Antimicrobial and Antioxidant Activities of *Hornstedtia leonurus* Retz. Extracts

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Abstract

The *n*-hexane, ethyl acetate and methanol extracts of the rhizome, leaf and stem of *Hornstedtia leonurus* Retz. from Malaysia were tested for antimicrobial and antioxidant activities. Antimicrobial activities were screened against three bacteria and two fungi by the broth microdilution assay, while antioxidant activities were analysed using DPPH radical scavenging assay. Most of the extracts exhibited weak to moderate antimicrobial activities (MIC 900 – 1800 µg/mL) except ethyl acetate extract of the rhizome with strong activity against *Candida albicans* (MIC 225 µg/mL) and *Aspergillus niger* (MIC 450 µg/mL) as well as methanol extract of the rhizome that inhibited activity of *Staphylococcus aureus* with MIC value of 450 µg/mL. Among nine extracts tested, *n*-hexane extract of the rhizome possessed moderate free radical scavenging activity with IC_{50} value of 59.60 ± 0.69 µg/mL compared to vitamin C (IC_{50} = 21.21 ± 1.25 µg/mL).

Keywords: *Hornstedtia leonurus* Retz.; broth microdilution assay; DPPH radical scavenging assay

1. INTRODUCTION

Nowadays, antimicrobial and antioxidant plant products have attracted interest many researchers due to the resistance of synthetic antibiotics against some microorganisms and side effect of synthetic antioxidant [1]. Natural antimicrobial compounds play an important role to preserve food and control human and plant diseases origin from microorganisms [1,2,3]. On the other hand, natural antioxidants can prolong the shelf-life of food products, slow down ageing process and treat human diseases such as atherosclerosis and cancer [2].

In Malaysia, Zingiberaceae or ginger family are often used as spices, food preservatives, colouring agents and cooking ingredients. Besides that, this species can provide health-promoting effects to relieve certain illnesses such as nausea, motion sickness, stomach-ache, asthma, diarrhoea, digestive disorder, vomiting, rheumatism, swelling, common cold and cough [4]. Many phytochemicals of these plants have been reported to have interesting biological activities include antifungal, antioxidant, insecticidal and anti-inflammatory activities [5]. There are also several reports regarding the Zingiberaceae extracts that have antimicrobial, antioxidant, anticancer and stimulated effect on the immune system [6].

There are more than ten species, including *H. scyphifera*, *H. ophiuschus*, *H. striolata*, *H. havilandii* and *H. phaeochoana* were found in Malaysia [7, 8]. The leaf of *Hornstedtia* species was consumed by the Javanese for flavouring, as well as to protect crops from insects by burning them. Furthermore, people in Kelantan used *H. macrocheilus* as an external application to reduce fever [9]. Previous study on the rhizome and flower oils of Malaysian *H. havilandii* revealed that both oils showed significant inhibitory activity against *Staphylococcus aureus* whereas flower oil exhibited moderate antibacterial activities towards *Escherichia coli* and *Pseudomonas aeruginosa*. In addition, both oils demonstrated very weak antifungal activities against *Candida albican* and *Candida glabrata* [8].

To the best of our knowledge, there has been no report on any bioactivities of *Hornstedtia* extract from Malaysia, or any other countries. Here, we are reporting the antimicrobial and antioxidant properties of the rhizome, leaf and stem extracts of Malaysian *H. leonurus* Retz.

2. MATERIALS AND METHODS

2.1 Plant Collection

The rhizome, leaf and stem of *H. leonurus* Retz. were collected in September 2012 from the forest in Universiti Teknologi MARA, Negeri Sembilan, Malaysia. A voucher specimen of SK 1770/10 for the plant was deposited at the Institute of Bioscience, Universiti Putra Malaysia.

2.2 Extraction of the Extracts

The dried and ground of rhizome, leaf and stem of *H. leonurus* Retz. were soaked for 48 hours using *n*-hexane, ethyl acetate and methanol successively. The mixture was filtered with Whatman filter paper and the filtrates were concentrated using rotary evaporator to obtain the extract. The dried extracts were placed in sample bottles and kept in the dark at -20°C until further use.

2.3 Broth Microdilution Assay

Minimum inhibitory concentration (MIC) values are used to assess the antimicrobial activity and evaluation are made using broth microdilution assay with some modifications [10]. Overnight cultures of a Gram-positive bacteria i.e. *Staphylococcus aureus* (ATCC 29737) and two Gram-negative strains; *Pseudomonas putida* (ATCC 49128), *Escherichia coli* (ATCC 10536), and two fungi, *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16888) were adjusted to McFarland turbidity standard (5%). The test samples (14.4 mg) were dissolved in DMSO (2.0 mL) to get 7200 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 µL) was added to each microplate well from row B to row H. The stock solutions of samples (100 µL) were added to microplate wells at row A and B. Then, the mixture of the samples and the sterile broth (100 µL) at row B was transferred to each microplate well in order to obtain a twofold serial dilution of the stock samples (concentration of 7200, 3600, 1800, 900, 450, 225, 112.5 and 56.25 µg/mL). The inoculate (100 µL) was added to each well. Plates were incubated at 37°C for 16-20 hours. Microbial growth was indicated by the presence of turbidity and a pellet at the bottom of the well. The MIC value was determined as the lowest concentrations that did not show any microbial growth [11]. The assay was performed in triplicate.

2.4 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The method was carried out as described by Tagashira and Ohtake [12] with some minor modifications. Each sample stock solution (1.0 mg/mL) was diluted to final concentrations of 500, 250, 125, 62.5, 31.3, 15.63 and 7.81 µg/mL in methanol. A total of 3.8 mL of 50 µM DPPH methanolic solution (1 mg/50 mL) was added to 0.2 mL of sample solution of different concentrations and allowed to react at room temperature for 30 minutes. After 30 minutes, the absorbance for the reaction of the mixture was measured at 517 nm by using UV-Visible spectrophotometer. Mixture of DPPH solution and methanol was used as the blank, whereas vitamin C was used as a standard antioxidant. The absorbance of the blank was measured immediately at 0 min. The percentage of inhibition was calculated as follows:

$$I\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

The IC_{50} value was determined as the concentration of each sample required to give 50% of the absorbance shown by the blank. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

2.5 Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD). Determination of IC_{50} was determined using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

The minimum inhibitory concentration (MIC) values of all extracts of *H. leonurus* Retz. are shown in Table 1. Classifications for the antimicrobial activity of the extracts were done by referring MIC values proposed by Magina *et al*. [13]. Thus, based on the MIC results, majority of the extracts showed weak to moderate activities with MIC values range 900-1800 µg/mL. Only two extracts which are REA and RM showed strong activities towards the tested microbial. REA exhibited potent activity against fungi *C. albicans* (MIC 225 µg/mL) and *A. niger* (MIC 450 µg/mL), while RM inhibited gram positive bacteria *S. aureus* with MIC value of 450 µg/mL.

Among the extracts, rhizome extracts possessed interesting antimicrobial potential compared to stem and leaf. Most of the rhizome extracts were active against *S. aureus, E. coli*, *C. albicans* and *A. niger* (MIC 225-900 µg/mL). Moreover, methanol extracts of each of three parts showed better antimicrobial activity than *n*-hexane and ethyl acetate extracts especially towards *S. aureus*, *E. coli*, *C. albicans* and *A. niger*. The growth of two tested fungi i.e. *C. albicans* and *A. niger* were prevented by more extracts where REA possessed the strongest inhibition towards both fungi followed by LEA, LM, RH, RM, SH and SM that possessed moderate antifungal activity and SEA that exhibited lower inhibition. LH extract portrayed moderate activity against *A. niger* but lower activity against *C. albicans*. Among the bacteria, *E. coli* and *P. putida* (Gram-negative bacteria) were more resistant compared to *S. aureus* (Gram-positive bacteria). This result was due to the present of barrier (antibiotics) at outer membrane of Gram-negative bacteria and the existing of the periplasmic space that contains enzymes that are able to break down foreign molecules. In addition, this bacterium was also able to reduce the cellular levels of antibiotics by having an efflux pump [13].

Table 1: Antimicrobial activity of *H. leonurus* Retz. extracts

Extracts[a]	Minimum inhibitory concentration (µg/mL)				
	Bacteria			Fungi	
	S. aureus	*E. coli*	*P. putida*	*A. niger*	*C. albicans*
LH	1800	1800	1800	900	1800
LEA	900	1800	1800	900	900
LM	900	900	1800	900	900
RH	900	900	900	900	900
REA	900	900	1800	450	225
RM	450	900	1800	900	900
SH	900	1800	1800	900	900
SEA	1800	1800	900	1800	1800
Streptomycin sulfate	56.25	56.25	56.25	nd	nd
Nystatin	nd	nd	nd	56.25	56.25

nd - not determined
[a]The tested extracts were LH: *n*-hexane leaf extract; LEA: ethyl acetate leaf extract; LM: methanol leaf extract; RH: *n*-hexane rhizome extract; REA: ethyl acetate rhizome extract; RM: methanol rhizome extract; SH: *n*-hexane stem extract; SEA: ethyl acetate stem extract; SM: methanol stem extract.
MIC ≥ 1600 µg/mL = weak, 600-1500 µg/mL = moderate and MIC ≤ 500 µg/mL = strong [13]

3.2 Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) scavenging assay is a rapid, simple and reproducible method to evaluate free radical scavenging activity. The scavenging of radical DPPH is detected by the decreasing of its absorbance at 517 nm [14]. The colour of DPPH radical will turn to yellow from purple when the odd electron of DPPH radical becomes paired with hydrogen from antioxidant compound [15].

Result of antioxidant activity of nine extracts of *H. leonurus* Retz. are tabulated in Table 2. All extract except RM, SEA and SM exhibited free radical inhibition more than 50%. Among the extracts, *n*-hexane extract of the rhizome showed the highest scavenging effect of 89.11 ± 1.03% at concentration 500 µg/mL with IC_{50} value of 59.60 ± 0.69 µg/mL compared to others. The antioxidant potential for this extract may be due to the synergistic effect of individual chemical compounds exist in the extract. However, this scavenging effect was lower than standard antioxidant, vitamin C which demonstrated 96.23 ± 0.00% inhibition with IC_{50} value of 21.21 ± 1.25 µg/mL.

Table 2: Antioxidant activity of *H. leonurus* Retz extracts

Extracts[a]	Percent inhibition at 500 µg/mL	IC_{50} Values (µg/mL)
LH	83.09 ± 1.29	98.33 ± 1.53
LEA	58.59 ± 0.16	300.97 ± 1.67
LM	64.92 ± 1.39	239.63 ± 0.64
RH	89.11 ± 1.03	59.60 ± 0.69
REA	51.65 ± 0.09	479.33 ± 1.15
RM	< 50	nd
SH	78.43 ± 1.63	122.67 ± 0.58
SEA	< 50	nd
SM	< 50	nd
Vitamin C	96.23 ± 0.00	21.21 ± 1.25

nd - not determined; Data represent mean ± SD of three independent experiments.
[a]The tested extracts were LH: *n*-hexane leaf extract; LEA: ethyl acetate leaf extract; LM: methanol leaf extract; RH: *n*-hexane rhizome extract; REA: ethyl acetate rhizome extract; RM: methanol rhizome extract; SH: *n*-hexane stem extract; SEA: ethyl acetate stem extract; SM: methanol stem extract.

4. CONCLUSION

The results obtained from this study revealed that several extracts especially from the rhizome of *H. leonurus* Retz has potential to be antifungal agents. The ethyl acetate extract of the rhizome has the potential to be used for treating diseases in plants caused by *A. niger* and treatment for candida infections caused by *C. albicans*. The extracts of

this plant also showed antioxidant activity, although rather mild. These extracts may be valuable natural antioxidant for increasing the shelf life of foodstuffs and for preventing cellular damage.

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REFERENCES

[1] Coisin, M., Burzo I., Stefan, M., Rosenhech, E. and Zamfirache, M.M. (2012). "Chemical Composition and Antibacterial Activity of Essential Oils of Three *Salvia* species, Widespread in Eastern Romania". *Analele Ştiinţifice ale Universităţii „Al. I. Cuza" Iaşis. II a. Biologie vegetală*. 58(1): 51-58.

[2] Baratta, M.T., Dorman, H.J.D., Deans, S.G., Figueiredo, A. C., Barroso, J.G. and Ruberto, G. (1998). "Antimicrobial and Antioxidant Properties of Some Commercial Essential Oils". *Flavour and Fragrance Journal*. 13: 235-244.

[3] Özkalp, B., Sevgi, F., Özcan, M. and Özcan, M.M. (2010). "The Antibacterial Activity of Essential Oil of Oregano (*Origanum vulgare* L.)". *Journal of Food, Agriculture and Environment.* 8(2): 272 - 274.

[4] Ling, L.R., Wahab, N.A. and Abidin, N.Z. (2005). "Cytotoxic Activity of Selected Zingiberaceae". *Malaysian Journal of Science.* 24: 207-212.

[5] Tg. Kamazeri, T.S.A., Abd Samah, O., Taher, M., Susanti, D. and Qaralleh, H. (2012). "Antimicrobial Activity and Essential Oils of *Curcuma aeruginosa*, *Curcuma mangga*, and *Zingiber cassumunar* from Malaysia". *Asian Pacific Journal of Tropical Medicine*. 202-209.

[6] Natta, L., Orapin, K., Krittika, N. and Pantip, B. (2008). "Essential Oil from Five Zingiberaceae for Anti Food-borne Bacteria". *International Food Research Journal.* 15(3): 337-346.

[7] Holtum, R.E. (1950). *The Zingiberaceae of the Malay Peninsula*, Volume 13, Garden's Bulletin Singapore, Singapore.

[8] Hashim, S.E., Sirat, H.M. and Yen, K.H. (2014). "Chemical Compositions and Antimicrobial Activity of the Essential Oils of *Hornstedtia havilandii* (Zingiberaceae)". *Natural Product Communications*. 9(1): 119-120.

[9] Burkill, I.H. (1966). *A Dictionary of Economic Products of the Malay Peninsula*, Volume 1, Ministry of Agriculture and Co-operatives, Kuala Lumpur.

[10] Gulluce, M., Sokmen M., Sahin, M., Sokmen, A., Adiguzel, A. and Ozer, H. (2004). "Biological Activities of the Essential Oil and Methanolic Extract of *Micromeria fruticosa* (L.) Druce ssp. *Serpyllifolia* (Bieb) PH Davis Plants from the Eastern Anatolia Region of Turkey". *Journal of the Science of Food and Agriculture.* 84: 735-741.

[11] Arias. M.E., Gomez. J.D., Cudmani. N.M., Vattuone. M.A. and Isla. M.I. (2004). "Antibacterial Activity of Ethanolic and Aqueous Extracts of *Acacia* aroma Gill. Ex Hook et Arn". *Life Sciences.* 75: 191-202.

[12] Tagashira, M. and Ohtake, Y.A. (1998). "New Antioxidative 1,3-benzodioxole from *Melissa officinalis*". *Planta Medica.* 64: 555-558.

[13] Magina, M.D.A., Dalmarco, E.D., Wisniewski, Jr A., Simionatto, E.L., Dalmarco, J.B., Pizzolatti, M.G. and Brighente, I.M.C. (2009). "Chemical Composition and Antibacterial Activity of Essential Oils of *Eugenia* species". *Journal of Natural Medicines.* 63: 345-350.

[14] Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995). "Use of A Free Radical Method to Evaluate Antioxidant Activity". *LWT- Food Science and Technology.* 28: 25-30.

[15] Heim, K.E., Tagliaferro, A.R. and Bobilya, D.J. (2002). "Flavonoid Antioxidants: Chemistry, Metabolism and Structure-Activity Relationship". *The Journal of Nutritional Biochemistry.* 13: 572-584.