# The potential of *Artemisia cina Berg ex Poljakov* as Antioxidant Agent

**E B E Kristiani[1], S Kasmiyati [1,3] and M M Herawati[2,3]**

[1] Biological Faculty, Universitas Kristen Satya Wacana, Jl. Diponegoro 52-60, Salatiga, Central of Java, 50711, Indonesia
[2] Faculty of Agriculture and Bussiness, Satya Wacana Christian University, Jl. Diponegoro 52-60, Salatiga, Central Java, 50711, Indonesia

[3]E-mail: betty.elok@uksw.edu, marinakartika@gmail.com

**Abstract**. This study aimed to determine the quercetin and kaempferol contents, and antioxidant activity of wild-type A. *cina* (TWN and KJT genotypes) and its polyploid mutant (J and M genotypes). KJT and TWN genotypes obtained from B2P2TOOT Tawangmangu. TWN genotype obtained directly from the land while KJT genotype through shoot culture. J genotype is a polyploid mutant obtained by inducing *A. cina* shoot culture with 100 mg/l colchicine for 48 hours. M genotype obtained by plant growth regulator induction with 2 mg/ml of benzyl adenine and 3 mg/ml of 2.4 D combination for 21 days. The sample prepared by the maceration method using solvent ethanol and ethyl acetate. The determination of quercetin and kaemferol content was using High-Performance Liquid Chromatography, while antioxidant activity was using the DPPH method with ascorbic acid as antioxidant agent standard. The quercetin content of TWN, KJT, J, and M were 0.0026, 0.0023, 0.0026, and 0.0030% while kaempferol were 0.0029, 0.0021, 0.0030, and 0.0028% respectively. The antioxidant activity showed an $IC_{50}$ value. The $IC_{50}$ of ethanol extracts TWN, KJT, J, and M were 26.3, 14.1, 13.7, and 13.3 mg/ml while ethyl acetate was 37.1, 14.9, 20.0, and 17.8 mg/ml respectively. The $IC_{50}$ of acetic acid was 0.014 mg/ml.

## 1. Introduction

*Artemisia* is one of the most abundant genera of the member of the Asteraceae family that received considerable interest in biological and pharmaceutical aspects. These genera have wide distribution and used in traditional medicine systems to treat various diseases. Several *Artemisia* species have investigated chemical constituent and physiological activities such as antioxidant, antimalarial, anticancer, antifungal, antiviral, and insecticidal. These biological activities have demonstrated to result from various medicinally critical secondary metabolites of the plant, such as terpenoids, flavonoids, coumarins, caffeoylquinic acids, and sterols [1-2]. The current research about Asteraceae plants shown that besides containing artemisinin, it also contained flavonoids, natural phenolic compounds that had potential antioxidant activity. Medicinal plants such as *Artemisia* with a high content of bioactive compounds are the potential to be used as natural antioxidant agents [3]. Chemical composition of several *Artemisia* species has investigated in inhibiting or quenching free radicals and reactive oxygen species and their relation of antioxidant activity [4]. Some *Artemisia* species that have studied for antioxidant activity and their chemical composition are *A. annua* [5-6], *A. chamaemelifolia* [7], *A. monosperma* [4], *A. absinthium* [8-9], *A. herba-alba* [10-12], *A.*

*campestris* [13,2], *A. sieberi*, *A. judaica* and *A. monosperma* [14]. Secondary metabolites such as flavonoids and artemisinin reported have played a role in antioxidant activity. The antioxidant activity of secondary metabolites (flavonoids, terpenoids, phenolics) caused by their ability to reduce the electron charge, donor the hydrogen, and chelate metal [15-16].

*A. cina* is one species of member the Asteraceae family. It belongs as weeds plant in the plateau of Indonesia. *A. cina* is an aromatic shrubby plant and has been extensively used in traditional medicine as an anthelmintic agent. *A. cina* also reported has biological activities as antitumor, antimalaria, and antibacterial [17-18]. The medicinally critical secondary metabolites of *A. cina* is L-santonin, artemisinin, monogynin, mibulactone, pseudosantonin, and desoxypseudosantonin [19]. One obstacle to using plants as a source of natural medicine is the low content of bioactive compounds produced by plants. The formation of secondary metabolites compounds by a plant is affected by genetic and environmental factors such as ploidy of chromosome, light intensity, altitude, and cultivation condition. Improvement of the number chromosome through artificial polyploid is a technique for increasing secondary metabolites production compounds of a plant. Artificial polyploid induction of *A. annua* using colchicine reported raising its artemisinin content [20-21]. This study aimed to determine the quercetin and flavonoid contents and antioxidant activity of wild-type *A. cina* (TWN and KJT genotypes) and its polyploid mutant (J and M genotypes).

Many diseases in the body are closely related to the oxidation process in cells. Information about Artemisia's antioxidant ability will open up opportunities for further excavation to obtain antioxidant agents to overcome various diseases

## 2. Material and Method

### *2.1. Material*

The *A. cina* plant, namely KJT and TWN genotypes, collected from B2P2TOOT Tawangmangu. TWN genotype received directly from the plantation land while KJT genotype through shoot culture. J genotype is a polyploid mutant obtained by inducing *A. cina* shoot culture with 100 mg/l colchicine for 48 hours. M genotype obtained by plant growth regulator induction with 2 mg/ml of benzyl adenine and 3 mg/ml of 2.4 D combination for 21 days. The plants identified at the Research Center of Biology in Indonesia, namely Herbarium Bogoriense. The collection plant (No. 001/2014/FPBUKSW/Koleksi) deposited in the FPB filed laboratory, Biological Faculty, UKSW.

### *2.2. Preparation of extract*

The whole aerial parts plant collected at the vegetative phase. All of the plant material was cleaned using tap water and then dried in the air-dried and oven for five hours at 40 $^{o}$C. The dry plant grind in a blender (Philip HR1538). As about 50 grams of powder sample macerated using solvent ethanol and ethyl acetate separately for 24 hours. After that, the extract filtered and concentrated using a rotary evaporator at 40 $^{o}$C (Rotavapor RE 100 Pro) using a vacuum (Eyela A-1000S).

### *2.3. Determination of quercetin and kaempferol*

HPLC analyzed quercetin and kaempferol according to [22] with slight modification. The pure quercetin and kaempferol used as standard compounds. The 2.5 g powdered sample was added with 25 ml methanol contained HCl 1% then sonicated for 30 minutes using Sonicator Krisbow DSA50-GL2-2,5L. The filtrate was filtrated and combined with the solvent like before up to 25 ml and 5 ml HCL 1.2 M. The mixture was refluxed for 2h. The extract was cooled to room temperature, re-sonicated for 3 minutes, then filtered using a 0.45 µm membrane filter. The filtrate was ready to be injected into the HPLC (Knauer Germany Series Smartline). The modification conditions of HPLC included the chromosorb column RP C18 (150x5 mm id), Knauer, the mobile phase was H3PO4 0.1%: acetonitrile (60:40), the flow rate was 1 ml/min, the volume of injecting was 20 µl, ambient temperature, and using UV 370 nm detector.

*2.4. Assay the activity of antioxidant*

The antioxidant activity of the extract determined using 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay [23] with a slight modification. The concentration series of extract analyzed were 0.0, 5,0, 10.0, 15.0, 20.0, and 25.0 mg/ml. Ascorbic acid (Merck 250) in concentration series 0.0, 5,0, 10.0, 15.0, 20.0, and 25.0 µl was used as standard antioxidant agent. In the reaction tube, extract as much as 1 mL added with DPPH 60 ppm in methanol as much as 2 ml. About 2 ml of DPPH (Sigma) 60 ppm in methanol prepared as a control. The mixture shakes using vortex until it thoroughly mixed then incubated in the dark for 30 minutes. The measurement of the mixture's absorbance used a UV-Vis spectrophotometer (Hitachi UV mini 1240) at 517 nm wavelength.The calculation of antioxidant activity using the equation antioxidant activity (%) = $((1 - A_{sampel})/A_{control})$ x 100%. Furthermore, the linear regression equation between extract concentration versus antioxidant activity is determined. The $IC_{50}$ value was calculated based on the linear regression equation antioxidant.

*2.5. Data analysis*

All experiments repeated three times. The data were analyzed the one-way analysis of variance (ANOVA) using the SAS program (version 9.1.3). If they're significantly different, The ANOVA followed by Duncan multiple range tests was used to compare the mean difference between samples, with significantly at $P < 0.05$.

## 3. Result and Discussion

*3.1. Quercetin and Kaempferol content*

Figure 1 was showed the quercetin and kaempferol contents in *A. cina* Berg ex Poljakov. KWN and T genotype were the wild types of sample plants, while J and M genotype were the mutant species.

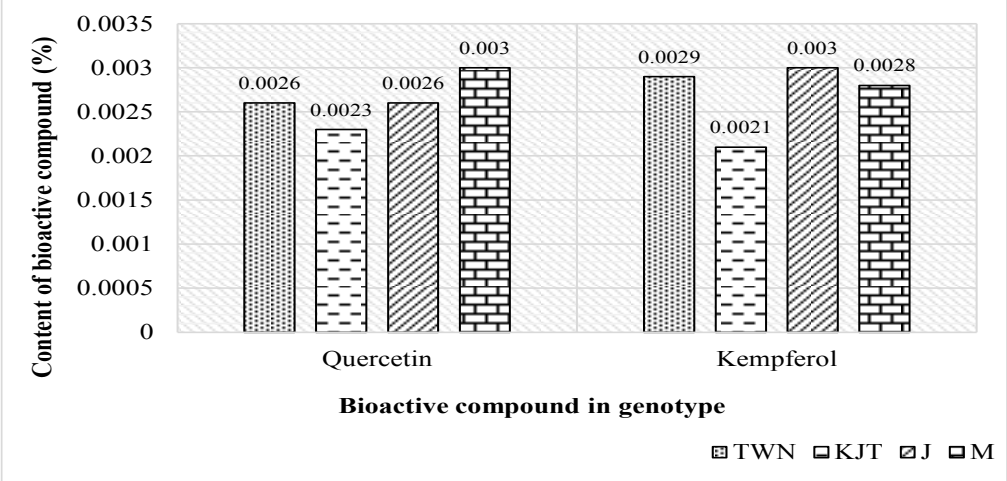


**Figure 1**. The quercetin and kaempferol content of wild type and polyploid mutant *A. cina* Berg ex Poljakov. TWN: wild genotype obtained from the plantation land; KJT: wild genotype obtained through shoot culture; J: polyploid mutant genotype obtained by inducing *A. cina* shoot culture with 100 mg/l colchicine for 48 hours. M: polyploid mutant genotype obtained by inducing *A. cina* to shoot culture with plant growth regulator 2 mg/ml of benzyl adenine and 3 mg/ml of 2.4 D combination for 21 days

There shown that shoot culture treatment to *A. cina* by inducing with a plant growth regulator that was the combination of benzyl adenine and 2.4 D increased the production of quercetin until 15% while inducing with increased the production of kaempferol until 13%.

Quercetin and kaempferol are secondary metabolites compound members of the flavonoid group, which is generally produced by plants. The accumulation of secondary metabolites in plants much variety. Based on the research result by [24] reported that the content of quercetin in *A. absinthium* L., *A. vulgaris* L., *A. austriaca* Jack, *A.verlotiorum* Lamotte, and *A. caucasica* Willd were 0.024, 0.0053, 0.0015, and 0.045 % while kaempferol content of them were 0.0036, 0.0037, 0.0004, 0.0053, and 0.0031 % respectively. The content of quercetin in *A. armeniaca, A. incana, A.tournefortiana,* and *A. scoparia* were 0.0223, 0.0013, 0.0101, and 0.0645 % while kaempferol content of them were 0.0036, 0.0, 0.00022, and 0.0262 % respectively [25]. At the same research, *A. haussknecbtii* has no both the two compounds.

Many factors could influence the secretion of secondary metabolites compounds, both extrinsic and intrinsic. Temperature and light, humidity, light, the supply of water and mineral are some of the extrinsic factors [26]. The quercetin content of A. annua varied depending on the habitat of growth, namely at the background polluted zone about 0.022 - 0.034 %, at the traffic polluted zone as about 0.035 – 0.044 %, at the industrial polluted zone as about 0.047 – 0.063 % while at the different altitude as about 0.028 - 0.032 % [27]. Colchicine is an alkaloid that may be applied to induce autotetraploid plants [28]. Colchicine could inhibit the division of plasma but does not inhibit the division of the nucleus. As a result of cell division, the number of chromosomes doubled. So, colchicine is a very effective substance for the polyploidy process.

*3.2. Antioxidant activity*

Many research found that quercetin and kaempferol have the ability as an antioxidant agent [29-31]. In the antioxidant process, the antioxidant agent gave a hydrogen atom to DDPH radicals and caused the color of DPPH from purple to yellow. The smaller the value of IC50, the higher the antioxidant ability. Fig. 2 and 3 showed the antibacterial activity of TWN, KJT, J, and M genotype that analyzed using DPPH assay.

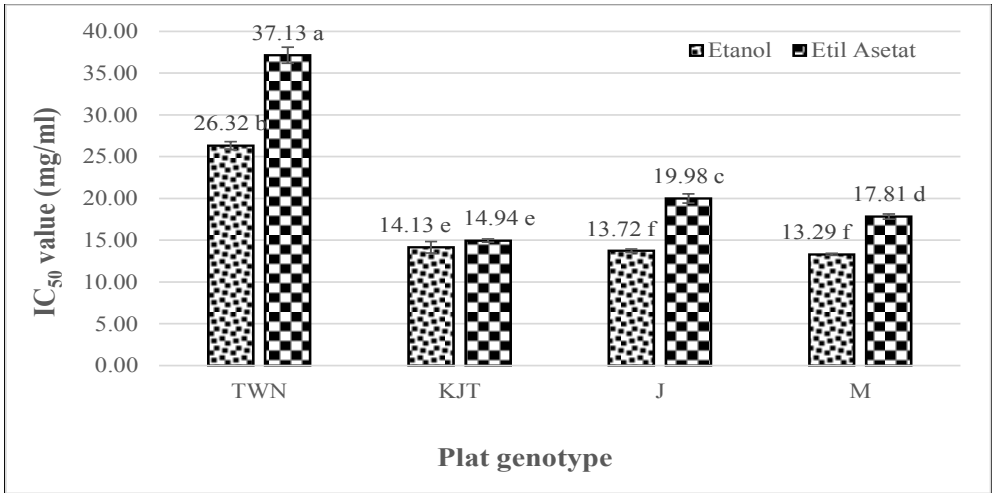


**Figure 2**. The antioxidant activity of ethanol and ethyl-acetate extract of wild type and polyploid mutant *A. cina* Berg ex Poljakov

The antioxidant activity expressed as an IC50 value. The IC50 value is the extract concentration needed to reduce 50% of free radical activity. The IC50 of TWN, KJT, J, and Methanol extracts were 26.3, 14.1, 13.7, and 13.3 mg/ml while ethyl acetate was 37.1, 14.9, 20.0, and 17.8 mg/ml respectively. The IC50 of acetic acid was 0.014 mg/ml.

As a member of phenolic compounds group, quercetin and kaempferol are compounds plentiful hydrogen. Their ability as an antioxidant caused by its ability to donate hydrogen atoms to oxidant compounds like DPPH to make it more stable in reduced form or because it is an ability as hydrogen donor which scavenge an active oxygen species [15,32]. In other words, flavonoids stabilize the reactive oxygen species (ROS) by reacting themselves with the reactive compound, namely the radical or oxidant compound [29]. The antioxidant properties of quercetin are due to three factors, namely, the group of catechol groups in the ring of beta, a double bond bounding, and a substitute of the hydroxyl group at 3 and 5 positions [33]. Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical.

In all genotypes, ethanol extract has significantly higher antioxidant activity than ethyl acetate extract (Fig. 2). It might cause many secondary metabolites, which are antioxidants are polyphenol compounds, so they tend to be polar. Flavonoids that are polyphenolic compounds [34] means that they tend to be polar. Ethanol is more polar than ethyl acetate so that the bioactive compounds carried in the ethanol extract are more than the ethyl acetate extract. *Artemisia annua* contains hydroxylated flavonoids that can extract in polar media, such as water or hydro-alcoholic solvents [35].

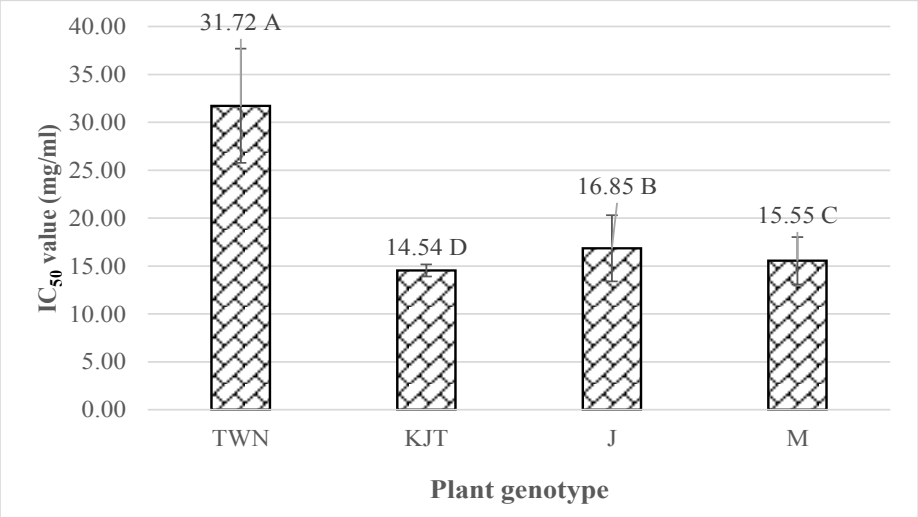


**Figure 3**. Comparison of the antioxidant activity of wild type and polyploid mutant *A. cina* Berg ex Poljakov extract.

Based on Figure 3, it has appeared that the multiplication of plants through shoot culture and induction both with colchicine and plant growth regulator might increase the antioxidant ability of plants. The antioxidant activity of KJT, J, and M (IC50 value is about 14 – 16 mg/ml) significantly more than TWN (IC50 value is 31 mg/ml). The increase of antioxidants may be because of the addition of colchicine and plant growth regulator being stressful for plants, which ultimately induced the formation of secondary metabolites included quercetin, kaempferol, and other secondary metabolites.

The role of secondary metabolites other than Q and K as antioxidant compounds seen in the KJT conditions. In KJT, although there was no increase in Q and K levels (Fig. 1), antioxidant activity increased significantly, which was probably influenced by other secondary metabolite compounds such as tannin or phenolic compounds (not calculated in this research). According to [36-38] were explained that not only flavonoids, phenolics, and tannins also have the ability as an antioxidant.

Ascorbic acid, as an antioxidant standard, shown robust activity (IC50 value of 14.6 µg/ml). The IC50 value of all sample *Artemisia cina* plants was higher than ascorbic acid. That is because the sample extract is a crude form result from maceration, not yet further purified. When it be related to the content of quercetin and kaempferol content, which total at the range 0.0045-0.0060%, then if the

extract is purified, the antioxidant activity can be increased by decreasing the IC50 value to tens of times lower than ascorbic acid.

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