported that endophytic fungi can produce the same compounds as that of host. This can be seen from the results that reported the production of taxol by the endophytic fungus *Taxomyces andreanae* isolated from *Taxus brevifolia* (Monika et al. 2020). *T. brevifolia* is a rare plant, so the discovery of the ability of endophytic fungi to produce compounds having similarity to their hosts can be used as a strategy for the discovery of compounds having medicinal values without compromising the existence of endangered or rare species.

Previous studies reported that some compounds isolated from *C. amboinicus* endophytic fungi exhibited cytotoxic activities (Astuti et al. 2016; 2020; 2021b). It has been observed that the ability of endophytes in producing secondary metabolites was influenced by many factors including light exposure (Kim et al. 2014; Soliman and Raizada 2018). Light also affected production of Taxol and Baccatin III in the *Taxus cuspidata* cell culture (Fett-Neto et al. 1995). Exposing natural light to the fermentation of *Eutypa linearis*, an endophytic fungus obtained from the leaves of *C. amboinicus* also influenced the metabolite production and thus its bioactivity (Gemantari et al. 2021). Previous studies reported that an N-containing substance isolated from endophytic fungus *A. rasikravindrae* isolated from the stem of *C. amboinicus* had cytotoxic activity against T47D and WiDr cancer cells (Astuti et al. 2021a). This study aimed to determine whether differences in fermentation conditions due to light exposure could influence secondary metabolites production of the fungal endophytic *A. rasikravindrae* and whether this also bring consequence on its bioactivity as antioxidant or anticancer.

MATERIALS AND METHODS

Materials

The reagents used in this study included ethyl acetate, chloroform, methanol, silica gel F_{254} purchased from Merck (Darmstadt, Germany). Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were purchased from Oxoid (Basingstoke, UK). Tris-HCl, quercetin dihydrate and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) purchased from Sigma-Aldrich (Saint Louis, USA). The cell culture growth media were DMEM (*Dulbecco's Modified Eagle Medium*) and RPMI (*Roswell Park Memorial Institute*), FBS (Fetal Bovine Serum, Gibco) as well as Fungizone and Penstrep (Sigma) media. Other cell culture materials were phosphate buffer saline (Sigma), trypsin-EDTA (Sigma), Doxorubicin, DMSO (Dimethyl Sulfoxide, Sigma), MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide, Sigma), and HCl pa (Merck).

Fermentation and extraction of *Arthrinium rasikravindrae*

The endophytic fungus was obtained from culture collection of Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia (Astuti et al. 2021a). The pure isolate of endophytic fungi (four plugs of 7 days old fungi grown on Potato Dextrose Agar) was cultured on Potato dextrose broth (PDB) containing 200 mL medium in 500 mL Erlenmeyer. The cultures were incubated in dark or exposed to natural light conditions, at room temperature, 25°C for 2 weeks, agitated at 120 rpm. The fermented cultures were filtered and the filtrate were extracted by liquid-liquid partition using ethyl acetate. The ethyl acetate (EtOAc) soluble layer was separated from the fermentation broth and then evaporated to dryness. The EtOAc extracts were kept at 4°C for further analysis.

Antioxidant activities

Radical scavenging activity was carried out using DPPH test with modifications (Alam et al. 2013). Preparation of DPPH test solution (2,2-diphenyl-1-picrylhydrazyl) was carried out in dark conditions. Ten milligrams of the extract was dissolved in 10 mL of methanol and the concentration series was made at final concentration of 250,125,62.5,31.25,15.63 µg/ml. Quercetin was used as positive control. One mL of sample or positive control solution, was mixed with 1 mL of 0.1 mM DPPH solution. The mixture was incubated for 30 minutes in dark conditions. The absorbance was measured at 517 nm. Each treatment was replicated three times. Radical scavenging activity was analyzed based on % of inhibition with the formula:

$$\text{Inhibition (\%)} = 1 - \left(\frac{\text{Absorbance Sample} - \text{Absorbance Blank}}{\text{Absorbance Control} - \text{Absorbance Blank}}\right) \times 100\%$$

Where:

Absorbance Sample = absorbance extract/quercetin and DPPH solution

Absorbance Blank = absorbance extract in serial concentration and methanol

Absorbance Control = absorbance methanol and DPPH solution

IC_{50} value is the concentration where DPPH as a free radical is reduced by 50% and analyzed based on the sample concentration plotted against % inhibition at each concentration point.

Cytotoxic activities

The cytotoxicity of the ethyl acetate extracts on cancer cells was performed using MTT assay (3-(4,5-dimethyl-thiazol-2-yl)-2,5-di-phenyltetrazolium bromide) against HeLa, MCF7, T47D, and WiDr cells (Ekowati et al. 2020). T47D, WiDr, and Hela cells were cultured in RPMI, MCF7 and Vero were cultured in DMEM. Vero cells were used as normal cell model. RPMI and DMEM were supplemented with 1% fungizone, 1% pen strep and 10% FBS. Cells at 80% confluence were detached using trypsin and harvested, seeded at a density of 5×10^3 cells/well. Cell suspension (100 µL) was added to each well of 96-well plate and grown to 80% confluence in a humidified incubator at 37°C, 5% CO_2 for 24 h. The media was discarded and replaced by adding new media containing various extract concentrations (500, 250, 125, 62.5, 31.25 µg/mL). Doxorubicin was used as positive control, medium only (without cells) were included as blank control. After 24 h incubation, the medium was removed and wells were washed with 1X warm PBS. Thereafter, 100 µL of medium containing 0.5 mg/ml (MTT) solution was added to each well, and the plate was incubated for another additional 4 h at 37°C. After that, 100 µL of 10% sodium dodecyl sulfate-0.01 N HCl were added to each well and incubated overnight at room temperature in dark conditions. The optical density was measured using a microplate reader (Biorad) at 595 nm. All experiments were performed in triplicate.

For the experiment using combination of extract with Doxorubicin, the extract concentration used was ½ IC_{50}. Doxorubicin at the concentration of ½ IC_{50} was added at different time points, overnight before, and concurrently with the addition of the extract.

The absorbance value (Abs) was determined and analyzed into % cell viability as followed:

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of treated cells} - \text{Absorbance of medium}}{\text{Absorbance of control untreated cells} - \text{Absorbance of medium}} \times 100\%$$

IC_{50} was calculated based on the plot of sample concentration versus % cell viability and the concentration value indicates 50% inhibition of cell growth.

Secondary metabolites analysis

Phytochemical analysis of ethyl acetate extract of endophytic fungi was conducted using thin-layer chromatography (TLC) and gas chromatography-Mass Spectrometry (GC-MS) analysis. TLC was performed with a stationary phase of Silica Gel 60 F_{254} (Merck) with a mobile phase of chloroform: ethyl acetate (7: 3 $^{v}/_{v}$). The TLC plate was observed under visible light, UV_{254}, and UV_{366} light, as well as using anisaldehyde-sulfuric acid spray reagent detection followed by heating the plate at 105°C in the hot air oven until development of spots. Volatile compounds from the stem ethyl acetate extract of *Coleus amboinicus*, ethyl acetate extract of endophyte fungi in dark and exposed to natural light conditions were analyzed using GC-MS-QP2010 (Shimadzu, Tokyo, Jepang). Sample (0.5 µL) dissolved in chloroform: methanol (1:1 $^{v}/_{v}$) was injected into GC-MS capillary column cross bond 100% dimethylpolysiloxane (30m x 0.25mm x 0,25µm). Ion source temperature was 200°C, injector temperature was 250°C, and column temperature was set 100 – 250°C, increased 10°C/min. Helium as carrier gas, column pressure of 100kPa, flow rate of 1.33 mL/minute. The electron ionization(EI) of the mass detector at 70eV. Identification of volatile compounds was performed using mass spectrum library data WILEY 7.LIB based on their retention time.

Statistical analysis

Data were analyzed statistically using One Way ANOVA test or Kruskal Wallis test (SPSS program version 16) depend on normality value and independent-sample t-test. Value of $p < 0.05$ was considered different significantly with 95% confidence level. The experiments were conducted triplicate and expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Effect of light exposure on secondary metabolites production

In this study, the fermentation culture treated at dark and natural light exposure was extracted using ethyl acetate, and then analyzed by TLC and GC-MS. Based on TLC data there were differences in phytochemical profiles between *A. rasikravindrae* extract both at dark and light exposure compared to stem extract (Figure 1). In addition, dark fermentation conditions showed more spots compared to light conditions as determined by anisaldehyde-sulfuric acid detection reagent (R_f 0.6-0.9) and UV_{366} light (R_f 0.55-0.85). Light exposure was reported by some studies to have an effect on the metabolites produced (Fett-Neto et al. 1995; Avalos and Estrada 2010; Kim et al. 2014; Soliman and Raizada 2018).

In order to examine the volatile components within the extracts, GC-MS analysis was also conducted (Figure 2). The results showed that there was a compound present in all extracts, namely methyl octadec-9-enoate (Tables 1, 2 and 3). This compound was reported to have antifungal properties and correlate with antioxidant and antibacterial activities (Abubacker and Deepalakshmi 2013; Bittencourt et al. 2015). It is interesting to note that underexposure to natural light, there was additional compound, which was similar to the stem extract, namely methyl palmitate (hexadecanoic acid, methyl ester). In line with the TLC profiles, GC-MS analysis also indicated that more compounds were produced in dark fermentation conditions. The differences in some compounds present in dark and light condition as well as the retention times suggested that natural light affect metabolites production. This study similar to that reported by Soliman and Raizada (2018), which showed that light suppresses the genes that synthesize taxol production and induces the expression of opsin genes for dark green pigments in an endophytic fungus (Soliman and Raizada 2018).

Table 1. The major chemical compounds of *Coleus amboinicus* stem ethyl acetate extract as analyzed by GC-MS

R. time	PA (%)	Structure name	SI	Molecular formula	MW (g/mol)
13.825	26.03	Hexadecanoic acid, methyl ester (CAS) methyl palmitate, methyl hexadecanoic, methyl n-hexadecane	96	$C_{17}H_{34}0_2$	270
14.702	33.24	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS) methyl linoleate	96	$C_{19}H_{34}0_2$	294
14.742	40.74	9-Octadecenoic acid, methyl ester (CAS) methyl octadec-9-enoate	97	$C_{19}H_{36}0_2$	296

Table 2. The major chemical compounds of ethyl acetate extract of *Arthrinium rasikravindrae* fermented in dark condition as analyzed by GC-MS

R. time	PA (%)	Structure name	SI	Molecular formula	MW (g/mol)
10.430	30.12	1,2-Ethanediol, 1-phenyl- (CAS) styrene glycol	90	$C_8H_{10}0_2$	138
10.610	27.62	1,2-Ethanediol, 1-phenyl- (CAS) styrene glycol	88	$C_8H_{10}0_2$	138
10.671	15.64	1,2-Ethanediol, 1-phenyl- (CAS) styrene glycol	90	$C_8H_{10}0_2$	138
13.314	25.02	Rosifoliol	82	$C_{15}H_{24}0_2$	220
14.738	1.61	9-Octadecenoic acid, methyl ester (CAS) methyl octadec-9-Enoate	97	$C_{19}H_{36}0_2$	296

Table 3. The major chemical compounds of ethyl acetate extract of *Arthrinium rasikravindrae* fermented exposed to natural light as analyzed by GC-MS

R. time	PA (%)	Structure name	SI	Molecular formula	MW (g/mol)
18.926	13.96	3,5-Dihydroxydecanoic acid .delta.-lactone , 2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pentyl- (CAS) 3,5-Dihydroo acid .delta.-lactone	95	$C_{10}H_{18}0_3$	186
22.108	46.05	Hexadecanoic acid, methyl ester (CAS) methyl palmitate, methyl hexadecanoic, methyl n-hexadecane	96	$C_{17}H_{34}0_2$	270
22.437	23.88	Hexadecanoic acid (CAS) Palmitic acid	94	$C_{16}H_{32}0_2$	256
23.883	16.11	9-Octadecenoic acid, methyl ester (CAS) methyl octadec-9-enoate	97	$C_{19}H_{36}0_2$	296

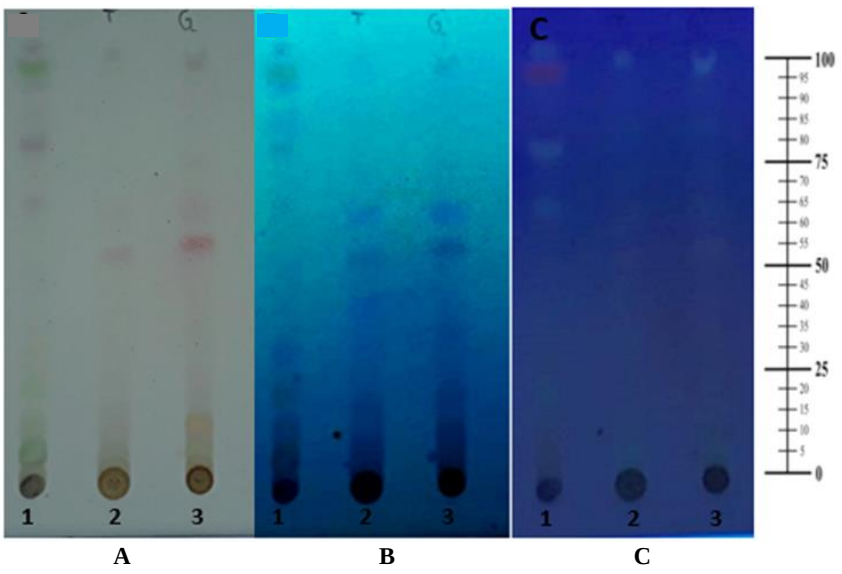

Figure 1. Thin Layer Chromatography (TLC) profiles of ethyl acetate extract of fermentation broth of *Arthrinium rasikravindrae* detected using anisaldehyde-sulphuric acid reagent **(**A) UV_{254} light (B) UV_{366} light (C) Stationary phase = Silica Gel 60 F_{254}; mobile phase = chloroform: ethyl acetate (7:3 $^{v}/_{v}$). 1. Stem extract of *C. amboinicus*. 2. Ethyl acetate extract of *A. rasikravindrae* exposed to light. 3. Ethyl acetate extract of *A. rasikravindrae* in dark condition

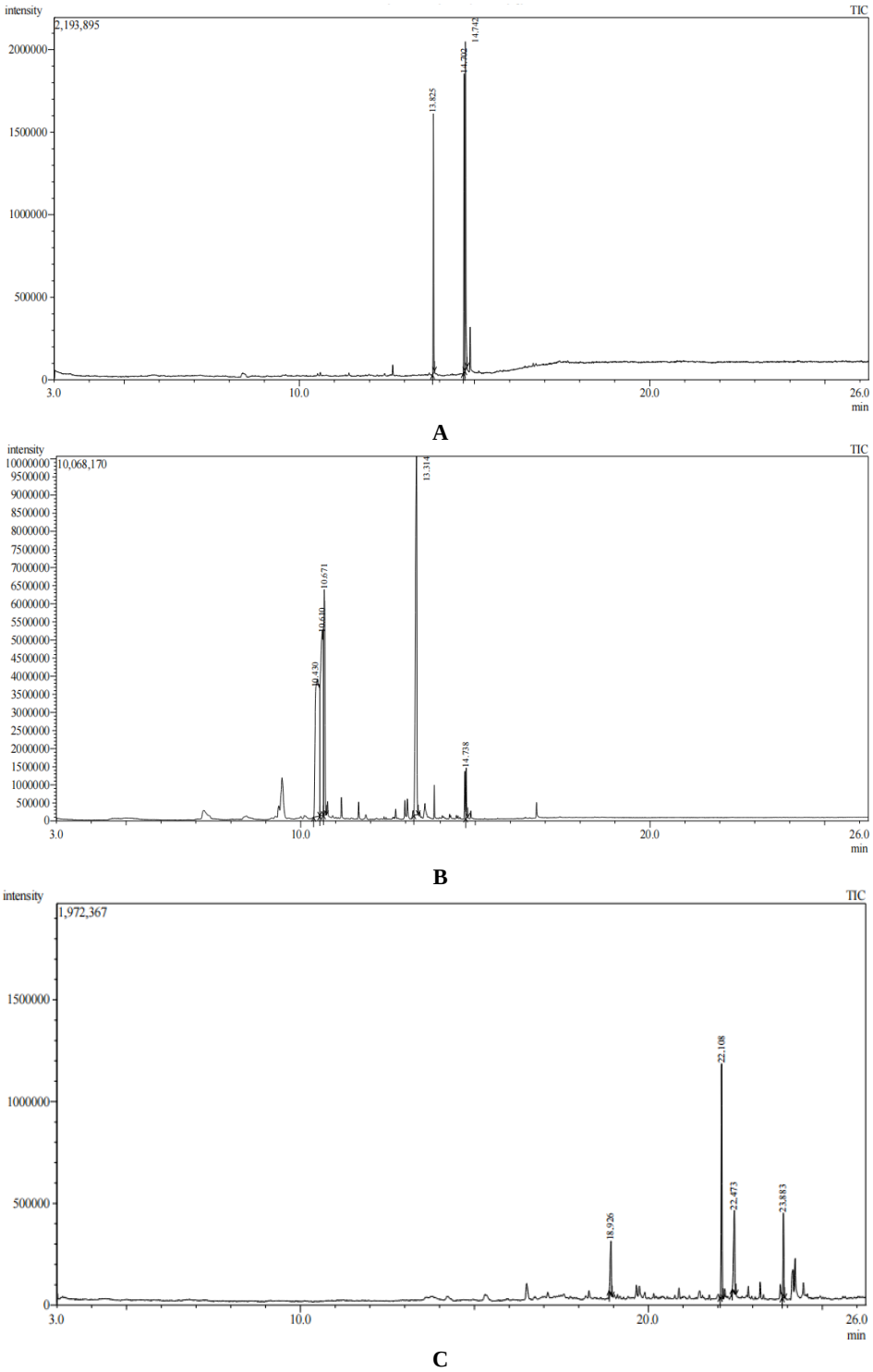

Figure 2. A. Gas Chromatography-MS Profile of *Coleus amboinicus* stem ethyl acetate extract, B. *Arthrinium rasikravindrae* fermented in dark condition, C. *A. rasikravindrae* fermented with natural light exposure

Antioxidant activities

This study attempts to examine whether the endophytic fungus *A. rasikravindrae* isolated from *C. amboinicus* also produced antioxidant compounds were conducted. It was found that the *A. rasikravindrae* fermented at dark conditions had IC_{50} of 66.36 ± 0.53 better than that fermented exposed to the natural light (556.91 ± 34.37) (Table 4). The antioxidant properties of dark fermentation extract were considered strong (50-100 µg/mL), while the extract exposed to light was categorized as weak (> 500 µg/mL) (Ansharullah et al. 2020). The extract from dark conditions mainly consists of styrene glycol, general flavoring agents used in foods, including condiments and seasonings (Arn and Acree 1998). The strong activity of

the extract under dark conditions could be contributed by the presence of rosifoliol, which represented 25.02% of the extract. This compound was reported to have antioxidant activity (Rosa et al. 2007). The ability of endophytic fungus *Arthrinium* sp. to produce antioxidant was also reported by Pansanit and Pripdeeveech (2018) who found the antioxidant activity of its ethyl acetate extract with an IC_{50} value of 28.47 µg/mL as determined by DPPH test (Pansanit and Pripdeevech 2018). The differences in antioxidant properties of the extract under dark and light conditions could be contributed by the effect of light on metabolite production. According to some literature, light exposure could affect the genes that play a role in the biosynthesis of bioactive compounds such as the taxol by the endophytic fungus SSM001 and aurofusarin and trichothecene by *Fusarium graminearum* (Kim et al. 2014; Soliman and Raizada 2018).

Cytotoxic activities

In this study, it was found that *A. rasikravindrae* isolated from *C. amboinicus* stem also exhibited cytotoxic activity. The fungi fermented under exposure to natural light produced compounds that were more potent than those in dark fermentation conditions. However, the level of cytotoxicity was considered weak (IC_{50}>100–1,000 µg/ml) (Ads 2020). IC_{50} extract obtained from fermentation with light exposure were 291.40 ± 2.34 µg/mL on WiDr, 336.80 ± 5.05 µg/mL on T47D, and 404.73 ± 3.46 µg/mL on Hela cell lines (Table 5). The IC_{50} values of the extract obtained from dark fermentation could not be determined in this study since its IC_{50} > 500 µg/mL, the maximum concentration used in this study. Similarly, IC_{50} values of both extract from light and dark fermentation conditions could not be examined towards normal Vero cells (> 500 µg/mL).

The better activity of extract exposed to the light as compared to the dark conditions could be contributed from the major volatile compounds present. A hexadecanoic acid compound (methyl palmitate) which was present at 46.05% on light fermentation condition extract was shown to be a good adjuvant for Sorafenib therapy on hepatocellular carcinoma cells (Breeta et al. 2021). Another hexadecenoic acid, palmitic acid (23.88%) compound was also reported to have cytotoxic activity (Harada et al. 2002). The results of the cytotoxic study indicated that ethyl acetate extract from the endophytic fungus *A. rasikravindrae* was also has the potential for further development. The potential of the endophytic fungi as a source of bioactive metabolites having chemotherapeutic values has been reviewed (Stierle and Stierle 2015). Several studies also have reported that endophytic fungus *Arthrinium sp*. had cytotoxic activity (Ebada et al. 2011; Bao et al. 2018; Su et al. 2020).

In this study, attempts to examine how the extract was capable of inducing cell death were determined by combining the extract obtained from light fermentation condition with doxorubicin. The combination treatment was carried out on WiDr which was found to have the best IC_{50} value. Extract at the concentration of ½ IC_{50} was combined with doxorubicin at the concentration of ½ IC_{50} which was added at two different time points. The data showed that adding the extract overnight after administration of doxorubicin resulted in more cell death compared to adding at the same time (Figure 3). The extract added overnight after doxorubicin treatment resulted in 15.42± 0.76 % of WiDr cell viability as compared to adding at the same time (34.44±2.83% cell viability). The viability of combined sample added at different time points resulted in more cell death as compared to administration of doxorubicin or the extract alone. It has been observed that doxorubicin-induced WiDR cell cycle arrest in the S and G2/M phase (Utami et al. 2020). Considering the higher level of cell death due to different time points of administration of extract towards doxorubicin suggested that doxorubicin may induced cell cycle arrest which then be abrogated by the presence of extract. Additional experiment using flow cytometry analysis or other methods is needed to confirm this finding. Checkpoint at G2 phase is an attractive target for anticancer therapy. Abrogating this G2 checkpoint prevents the cells to repair DNA damage and forces them to enter mitotic catastrophe. Checkpoint inhibition has grown to be the target for novel drug development with some of them have been in phase I/II clinical trials (Bucher and Britten 2008).

Table 5. Cytotoxicity of *Arthrinium rasikravindrae* ethyl acetate extract under dark and light conditions, expressed as IC_{50} against several cancer cell lines

Cells	IC_{50} (µg/mL)		
	Ethyl acetate extract, dark condition	**Ethyl acetate extract, light condition**	**Doxorubicin**
Hela	>500	404.73 ± 3.46	4.33± 0.12
T47D	>500	336.80 ± 5.05	1.98± 0.11
WiDr	>500	291.40 ± 2.34	2.52± 0.14
MCF-7	>500	>500	3.30± 0.21
Vero	>500	>500	7.55± 0.83

Note: All data are presented as mean ± SD (n=3) $p<0.05$

Table 4. DPPH scavenging activity of *Arthrinium rasikravindrae* ethyl acetate extract under dark and light conditions, expressed as IC_{50} values

Samples	**IC_{50} (µg/mL)**
Ethyl acetate extract from fermentation broth under dark condition	66.36 ± 0.53*
Ethyl acetate extract from fermentation broth exposed to natural light	556.91 ± 34.37*
Quercetin	1.365 ± 0.022*

Note: All data are presented as mean ± SD (n=3); * statistically significant different $p<0.05$

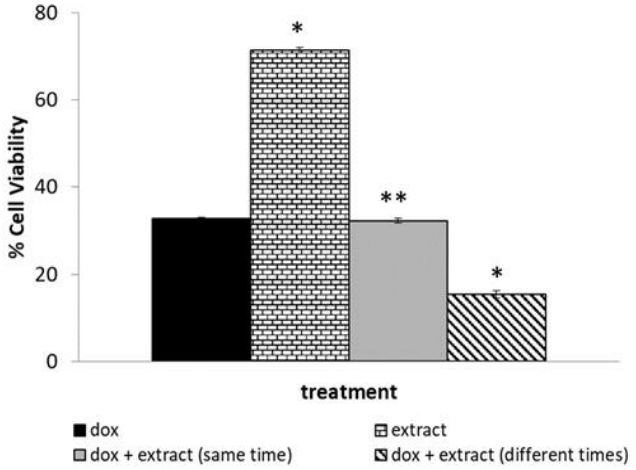

Figure 3. Cytotoxic combination test of *Arthrinium rasikravindrae* ethyl acetate extract from light fermentation condition with doxorubicin on WiDr cells, expressed as % cell viability. * statistically significantly different with dox; **statistically no significant difference with dox. All data are expressed as mean ± SD (n=3) $p<0.05$

Doxorubicin is a cytostatic drug that is well known for its use in the treatment of various cancers (Lüpertz et al. 2010). However, due to its instability for oral administration and its side effect, doxorubicin is not the primary choice for colon cancer chemotherapy, although due to its cost-effectiveness, this agent is still considered a drug of choice (Sonowal et al. 2017). The mechanism of doxorubicin towards its pro-apoptotic effect is by disrupting the function of DNA and caused DNA damage. It is also mentioned that doxorubicin can intercalate into the DNA double helix, inhibiting topoisomerase II and cross-linked DNA strands (Gewirtz 1999). Doxorubicin initiated human colon cell cycle arrest, which can occur either at G0/G1 or G2 phase, and this mechanism of cell cycle induced arrest could be dependent or independent p53 (Lüpertz et al. 2010). The p53 is a transcriptional factor that can be activated by genotoxic stress. It regulates multiple cellular responses which are involved in cell cycle control, DNA repair, and apoptosis (Vousden and Lu 2002).

Based on the results of the present study, natural light had an effect on secondary metabolites production of the endophytic fungi *Arthrinium rasikravindrae.* The differences in secondary metabolites profiles influenced its bioactivity as antioxidant and anticancer.

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