Bali Medical Journal (*Bali Med J*) 2017, Volume 3, Number 3 (IBL Conference 2017 Special Issue): S80-S83
P-ISSN.2089-1180, E-ISSN.2302-2914

Published by DiscoverSys

Ethanolic extract of the powder of red earthworm (*Lumbricus rubellus*) obtained from several organic farmlands in Bali, Indonesia: Analysis of total phenolic content and antioxidant capacity

Ni Wayan Sucindra Dewi,[1*] Agung Nova Mahendra,[1,2] Gustu Widi Kencana Putra,[3] I Made Jawi,[1] Dewa Made Sukrama,[4] Ni Luh Kartini[5]

ABSTRACT

Background: Red earthworm (*Lumbricus rubellus*) has been used for centuries as an ingredient in Ayurvedic therapy and traditional Chinese medicine (TCM). The worm is used in the treatment of inflammation, hematological disorders, fever, hepatic disorders, joint pain, high blood pressure, and several other indications. To our knowledge, there is no data on the total phenolic content and antioxidant capacity of *L. rubellus* obtained from Bali.

Methods: Ethanolic extract of *L. rubellus* powder was made in The Laboratory of Division of Drug Development and Laboratory Animal, Integrated Biomedical Unit, Faculty of Medicine, Udayana University. Total phenolic content and antioxidant capacity were investigated in The Laboratory of Food Analysis, Udayana University. Total phenolic content was determined using Folin-Ciocalteu method. Meanwhile, antioxidant capacity was determined using DPPH method.

Results: We found that ethanolic extract of *L. rubellus* powder had total phenolic content of 1016.31 mg/100 g gallic acid equivalent (GAE), and exhibited IC 50% of 12.33 mg/mL.

Conclusion: We conclude that ethanolic extract of *L. rubellus* powder contains phenolic acid and shows an antioxidant effect *in vitro*. *L. rubellus* powder can be potentially used as a natural antioxidant source to treat disorders associated with inflammation and oxidative stress.

Keywords: *Lumbricus rubellus* powder, ethanolic extract, total phenolics, antioxidant capacity
Cite This Article: Dewi, N.W.S., Mahendra, A.N., Putra, G.W.K., Jawi, I.M., Sukrama, D.M., Kartini, N.L. 2017. Ethanolic extract of the powder of red earthworm (*Lumbricus rubellus*) obtained from several organic farmlands in Bali, Indonesia: Analysis of total phenolic content and antioxidant capacity. *Bali Medical Journal* 3(3): S80-S83. DOI:10.15562/bmj.v3i3.730

Department of Pharmacology and Therapy,[1] Division of Drug Development and Laboratory Animal, Integrated Biomedical Laboratory Unit, Faculty of Medicine,[2] Laboratory of Food Analysis, Faculty of Agricultural Technology,[3] Department of Microbiology, Faculty of Medicine,[4] Faculty of Agriculture,[5] Udayana University, Bali, Indonesia

*Correspondence Author: Ni Wayan Sucindra Dewi, M.D., Faculty of Medicine, Udayana University, Bali, Indonesia
sucindradewi@unud.ac.id

Received: 2017-07-03
Accepted: 2017-07-15
Published: 2017-07-17

INTRODUCTION

Earthworms have a long history of uses in Hindu, Chinese, Japanese, Vietnamese, Korean, and Arabic cultures as a source of food and medicine.[1] Di Long "earth dragon" extract in traditional Chinese medicine (TCM) is made from red earthworms (*Lumbricus rubellus*). This TCM preparation is used empirically to treat high fever with convulsion, inflammatory joint pain, and swollen.[2] The authors in present study observe that the oral preparations of *L. rubellus* dry powder have been used empirically in the Bali province, Indonesia, to treat diabetes, stroke, joint pain, and several other conditions such as hypertension and typhoid fever or infection. Thus, similar uses of medicinal preparations made from *L. rubellus* are also found in Bali, Indonesia.

The aforementioned pathological conditions are known to be associated with inflammation and oxidative damage.[3,4] Phenolic compounds are a group of secondary metabolites known to be capable of scavenging free radicals that may contribute to oxidative damage.[5] The administration of these compounds is expected to mitigate the harmful effects of free radicals to human health.

To our knowledge, no study on the total phenolics and antioxidant activity of *L. rubellus* powder ethanolic extract from organic farmlands in Bali had ever conducted before. Therefore, this study was aimed to investigate the total phenolic content and antioxidant activity of *L. rubellus* powder ethanolic extract.

MATERIALS AND METHODS

L. rubellus Powder Production and Extraction

Red earthworms (*Lumbricus rubellus*) were obtained from several organic farmlands in several regencies in Bali. These organic farms are managed by Bali Organic Association (BOA). Red earthworm powder was made in Faculty of Agriculture, Udayana University. Red earthworm bodies were washed with tap water to remove the mucus on the outer surface of the bodies. The bodies were macerated in distilled water for 6-8 hours and then re-washed with clean distilled water to remove the dirt. The bodies were dried using the oven at a constant temperature of 40°C for three days and

blended into a dry powder. The powder was then processed to produce ethanolic extract in Laboratory of Pharmacology and Therapy – Division of Drug Development and Therapy, Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University. *L. rubellus* powder was macerated with 80% ethanol for 48 hours to remove the rest of the water content in the powder. The crude extract was then made using a rotary vacuum evaporator.

Quantification of Total Phenolic Content

Determination of total phenolic content in the present study was based on Folin-Ciocalteu method.[6] The sample for as much as 100 mg was dissolved in particular volume using 85% methanol. The solution was then vortexed and filtrated. Standard solution and filtrate (0.1 mL for each solution) were mixed with Folin-Ciocalteu reagent with the same volume and vortexed. The resulting solutions were allowed to rest for 6 minutes. The next step was adding 0,8 mL of 5 % Na_2CO_3 to neutralize the oxidative reaction of Folin-Ciocalteu reagent. The total volume of the solution was 1 ml. This solution was then vortexed and allowed to rest for 30 minutes. The absorbance of the resulting blue color was measured at 760 nm. Total phenolic content in the ethanolic extract, using Gallic acid as a calibration standard, was expressed as Gallic Acid Equivalent (GAE) per gram of dry weight of the sample. Gallic acid linear regression curve was then made by inputting absorbance value in y-axis and concentration in the x-axis. The formula y = ax + b can be determined by drawing a straight line across the coded points.

Quantification of Antioxidant Activity

The antioxidant activity quantification was adopted from Aldarraji et. al. (2013) with slight modification.[7] Different concentrations of the samples were prepared in methanol at five concentrations (20, 40, 60, 80, and 100 mg/mL). DPPH solution at the final concentration of 40 ppm was prepared in methanol by adding 0.004 g. One hundred milliliters of each concentration in methanol was added to 700 mL of DPPH solution. The mixture was shaken and incubated for 30 min in the dark at room temperature. The absorbance was read spectrophotometrically at 517 nm. The control solution was prepared by following the same method without the sample. Gallic acid was used as a positive control standard. Inhibition of DPPH free radical in percent was evaluated according to the formula: Inhibition (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$, where A control represents the absorbance of the control reaction (containing all reagents except the test compounds), A sample represents the absorbance of test samples. Gallic acid was used as positive control.

The percentage of inhibition obtained from the sample was made into a graph by inputting sample concentration and percentage of inhibition in x and y-axis (as absis and ordinate), respectively. Linear regression equation (y = ax + b) was obtained by drawing a straight line at the meeting point of absis and ordinate. Inhibition concentration 50 % (IC50%) can then be determined based on this equation.[8] The antioxidant activity was expressed as the Antioxidant Activity Index (AAI), calculated as: [Final concentration of DPPH in the blank (ppm)/ IC50 (ppm)] x 100. This method was chosen because it provides a constant value, independent of the DPPH concentration and sample used.[9] According to the scale proposed by Scherer and Godoy (2009), namely, poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when 0.5 < AAI < 1.0, strong antioxidant activity when 1.0 < AAI < 2.0 and very strong antioxidant activity when AAI > 2.0.[10]

RESULTS

Total Phenolic Content

The standard Gallic acid prepared with concentrations of 2, 4, 8, 16 and 20 ppm, read at 760 wavelengths, results in absorbance as shown in Table 1. The absorbance value is inserted on the y-axis, and the concentration on the x-axis produce linear regression curve and the equation y = 0.047x + 0.0635 (figure 1). The value of y obtained is 0.198, by inserting the value of y in the equation, result in 1016.31 mg / 100g GAE phenolic compounds. Using Folin-Ciocalteu method for phenolic assay, the present study found that the total phenolic content of L. *rubellus* powder ethanolic extract was 1016.31 (mg/100g GAE) or in other words, there is 1016.31 mg Gallic acid per 100 gr extract.

Table 1 **The result of absorbance measurement of standard solution of Gallic acid at 760 nm wavelength with UV-Vis spectra**

Concentration ppm	Absorbance
0,0	0,000
2,0	0,199
4,0	0,280
8,0	0,453
16,0	0,792
20,0	1,007

Table 2 IC (%) value at various concentrations of the sample

Concentration mg/mL	Absorbance	IC %
0,00	0,969	30,37
4,18	0,932	34,29
12,54	0,837	44,34
16,72	0,700	58,84
20,90	0,605	68,89

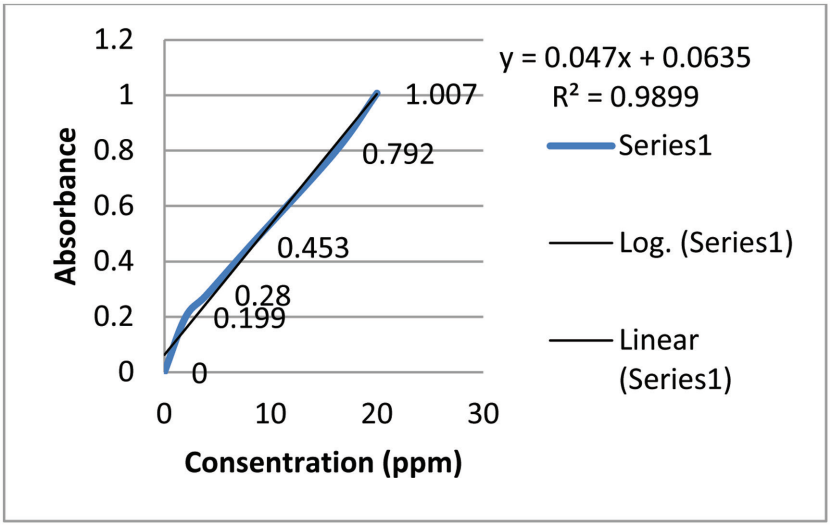

Figure 1 Gallic acid calibration curve in Folin-Ciocalteu reagent

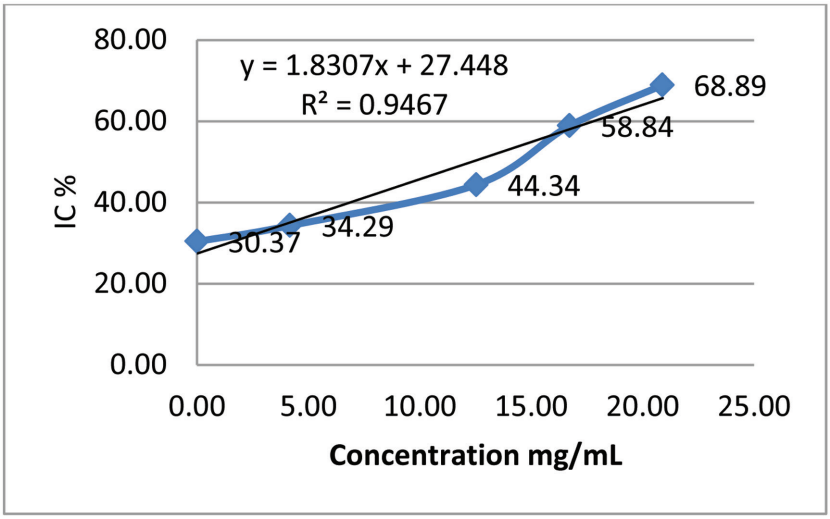

Figure 2 The relationship of IC value and concentration of sample in reducing free radical of DPPH 0.1 mM

Antioxidant Activity

Samples were made with various concentrations and read their absorbance at 517 nm wavelength. The IC value of the sample is calculated by the Inhibition (%) formula = $[(A_{control} - A_{sample})/A_{control}] \times 100$, and the results shown in Table 2. The IC50% value is calculated by the linear equation (y = 1,8307x + 27,448) obtained from relationship curve between IC and the concentration of the sample in reducing the free radical of DPPH (figure 2). The y-value of 50% is included in the equation so that the value of x is 12, 33 mg / mL. At concentration 12.33 mg / mL ethanolic extract of earthworm able to ward of DPPH free radical as much as 50%. In other words, IC50% ethanol extract earthworm is 12.33 mg / mL (12330 ppm).The AAI value is 0.32% (AAI <0.5), so it can be said that ethanolic earthworm extract has weak antioxidant activity.

DISCUSSION

Total Phenolic Content

Ethanolic extract of *Lumbricus rubellus* in our study showed a notable amount of phenolic compounds. Phenolic compounds are highly soluble in ethanol.[11] The present study used 80% ethanol based on the research done by Aldarraji et. al. (2013).[7] Since we used a high concentration of ethanol to macerate the sample, we had been successfully extracting phenolic compounds from earthworm sample obtained from organic farmlands in Bali.

Lumbricus rubellus ethanolic extract in our sample showed higher total phenolic content than that of Aldarraji et. al. (2013). This finding may be explained by the effect of the different parameter in extraction and living site or biology of the worm. Apart from methanol concentration in water, different parameters affect the extraction of phenolic compounds such as particle size and some extraction stage and the presence of interfering substances.[12] Various factors affected the biology of earthworms, these different factors were physical, chemical and biological properties of soil, types of plant litter, animal's dung and type of microorganisms and nematodes.[7] Earthworm also have the capacity to accumulate and concentrate large quantities of inorganic and organic pollutant.[13] Due to our result ethanolic extract of *L. rubellus* can be a potential source of the phenolic compound.

Antioxidant Activity of Ethanolic extract of *Lumbricus rubellus*

Ethanolic extract of *Lumbricus rubellus* in our study showed IC 50% amount 12,33 mg/mL and had weak Antioxidant Activity Index (AAI). Ethanolic extract of earthworm *L. rubellus* in our study has some amount of phenolic while, on the other hand, it has weak antioxidant activities index. These result caused by many factors. First, we used ethanol as solvent. Zhao et. al.found that differences in the DPPH radical scavenging activity of the extraction resulted from the differences in selectivity of solvents for extracting specific phenolic groups with various DPPH radical quenching activity.[14] Meanwhile, a study by Aldarraji et. al. showed methanolic extract has lower inhibition

Published by DiscoverSys | *Bali Med J* 2017; 3(3): S80-S83 | doi: 10.15562/bmj.v3i3.730

capacity (IC) than ethanolic extract in the same sample. Secondly, Zhao and Yu, state the extraction condition may affect the efficiency of antioxidant activity.[15] Thirdly the extracts may include nonphenolic materials such as sugar, protein, pigments and an organic acid which can interrupt throughout the antioxidant assessment and interfere with Folin-CIO cal TEU essay leading to overestimation of total phenolic content.[16] Finally, Folin-Ciocalteu method cannot quantify and determine all phenolic compounds in the samples.[17] Ethanolic extract of *L. rubellus* in our study can be potential use as a natural antioxidant.

CONCLUSION

The present study showed that ethanolic extract of *L. rubellus* powder contains notable amounts of phenolic acid and shows an antioxidant effect *in vitro*. *L. rubellus* powder can be potentially used as a natural antioxidant source to treat disorders associated with inflammation and oxidative stress.

ACKNOWLEDGEMENT

The authors would like to thank I Gede Wiranata, S.Si. (Technician, Division of Drug Development and Laboratory Animal, Integrated Biomedical Laboratory Unit, Faculty of Medicine), I B Ketut Widnyana Yoga, STP. Msi. (Laboratory of Food Analysis, Faculty of Agricultural Technology) for their assistances.

REFERENCES

1. Prabha L, Shathya S. Earthworm-An alternative approach to biomedicine. Int J Curr Sci. 2014;13: 6 – 8.
2. Cooper EI, Balamurugan M, Huang CY, Tsao CR, Heredia J, Ponzetta MT, Paoletti MG. Earthworm dilong: ancient, Inexpensive, Noncontroversial models may have clarify approaches to integrated medicine emphasizing neuroimmune system. Evidence-Based Complimentary and Alternative Medicine. 2012; 2012.
3. Hunter P. The inflammation theory of disease, the growing realization that chronic inflammation is crucial in many diseases opens new answer far treatment. CMBO. 2012;13(11): 968–970.
4. Huy LAP, He H, Huy CP. Free radical antioxidant in disease and health. International Journal of Boiomedical Science. 2008; 4(2): 89-96.
5. Katalinic V, Možina SS, Skroza D, Generalic I, Abramovic H, Miloš M. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia). Food Chem. 2010; 119: 715 – 23.
6. Wang BJ, Liu CT, Tseng CY, Yu ZR. Antioxidant activity of Bupleurum kaoi Liu (Chao et Chuang) fractions fractionated by supercritical CO_2. Lebensmittel-Wissenschaft and Technologie - Food Science and Technology. 2005; 38(3):281 – 87.
7. Aldarraji QM, Halimoon N, Majid NM. Antioxidant activity and total phenolic content of earthworm paste of *Lumbricus rubellus* (red worm) and *Eudrilus eugenia* (African night crawler). J Entomol Nematol. 2013; 5(3):33 – 7.
8. Burda S and Oleszek W. Antioxidant and antiradical activities of flavonoid. J Agric Food Chem. 2001; 49(6): 2774-9
9. Faustino H, Gil N, Baptista C, Duarte AP. Antioxidant Activity of Lignin Phenolic Compounds Extracted from Kraft and Sulphite Black Liquors. Journal molecules. 2010; 15: 9308-9322.
10. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem. 2009; 112: 654-658.
11. Spigno G, Tramelli L, De Faveri DM. Effect of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenols. J. Food En. 2007; 81(1): 200-208.
12. Nepote V, Grosso NR, Guzman CA. Optimization of extraction of phenolic antioxidants from peanut skins. J. Sci. Food Agric. 2005; 85: 33-38.
13. Maity S, Roy S, Chaudhury S, Bhattacharya S. Antioxidant respone of the earthworm lampito mauritii exposed to Pb and Zn contaminated soil. Journal envpol. 2007; 03: 005.
14. Zhao H, Dong J, Lu J, Chen J, Li Y, Shan L. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). J. Agric. Food Chem. 2006; 54: 7277-7286.
15. Zhou K and Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT-Food Sci. Technol. 2004; 37: 717-721.
16. Ronald L, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chemi. 2005; 53: 4290-4302.
17. Naczk M and Shahidi F. Extraction and analysis of phenolics in food. J. Chromato. A. 2004; 1054: 95-111.