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NMR metabolomics for identification of adenosine A1 receptor binding compounds from *Boesenbergia rotunda* rhizomes extract

Nancy Dewi Yuliana [a,b,c,*], Slamet Budijanto [b,c], Robert Verpoorte [a], Young Hae Choi [a]

[a] Natural Products Laboratory, Institute of Biology, Leiden University, 2300 RA Leiden, The Netherlands
[b] Department of Food Science and Technology, Bogor Agricultural University, IPB Darmaga Campus, PO Box 220, Bogor 16680, Indonesia
[c] Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, Jalan Puspa No. 1, IPB Darmaga Kampus, Bogor 16680, Indonesia

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ABSTRACT

Ethnopharmacological relevance: *Boesenbergia rotunda* Linn. (Zingiberaceae) is traditionally used in many Asian countries as medicine for stomach pain and discomfort, viral and bacterial infection, inflammation, and as diuretic agent.
Aim of the study: The study aimed to identify adenosine A1 receptor binding compounds from *Boesenbergia rotunda* rhizome extract by using comprehensive extraction coupled to the NMR metabolomics method.
Materials and methods: Dried and powdered *Boesenbergia rotunda* rhizomes were extracted with the comprehensive extraction method to obtain several fractions with different polarity. Each fraction was divided into two: for NMR analysis and for adenosine A1 receptor binding test. Orthogonal projection to the least square analysis (OPLS) was used to study the correlation between metabolites profile and adenosine A1 receptor binding activity of the plant extracts. Based on Y-related coefficient and variable of important (VIP) value, signals in active area of OPLS loading plot were studied and the respective compounds were then elucidated
Results and discussions: Based on OPLS Y-related coefficient plot and variable of importance value plot, several characteristic signals were found to positively correlate to the binding activity. By using 1D and 2D NMR spectra of one of the most active fraction, pinocembrine and hydroxy-panduratin were identified as the possible active compounds. Two signals from ring C of pinocembrine flavanone skeleton with negative coefficient correlations possibly overlapped with those of non-active methoxylated flavanones which were also presence in the extract. NMR based metabolomics applied in this study was able to quickly identify bioactive compounds from plant extract without necessity to purify them. Further confirmation by isolating pinocembrine and hydroxy-panduratin and testing their adenosine A1 receptor binding activity to chemically validate the method are required.
Conclusion: Two flavonoid derivatives, pinocembrine and hydroxy-panduratin, have been elucidated as possible active compounds bind to adenosine A1 receptor. Flavonoid was reported to be one of natural antagonist ligand for adenosine A1 receptor while antagonistic activity to the receptor is known to associate with diuretic activity. Thus, the result of this research supports the traditional use of *Boesenbergia rotunda* rhizome extract as diuretic agent.

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1. Introduction

Boesenbergia rotunda (Linn.) Mansf. or *Boesenbergia pandulata* (Roxb.) Schltr. (Zingiberaceae) is widely used as a spice in some Asian countries such as Indonesia, Malaysia, and Thailand. It is also used as traditional medicine for stomach pain and discomfort, viral and bacterial infection, inflammation, and as diuretic agent. A number of chalcones and flavonoids derivatives isolated from this plant has been reported as responsible compounds for the aforementioned medicinal uses (Abdelwahab et al., 2011; Bhamarapravati et al., 2006; Ching et al., 2007; Kiat et al., 2006; Mahmood et al., 2010; Morikawa et al., 2008; Tuchinda et al., 2002) except for diuretic activity. Antagonistic binding activity to adenosine A1 receptors have been reported to be associated with diuretics activity (Modlinger and Welch, 2003). Flavonoids form is a group of natural products which have been studied the most for its antagonistic activity to this receptor (Ji et al., 1996; Yuliana et al., 2009).

In this paper, the correlation of NMR signals of metabolites present in *Boesenbergia rotunda* extracts obtained from comprehensive extraction with its adenosine A1 receptor binding activity

* Corresponding author at: Department of Food Science and Technology, Bogor Agricultural University, IPB Dramaga Campus, Bogor 16680, Indonesia. Tel./fax: +62 251 8626725.
E-mail address: nancy_dewi@ipb.ac.id (N.D. Yuliana).

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was studied. The aim was to quickly identify which compounds have strong correlation to the receptor binding activity. This combination of comprehensive extraction and NMR metabolomics approach has been successfully applied to identify seven methoxy flavonoids from *Orthosiphon stamineus* which have binding activity to the receptor (Yuliana et al., 2011b). The method offers many benefits as compared to bioassay guided fractionation. Compounds that are important for the tested bioactivity can be easily identified to be further studied while common compounds that may cause false positive can be discarded at very early stage (Yuliana et al., 2011a).

2. Materials and methods

2.1. Plant material extraction

Dried *Boesenbergia rotunda* rhizomes were purchased from a traditional market in Bandung, Indonesia and were identified by one of the author (N.D. Yuliana). The voucher specimen was stored at Natural Products Laboratory, Leiden University. The rhizomes were powdered and subjected to comprehensive extraction with protocol as follow: 0.70 g of *Boesenbergia rotunda* powder was mixed with 0.05 g Kieselguhr, packed into stainless steel extraction column (L=4.00 cm, d=1.80 cm). The column was closed at both ends with fat free cotton and connected to a Waters 600E pump (Waters, Milford, MA). Organic solvents and filtered millipore water (500 mL each) were ultrasonicated and degassed before use. The combination of solvents used was *n*-hexane (A), acetone (B), and acetone–water 1:1 (C). The solvent was continuously delivered into the column in gradient (see Table 1). The fractions were collected in 10 mL tubes every 2 min with an automatic fraction collector and every 2 samples were combined to obtain 17 fractions at the end of extraction. The extraction was performed in 3 replicates. From each extraction 4 ml was sampled for bioassay, other 12 ml for NMR. All were dried under N_2 and put overnight in freeze drier before analysis. Concentration of the extracts for the bioassay and NMR were adjusted to 1.4 mg/ml DMSO and 5–10 mg/ml MeOD, respectively.

Table 1
Comprehensive extraction scheme.

Time (min)	Gradient	Flow rate (ml)
0–12	A 100%	4
12–32	A 100%–B 100%	4
32–44	B 100%	4
44–64	B 100%–C 100%	4
64–80	C 100%	4

2.2. NMR measurement and data analysis

NMR measurements were performed according to Kim et al. (2010). The solvent used was MeOD. The ^{1}H NMR spectra were automatically reduced to ASCII files. Bucketing was performed by AMIX software (Bruker, Karlsruhe, Germany). Spectral intensities were scaled to total intensity and reduced to integrated regions of equal width (0.04) corresponding to the region of δ 0.3–10.0. The regions of δ 4.75–4.90 and δ 3.28–3.34 were excluded from the analysis because of the residual signal of D_2O and MeOD, respectively. Orthogonal projection to the latent structure (OPLS) analysis were performed with the SIMCA-P software (v. 12.0, Umetrics, Umeå, Sweden) with scaling based on the Pareto method.

2.3. Adenosine A1 receptor bioassay

The assay was performed as previously described (Chang et al., 2004) except that the volume of the total mixture in the assay was 200 μL. The radioactive ligand used for the assay was 0.4 nM [3H] DCPCX (8-cyclopentyl-1,3-dipropylxanthine) (K_d=1.6 nM). Membranes were prepared from Chinese hamster ovary (CHO) cells stably expressing human adenosine receptors by a method previously described (Dalpiaz et al., 1998). Non-specific binding was determined by using 10 μM CPA (N6-cyclopentyladenosine). The mixture consisting of 50 μL [3H] DPCPX, 50 μL CPA/50 mM Tris–HCl buffer/test compounds in different concentrations, 50 μL 50 mM Tris–HCl buffer pH 7.4, and 50 μL of membrane was incubated at 25 °C for 60 min and then filtered over a GF/B Whatman filter under reduced pressure. The filters were washed three times with 2 mL ice-cold 50 mM Tris/HCl buffer, pH 7.4, and 3.5 mL scintillation liquid was added to each filter. The radioactivity of the washed filters was counted by a Hewlett-Packard Tri–Carb 1500 liquid scintillation detector. Non-specific binding was determined in the presence of 10–5 M CPA.

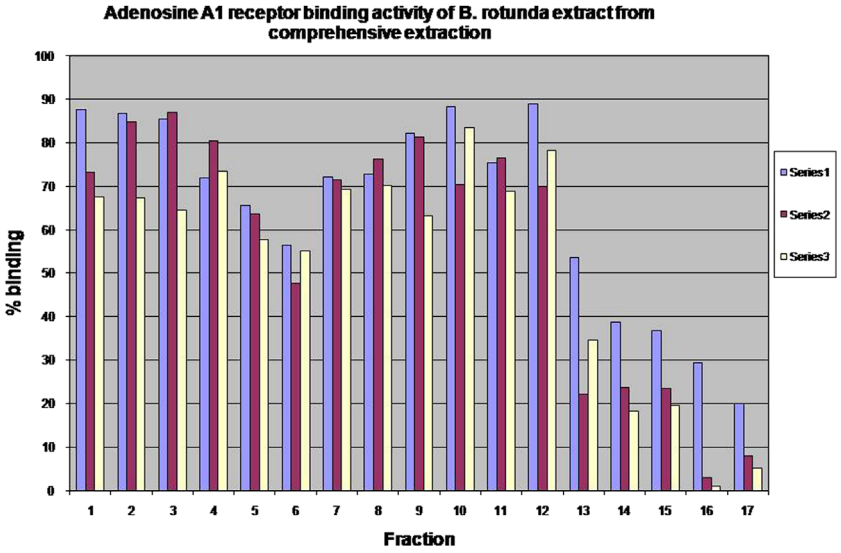

Fig. 1. Adenosine A1 receptor binding activity profile of *Boesenbergia rotunda* fractions obtained from comprehensive extraction.

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