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# Antioxidant Activity of Extract Cantigi (*Vaccinium varingiaefolium*) (Bl.) Miq and Its Major Compound of GC MS NIST Library Analysis

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**ABSTRACT**

Antioxidant are an important topic in various disciplines especially in the fields of medicine and health. The theory about compounds of radicals, free radicals and antioxidants is growing most diseases are preceded by excessive oxidation reactions in the body. *Vaccinium varingiaefolium* (Blume) Miq. belongs to Ericaceae family and elucidate its major compound contribute to ease free radicals as an antioxidant effect to human body. The DPPH (2,2-diphenyl-1-pikrilhidrazil) method is one test to determine antioxidant activity. The DPPH method provides information on the reactivity of compounds tested with a stable radical. The spectrum of unknown component was compared with the spectrum of NIST library. The name molecular weight and structure of the component of the test material were as retained. The experiment shows it has moderate anti-oxidant activity, the methanol extract have 15 compounds that have similarity more than 70% with NIST library whereas 1 compounds formula cannot be detected in databases. There are five major compounds with percentage above 8%. Four compounds mostly in the form of fatty acid methyl ester (FAME) in plant. There are limited data of the of biological activities of those compounds. Some paper said that fatty acid in plant used for biodiesel. One of interesting compound is Dichloroacetic acid (DCA). Latest study shows that DCA have potential in suppressing cell proliferation in cancer cells however some study found its carcinogenic effect.

***Keywords***: *antioxidant Cantigi, Vaccinium varingiaefolium (Blume), Miq, GCMS*

## 1. INTRODUCTION

This Indonesia has a plenty of endemic plants which spread out over the nation. One of them is *Vaccinium varingiaefolium* (Blume) Miq. belongs to Ericaceae family. The local name of this plant is Mentigi or Manis Rejo, in different region for example in the West Java this plant famous by the name of purple Cantigi. This endemic plant has a similarity with billberry (V. *myrtillus*) and blueberry (V. *corymbosum*). Even this mentigi is to be called "the bilberry of Java" . The group of bilberry and blue berry rich of antioxidant. Cantigi who one of types of bilberry extract may have antioxidant activity. In Java, this plant grows well in the area closed to Sulphur vents or volcano region [1]. Cantigi (*Vacinium varingaefolium*) (Bl.) Miq) is a plant that grows on the island of Java naturally and only in high agricultural areas above 1,000 above the sea level. This plant is known as a plant that withstands various grips, most of which is information about the origin of typical craters on land [2]. Extract ethanol 70% of Cantigi stem have analgetic effect on mouse at dose 1200 mg/kg body weight [3]. At the same dose, it also has anti-inflammatory activity in the mouse, the doze has reduced the plantar induce by carrageenan. The inflammatory test was performed using carrageenan for induction inflammatory effect [4].

Today's antioxidants are an important topic in various disciplines. Especially in the fields of medicine and health, the theory of compounds of radicals, free radicals and antioxidants is growing. This is based on the understanding that most diseases are preceded by excessive oxidation reactions in the body [5]. Cantigi (*Vacinium varingaefolium*) (Bl.) Miq) has 2 color leaves in the apex its color is red to purple while the older leaves color is green. In plant red or purple color may rich of anthocyanin as the flavonoid it may effective for antioxidant.

Cantigi has not been widely studied as a medicinal plant. In this study the methanol extract of the cantigi apex leaves that have red color was examined as the antioxidant activity and the metabolites contained in the extract were examined with GCMS.

## 2. METHOD

### Free Radical Damping Method (DPPH)

The DPPH (2,2-diphenyl-1-pikrilhidrazil) method is one test to determine antioxidant activity. The DPPH method provides information on the reactivity of

compounds tested with a stable radical. So that if DPPH is used as reagent in free radical capture tests it is sufficiently dissolved and will be stable for several years if stored in dry conditions [6]. The cantigi has not been test as an antioxidant. The flavonoid compound in its leaves could have antioxidant effect.

**GCMS**

The spectrum of unknown component was compared with the spectrum of NIST library. The name molecular weight and structure of the component of the test material were as retained.

from the leaves of the plant cantigi (*Vacinium varingaefolium*) (Bl.) Miq). Extraction method is done by percolating simplicia which has been dried under the sunlight. The anti-oxidant effect was tested by DPPH immersion method. [6] The principle of DPPH free radical reduction method is the capture of hydrogen by DPPH from antioxidants. Based on the reduction of colored DPPH free radicals by free radical inhibitors which involves measuring the decrease in DPPH absorption at its maximum wavelength, which is comparable to the concentration of free radical inhibitors added to the DPPH solution. This activity is expressed as an effective concentration EC50 or inhibitory concentration IC50, which is the concentration of an antioxidant which can cause 50%

$$\% \text{ inhibition} = \frac{(\text{absorbance blank} - \text{absorbance sample})}{100\%} \times$$

DPPH to lose the radical character or concentration of an antioxidant which gives a 50% percent inhibition. [7].

**Extraction**

25g of finely ground (100 mesh) and sieved dry reddish dry leaves (simplicial) was dissolved with 12.5mL of methanol p.a, put into a closed vessel for at least 3 hours. Filtrate from percolator ware collected in bottle with dripping flow rate1mL per minute after overnight immersion. The concentrated filtrate with rotary evaporator at 60°C (Heidolph), Finally using water bath for final concentration process.

**Anti Oxidant**

The DPPH (2,2-diphenyl-1-pikrilhidrazil) method is one test to determine antioxidant activity. The DPPH method provides information on the reactivity of compounds tested with a stable radical. So that if DPPH is used as reagent in free radical capture tests it is sufficiently dissolved and will be stable for several years if stored in dry conditions. [6]. DPPH provides strong absorption at a wavelength of 515 nm with dark violet colors. Catching free radicals causes electrons to become pairs which then causes color loss which is proportional to the number of electrons taken [7]. The serial extracts were tested using spectrophotometry Optizen model 5U4708-12606-00. The test steps are as follows:

- **Measure the maximum wavelength absorbance of DPPH**

As much as 2mL of 0.2mM DPPH solution were put into the test tube and then added 2mL of methanol p.a shake until homogeneous then poured into the insert cuvette into a UV-Vis spectrophotometer with a wavelength of 400-800nm [8]

- **Blank solution**

A 3mL DPPH 0.2mL solution was put into a test tube, 3mL methanol p.a was added then cover the test tube with aluminum foil, then shaken until homogeneous and measured with a UV-Vis spectrophotometer at wavelength with maximum DPPH absorption [9]

A series of concentrations of 20, 40, 60, 80 and 100 ppm of methanol extract were made. Each 3mL test solution was put into a test tube. Added 3mL DPPH 0.2mM solution, then homogenized then incubated at 37°C in a dark room for 30 minutes.

- **Vitamin C Comparison Solution**

The concentration of 2, 4, 6, 8, 10 ppm was made. each concentration of the vitamin C comparison solution is 3mL, homogenized and then incubated at 37°C in a dark room for 30 minutes. As much as 5mg of vitamin C powder was dissolved with 50mL of methanol p.a in a 50mL Each concentration of vitamin C standard on solution as much as 3mL, homogenized then incubated at 37°C in a dark room for 30 minutes. [10]

- **IC50 (inhibitory concentration**

The parameters commonly used to interpret the results of the antioxidant activity test using the DPPH method are Inhibition concentration (IC50) or often called IC50 value, which is concentration which causes a 50% loss of DPPH activity to calculate IC50 values. Percentage of inhibition can be calculated using the following formula;

The sample concentration and percent inhibition obtained were plotted on the x and y axes in the linear regression equation, respectively. This equation is used to determine the IC50 value of each sample stating with the value of y of 50 and the value of x to be obtained as IC50.

Table 1. Antioxidant Categories Based On IC50 Value

| Category | Nilai IC50 |
|---|---|
| Very Strong | < 50 |
| Strong | 50 -100 |
| Moderate | 101-150 |
| Weak | 151 - 200 |

**Major Compounds analysis**

Analysis of extract content was performed on gas chromatography tools Agilent Technologies 6890N and Mass Spectrometry Agilent Technology 5975B with MSD detector. The column used by Agilent 19091S-433 32 325 C Max HP-5% Phenyl Methyl Siloxane Capillary 30.0 m x 250 µm x 0.25 µm nominal. Gas carrier 1mL per min, Split 10: 1. MSD Software Turbomass Detector 5.2 inject sample: 2 µl. Oven temperature program 110°C up to 200°C at the rate of 10°C/min -no hold: up to 280°C at the rate of 5°C/min 9 min hold: Injector temperature 250°C. Total GC running time 36 min.

**MS Program**

Library used NIST Version Year 2005: Inlet line temperature 200°C. Electron energy 70 eV: Mass Scan (m/z) : 45 45D : Solvent Delay : 0-2 min: total MS running time 36 min. Interpretation on GC mass spectrum was conducted using the database of National

Institute Standard and Technology having more than 62 patterns. The spectrum of unknown component was compared with the spectrum of NIST library. The name molecular weight and structure of the component of the test material were as retained.[11]

## 3. RESULTS AND DISCUSSION

### A. Extraction

Extraction is done by cold method of percolation, this method uses an always new solvent which is repeatedly added and flows through filter paper and flannel, the weight of the sample used is 25g of dried leaf simplicia, methanol used in extracting approximately 300mL and 250mL of yield then concentrated by evaporating the liquid with a rotary evaporator and evaporated in the water bath until a thick extract of 7,524g was obtained, the extraction process took 3 to 4 days. Percent of yield obtained was 30,09%b/b.

### B. Anti-oxidant activity

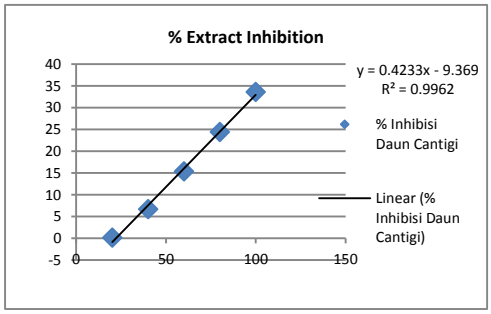


Figure 1. Antioxidant Activity of *Vaccinium Varingiaefolium* (Blume) Miq

Table 2. Antioxidant of *Vaccinium Varingiaefolium* (BLUME) Miq

| Extract Concentration Cantigi of Leave (ppm) | Antioxidant Activity (%) |
|---|---|
| 20 | 0,12 |
| 40 | 6,69 |
| 60 | 15,35 |
| 80 | 24,36 |
| 100 | 33,61 |

Table 3. IC50 Antioxidant of *Vaccinium Varingiaefolium* (BLUME) Miq

| Sample | IC50 (µg/mL) |
|---|---|
| *Vaccinium varingiaefolium* (Blume) Miq. | 141,22 |
| Vitamin C (control positif) | 6,97 |

The amount of antioxidant activity is characterized by the number IC50, which is the concentration of the sample solution needed to inhibit 50% of DPPH free radicals. The test results showed cantigi leaf extract as an antioxidant with IC50 141,22µg/mL. IC50 value of cantigi leaf extract is smaller than vitamin c with IC50 value of 6.97µg/mL. This shows that cantigi leaf extract has a moderate category of antioxidant activity which has an IC50 value of less than 150 µg/mL and vitamin c has a strong category of antioxidant activity which has an IC50 value of less than 50 µg/mL.

Testing of antioxidant activity at various concentrations showed that higher concentrations showed higher antioxidant activity but when compared to vitamin C cantigi leaves had lower antioxidant activity. The other study of antioxidant activities if cantigi find that its fruits has strong antioxidant activities. Inhibition concentration of cantigi fruit was 44,994 ppm while this study finding the apex leaves of red to purple of cantigi leaves was 141,22 ppm (mg/mL). The antioxidant activities in cantigi fruits stronger than its apex leaves. [12]

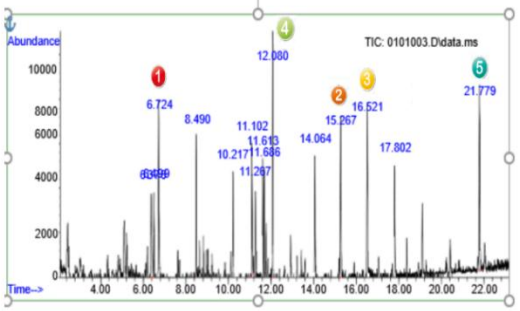


Figure 2. Structure of Cantigi (*Vacinium Varingaefolium*) (Bl.) Miq) Methanol Extract

1 Dodecanoic acid methyl ester
2 Nonadecane
3 Hydroxylamine o-decyl
4 S-[N-[Adamantyl]amidino]methyl hydrogen thiosulfate
5 Dichloroacetic acid decyl ester

From methanol crude extract analysis with GC MS appear 15 peaks pattern represent of 15 compounds. There are 5 compounds major peak that have similarity more than 70% compare with NIST library. The 4 Highest figure profile as follow:

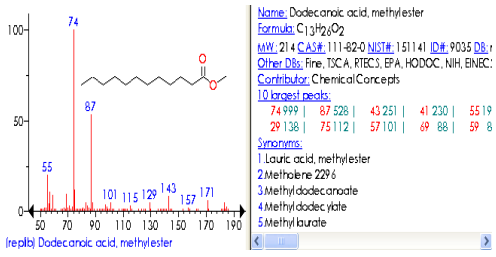


Figure 3. Dodecanoic Acid Methyl Esther Cromatogram

Each molecule has the same fragmentation properties with the same ionization conditions. Patterns in the

spectrum are compared with patterns in the NIST library. Dodecanoic acid methyl Esther fragmentation spectra have similarities with 5 other molecules namely lauric acid methyl ester, Methylene, Methyldodecylate, Methyllaurate. The most stable fragment of all has 74 mw.

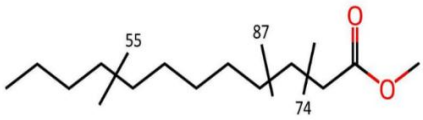


Figure 4. Dodecanoic Acid Methyl Esther Fragmentation Sites

The fragmentation of Dodecanoic acid methyl Esther shown in figure 4. Two fragmentation near functional group and the one was at the hydrocarbon tail. There are three fragmentation with the molecular size 55, 87 and 74. The mass were match with the fragmentation site in the figure above.

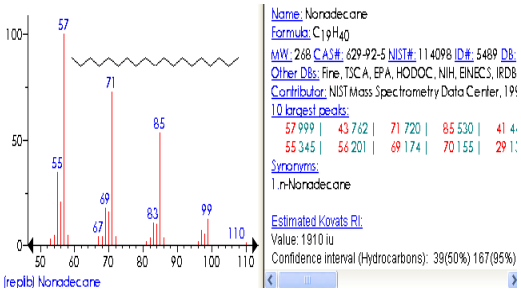


Figure 5. Nonadecane Chromatogram

In the nonadecane fragmentation pattern the most stable compound at 57 m/z. Nonadecane is a series of hydrocarbon molecules weighing 268 m/z. Nonadecane not appear as the intact molecule probably due to degradation during mass spectrum fragmentation.

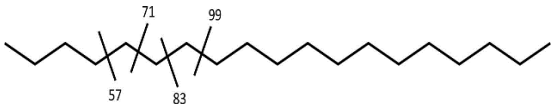


Figure 6. Nonadecane Fragmentation Sites

Nonadecane as hidrocarbon render to fragmentation. Many fragmentaion happeneed in the edge of hidrocarbon chain.

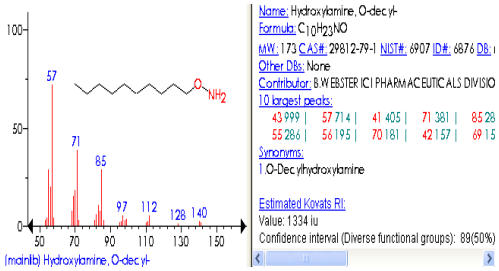


Figure 7. Hydroxylamine O-Decyl Chromatogram

Hydroxylamine o-decyl is a hydrocarbon that has an amine group with 10 carbon chains. The most stable fragmentation fragmentation pattern has molecular weight 57.

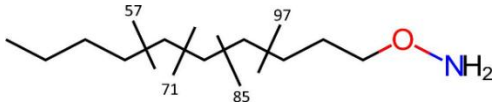


Figure 8. Hydroxylamine O-Decyl Fragmentation Sites

The most fragile bond were hidrocarbon in Hydroxylamine o-decyl stucture. Many fragmentation of hidrocarbon structure locate in the middle of metabolit.

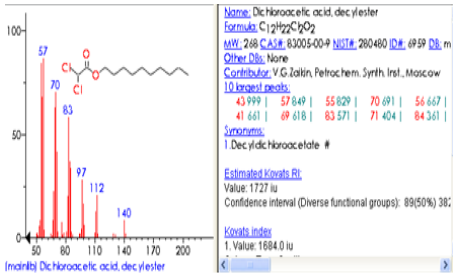


Figure 9. Dichloroacetic Acid Decyl Ester Chromatogram

Dichloroacetic acid decyl ester is synonymous with decyl chloroacetate with a molecular weight of 268 m / z having 2 chlorine groups in the ester functional group. The most stabile fragmentation patterns have molecular weight 57.

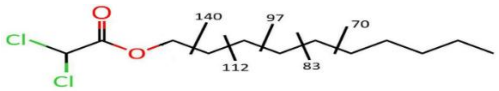


Figure 10. Dichloroacetic Acid Decyl Ester Fragmentation Sites

## DISCUSSION

Table 4. The Five Compound Highest Peak

| No | Name | Formula | Qual | % Max |
|---|---|---|---|---|
| 1 | Dodecanoic acid methyl ester | C13H26O2 | 83 | 8.98 |
| 2 | Nonadecane | C19H40 | 72 | 9.87 |
| 3 | Hydroxylamine o-decyl | C10H23NO | 47 | 9.87 |
| 4 | S-[N-[Adamantyl]amidino] methyl hydrogen thiosulfate | Undetected | 71 | 10.83 |
| 5 | Dichloroacetic acid decyl ester | C12H22Cl2O2 | 35 | 11.86 |

The previous research shows that cantigi fruits has strong anti-oxidant activities[14]. The compound in table 4 were mostly in form of fatty acid methyl ester (FAME) in plant. There are limited data of the of biological activities of those compounds. Some paper said that fatty acid in plant used for biodiesel.

Dichloroacetic acid suggest have potential inhibit neuroblastoma growth [13]. Dichloroacetic acid (DCA) reverses reprogramming in tumor cells switch cytoplasmic glucose metabolism to mitochondrial oxidative phosphorylation.

Various concentrations of DCA on A2780 cells resulted in decreased expression of UCP2, a metabolic switch from glycolysis to mitochondrial. [14]. DCA upregulated the expression of activating transcription factor 3 (ATF3) ($p < 0.001$) in cervical cancer cells. Overexpression ATF3 suppress cell proliferation and induced apoptosis ($p< 0.001$), the mechanism of DCA suggesting that tumor suppressor protein 53 (P53) was responsible for ATF3-mediated anti-tumorigenesis in DCA-treated cervical cancer [15].

One of Cancer cells suppressive factor through selective elevation of intracellular reactive oxygen species (ROS). Liposomal formulation of dichloroacetic acid (DCA) and metal–organic framework (MOF)- Fe2+ (MD@Lip) can efficiently stimulate ROS- mediated cancer cell apoptosis in vitro and in vivo [16]. However early-life exposure to DCA may be as carcinogenic as life-long exposures, potentially via epigenetic-mediated effects related to cellular metabolism [17].

## 4. CONCLUSION

*Vaccinium varingiaefolium* (Blume) Miq apex has moderate antioxidant effect and potential as tumor suppressor. The anti-oxidant in the fruits stronger than in the apex of reddish cantigi leaves.

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