Antioxidant Activity of Methanol Extract *Tetracera scandens* L Merr Predicted Active Compound of Methanol Extract with GCMS NIST Library

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Abstract. Excessive exposure to free radicals can increase the risk of premature aging and several diseases, such as heart disease, cancer, and dementia. Therefore, the body needs antioxidants to fight the effects of exposure to these free radicals. One of the plants used as traditional medicine is *Tetracera scandens* L which comes from the Dilleniaceae family. Various parts of the *T. scandens* L plant have been used in traditional medicine such as lowering high blood pressure, treating gout and hepatitis. This study tested the anti-oxidant effect of *T. scandens* with the DPPH free radical reduction method. The active substance content in the extract was viewed by GCMS compared to the existing NIST library. The results showed that the methanol extract had very strong anti-oxidant activity, IC50 value of 47.82 µg/mL. The results of GCMS contained 14 kinds of compounds and the largest content was that the most compounds were Decanedioic acid, bis (2-ethylhexyl) ester which had 68% similarity with the data base collected as much as 21% of the total detected compounds with a retention time of 21.782 minutes. The second largest compound, 7-Amino-7H-S-triazolo [5,1-c]-S-triazole-3-thiol which has 40% similarity with an abundance of 14.098% peaks appeared at the retention time of 12.082. The third compound is Heptacosane which has 87% similarity with the data base that appeared at 15.269 minutes. Eleven other compounds had an abundance of below 10%. *Tetracera scanden* is potential as an antioxidant because it has very strong anti-oxidant activity.

Keywords: *Tetracera scandens* L. Merr., anti-oxidant, IC50, GCMS.

1. Introduction

Free radicals are atoms, groups of atoms and molecules that have one or more paired electrons, causing free radicals to be unstable and highly reactive. To achieve atomic or molecular stability, free radicals will react with surrounding molecules in order to gain electron pairs. This reaction will continue in the body and if not stopped, it will cause various diseases such as cancer, premature aging, and heart disease [1]. To reduce free radical activity, antioxidants are needed. Antioxidants are molecules that can donate electrons to free radical molecules, so they can stop the chain reaction [2]. One of the plants used as traditional medicine is *Tetracera scandens* L merr which comes from the Dilleniaceae family. *Tetracera scandens* is a green plant and is found widely in India, Indonesia, China, Myanmar, Philippines, Thailand, Malaysia and Vietnam. *T. scandens* L contains flavonoid derivatives such as quercetin, kaempferol, apigenin, luteolin, and myricetine. Various parts of the *T. scandens* L plant have been used in traditional medicine such as lowering high blood pressure, treating gout and hepatitis. In Indonesia, the stem of *T. scandens* L is drunk and used as a cough medicine [3]. In previous studies, extracts of *T. scandens* L have been reported to exhibit potential inhibition of Xanthine oxidase (XO) activity in a...
concentration-dependent manner in vitro [4]. Five is flavonoids, namely genistein and its derivatives 3,5-diphenylgenistein, 6,8-diprenylgenistein, derron and alpium isoflavones isolated from the leaves of T. scandens L. have been shown to provide significant glucose uptake effect and insulin stimulation in vitro shows great potential in management diabetes [3]. In this study an antioxidant test was carried out on the extract of T. scandens L using DPPH for free radical model and the percolation method for extraction method. This research is expected to provide an overview of the antioxidant activity of the leaves of T. scandens L methanol extract then running in GCMS to see the prediction of their metabolite.

2. Materials and Methods
Leaves of Tetracera Scanden L Merr were determined at the Biology Center of LIPI (Indonesian Institute of Sciences) in the Botanical Sector of Cibinong, Bogor. A total of 2.2 kg of leaves were weighed then washed and then aerated without direct sunlight for ± 7 days until a shrinkage of 10%. After sorting again then mashed using a blender. The pulvirese leaves powder can be sifted with 40 mesh and then weighed. T. scandens L. leaf extract was prepared using the percolation method [5].

2.1. Extraction
The pulvirese leaves were put in a closed vessel as much as 125 grams and moisten with 62.5 mL of methanol for at least 3 hours. The percolator was prepared by putting cotton and filter paper in it, prepare a hose and a container for the percolating results. The simplicial that has been soaked into the percolator were transfer little by little while each time being pressed carefully. The liquid was poured into percolator until the liquid starts dripping and on top of the simplicial there is still a layer of filter liquid. Close the percolator tap and incubated for 24 hours. Allow the liquid to drip at a rate of 1 mL per minute. Repeatedly add enough methanol so that there is always a layer of filter liquid on top of the pulvirese leaves until 500 mL is obtained. The extract were transferred it to a vessel, cover and leave for 1-2 days in a cool place, protected from light to settle insoluble materials, filter using paper filter then put it into the rotary evaporator to evaporate until ± 67 mL remains transfer the extract to a steam cup to evaporate until a thick extract is obtained [5].

2.2. Antioxidant activity test
Antioxidant activity test were perform by adding serial concentration of extract 10, 20, 30, 40, 50, 60, 70, 80, 90 dan 100 ppm. 2 mL of each were put into the test tube then mixed with 0.4 mM DPPH solution and then homogenized, then incubated in a dark room for 30 minutes. The positive controls were vitamin C concentration 1, 3, 5, 7, 9 ppm dilute with methanol p.a. Negative control was methanol. First of all, before measurement determined the maximum wavelengths of DPPH. A total of 2 mL of 0.4 mM DPPH solution was put into a test tube then 2 mL of methanol p.a was added, covered with aluminium foil, homogenized then poured into a cuvette and measured at a wavelength of 400-800 nm using a UV-Vis spectrophotometer. The maximum wavelength was applied to measure each of serial extract concentration mixed with DPPH solution. The parameter commonly used to interpret the results of the antioxidant activity test using the free radical reduction method is the value of the efficient concentration (EC50) or often called the IC50 value, which is the concentration that causes a loss of 50% of DPPH activity.

2.3. 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 (Helium) 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 [6]. 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 (NIST). 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 [6].

3. Result and Discussion

The extraction was carried out using the percolation method. The solvent used is methanol which is polar because it can dissolve the antioxidant components in the pulverised *T. scandens* L. leaves, namely flavonoids and polyphenols which are polar.

The extraction process was carried out for 24 hours, using solvent as much as 700 mL. The extraction yield was 500 mL. The extraction results are then concentrated with a rotary evaporator at a temperature of 65°C until the solvent evaporates until a concentrated liquid extract is obtained, then the filtrate extract is steamed with water bath until concentration extract is obtained and a concentrated extract yield is of 14.77 grams (11.82%).

![Figure 1. Scanning the maximum wavelength of DPPH with spectrophotometer](image)

The wavelength used for quantitative analysis is the wavelength where the maximum absorption occurs. The maximum absorption wavelength is done by making a relationship curve between the absorbance and the wavelength of a standard solution at a certain concentration. The curve shows high peak of absorbance stand at $\lambda$ maximum 515 nm.

DPPH is a free radical that is stable at room temperature and is often used to assess the antioxidant activity of several compounds or extracts of natural ingredients. The interaction of antioxidants with DPPH either by electron transfer or hydrogen radicals on DPPH will neutralize the free radical character of DPPH. The principle of the DPPH test was changing the colour of complex form of DPPH and its constituent that attach to reactive site in its molecule. The changes of colour absorb different wavelength. The absorbance measure of 515 nm using a spectrophotometer. DPPH radicals with organic nitrogen structure in the middle are stable free radicals with a dark purple colour which when reduced to non-radical forms by antioxidants becomes yellow.
Table 1. Inhibition of *Tetracera scandens* L.

| Extract Concentration (µg/mL) | Absorbance 1 | Absorbance 2 | % inhibition 1 | % inhibition 2 | IC$_{50}$ 1 | IC$_{50}$ 2 | Mean IC$_{50}$ |
|------------------------------|--------------|--------------|----------------|----------------|-------------|-------------|---------------|
| 10                           | 0.90         | 0.90         | 10.45          | 8.64           |             |             |               |
| 20                           | 0.73         | 0.72         | 19.03          | 19.29          |             |             |               |
| 30                           | 0.61         | 0.62         | 32.34          | 30.93          |             |             |               |
| 40                           | 0.55         | 0.54         | 39.16          | 39.57          |             |             |               |
| 60                           | 0.32         | 0.32         | 63.91          | 64.52          |             |             |               |
| 70                           | 0.20         | 0.20         | 77.44          | 77.71          |             |             |               |
| 80                           | 0.11         | 0.11         | 87.45          | 87.47          |             |             |               |

The results of the antioxidant activity test showed that the extract of pulverised leaves *T. scandens* L had antioxidant activity. The antioxidant activity of the *T. scandens* L leaf extract has an IC$_{50}$ value of 47.82 µg/mL which is classified as very strong antioxidant activity. Meanwhile, standard vitamin C has an average IC$_{50}$ value of 6.91 µg/mL which is also classified as very strong antioxidant activity. The activities of *T. scandens* L leaves extract and vitamin C both have very strong antioxidant activity, however, vitamin C has a stronger antioxidant activity than *T. scandens* L leaves extract.
Table 2. Anti-oxidant activity of vitamin C

| Positive Control (µg/mL) | Absorbance | % Inhibition 1 | % Inhibition 2 | IC<sub>50</sub> 1 (µg/mL) | IC<sub>50</sub> 2 (µg/mL) | Mean IC<sub>50</sub> (µg/mL) |
|--------------------------|------------|----------------|----------------|--------------------------|--------------------------|-----------------------------|
|                          | Blanc 1    |                |                |                          |                          |                             |
|                          | Blanc 2    |                |                |                          |                          |                             |
| 1                        | 0.81       | 6.23           | 5.12           | 6.96                     | 7                        | 6.98                        |
| 3                        | 0.71       | 17.89          | 17.59          |                          |                          |                             |
| 5                        | 0.56       | 34.64          | 33.91          |                          |                          |                             |
| 7                        | 0.40       | 53.57          | 53.37          |                          |                          |                             |
| 9                        | 0.31       | 63.74          | 63.51          |                          |                          |                             |

**Figure 4.** Antioxidant activity of Vitamin C (experiment 1)

y = 7.5346x - 2.4539
R² = 0.9912

**Figure 5.** Antioxidant activity of Vitamin C (experiment 2)

y = 7.6282x - 3.4318
R² = 0.9917

**Figure 6.** The chromatogram of GCMS *Tetracera scanden* L.

There are 14 compounds detected in 14 different retention time. Each retention time have 3 similarities with NIST databased with 3 different qualification. In this research we select the most similar
one amongst 3 other structure predicted available from database result. Highest peak which mean that it has highest concentration in the methanol solution of the extract.

Table 3. Metabolites name from GCMS result

| Peak | Retention Time (minute) | Metabolite Name | % of Total | Qual |
|------|-------------------------|-----------------|------------|------|
| 1    | 6.402                   | Anthracene, tetradecahydro-, alpha,8a.alpha.,9a.beta.,10a.alpha.) | 5.17%      | 9    |
|      |                         | Pentamethylenzoic acid |            | 9    |
|      |                         | 5-Isoindolinecarboxylic acid, 1,3-dioxo-, nonyl ester | 7          |  |
| 2    | 6.735                   | Dodecanoic acid, methyl ester | 7.09%      | 91   |
|      |                         | Dodecanoic acid, methyl ester | 91         |  |
|      |                         | Tridecanoic acid, methyl ester | 78        |  |
| 3    | 11.102                  | Pentadecanoic acid, 14-methyl-, methyl ester | 6.37%      | 97   |
|      |                         | Hexadecanoic acid, methyl ester | 97        |  |
|      |                         | Pentadecanoic acid, 14-methyl-, methyl ester | 96        |  |
| 4    | 11.268                  | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | 3.47%      | 81   |
|      |                         | 3,5-Di-tert-butyl-4-trimethylsiloxytoluene | 52        |  |
|      |                         | alpha-[2-Pyridyl]-2-tert-butyl-4-quinolinemethanol | 38        |  |
| 5    | 11.615                  | Cyclopentanepropanoic acid, 2-methyl-3-oxo-, methyl ester, trans-(+,-) | 4.18%      | 35   |
|      |                         | 9-Methyl-Z-10-pentadecen-1-ol | 32        |  |
|      |                         | Sulfurous acid, cyclohexylmethyl ethyl ester | 25        |  |
| 6    | 11.686                  | Sulfurous acid, cyclohexylmethyl octadecyl ester | 4.15%      | 38   |
|      |                         | Sulfurous acid, cyclohexylmethyl hexadecyl ester | 37        |  |
|      |                         | Allyldimethyl(prop-1-ynyl)silane | 37        |  |
| 7    | 12.082                  | 7-Amino-7H-S-triazolo[5,1-c]-S-triazole-3-thiol | 14.10%     | 40   |
|      |                         | Thiosulfuric acid (H2S2O3), S-(2-(cyclopropylamino)-2-iminoethyl) ester | 33        |  |
|      |                         | Thiosulfuric acid S-[2-[5-[10-toloxyl]pentyl]amino]propyl ester | 25        |  |
| 8    | 14.063                  | Eicosane | 3.77%      | 74   |
|      |                         | Hexadecane | 72        |  |
|      |                         | 1-Iodo-2-methylundecane | 72        |  |
| 9    | 15.269                  | Heptacosane | 11.11%     | 87   |
|      |                         | Hexatriacontane | 86        |  |
|      |                         | 10-Methylnonadecane | 86        |  |
| 10   | 16.519                  | Eicosane | 7.08%      | 86   |
|      |                         | Tetraatriacontane | 83        |  |
|      |                         | Heptacosane | 83        |  |
| 11   | 17.802                  | Tetratetracontane | 5.08%      | 90   |
|      |                         | Heptacosane | 90        |  |
|      |                         | Heptadecane, 2,6,10,15-tetramethyl | 90        |  |
The retention times varied from 6 to 22 minutes. The compound consists of hydrocarbon with some functional mostly in ester forms. Molecular weight ranges from 144 to more than 600. The fourteen highest peak which have similarities with data base that shown in the chromatogram above were more detail shown in the table below.

### Table 4. Compound name GCMS result [7,8]

| Peak | Retention Time (minute) | Compound Name | Compound Structure | Molecular weight (g/mol) | % of Total | Qual |
|------|-------------------------|---------------|--------------------|--------------------------|------------|------|
| 1    | 6.402                   | Anthracene, tetradecahydro-| C_{14}H_{24}          | 192.34                  | 5.173%     | 9    |
|      |                         | tetrahydro-| , lpha., 8a, alpha., 9a, beta, |                |            |      |
|      |                         |              | , 10a, alpha         |                          |            |      |
| 2    | 6.735                   | Dodecanoic acid, methyl ester | C_{12}H_{26}O_{2}     | 214.34                  | 7.093%     | 91   |
| 3    | 11.102                  | Pentadecanoic acid, 14-methyl-, methyl ester | C_{17}H_{34}O_{2}     | 270.45                  | 6.373%     | 97   |
| 4    | 11.268                  | Benzenepropanoic acid, 3,5-bis-dimethylethyl)-4-hydroxy-, methyl ester | C_{18}H_{28}O_{3}     | 292.4                   | 3.472%     | 81   |
| 5    | 11.615                  | Cyclopentane propanoic acid, 2-methyl-3-oxo-, methyl ester, trans- | C_{11}H_{12}O_{3}     | 144.17                  | 4.182%     | 35   |
| 6    | 11.686                  | Sulfurous acid, cyclohexylmethyl octadecyl ester | C_{24}H_{40}O_{5}     | 430.7                   | 4.149%     | 38   |
Free radicals may cause chain reaction inside the living cell the reaction may course damage in the tissue of living cells. Free radical come from inside human produce during biochemical activities in organelles reaction that produce free electrons. Free radicals may also come from outside human body such as pollutants, heavy metal, smoke or irradiation. Human body have neutralised mechanism to stop excessive reaction of free radicals [9]. Glutathione is one of native strong of anti-oxidant produce in the body. In the case of supply the native antioxidant in the body not sufficient to neutralise free radicals the stress of oxidation happened [10]. Oxidative stress triggered some disease such as cancer, premature aging, skin and lung damage, hearth disease. Anti-oxidant intake help body prevent further damage and nourishment native antioxidant [11].

*T. scandens* L potential as an anti-oxidant because it have strong activities against free radicals. The ethanol extract also active against virus and cancer cells [12]. Methanol extract effective for type 2 diabetes [13, 14]. In Indonesia tribe from *Tetracera scandens* used to heal diarrhoea [15]. Protective effect of *T. scandens* L. leaf extract against CCl4-induced acute liver injury in rats [16]. Secretory tissue commonly produces many compounds. In *T. scandens* L stem appears 2 kind of secretory structure glandule trichome and secretary idioblast cell. Glandula trichome located in epidermis. The secrete rich of alkaloid, flavonoid, terpenoids and phenols [16]. There are some other compounds detect in *T. scandens* L methanol extract some them genistein, botulinic acid, stigma sterol, kaempferol, quercetin,
derrone, hipoletin, isoscutellarein [17]. Genistein stimulate glucose uptake [18]. In GCMS result those compounds not appear. The different method and solution might have different result. In GCMS mostly separate the extract solution who dissolve in certain solution and carried by helium gas. The fragmentation during the ionization process might break the intact structure into pieces. The fragmentation pattern depends on molecule stability.

4. Conclusion
Methanol extract of *Tetracera scanden* is potential as an antioxidant because it has very strong antioxidant activity with IC50 value of 47.82 µg/mL.

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