

Exploring anticancer activity of wild and polyploid mutant of *Artemisia cina*

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Abstract. Kasmiyati S, Kristiani EBE, Herawati MM, Rondonuwu FS. 2021. Exploring anticancer activity of wild and polyploid mutant of *Artemisia cina*. *Biodiversitas* 22: 1227-1234. The research aims were to explore the anticancer compounds in wild type (W) and polyploid mutant (P) of *Artemisia cina* Berg ex Poljakov using NIR and compare their cytotoxicity and selectivity on WiDR colon cancer and HTB183 lung cancer cell lines and Vero normal cell line. The W was obtained from B2P2TOOT Tawangmangu, Indonesia while P was by inducing *A. cina* shoot culture with 100 mg/l colchicine for 48 hours. They were extracted with hexane using the soxhletation method for 6 hours. The anticancer compounds were detected using NIR. The cytotoxic activity was determined using MTT assay, with Doxorubicin (D) as a control. The calculation of IC₅₀ value used SPSS16 with a Probit analysis. The P contained the determined four compounds while the W contained no rutin. The IC₅₀ values of W, P, and D were 295.5, 84.1, and 0.5 µg/mL on WiDr, 322.4, 128.6, and 39.9 µg/mL on HTB 183, whereas on Vero were 10⁴, 315.6, and 295.5 µg/mL respectively. The selectivity indexes of W, P, and D were 34, 4, and 91 on WiDr, while HTB 183 was 31, 3, and 7, respectively. The P contained artemisinin, quercetin, kaemferol, and rutin, while the W contained no rutin. The cytotoxicity of both plants was less than doxorubicin. Both plants were selective on WiDR and HTB 183.

Keywords: *Artemisia*, cancer cell lines, cytotoxicity, selectivity indexes, anticancer compounds

Abbreviations: B2P2TOOT: Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional; TLC: Thin Liquid Chromatography; HPLC: High-performance liquid chromatography; GC/LCMS: Gas Chromatography/Liquid Chromatography/Mass Spectrophotometry; NIR: Nera Infrared

INTRODUCTION

Cancer is known as the second killer sickness in the world. Cases of cancer increase every year. International Agency for Research on Cancer (IARC) reported that in 2018, the number of cancer cases in the world increased with 18.1 million new cases, and 50% of them caused deaths. This condition spurred the medical world to get new therapies to overcome the disease. Natural products, especially natural plants, have been used to prevent and treat various diseases for thousands of years. A lot of research showed that compounds from natural products, which are usually called secondary metabolites, have anticancer therapeutic properties and show lower toxicity than chemotherapy (Almehdar et al. 2012; Arullappan et al. 2013; Kashani et al. 2012; Akter et al. 2014).

Many secondary metabolites have shown phytopharmaceutical activity including alkaloids, carotenoids, and polyphenolic compounds such as flavonoids, tannins, curcumin, stilbenes, phenolic acids, resveratrol and gallo catechin, brassinosteroids (Greenwell and Rahman 2015; Abotaleb et al. 2019). Terpenoids, flavonoids, phenols, and alkaloids were compounds that have potential as anticancer agents (Baikar and Malpathak 2010). In general, the accumulation of secondary

metabolites in plant organs varies (Croteau et al. 2000).

Many ways can be used to determine the content of secondary metabolites both qualitative and quantitative. Phytochemical screening uses specific reagents to confirm qualitatively the presence of compounds such as alkaloids, flavonoids, steroids/terpenoids, saponins, and others. GCMS is used to detect qualitatively the volatile compounds while LCMS is for both volatile and non-volatile compounds. TLC, HPLC, NIR can be used to detect a compound qualitatively and quantitatively. Many countries had made their respective regulations in controlling the quality of herbal medicine for both export and import markets. In this regard, a universal analytical tool with sufficient accurate results is needed for the analysis of phytochemical compounds in the industry of food, agriculture, the field of medicine and, and herbal samples (Liu et al. 2016; Mia et al. 2019).

Plants are the major source of herbal medicines that are currently widely consumed, and the demand for medicinal plants is huge because their consumption is increasing from time to time and extends throughout the world. According to Salma et al. (2016), the disadvantage of using conventional propagation of medicinal plants is the low bioactive content and both biotic and abiotic environmental factors influence its production. Polyploidization is a

strategy that can be developed to enhance secondary metabolite production of medicinal plants. Polyploidization often causes the emergence of novel characteristics that are not present in the diploid parent (Caruso et al. 2013). Polyploidization is considered the major driving force in speciation and an important evolutionary factor in forming new plant species, including the creation of commercially cultivated plant species (Salma et al. 2017; Parida and Misra 2015).

According to Parida and Misra (2015), the frequency of polyploidy in flowering plants is relatively high. Polyploidization in plants can occur naturally or artificially through induction with mutagens. Polyploidization in medicinal plants can change the biosynthesis of secondary metabolites both in quality and quantity (Salma et al. 2016). Many studies on the artificial polyploidization of medicinal plants have been conducted to investigate their role in enhancing active compounds of pharmaceutical importance. The reports of numerous polyploid medicinal plants with enhances in bioactive compound cover *Papaver somniferum* (Mishra et al. 2010), *Tanacetum parthenium* (Majdi et al. 2010), *Centella asiatica* (Kaensaksiri et al. 2011), *Echinacea purpurea* (Abdoli et al. 2013; Nilanthi and Yang 2013), *Solanum bulbocastanum* (Caruso et al. 2013), *Thymus persicus* (Tavan et al. 2015), *Artemisia cina* (Herawati et al. 2015), *Pogostemon cablin* (Yan et al. 2016), *Stevia rebaudiana* Bertoni (Zhang et al. 2018), *Artemisia annua* (Banyai et al. 2010; Yunus et al. 2018), and *Cannabis sativa* (Parson et al. 2019).

Artemisia belongs to the Asteraceae family, which usually is used as medicinal plants. For a long time, *Artemisia annua* has been used in China for traditional treatments of various diseases. Previous studies have shown that these bioactive compounds in *Artemisia* have antimicrobial, antimalarial, antioxidant, anthelmintic, and anticancer effects (Koul et al. 2017). The phytopharmaceutical activity of secondary metabolites of *Artemisia* was very potential, but its content was small. Tetraploid induction of this plant was able to increase the artemisinin content up to three times compared to the diploid plant (Herawati et al. 2015). The increasing levels of artemisinin may allow changes in the level of other secondary metabolites. The potential anticancer activity of various *Artemisia* species has been widely studied. Various species of *Artemisia* that have been tested for their anticancer potential include *A. princeps* var. *orientalis* (Choi et al. 2013), *A. armeniaca* (Mojarrab et al. 2013), *A. sieversiana* (Tang et al. 2015), *A. sacrorum* (Yuan et al. 2016), *A. nilagirica* (Gul et al. 2017), *A. turcomanica* (Mousavi et al. 2018), *A. vulgaris* (Lian et al. 2018), *A. scoparia* (Moulaie et al. 2018), *A. absinthium* (Koyuncu 2018), *A. lactiflora* (Kulprachakarn et al. 2018), *A. herba-alba* (Mohammed et al. 2019), and *A. annua* (Isani et al. 2019). The anticancer potential of various *Artemisia* species was tested in the form of extracts or fractions from methanol (Choi et al. 2013; Lian et al. 2018; Koyuncu 2018; Mohammed et al. 2019), ethanol (Tang et al. 2015; Yuan et al. 2016; Kulprachakarn et al. 2018), hexane and ethyl acetate (Mojarrab et al. 2013; Gul et al. 2017), and silver nanoparticles (AgNPs) (Mousavi et al. 2018;

Moulaie et al. 2018). The anticancer activity tests of various *Artemisia* species that these researchers have carried out include human hepatoma cancer cells, HeLa cervical cancer cells, breast cancer cells, human leukemic cancer cell lines, colon cancer cells, gastric cancer cell lines, and lung cancer cells. The research aims were to determine the anticancer compounds in wild type (W) and polyploid mutant (P) *Artemisia cina* Berg ex Poljakov using NIR and compare their cytotoxicity and selectivity on WiDR colon cancer and HTB183 lung cancer cell lines and Vero normal cell line.

MATERIALS AND METHODS

General experimental procedures

The wild type of *A. cina* plant was collected directly from the plantation land of B2P2TOOT Tawangmangu, Indonesia. The polyploid mutant genotype is a polyploid mutant obtained by inducing *A. cina* shoot culture with 100 mg/l colchicine for 48 hours. The plants were identified at the Herbarium Bogoriense, Research Center for Biology, Bogor, Indonesia. The collection plant (No. 001/2014/FPBUKSW/Koleksi) was deposited in the field laboratory of Faculty of Agriculture and Business, Universitas Kristen Satya Wacana, Salatiga, Indonesia.

The extraction process of plants was performed in Laboratory of Biochemistry and Molecular Biology, Faculty of Biology, Universitas Kristen Satya Wacana, Salatiga, Indonesia. The cancer cell lines namely WiDr and HTB-183 and normal cell lines Vero were obtained from Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. The pure chemicals artemisinin and quercetin were purchased from Aldrich (USA), kaemferol (EMD Millipore Corp., USA), while the rutin was from Sigma (USA). The other chemicals were purchased from Sigma including sodium dodecyl sulfate (SDS), phosphate buffer saline (PBS), absolute ethanol, and hexane, while, RPMI-1640 medium, fetal bovine serum (FBS), and trypsin-EDTA 0.25% were purchased from Gibco (USA). Doxorubicin hydrochloride was obtained from Haarlem (Holland), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) ultra-pure from Bio Basic Canada Inc. (Canada), while Dimethylsulfoxide (DMSO) was from Merck (USA).

Procedures

Preparation of extract

The extracts were made by soxhlet extraction using hexane as a solvent. An amount of 10 grams of sample powder were wrapped in filter paper, then were put in a series of soxhlet apparatus using 250 mL of hexane solvent (Merck). The process was carried out for 6 hours. The extract was obtained and concentrated using a rotary evaporator (Rotavapor R-114 Buchi) with a vacuum pump (Eyela A-1000S).

Cytotoxic assay of extract

The determination of the cytotoxicity limit of the extract used the MTT method with slight modification²

with a minor modification. In this study, the stop solution of formazan formation was not DMSO but SDS. Furthermore, the incubation process was done overnight. About 100 µl cell suspensions containing 1×10^4 cells/wells (WiDr/HTB-183/Vero cells) were put into a 96-well microplate and incubated for 24 hours in a 5% CO₂ incubator at 37°C. The plate was passed out from the incubator, and the medium was discarded. The series of treatment concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL) were dropped to the well according to a predetermined design. In each test time Doxorubicin, with concentration of 300, 150, 75, 37.5, 18.75, 9.375, and 4.6875 µg/mL, is always included and cell control and control medium are prepared. The dish was re-incubated in a 5% CO₂ incubator at 37 ° C for 24 hours. After this, the medium was dropped again. Then 100 µl of culture medium containing MTT of 5 mg/mL was added and incubated for 3 hours in a 5% CO₂ incubator at 37 ° C for 24 hours. After that period, 100 µl of 10% SDS in 0.1N HCl was added as a stopper. The plate was then wrapped in aluminum foil and allowed for one night at room temperature. After one night, the absorbance of each well was calculated on the ELISA reader. The absorbance measurement results are converted to the percentage of living cells (cell viability) using the formula Viability cell (%) = ((Measured absorbance – medium's absorbance) / (cell absorbance – medium's absorbance)) x 100%. The calculation of IC₅₀ value used software of SPSS 16.0 for Windows at Probit analysis.

Calculating of Selectivity Index (SI)

The SI value was obtained by dividing the IC₅₀ values of anticancer compounds in the normal cells Vero found in the cancer cells.

Identification of anticancer compounds

The anticancer compounds detected in this study were artemisinin, quercetin, kaempferol, and rutin. They were analyzed using Near-Infrared Spectrophotometer (NIRFlex N-500). The extract was placed into a sample container. NIR spectroscopy was started with measuring the solvent, followed by the standard compound or sample. The standard compound and sample were dissolved in the solvent. Then the measurement of the test sample was done. The artemisinin, quercetin, kaempferol, and rutin contents were measured using HPLC according to Tokusoglu et al. (2003) method with modification. The HPLC conditions for separation of flavonoid compounds (quercetin, kaempferol and rutin) were using the Chromosorb Column RP C18 (150 x 5 mm id - Knauer), H₃PO₄ 0.1%: acetonitrile (60:40) as the mobile phase, at the flow rate of 1 mL/min, with the volume of injection of 20 µl, ambient temperature, and UV 370 nm detector. Meanwhile, for artemisinin separation, the condition of HPLC was modified in using the Chromosorb column RP C18 (150 x 5 mm id – Knauer), buffer phosphate of 0,01 M

pH 7: methanol (55:45) as the mobile phase, the volume of injection was as much as 20 µl, ambient temperature, and UV 260 nm as detector. The quantification of artemisinin, quercetin, kaempferol, and rutin content in *A. cina* sample extracts was determined using a calibration standard curve of artemisinin, quercetin, kaempferol, and rutin pure compounds.

Statistical analysis

The data from ELISA readers were analyzed using SPSS 16 with Probit analysis to determine the IC₅₀ value. The IC₅₀ presented as a mean ± standard error. The differences in IC₅₀ values between diploid, polyploid mutant, and doxorubicin on the cell line were analyzed statistically using one-way analysis of variance (ANOVA) using the SAS ver. 9.1.3, followed by Tukey test to compare the mean difference between samples (P > 0.05). All experiments were conducted in five replicates.

RESULTS AND DISCUSSION

The anti-cancer compounds of hexane extract of *Artemisia cina*

This research studied the presence of anticancer bio-marker compounds in the hexane extracts of *A. cina*. There are two kinds of *A. cina* studied namely the wild type and the polyploid mutant (Figure 1). The polyploid mutant type of *A. cina* was obtained using colchicine as mutagens. The wild type and polyploid mutants of *A. cina* plants showed differences in growth and leaf morphology (Figure 1).

The anti-cancer compounds of hexane extract of studied polyploid mutant *A. cina* were artemisinin, quercetin, kaempferol, and rutin (Figure 2). The detection of these compounds was using Near Infrared (NIR) Spectrophotometer. The wild-type *A. cina* plants showed the same peak with artemisinin at 4324, 5136, 5678, and 5776 Hertz. It was also shown the same peak at 4524 Hertz with quercetin and kaempferol. It means that the wild-type plants of *A. cina* contain artemisinin, quercetin, and kaempferol. Unlike the wild-type plant, the polyploid mutant plants of *A. cina* showed the same stiff peak with rutin at 5136 Hertz.

The similarity of the peak between this plant with quercetin and kaempferol was at 3156 and 7070 Hertz while with artemisinin was at 4324, 5136, 5678, and 5776 Hertz. It means that the polyploid mutant plants of *A. cina* besides contain anticancer biomarker compounds artemisinin, quercetin, and kaempferol also contain rutin. The flavonoid compounds identified in this study were artemisinin, quercetin, kaempferol, and rutin. The NIR analysis showed that both *A. cina* plants contain artemisinin, quercetin, and kaempferol unless rutin was found in a polyploidy mutant plant only (Table 1).



Figure 1. The different growth response and leaf morphology of the two genotypes of *Artemisia Cina* plants. The wild genotypes (A) were diploid and polyploid mutant (B). Bar = 4 cm

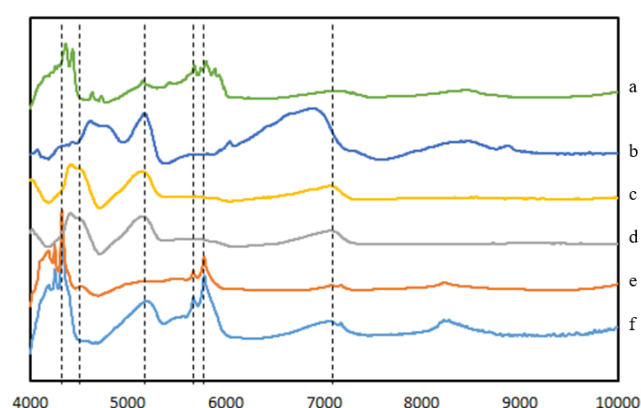


Figure 2. NIR spectra of pure artemisinin (a), pure rutin (b), pure quercetin (d), pure kaempferol (d), wild type of *Artemisia Cina* (e), polyploid mutant of *A. Cina* (f).

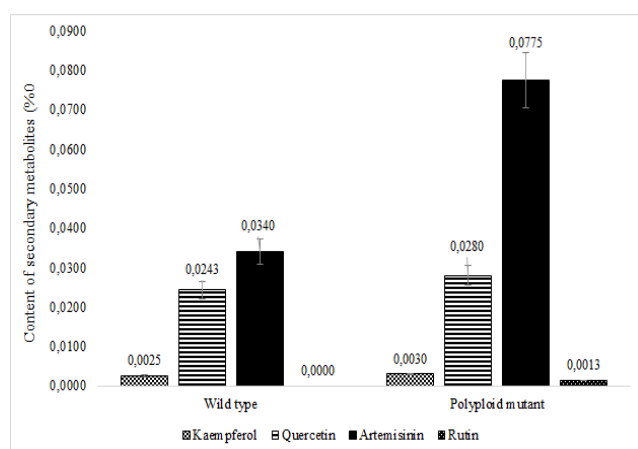


Figure 3. The quercetin, kaempferol, rutin and artemisinin content of wild type (W) and polyploid mutant (P) *Artemisia Cina* was measured by HPLC method

Apart from being detected by NIR spectrophotometer method, the secondary metabolites contained in wild-type *A. Cina* and polyploid mutants plants were also determined quantitatively by the HPLC method. Figure 3 shows the content of four secondary metabolites measured in *A. Cina* wild type and polyploid mutant including 3 types of flavonoid compound (kaempferol, quercetin and rutin) and artemisinin as determined by the HPLC method. Based on the results shown in Figure 3, it can be concluded that the content of kaempferol, quercetin, artemisinin and rutin in wild *A. Cina* and polyploid mutant were different. The *A. Cina* polyploid mutant showed higher content of kaempferol, quercetin, rutin and artemisinin than wild type. Even for wild *A. Cina*, the rutin content was not measured, this was consistent with the detection results using the NIR spectrophotometer method.

The cytotoxicity of hexane extract of *Artemisia Cina* on the WiDr and HTB 183 cell lines

Based on ANOVA analysis, the cytotoxicity of hexane extract of *A. Cina* polyploid mutant plant on the WiDr and HTB 183 cell lines was significantly stronger than the wild-type plant, but less than Doxorubicin cancer drug (Table 2). The cytotoxicity of the polyploid mutant plant (IC_{50} was 84 $\mu\text{g/mL}$ on WiDr cell lines and 128 $\mu\text{g/mL}$ on HTB 183 cell lines) tended to be stronger than wild type plants (IC_{50} was about 300 $\mu\text{g/mL}$ on both of tested two cancer cell lines). However, that strength was still lower compared to the cytotoxicity of doxorubicin that was sixteen-fold weaker on WiDr cancer cell lines and three-fold lower on HTB 183 lung cancer cell lines.

The selectivity index of hexane extract of *Artemisia Cina* on the WiDr and HTB 183 cell lines

Based on IC_{50} values between two cell lines, we can calculate the toxic selectivity of an agent on a particular cell line against another cell line. The selectivity index of both studied plants on WiDr colon cancer and HTB 183

lung cancer toward Vero normal cell was shown in Table 3. Both types of plants had a selectivity index of more than two-point, so did Doxorubicin.

Discussion

The phytochemical, bioactive non-nutrient compounds usually caused the pharmacological activities of plants. Flavonoids are one of the phytochemical compounds from a group of phenolic compounds (Kashani et al. 2012). They could be found in many organs of plants, maybe on roots, barks, seeds, leaves, or fruits. Based on many in vitro and in vivo studies, flavonoids have anticancer activity to induce apoptosis of cancer cells, as agents of antioxidant, immunomodulatory, anti-inflammatory that supporting and restoring the normal functions of cells (Kopustinskiene et al. 2020; Panche et al. 2016). This research studied the presence of anticancer bio-marker compounds in the hexane extracts of two kinds of *A. cina* that were the wild type and the polyploid mutant using NIR spectrophotometer.

Table 1. The anticancer biomarker compounds content of *Artemisia cina* based on the NIR spectra

Plant	Compounds detected			
	Artemisinin	Quercetin	Kaempferol	Rutin
Wild type	+	+	+	-
Polyploid mutant	+	+	+	+

Table 2. Comparison of the cytotoxicity of hexane extract of *Artemisia cina* polyploid mutant and wild type plants on WiDr and HTB 183 cancer cell lines and Vero normal cell lines

Plant	IC ₅₀ value on cell lines (µg/mL)		
	WiDr	HTB 183	Vero
Wild type	295.5±8.81 B	322.4±3.51 B	10.000.5±70.71 A
Polyploid mutant	84.1±0.59 CD	128.6±3.04 C	315.6±78.67 B
Doxorubicin	0.5 ± 0,02 E	36.6±0.61 DE	295.5±2.52 B

Note: WiDr colon cancer cell lines; HTB 183 lung cancer cell lines; Vero normal cell lines; The value represents the mean ± standard deviation (SD) (n=5); Mean values in the same column and row having different letters differ significantly were analyzed using one way ANOVA and followed by Tukey test to compare the mean difference between samples ($p > 0.05$).

Table 3. Selectivity Index of hexane extract of *Artemisia cina* polyploid mutant and wild type plants on WiDr and HTB 183 cancer cell lines against Vero normal cell lines

Sample	SI value on cancer cell lines	
	WiDr	HTB183
Wild type	34	31
Polyploid mutant	4	3
Doxorubicin	591	7

NIR Spectroscopy measures the absorption of the chemical bonds C-H, O-H, and N-H, the main bound of a compound in the near-infrared wavelength range. The absorption spectra of each compound are unique which are commonly referred to as fingerprints (Prieto et al. 2017; Liu et al. 2016). Based on these properties, this study determines the presence of the artemisinin, quercetin, kaempferol, and rutin using the fingerprint of the standard compound of the concerned compound. Several researchers reported this technique in their research. Wulandari et al. (2020) suggested that combination of NIR and multivariate calibrations methods can be used to detect flavonoids selectively and in the way of good validation. The combination of NIR with absorption pattern recognition allows this tool for qualitative and quantitative analysis rapidly and to detect groups of organic compounds (Liu et al. 2016) of various compounds such as foodstuffs, agricultural products, pharmaceutical products, etc. This method has several advantages including no need for reagent, rapid detection, non-destructive preparation, and ecofriendly (Wulandari et al. 2020; Ma et al. 2019; Liu et al. 2016; Patchava et al. 2015). In this study, the samples were extracted for the purpose of cytotoxicity assay.

For the purpose of quantifying the levels of the compound, analysis was carried out using HPLC. Today, HPLC is recognized as a sophisticated detection tool for the qualitative and quantitative purposes of various compounds, including medicinal compounds (Rao et al. 2015). Like NIR, HPLC is also a tool for fingerprint detection in the quality of herbal plants (Fan et al. 2006). Several researchers reported the use of HPLC for the characterization and quantification of the compounds quercetin, kaempferol, routine, and artemisinin. The recovery using HPLC for analysis of kaempferol and quercetin of leaves, flowers, and fruits methanol extracts of *Moringa oleifera* was about 95-99% (Meghani et al. 2018).

In general, the type and amount of extracted compounds depend on the polarity of the used solvent (like dissolve like principle). Quercetin, kaempferol, and rutin belong to the flavonoids compounds. They were polyphenolic compounds, while artemisinin is a sesquiterpene. In this study, we used hexane as a solvent to extract these compounds because based on their structure, it is possible for these compounds to be extracted in hexane. Many researchers reported that they found artemisinin and flavonoid in hexane extract although less than other more polar extracts while (Rivai et al. 2019) reported that there is no flavonoid, phenol, tannin, or alkaloid compared to acetone and ethanol extract hexane extract of bay leaf among the four analyzed compounds, the wild type genotype contained three of them namely artemisinin, quercetin, and kaempferol, while the polyploid genotype contained four of them, including rutin.

The four metabolites measured in these studies are classified as secondary metabolites which are generally produced by plants, except for artemisinin which plant members of the genus *Artemisia* specifically produce. According to Ramakrishna and Ravinshanka (2011), the production of secondary metabolites in plants is influenced by both external and internal factors. Several external

factors that affect the production of secondary metabolites are temperature, light, humidity, water availability and nutrient supply. One of the internal factors that influence the production of secondary metabolites is plant genotype. The increased production of secondary metabolites through polyploid induction has been carried out in various plants. In contrast to the results of this study, Caruso et al. (2013) reported that polyploidization (tetraploid) in *Solanum bulbocastanum* did not significantly increase the production of secondary metabolites in the form of phenylpropanoids, tryptophan, tyrosine, and α -chaconine compared to their diploid parents, but only significantly increased their saponin content. Parson et al. (2019) also reported that polyploidy in *Cannabis sativa* did not increase the production of all secondary metabolites that were targeted. Polyploidization in *Cannabis sativa* only changed the secondary metabolite profile and increased cannabidiol up to 9%, but did not significantly increase tetrahydrocannabinol. Parida and Misra (2015) reported that polyploidization in plants that occurs naturally or is artificially induced increases primary metabolism, secondary metabolism (such as biosynthesis of terpenoids, phenylpropanoids, flavonoids, and alkaloids), and constituents of bioactive compounds. The artificial polyploidization using the colchicine mutagen in *Artemisia annua* (Banyai et al. 2010; Yunus et al. 2018) and *Artemisia cina* (Herawati et al. 2015) is reported to increase the content of artemisinin. However, the content of secondary metabolites in polyploid plants is not always higher than its diploid parents. According to Parida and Misra (2015) the lack of superiority of polyploid plants compared to diploid can be related to several things, including the time needed for adjustment, adaptation and evolution after the induction of genomic changes by polyploidization and the fact that the optimal level of ploidy among species varies and is very important. Therefore, polyploidization as a strategy to increase secondary metabolite production cannot be generalized to all medicinal plants.

Phytochemical screening of flavonoid and phenolic compounds showed positive in hexane extract of *Trigonella foenum-graecum* leaves (Sambandam et al. 2016), *Morus nigra* leaves (Mallhi et al. 2018). The presence of quercetin and kaempferol in hexane extract was reported by Hardinsyah et al 2019 in a study of mature gandaria leaves. The content of quercetin, kaempferol, and rutin on five Turkish *Artemisia* species, quercetin was found in almost all species except in *A. haussknechtii*, kaempferol was not found in *A. incana* and *A. haussknechtii*, while rutin was only found in *A. Haussknechtii* (Kursat et al. 2015). Identification of *A. absinthium* leaf (Lee et al. 2003) and *A. dracunculus* aerial parts (Ibrahim 2017). showed that they contained quercetin and kaempferol. Many researchers reported the presence of artemisinin in hexane extract of various species of *Artemisia*. *A. annua* plant contained artemisinin between 0.07% and 0.45% based on dry mass (Numonov et al. 2019). The yield of artemisinin in between hexane, methanol, and ethyl acetate extract was not significantly different by Microwave-assisted extraction (Misra et al.

2014; Soktoeva et al 2013) but significantly different in extraction using soxhlet (Misra et al. 2014). That finding was consistent with Croteau (2000) statement that the kind and accumulation of secondary metabolites in plant organs varies. Mannan et al (2010) reported on the results of the analysis of artemisinin concentrations in flowers, leaves, roots, and stems of *A. annua* and 14 other species of *Artemisia* using the HPLC method. Based on the artemisinin analysis on the 15 *Artemisia* species, it was shown that the artemisinin concentration in plant organs was found in the order of flowers > leaves > stems > roots.

Many research represented that those compounds from *Artemisia* shown anticancer activity (Crespo-Ortiz & Wei 2012; Zhu et al. 2013; Dixit 2014; Yuan et al. 2016). The differences in phytochemical compounds will cause different phytopharmacological activities (Almehdar et al. 2012; Arullappan et al. 2013; Kashani et al. 2012). It seems in this study that the differences in rutin content between wild-type and polyploid mutant plants significantly caused the difference in their anticancer ability. The cytotoxicity of wild-type extracts against the two test cancer cells was very low and was not significantly different from the cytotoxicity of normal Vero cells. The polyploid mutant plant that contained rutin had cytotoxic on cancer cell lines significantly stronger than the wild type, in this case, both to WiDr colon cancer and HTB 183 lung cancer cell lines (Table 2). Rutin isolated from *Triticum aestivum* Straw caused significantly increased levels of enzymes involved in oxidative stress and chemopreventive activity on mice induced by carcinogenic compound DMBA (Dixit 2014). It could suggest that the rutin content on the polyploid mutant plant of *A. cina* was a synergy with other flavonoids compounds, so it increased its cytotoxic activity.

Another parameter to predict the potency of a plant as an anticancer agent is the Selectivity Index value. The Selectivity Index (SI) is the selectivity of the cytotoxic properties of an anticancer agent against a cancer cell compared to other cells, in this study, a normal cell. The greater the SI value the more selective it is. An agent with SI of more than 2 points is considered to have high selectivity (Badisa et al. 2006). Both of two studied plants had high selectivity on both studied cancer cell namely WiDR colon cancer (SI were 34 of wild type and 4 of polyploid mutant) and HTB183 lung cancer cell lines (SI were 31 of wild type and 3 of polyploid mutant) because their SI were more than 2 points. A drug candidate could be considered to have therapeutic potential if it has SI higher than standard anticancer drugs. In this study, extract hexane of wild type of *A. cina* Berg ex Poljakov is considered to have therapeutic potential against lung cancer because its SI (31 points) was higher than SI of doxorubicin, the standard anticancer drug.

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