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Cytotoxic Activity of the Indonesian Fern *Angiopteris angustifolia* C. Presl and Liverwort *Mastigophora diclados (Birs. ex Web) Nees* Against Breast Cancer Cell Lines (MCF-7)

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Abstract

Breast cancer is the most frequent cancer among women worldwide, and it is also the main cause of death from cancer. Fern *Angiopteris angustifolia* C. Presl and liverwort *Mastigophora diclados (Birs.ex Web) Nees* are the plants that grow in Indonesia that are limited explored for their phytochemical and biological activity. This study aims to evaluate the cytotoxic effect of ethanolic extracts leaves of *A. angustifolia* and *M. diclados* against breast cancer cell lines (MCF-7). The MTT assay was used to determine cytotoxic activity, which revealed that the ethanol extract of *A. Angustifolia* and *M. diclados* exhibited cytotoxic activity with an IC_{50} value of 121.8 ± 13.3 and 29.2 ±1.4 µg /mL, respectively.

Keywords : *Angiopteris angustifolia*, breast cancer cell line (MCF-7), cytotoxicity, *Mastigophora diclados*, MTT assay.

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1. INTRODUCTION

According to the International Agency for Research on Cancer (IARC) data, breast cancer is a malignant tumor that is now the most often diagnosed cancer worldwide. Based on GlOBOCAN 2020, it is estimated that about 19.292.789 cases of cancer and 11.7% of these cases are breast cancer (WHO, 2020a). In Indonesia, over 396.914 cancer cases in both sex males and females, breast cancer is the most common cancer diagnosed (16.6%), followed by cervix uteri (9.2%) and lung cancer (8.8%) (WHO, 2020b).

Immunotherapy, chemotherapy, radiotherapy, tumor surgery, photodynamic therapy, stem cell transformation, cancer vaccines, and a combination of treatments can all treat cancer. These treatments are occasionally nevertheless accompanied by a slew of negative side effects. Much effort has been put into reducing the hazardous side effects of cancer treatment and expanding the search for new target medications. With their vast bioactive secondary metabolites, plants are the most promising source for more effective and less hazardous cancer drugs (Iqbal et al., 2017).

Ferns are the second most varied vascular plant that contains about 11000 different species. (Dai et al., 2020). The ferns with attractive green leaves are widely used as ornamental plants. *Angiopteris* is a fern that belongs to the Marratiaceae Family (King-fern family), which is distributed from Madagascar to Seychelles and southern India and Pacific islands as far east as Pitcairn. Certain rhizomes of *Angiopteris* species are consumed for their starch in Southeast Asia. In Polynesia, *Angiopteris rhizomes* are used for flavouring rice. In India, *Angiopteris* stems make ruschshi an intoxicating drink (Maarten et al., 2017). In

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China, *Angiopteris* species were used to treat snake bites, cough, rheumatic and arthralgia pain, and other diseases (Chen et al., 2010a). Some secondary metabolites such as lactones, coumarins, and triterpenoids have been found in *Angiopteris* species (Chen et al., 2010; Chen et al., 2010b; Yu et al., 2009). *Angiopteris* species have been shown to exhibit anti-adipogenic, anti-inflammatory (Lamichhane et al., 2020), antibacterial, anti-fungal (Khan & Omoloso, 2008), and hypoglycemic effects (Hoa et al., 2009). It was also found that *Angiopteris* species have cytotoxic activity against human hepatoma SK Hep-1 cells (Chen et al., 2010) and HeLa, K562, and KB cell lines. (Yu et al., 2009).

Liverworts are a diverse phylum of small, herbaceous, terrestrial plants containing about 500 species in 391 genera (Goffinet & Shaw, 2009). With their oil bodies, liverworts have been accounted for to contain different auxiliary metabolites responsible for some biological activities (Asakawa et al., 2013). *Mastigophora diclados* are epiphytic bryophytes found in tropical places worldwide, where they form thick colonies in montane forest habitats. (Ah-Peng et al., 2017). *Mastigophora diclados* has been reported to contain herbertane-sesquiterpenes, eudesmanolides, chlorinated cyclic bis(bibenzyl), ent-trachylobane diterpenoids (Hashimoto et al., 2000; Komala et al., 2010; Leong & Harrison, 1997). Herbertane-sesquiterpenes are major components of the *M. diclados* which have been known to exhibit cytotoxicity, antimicrobial and antioxidant (Komala et al., 2010), neurotrophic (Fukuyama & Asakawa, 1991), inhibitions of NO production of lipopolysaccharide (LPS)-stimulated RAW 264.7 cells (Harinantenaina et al., 2007).

Both ferns *A. angustifolia* and liverwort *M. diclados* are easy to grow in Indonesia, but the exploration of their chemical components and biological activity is still limited. To discover more effective cancer drugs from natural sources, in this research, we investigate the cytotoxic activity of both Indonesian fern *A. angustifolia* and liverwort *M. diclados* against breast cancer cell line (MCF-7).

The previous investigation reported that fern *A. angiopteris* is neglected phytochemically and biologically activity. Meanwhile, *M. diclados* have been reported to contain herbertane-sesquiterpenoids as major components that are active against HL-60 dan KB cell lines (Komala et al., 2010). This research determines the cytotoxic activity of ethanol extract of *A. angiopteris* and *M. diclados* using the MTT colourimetric assay.

2. MATERIALS AND METHODS

Materials

A. angustifolia was collected from Bogor, Indonesia, and *M. diclados* was collected from Slamet Mount Central Java, Indonesia in 2012. Both samples were determined in Herbarium Bogoriense, Research Center for Biology-Indonesian Institute of Sciences (LIPI) and stored in the Faculty of Health Sciences Laboratory.

The human breast cancer, MCF-7 cell lines (ATCC), RPMI 1640, (Gibco, USA), PBS (Gibco, Jerman), Penicillin-streptomycin (Gibco, USA), FBS (Sigma-Aldrich Inc, Jerman), Trypsin EDTA 5% (Sigma-Aldrich Inc, Jerman), MTT (Sigma-Aldrich Inc, Jerman), Trypan Blue Stain 0.4% (Sigma-Aldrich Inc, Jerman), DMSO (AppliChem, USA).

Extraction

The leaves of A. *angustifolia and M. diclados* were air-dried at room temperature after the collection. The dried leaves of *A. angustifolia* (40.3g) and *M. diclados* (90 g) were ground and extracted using ethanol technical grade to give 3.8 g (9.4% yield) and 6.0 g (6.7% yield) crude extract, respectively.

Cytotoxicity assay

MCF-7 cell lines were cultured and maintained in RPMI 1640 media, supplemented with 10% FBS at 37°C and 5% CO_2. 100 µL of MCF-7 cells were seeded at a density of $5x10^3$cells/well in 96-well plates. The culture was incubated for 48 hours in a 5% CO_2 incubator at 37^0C. By serial dilution, different concentrations of the extracts of *A. angustifolia* and *M. diclados* (200, 100, 50, 25, 12.5, 6.25. 3. 125 µg/mL) were prepared from the stock solutions. 200 µl of the medium containing extract was added and incubated for 24 hours. The cytotoxic assay was carried out according to the method that was described by Mosmann, *et al*. (Mosmann, 1983). As a control, the untreated cell was used. Each concentration of treated cells, untreated cells, and blank was tested in triplicate. Absorbance was read at optical density 540 nm. The IC_{50}

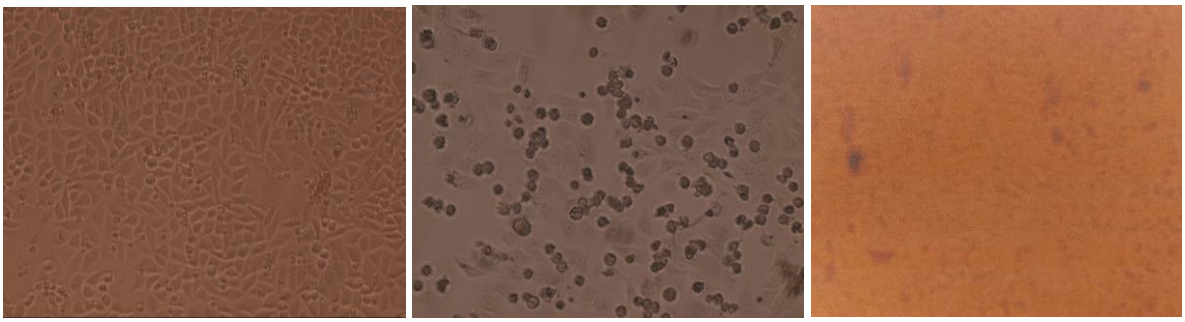
was measured as the concentration of the extract that causes 50% inhibition in cell viability towards the MCF-7. Cell viability (%) was calculated by equation 1:

$$Cell\ Viabilty\ (\%) = \frac{As-Ab}{Ac-Ab} x\ 100\ \% \qquad (1)$$

Where As is absorbance of sample, Ac is absorbance of control, and Ab is absorbance of blank.

3. RESULTS AND DISCUSSION

The air-dried leaves of *A. angustifolia* (40.3 g) and *M. diclados* (90 g) were extracted using ethanol to give 3.8 g (9.4 % yield) and 6.0 g (6.7 % yield) crude extracts, respectively. To explore the potential of the Indonesian plants as sources of a new anti-cancer drug, we are evaluating the cytotoxic activity of the Indonesian fern *A. angustifolia* and liverwort *M. diclados* against breast cancer cell line (MCF-7). The assay was conducted using the MTT method. Morphology of the MCF-7 cell line was performed in Figure 1. Calculation of IC_{50} values indicates that both fern *A. angustifolia* and liverwort *M. diclados* extracts can inhibit the breast cancer cell line (MCF-7), with IC_{50} values are 121.8 ± 13.3 and 29.2 ±1.4 µg /mL, respectively (Figure 2, and Table 1).



(a) (b) (c)

Figure 1. (a) Morphology of MCF-7 cell line in RPMI medium after incubated for 48 hours, (b)Morphology of MCF-7 cell line after treated with 200 µg/mL of *A. angustifolia* for 24 hours, (c)Morphology of MCF-7 cell line after treated with 200 µg/mL of *M. diclados* for 24 hours.

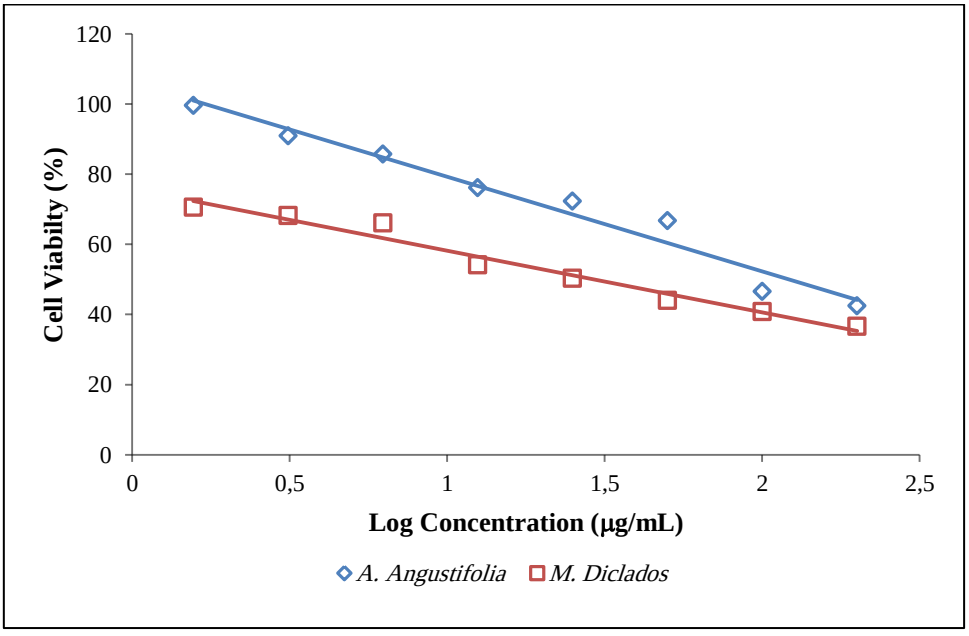


Figure 2. Correlation between log concentration of ethanol extract of *A. angustifolia* and *M. diclados* cell viability (%) of MCF-7 cell line.

Table 1. IC_{50} values of the ethanol extract of *A. angustifolia* and *M. diclados* against breast cancer cell line (MCF-7)

Extract	IC_{50} (µg/mL)
A. angustifolia	121.8± 13.3
M. diclados	29.2 ±1.4

IC_{50} values are represented as mean ± SD (n=3)

Cytotoxic activity was categorized based on the NCI and Geran protocol. The extracts with an IC_{50} of less than 20 µg/mL are highly cytotoxic, extracts with an IC_{50} of 21 to 200 µg/mL are moderately cytotoxic, extracts with an IC_{50} of 201 to 500 µg/mL are weakly cytotoxic, and extracts with an IC_{50} of more than 501 µg/mL are non-cytotoxic (Nguyen et al., 2020). Therefore, ethanol extract of both the Indonesian fern *A. angustifolia* and liverwort *M. diclados* are considered to have moderately cytotoxic activity.

The literature search shows no previous cytotoxicity studies of fern *A. angustifolia*. However, other *Angiopteris* species, such as *A. palmiformis*, possess triterpenoids with cytotoxic effects against human hepatoma SK Hep-1 cells (Chen et al., 2010). Lactone compounds of *A. caudatiformis* were active against HeLa, K562, and KB cell lines (Yu et al., 2009). It is predicted that the cytotoxic action of *A. angustifolia* against the MCF-7 cell line is related to its triterpenoid content. Lactone components with the potential to have cytotoxic activity have been found in abundance in the genus *Angiopteris* (Chen et al., 2010a; Yu et al., 2009). The chemotaxonomic theory states that the plant can have similar chemical components within one genus. It is probable that these lactone compounds were also found in *A. angiopteris* and are responsible for its cytotoxicity (Umoh, 2020).

Our previous research reported that the liverwort *M. diclados* is rich in herbertanes-sesquiterpenoid (Komala et al., 2010; Komala et al., 2011; Komala et al., 2016). The cytotoxicity of these herbertane-sesquiterpenes has been demonstrated against the HL-60 and KB cell lines (Komala et al., 2010). It is predicted that the cytotoxic activity of the Indonesian liverwort *M. diclados* against the MCF-7 cell line was also due to its herbertane-sesquiterpene content.

4. CONCLUSION

Ethanol extract of the Indonesian fern *A. angustifolia* and liverworts *M. diclados* were both reported to be potent against breast cancer cell lines with the IC_{50} value of 121.8 ± 13.3 and 29.2 ±1.4 µg /mL, respectively. Triterpenoid and possible natural lactones content were predicted as the secondary metabolite responsible for cytotoxic activity of *A. angustifolia*. Meanwhile and herbertane-sesquiterpenoids content in *M. diclados* were considered as the chemical component that causes cytotoxic activity.

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