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Preparation and Antioxidant Activity of Methanol Extract of *Myrmecodiarumphii* Becc

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**ABSTRACT**

Ant-plant *Myrmecodiarumphii* Becc. usually used as traditional Papuan medicine. In the present paper, we provide for the first time the cytotoxicity and antioxidant potential of *M. rumphi* Becc. This study reported cytotoxic and antioxidant activities of *M. rumphi* Becc. by using maceration method for three times in every 24 hours and evaporated to obtain 77 g reddish brown concentrated. All samples were partitioned or fractionated by dichloromethane, ethyl acetate, and methanol fraction, respectively, to obtain 1 to 7 fraction. Cytotoxicity was determined using Brine Shrimp Lethality Test (BSLT) method while the antioxidant activity using 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay. The most active fraction from BSLT test is fraction 7 with LC₅₀ is 0.4752 µg/ml. DPPH assay shows that fraction 5 was the most potentials antioxidant with 92.6601% radical scavenging activity. From this result indicated that the local ant-plant (Sarang Semut plant) from Merauke are high potential as a source of antioxidant.

**Keywords:** ant-plant; *Myrmecodiarumphii* Becc.; BSLT method; DPPH assay; antioxidant

**INTRODUCTION**

Free radicals, oxygen radicals, and other reactive oxygen species are produced either from normal cell metabolism in situ or from external. Overproduction of free radicals that cannot gradually be destroyed, called oxidative stress, may cause oxidative damage in the human body, eventually leading to chronic and degenerative illness such as cancer, autoimmune disorder, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative disease⁴⁻⁻⁵⁻⁻³.

Antioxidants have been considered essential for preventing cell damage by scavenging deleterious free radicals⁴. Antioxidants are very important in health care to prevent and scavenge free radicals and the damage caused by reactive oxygen species; alleviate chronic disease and degenerative ailments such as cancer, autoimmune disorders, hypertension, atherosclerosis; and delay the aging process⁵⁻⁻⁶. Endogenous and exogenous antioxidants are used to neutralize free radicals and protect the body from free radicals by maintaining the redox balance⁷.

Many plants, vegetables, spices, and herbs contain important natural substances such as antioxidant. Some studies have done with antioxidant activity to obtain some new sources of natural antioxidants to be used in food, cosmetics, medicine, and other purposes ⁶.

Several studies about plants from Rubiaceae family showed that this family produced natural substances such as antioxidant. Ethanol extract of leaves of *Borreriverticillita* Linn (Rubiaceae) may serve as a promising source of the antioxidant agent as well as being helpful in the treatment of ailments resulting from free radical damage⁸. The methanol extract of *Psychotriagriffithii* and *Hydnophytumformicarum* showed strong DPPH radical scavenging activity with IC₅₀ values of 14.0 and 22.4 µg/ml, respectively⁹. Methanol extract of Lady’s Bedstraw (*Galiumverum* L., Rubiaceae) herb from two different location in Serbia, Mt. Zlatar and Veternik, expressed very strong scavenger activity, with IC₅₀ 0.05 and 0.54 µg/ml, respectively¹⁰. *Sambucusnigra* flower, leaves, and bark are
extraordinarily rich in antioxidants and have frequently been used in traditional medicine and healing\textsuperscript{11}.

\textit{Myrmecodia} and \textit{Hydnophytum} genus, belonging to family \textit{Rubiaceae}, also known as ant-plant or Sarang Semut plant, is widely used in Papua as herb with a broad range of therapeutic values, which is used to enhance immunity, treat gout, rheumatic, and tumor. There are 45 species of \textit{Myrmecodia}dan 26 species of \textit{Hydnophytum} which have an association with ants \textsuperscript{12}. Several studies have done to obtain their bioactivity. The crude hexane, dichloromethane, ethyl acetate, and methanol extract of \textit{Hydnophytumformicarum} Jack. showed such activities against many Gram-positive and Gram-negative bacteria, antioxidant potency, and had the ability to increase lymphocyte proliferation by increasing concentration \textsuperscript{13,14}. Extract of ant-plant have the capability to inhibit the growth of HeLa and MCM-B2 cells\textsuperscript{12}. Plant extract of \textit{Myrmecodiataruberosa} Jack and its fractions revealed the significant potency of this plant as an immunomodulator and may act as co-chemotherapy in Dox use \textsuperscript{15}. Papua’s \textit{M. pendants} exhibited a high potential antitumor activity in human oral tongue squamous cell carcinoma through induction of p27Kip1 and suppression of cyclin E \textsuperscript{16}.

Tomer is one of the villages in Merauke, Papua which has different species of ant-plant (Sarang Semut plant) which is often used by local people as traditional medicine. Taxonomy identification shows that this local ant-plant (Sarang Semut plant) was \textit{Myrmecodia rumphi} Becc. To the best of our knowledge, there are no data available on the bioactivity of \textit{M. rumphi} Becc. In this study, we are focusing on the cytotoxicity and antioxidant capacity of \textit{M. rumphi} Becc. using Brine Shrimp Lethality Test (BSLT) method and 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay.

\textbf{MATERIALS AND METHOD}

\textbf{Plant Material}

Ant-plants were collected from Tomer village, Merauke, Papua, the east of Indonesia and identified as \textit{Myrmecodia rumphi} Becc. A voucher specimen was deposited at the Biodiversity Center of Papua University, Papua. Figure 1 shows the morphology of \textit{M. rumphi} Becc. in the study.

\textit{Preparation and Partition of Methanol Extract}

Hypocotyl tubers of ant-plants were peeled, washed, thinly sliced, dried in the sunshine, and ground to obtain finely powdered sample about 3.36 kg. The sample was macerated in methanol for three times in every 24 h and evaporated using rotary vacuum evaporator to obtain 77 g reddish brown concentrated extract. Samples were partitioned by dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) solvent, respectively, using vacuum liquid chromatography. Thin layer chromatography (TLC) test showed the TLC profile of each fraction. Fractions showing the same spot and retention factor (Rf) were merged, so obtained Fraction 1 to 7.

\textit{Brine Shrimp Lethality Test (BSLT)}

Brine shrimp lethality test was used to predict the cytotoxic activity of MeOH and Fraction 1 to 7 by a method described previously\textsuperscript{17}. For the experiment, 1 mg of each extract saw dissolved in 1 ml of dimethylsulfoxide (DMSO) and solutions of varying concentration (1000, 500, 250, 125, 62.5 \( \mu \)g/ml) were obtained by the serial dilution technique using simulated seawater. The solutions were then added to the pre-marked vials containing 10 live brine shrimp nauplii in simulated water. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. The mortality endpoint of this bioassay was defined as the absence of controlled forward motion during 30 s of observation. From this data, the percent of lethality of the brine shrimp nauplii for each concentration was calculated. An approximate linear correlation was observed when the logarithm of concentration versus percentage of mortality was plotted and the value of LC\textsubscript{50} was calculated using Microsoft Excel 2010 \textsuperscript{17}.

\textit{2,2-diphenyl-2-picrylhydrazyl (DPPH) Assay}

Free radical scavenging activity of extracts was determined by a method described previously Bang, T.H., Suhara, H., Doi, K., Ishikawa, H., Fukami, K., Parajuli, G.P., Katakura, Y., Yamashita, S., Watanabe, K., Adhikari, M.K., Manandhar, H.K., Kondo, R., Shimizu, K. in their study\textsuperscript{18}. DPPH radicals have an absorption maximum at 515 nm; upon reduction by an antioxidant, the solution color fades and the reaction progress is easily monitored by a spectrophotometer (UV Vis Thermo Scientific Genesis). Determination procedures were as follow: 6 x 10\textsuperscript{-5} M DPPH solution was prepared by dissolve 0.24 mg DPPH powder in 10 ml MeOH. A methanolic solution of the sample was prepared by dissolve 10 mg samples in
1 ml MeOH. 33.3 µl of the sample solution was mixed with 1 ml DPPH solution; after 20 min incubation for at 37°C, absorbance decrease of the mixture was monitored at 515 nm (A.). Blank sample with 33.3 µl MeOH in the above DPPH solution was prepared and measured daily at the same wavelength (A.). The experiment carried out in triplicate. Radical scavenging activity was calculated using the following formula:

\[ \text{Inhibition rate} (\%) = \frac{A_s - A_d}{A_s} \times 100 \]  

RESULTS AND DISCUSSION

Cytotoxicity

*Brine shrimp lethality test* was used to determine the cytotoxic activity of MeOH and Fraction 1 to 7. LC\(_{50}\) values obtained from brine shrimp lethality test (Table 1) are 0.9868, 6.4679, 43.0450, 47.8253, 80.9516, 163.3445, 81.0581, 0.4841 µg/ml for MeOH extract and 1 to 7 fraction, respectively.

### Table 1. The results of cytotoxic activity of MeOH extract and fraction 1 to 7.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Merged Fraction</th>
<th>LC(_{50}) (µg/ml)</th>
<th>Regression Equation</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>MeOH extract</td>
<td>0.99</td>
<td>y = 0.1737x + 0.5010</td>
<td>0.9542</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>fraction DCM 1</td>
<td>6.47</td>
<td>y = 0.2436x + 0.3025</td>
<td>0.9464</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>fraction DCM 2</td>
<td>43.04</td>
<td>y = 0.4357x - 0.2119</td>
<td>0.9223</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>fraction DCM 3 to 4</td>
<td>47.83</td>
<td>y = 0.4208x - 0.2068</td>
<td>0.9614</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>fraction DCM 5 to 7 and EtOAc 1</td>
<td>80.95</td>
<td>y = 0.5568x - 0.5625</td>
<td>0.9310</td>
</tr>
<tr>
<td>Fraction 5</td>
<td>fraction EtOAc 2 to 4</td>
<td>163.34</td>
<td>y = 0.641x - 0.9186</td>
<td>0.9705</td>
</tr>
<tr>
<td>Fraction 6</td>
<td>fraction EtOAc 5 to 8</td>
<td>81.06</td>
<td>y = 0.5252x - 0.5025</td>
<td>0.9570</td>
</tr>
<tr>
<td>Fraction 7</td>
<td>fraction MeOH 1 to 5</td>
<td>0.48</td>
<td>y = 0.1609x + 0.5507</td>
<td>0.9379</td>
</tr>
</tbody>
</table>

All fractions resulting in LC\(_{50}\) less than 1000 µg/ml were considered significantly toxic towards brine shrimp and clearly indicate the presence of the potent bioactive compound. MeOH extract and MeOH fraction are more active than other fractions. MeOH extract of *M. rumphii*Becc. (LC\(_{50}\) 0.99 µg/ml) is more active than MeOH extracts of *Alternantherasessilis* Linn. (LC\(_{50}\) 19.825 µg/ml), *Amaranthustricolor* (LC\(_{50}\) 28.319 µg/ml), *Benincasahispida* (LC\(_{50}\) 45.187 µg/ml), *Chenopodium album* (LC\(_{50}\) 10.000 µg/ml), *Corchorusolitorius* (LC\(_{50}\) 26.254 µg/ml), *Diplaziumesculentum* (LC\(_{50}\) 18.561 µg/ml), *Enhydractfluans* (LC\(_{50}\) 10.522 µg/ml), *Glinusoppositifolius* (LC\(_{50}\) 27.650 µg/ml), *Ipomeaaquatica/ Ipomea alba* (LC\(_{50}\) 32.668 µg/ml), *Lagenariasiceraria* (LC\(_{50}\) 35.835 µg/ml), *Nymphaeanouchalli* (LC\(_{50}\) 21.864 µg/ml), *Portulacagrandifolia* (LC\(_{50}\) 11.647 µg/ml), *Spinaciaoleracea* (LC\(_{50}\) 60.323 µg/ml), *Xantiumindicum* (LC\(_{50}\) 8.447 µg/ml) edible vegetables from Bangladesh.

19. Besides, MeOH extract of *M. rumphii*Becc. are more active than MeOH extract of *Dilleniaindica* Linn. bark with LC\(_{50}\) 45.32 ± 2.13 µg/ml 20. All fractions show moderate to potentials cytotoxic activity and could serve for further ethnobotanical and phytochemical research to find the possible relationship between brine shrimp lethality and plant bioactivity.

Antioxidant Activity

Antioxidant activity of the extracts was determined using 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay. The antioxidant capacity (DPPH radical scavenging activity and discoloration) of all fractions of MeOH extract of *M. rumphii*Becc. is presented in Table 2.
Table 2. Discoloration of DPPH solution before and after added sample, absorbance, and antioxidant activity by DPPH scavenging activity (%) of MeOH and Fraction 1 to 7 of *M. rumphii* Becc.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Discoloration</th>
<th>Absorbance</th>
<th>DPPH Scavenging (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before <em>a</em></td>
<td>After <em>b</em></td>
<td></td>
</tr>
<tr>
<td>MeOH extract</td>
<td>purple</td>
<td>purple</td>
<td>0.064</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>purple</td>
<td>purple</td>
<td>0.687</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>purple</td>
<td>purple</td>
<td>0.661</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>purple</td>
<td>purple</td>
<td>0.607</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>purple</td>
<td>light yellow</td>
<td>0.617</td>
</tr>
<tr>
<td>Fraction 5</td>
<td>purple</td>
<td>light yellow</td>
<td>0.052</td>
</tr>
<tr>
<td>Fraction 6</td>
<td>purple</td>
<td>light yellow</td>
<td>0.060</td>
</tr>
<tr>
<td>Fraction 7</td>
<td>purple</td>
<td>purple</td>
<td>0.069</td>
</tr>
</tbody>
</table>

When DPPH reacts with an antioxidant compound, its stable purple color will be changed to a light yellow color of diphenyl-picrylhydrazine. The result shows that MeOH extract and fraction 5 to 7 have antioxidative activity. The highest DPPH scavenging activity is shown by Fraction 5, followed by Fraction 6, MeOH extract, and Fraction 7 with of 91.58, 90.98, and 90.28 %, respectively. Fraction 5 (EtOAc extract) with 92.66% scavenging is more active than EtOAc extract of *Hydnophytum formicarum* Jack. with 83.31% scavenging. Besides that, Fraction 7 (MeOH extract) with 90.28% scavenging is more active than MeOH extract of *Hydnophytum formicarum* Jack., *Origanum syriacum*, *Zingiber officinal*, and *Thymus syriacus* with 83.31%, 69.25%, 47.42%, and 45.75% scavenging, respectively. DPPH scavenging of methanol extract of other plants compared to *M. rumphii* Becc.

DPPH inhibition of ethanol extract of *Borreriai verticillata* Linn (EEBV), hydroalcoholic extract of *Rheum emodi* Wall.exMeissn (HERE), hydroalcoholic extract of *Sapindus mukorossi* Gaertn. (HESM), standard Ascorbic acid and Gallic acid for 322 µg/ml concentration were calculated by interpolation from the data of concentration and percentage of scavenging. Results show that MeOH extract of *M. rumphii* Becc. (MEMR) is similar to EEBV to scavenge DPPH radicals and more active in scavenging DPPH radicals than HERE and HESM. Compared to standards of antioxidant, MEMR is active enough as DPPH radical scavenger.

The screening of cytotoxicity and antioxidants derived from natural sources will encourage researchers to identify compounds as suitable antioxidants and antitumor. The cytotoxic activity fractions indicate the presence of potentials bioactive compounds. Natural extracts with proven antioxidant activity are usually composed with their phenolic moiety. The search antioxidant activity should be continued with the phytochemicals and possible substance with a wide range of pharmacological activities.

**CONCLUSION**

The most active fraction from BSLT test is fraction 7 with LC50 is 0.4752 µg/ml. DPPH assay shows that fraction 5 was the most potentials antioxidant with 92.66% radical scavenging activity. This indicated that the local ant-plant (*Sarang Semut* plant) from Merauke are high potential as a source of antioxidant and antitumor. Information of this study should be a valuable reference for future studies on antioxidant, antitumor, and others bioactivities of *M. rumphii* Becc.

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**Ethical Research:** taken from university and the agreement with respondent

**REFERENCES**


