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Potent water extracts of Indonesian medicinal plants against PTP1B

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ABSTRACT

Objective: To examine the potent of water as a solvent agent in the preparation of traditional herbal medicine.

Methods: Water extracts of 18 plants were prepared through reflux and examined (25 μg/mL) to evaluate their possibility for inhibiting protein tyrosine phosphatase 1B (PTP1B). The determination of IC_{50} values was performed for the samples possessing more than 80% inhibition. Meanwhile, those exhibiting IC_{50} values more than 7.0 μg/mL were further profiled for their chemical constituents through nuclear magnetic resonance (NMR) measurement.

Results: About 44% (8) of the examined samples showed more than 80% inhibition against PTP1B. The water extracts of *Elephantopus scaber*, *Helicteres isora* aerial parts, *Elaeocarpus grandiflorus* (*E. grandiflorus*) fruits, *Melaleuca leucadendron* leaves, and *Quercus infectoria* gum had IC_{50} values ranging from 2.05 to 6.90 μg/mL. Meanwhile, *Andropogon nardus* and *Centella asiatica* were at the area of δ 3.0–4.0 ppm. Further, the ^{13}C NMR observation of samples possessing the most intensive signals in their proton NMR *Cinnamomum burmannii* and *E. grandiflorus* showed the peaks at the area of δ 60–90 ppm as the supportive evidence for sugar group signals. Intriguingly, a disaccharide from *E. grandiflorus* could be an active inhibitor towards PTB1B.

Conclusions: In contrast to the mainstream solvents currently used in modern herbal manufactures especially Jamu medicine in Indonesia, pure-water-extracted materials should be reconsidered and could be reemerged for future studies and for the manufacture of herbal medicines. In addition, the activity of Jamu components should be confirmed that their antidiabetes and antiobesity activities could be through the inhibition of PTP1B.

1. Introduction

Traditional medicines *e.g.* medicinal plants have been embedded in human civilization. Indonesian herbal preparation, locally called Jamu medicine, remains popular to date. Its preparation was originally done by crushing or squeezing or boiling herbal materials in water. In the past, people and traditional societies mainly employed water as the extracting solvent [1]. In this way, water soluble matters became the main constituent of traditional medicines. In two decades, Jamu has been formulated and prepared in modern dosage forms. Jamu industries have also recently been thriving very well. At the main time, Jamu medicines industries have been thriving very well in two decades. As revealed by Indonesian Ministry of

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Health through health basic research report, 49% of 240 million Indonesian population is Jamu users [2].

The introduction of organic solvents for herbal extraction and support of official guiding monographs [3,4], however, has indirectly encouraged the ethanol usage in industrial field as well as in academic activities. Indeed, this became a hall mark of an era where a vast majority of registered Jamu products in the market are prepared from ethanol extract (ethanol 50%–96%). The supportive advantages of using ethanol are in view of its fast drying process, less toxic content, and simplicity to avoid microbial growth in comparison to water. This phenomenon, as a consequence, has marginalized the water utility in herbal extraction in the last two decades. Previously, Jamu was prepared mainly as recenter paratus by infusion or decoction [5], in which water soluble matters were the main component. In other words, representing herbal medicines with organic solvent extracts is most likely not factual and could be biased from its historical traditions.

To confirm the nature of traditional preparation of herbal medicines through water solvent extraction, 18 water extracts referred in traditional formulation records of Jamu components for antidiabetes or antiobesity: *Abrus precatorius* L. (*A. precatorius*), *Acarus calamus* L. (*A. calamus*), *Andropogon nardus* L. (*A. nardus*), *Centella asiatica* L. (*C. asiatica*), *Coriandrum sativum* L. (*C. sativum*), *Elaeocarpus grandiflorus* Smith (*E. grandiflorus*), *Elephantopus scaber* L. (*E. scaber*), *Helicteres isora* L. (*H. isora*), *Imperata cylindrica* (L.) P. Beauv (*I. cylindrica*), *Kaempferia galanga* L. (*K. galanga*), *Melaleuca leudendron* L. (*M. leudendron*), *Myristica fragrans* (Houtt.) (*M. fragrans*), *Nigella sativa* L. (*N. sativa*), *Pandanus amaryllifolius* Roxb. (*P. amaryllifolius*), *Parkia roxburghii* G. Don. (*P. roxburghii*), *Phyllanthus niruri* L. (*P. niruri*), *Quercus infectoria* (Oliver) (*Q. infectoria*), and *Trigonella foenum-graecum* L. (*T. foenum-graecum*) were submitted for test against protein tyrosine phosphatase 1B (PTP1B) as a molecular target model. PTP1B is a validated negative insulin regulator in its main targeted organs, *i.e.* lever, kidney, and skin [6–8]. Meanwhile, excessive PTP1B in brain will impair the signaling of leptin, a major appetite regulator [9,10]. Here, the samples showing more than 80% inhibitory activities were further determined for their IC_{50} values. To observe chemical constituent groups which could be possible corresponding for this bioactivity, a chemical profiling was conducted by using proton and carbon nuclear magnetic resonance [$^{1}H/^{13}C$ nuclear magnetic resonance (NMR)]. NMR spectrometry is a reliable and comprehensive method for being able to provide a "holistic view" [11] compared to the other customized analytical methods such as liquid or gas chromatography, infrared spectrometry, and mass spectrometry. The area of diagnostics, *i.e.* amino acids (δ 2–2.8 ppm), sugars (δ 3–4 ppm), and aromatic regions (δ 6–8 ppm) that NMR allows us to specifically distinguish the peaks of interest. It is robust, no need for calibration, indestructible towards analyses, and efficiency *e.g.* 10 min for data acquisition. In a course of studies for finding PTP1B inhibitors, water extract of *Blumea balsmifera* leaves, *Cinnamomum burmannii* (*C. burmannii*) barks, *Syzygium aromaticum* fruits, and *Syzygium polyanthum* leaves exhibited some potent activities with IC_{50} values of 2.68, 6.25, 4.07, and 3.14 μg/mL, respectively [12]. Moreover, they have been recorded in Jamu formulations for the treatment of diabetes and obesity as well [13,14]. In this paper, we also report their chemical constituent profiles based on NMR spectra.

2. Materials and methods

2.1. Herbal materials

The herbal materials used in this study (Table 1) were purchased from Pasar Genteng Surabaya and authenticated by one of the authors (Dr. Subehan). The voucher specimens were preserved in Museum of Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Toyama, Japan.

2.2. Preparation of test solutions

Each medicinal herb (25–150 g) was cut into small pieces and extracted with water (150–400 mL, 1-h reflux, 100 °C, ×2). After filtration, the water solution was concentrated under a reduced pressure, followed by lyophilization, to result in a water extract. All extracts were stored under −80 °C before being in use. For the PTP1B inhibitory assay, the extracts were dissolved in distilled water at the concentration of 1 mg/mL and aliquots of the solution were added to each well. The final concentration for each sample was 25 μg/mL.

2.3. PTP1B inhibitory assay

The assay was performed in 96-well clear polystyrene microplate (Corning, NY, USA) according to Cui *et al.* with slight modifications [21]. Each well contained 0.05 μg PTP1B (Enzo Life Sciences, Farmingdale, NY), 2 mmol/L *p*-nitrophenyl phosphate (pNPP; Wako Pure Chemical Industries, Osaka, Japan), and 50 mmol/L citrate buffer containing 0.1 mmol/L NaCl, 1 mmol/L dithiothreitol, and 1 mmol/L ethylenediamine-N,N,N′,N′-tetraacetic acid. The final volume of the mixture was 200 μL. The reaction was initiated by the addition of pNPP, incubated at 37 °C for 30 min, and terminated with the addition of a stop solution (10 mol/L NaOH). The amount of *p*-nitrophenol produced was estimated by measuring the absorbance at 405 nm using a Perkin-Elmer HTS 7000 bioassay reader. To identify the level of nonenzymatic substrate hydrolysis, the absorbance of wells only containing buffer and pNPP were measured for correction. The difference between full enzymatic activity and the correction was set arbitrarily to 100% activity. The percent residual activity was calculated using the following formula:

$$\text{Residual activity } (\%) = \frac{[\text{Abs(full enzymatic)} - \text{Abs(test sample)} - \text{Abs(correction)}]}{[\text{Abs(full enzymatic)} - \text{Abs(correction)}]} \times 100$$

where Abs(full enzymatic) was the absorbance of *p*-nitrophenol liberated by the enzyme in the system without a test sample in contrast to Abs(test sample). The assays were performed in triplicate for all samples. The recognized phosphatase inhibitors, RK-682 (purity 98%; Enzo Life Sciences) [22] and ursolic acid (purity 90%; Tokyo Chemical Industry, Tokyo, Japan) [23] were used as the positive controls.

2.4. Statistical analyses

IC_{50} values were obtained by calculating the linear regression using MS Excel. A number of statistical differences were then

compared using ANOVA with Tukey's *post-hoc* test. P values < 0.05 were considered to be significant.

2.5. NMR measurement

NMR spectra were measured using a JEOL JNM-LA400 spectrometer. Chemical shifts were given in the δ scale. Sample for measurement was 15–20 mg and dissolved up to 0.8 mL with D_2O (Cambridge Isotope Lab, UK). The NMR chemical shifts in the spectra were referenced to 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid at 0.00 ppm. For ^{1}H NMR measurement, each spectrum consisted of 96 scans of 32768 points. On the other hand, ^{13}C NMR of *Eucalyptus floribunda* and *C. burmannii* measurements consisted of 43478 and 28553 scans, respectively.

3. Results

3.1. PTP1B inhibitory activity

Among the extracts listed in Figure 1, *A. nardus* (85.2%), *C. asiatica* aerial parts (83.3%), *E. grandiflorus* fruits (96.4%), *E. scaber* leaves (96.4%), *H. isora* gum (95.9%), *Melaleuca leucadendron* (*M. leucadendron*) leaves (96.0%), *P. niruri* (83.3%), and *Q. infectoria* (94.7%) exhibited more than 80% inhibition at the concentration of 25 μg/mL. Meanwhile, the positive controls RK-682 and ursolic acid exhibited 98.8% inhibition.

In contrast, *P. roxburghii* (65.4%) extract showed a moderate activity. The extracts of *A. precatorius* (6.6%), *A. calamus* (21.6%), *C. sativum* (12.1%), *I. cylindrical* (18.1%), and *P. amaryllifolius* (17.6%), meanwhile, exhibited mild activities (2.3%–21%). Their tested solutions formed darker color producing higher absorbance values compared to the blank solutions, *K. galanga*, *N. sativa* and *T. foenum-graecum* seed that showed minus inhibitions, ranging from −14.2% to −0.6%. However, the negative values were justified as the inactive samples. This indicates that the activity mechanisms of these recorded components, both as antidiabetes and as obesity remedies, might be performed through other molecular targets or pathways.

Further IC_{50} examination of the samples showing more than 80% inhibitions, they demonstrated a dose dependent inhibitions with IC_{50} values ranging from 2.05 to 13.20 μg/mL (Table 2). In particular, *M. leucadendron* was the most potent sample demonstrating the IC_{50} value equal to that of the positive control RK-682 (2.05 μg/mL) and more potent compared to ursolic acid (IC_{50} = 9.11 μg/mL). Interestingly, those potent samples were made up 44% of examined samples. Hence, their traditionally reported antidiabetes and obesity activity could be due to PTP1B inhibition.

3.2. Analyses of chemical composition of water extract

To observe the chemical constituents that could be possible to be a corresponding constituent to inhibitory activity, NMR measurement was conducted towards those potent samples. The area of diagnostics *i.e.* amino acids, sugars, vinyl, and aromatic chemical shift zones, both ^{1}H and ^{13}C NMR spectra that allowed us to specifically distinguish the peaks of interest. According ^{1}H NMR spectra, those extracts exhibited some dominant peaks ranging from δ 3–4.2 ppm (Figure 2).

Therefore, most of active samples showed the peaks of oxygenated methylene protons called as sugar zone. Although *Blumea balsamifera* and *H. isora* spectra showed some aliphatic signals at δ 1.2–2.6 ppm and vinyl peak signals at δ 6–7 ppm which were attributable as aliphatic and aromatic of amino acid signals, these peaks exhibited several short intensities which could be present in low quantity. For this reason, sugars must be present in much higher concentration in all active samples and contributed significantly to their activity. Meanwhile, *E. scaber*, *A. nardus*, and *M. dendron* extracts had some comparable intensities in both sugar and amino acid peak signals (δ 1.2–2.5 ppm) (Figure 2b, c and f). In an aqueous extract, sugars were found usually including fructose, sucrose, and glucose. Differently, amino acids frequently present are arginine, alanine, glutamate, serine, glutamine, asparagine, histidine, proline, and valine. The available organic acids included malic, ascorbic, citric, and succinic acid [24].

Considering our experiences that sugars normally have a low signal intensity in ^{13}C NMR measurements, we then chose

Table 1
Indonesian medicinal plants, used part and usage or indication.

Species	Family	Voucher	Used part	Recorded usage or indication	Reference	Extraction yield (%)
A. precatorius	Fabaceae	24925	Leaves	Antidiabetes	[14]	9.5
A. calamus	Acaroceae	24923	Roots	Antidiabetes	[15]	14.5
A. nardus	Poaceae	24905	Leaves	Antidiabetes and antiobesity	[13]	6.4
C. asiatica	Apiaceae	24919	Aerial parts	Antidiabetes	[14]	22.0
C. sativum	Apiaceae	24901	Fruits	Antiobesity and hyperlipidemia	[14]	7.6
E. grandiflorus	Alaeocarpaceae	24916	Fruits	Antidiabetes	[16]	3.3
E. scaber	Asteraceae	24931	Aerial parts	Antiobesity and hyperlipidemia	[13]	16.7
H. isora	Malvaceae	24922	Gum	Antidiabetes	[17]	10.2
I. cylindrica	Poaceae	24918	All parts	Antiobesity and hyperlipidemia	[14]	7.5
K. galanga	Zingiberaceae	24905	Rhizomes	Antidiabetes, antiobesity and hyperlipidemia	[13,14]	6.7
M. leudendron	Myrtaceae	24917	Leaves	Antiobesity and hyperlipidemia	[13]	3.4
M. fragrans	Myrtaceae	24904	Seeds	Antiobesity and hyperlipidemia	[13,18]	9.3
N. sativa	Ranunculaceae	24902	Seeds	Antihiperlidemia	[19]	13.6
P. amaryllifolius	Pandanaceae	24911	Aerial parts	Diabetes mellitus	[18]	3.9
P. roxburghii	Fabaceae	24929	Seeds	Antiobesity and hyperlipidemia	[13]	11.2
P. niruri	Euphorbiaceae	24930	Aerial parts	Antiobesity and hyperlipidemia	[13]	14.1
Q. infectoria	Fagaceae	24924	Fruits	Antiobesity and hyperlipidemia	[13]	59.1
T. foenum-graecum	Fabaceae	24914	Seeds	Antidiabetes	[20]	1.7

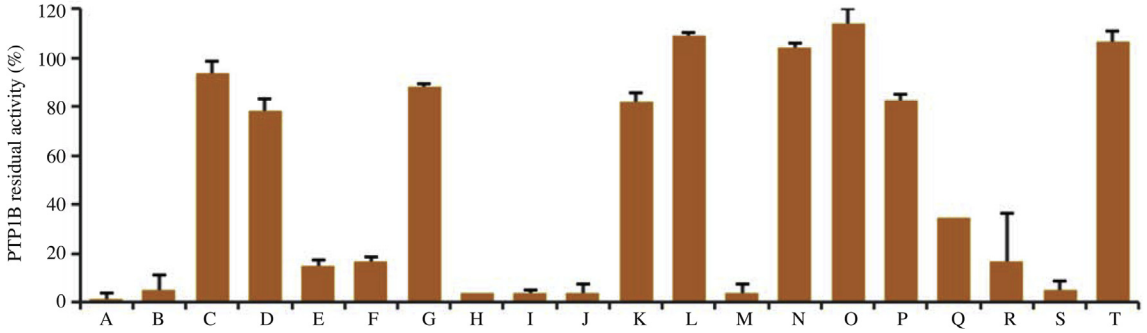

Figure 1. PTP1B residual activity due to the effect of water extract of Indonesian medicinal plants at a concentration of 25 μg/mL. RK-862 and ursolic acid were used as the positive controls. Residuals higher than 100% were related to a darker tested solution resulted by three samples. Data points represent mean ± SEM; n = 3. A: RK-862; B: Ursolic acid; C: *A. precatorius*; D: *A. calamus*; E: *A. nardus*; F: *C. asiatica*; G: *C. sativum*; H: *E. grandiflorus*; I: *E. scaber*; J: *H. isora*; K: *L. cylindrica*; L: *K. galanga*; M: *M. leudendron*; N: *M. fragrans*; O: *N. sativa*; P: *P. amaryllifolius*; Q: *P. roxburghii*; R: *P. niruri*; S: *Q. infectoria*; T: *T. foenum-graecum*.

Table 2

Samples with ≥ 80% inhibition and their IC_{50} values.

Samples	IC_{50} (μg/mL)
A. nardus	10.63
C. asiatica	13.20
E. grandiflorus	6.90
E. scaber	2.64
H. isora	3.49
M. leucadendron	2.05
P. niruri	10.99
Q. infectoria	4.68
RK 682	2.05
Ursolic acid	9.11

C. burmannii and *E. grandiflorus* samples possessing intensive ^{1}H NMR spectra to measure. Their spectra confirmed the presence of sugar carbon signals at δ 60–90 ppm. Although ^{13}C NMR of *C. burmannii* showed three peaks between δ 90–105 ppm and two short peaks at δ 145 and 152 ppm, respectively, these peaks should be the part of sugar structures (Figure 3). Interestingly, ^{1}H and ^{13}C NMR spectrum of *E. grandiflorus* clearly revealed eleven carbon signals at δ 60.1, 62.9, 63.2, 64.2, 64.7, 70.0, 71.8, 73.2, 73.4, 74.8, 77.2, 82.2, and 104.2 ppm (Figure 3d), which were similar to those of sucrose [25]. Moreover, its ^{1}H NMR signal at δ 5.2 ppm (Figure 3c, arrow) also showed a good agreement [11,24].

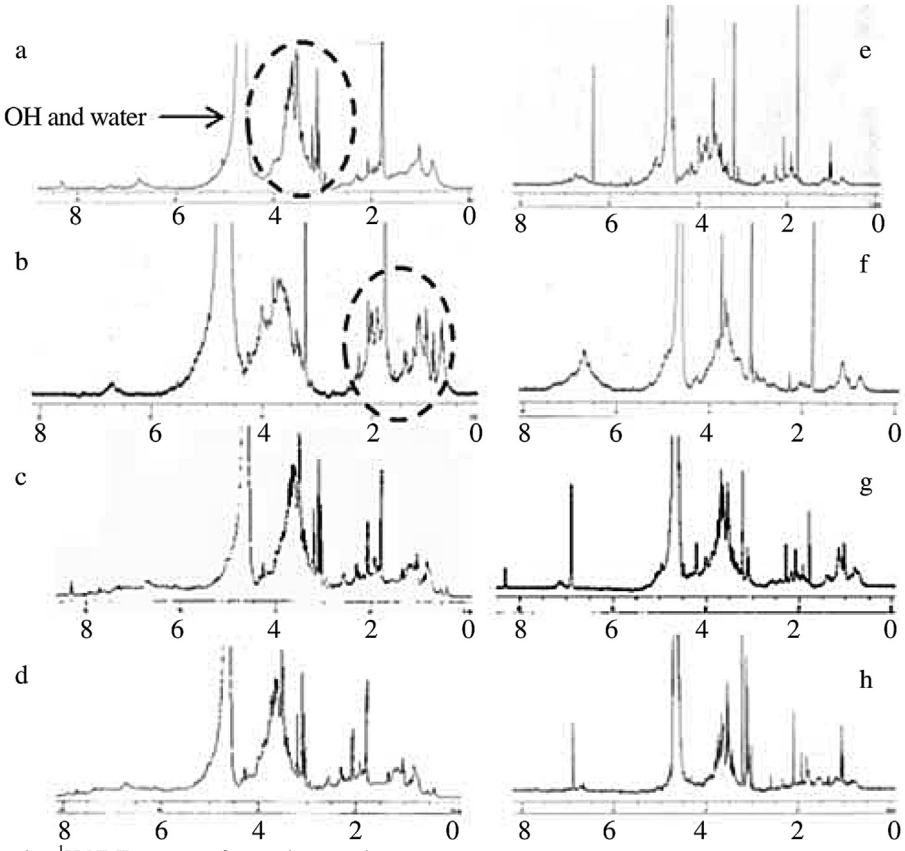

Figure 2. The representative ^{1}H NMR spectra of several water plant extracts. ^{1}H NMR spectrum of a: *A. nardus*; b: *E. scaber*; c: *P. niruri*; d: *Syzygium aromaticum*; e: *Blumea balsamifera* leaves; f: *H. isora* fruits; g: *M. leucadendron*; h: *Syzygium polyanthum* leaves. All samples showed sugar proton signals at δ 3.0–4.5 ppm. It was only *E. scaber* that showed a comparable intensity of amino acid signals at δ 1.5–2.5 ppm. Internal standard (3-(trimethylsilyl) propionic-2,2,3,3-d4 acid).

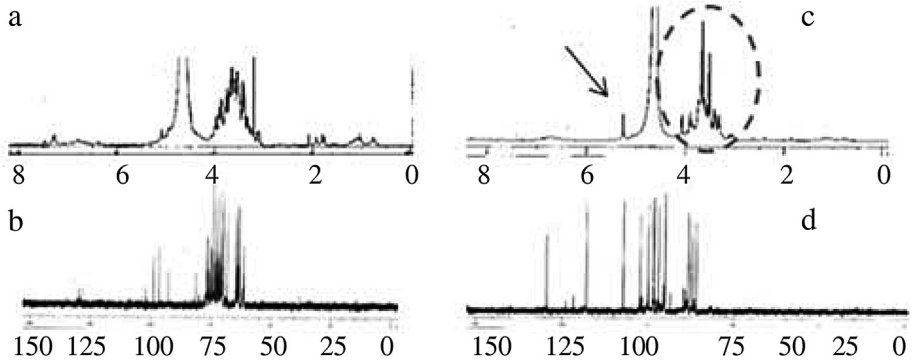

Figure 3. The representative NMR spectra of water extracts of *C. burmannii* and *E. grandiflorus*.
a and b: ^{1}H and ^{13}C NMR spectra of *C. burmannii* leaves; c and d: ^{1}H and ^{13}C NMR spectra of *E. grandiflorus* fruits.

Therefore, on the basis of both NMR spectra the compound was deduced as a disaccharide which could be a succrose. The sugar remarkably showed 95% purity according to this 1D NMR analyses. Hence, this simple sugar should be the main contributor towards its extract activity. However, this case should not be taken into account for *in vivo* case immediately.

4. Discussion

This study was embarked from a very simple hypothesis that water extracts contain a group of polar compounds rather than secondary metabolites. Thus, based on the fact, traditional preparation have been relaying on water extraction. The water extracts of all 18 samples of Indonesian medicinal plants recorded in Jamu formulations or reported as antidiabetes or antiobesity were examined for their ability to inhibit PTP1B-mediated dephosphorylation of pNPP. Based on NMR analyses, it is convincing that instead of secondary metabolites, the activity of original Jamu medicines is due to the significant contribution of sugars or synergism or plausible effect among sugars, amino acids, and organic acids. Hence, water extracted herbal medicine has a distinguished paradigm and secondary metabolite based on Jamu medicine is another paradigm.

To date, the reports on the activities of carbohydrate class on antidiabetes related target via the inhibitory of PTP1B are still limited. It is just recently reported that saccharide from *Astragalus membranaceus* inhibits PTP1B and decreases PTP1B expression via relieving endoplasmic reticulum stress [26]. Saccharides from *Morus alba* leaves exhibited the hepatic glucose metabolism and insulin signaling by inhibiting the expression of PTP1B [27]. Acidic proteoglycan from fungi *Ganoderma lucidum* shows the competitively inhibiting PTP1B [28,29]. However, rather than saccharides, micromolecules have been becoming an interest in hitherto PTP1B inhibitor discoveries [30–32]. Thus, carbohydrates could also be a promising candidate to explore in future studies. Previously, we demonstrated the pairs of organic and water extracts in total of 56 extracts [12]. Of these extracts, 9 water extracts and 11 methanol extracts showed more than 70% inhibition with IC_{50} values ranging from 2.05 to 13.4 μg/mL. From the methanol extract, we isolated the active secondary metabolite type comprising sequiterpens, triterpenes, and phloroglucinols. Hence, in our current study, the water extracts activity was contributed significantly by sugars and polar matters. Unfortunately, the use of pure water as a solvent is almost absent in the official guidances for industrial scale issued by Indonesian Drug and Food Control Agency [33]. Therefore, to some extent it should be revised based on Jamu historical preparations.

On the basis of the findings, water soluble extracts should not be ignored from utilities. It can be noted here that the paradigm of representing traditional medicine preparations with organic solvents seem to be inappropriate. For modern herbal manufacturers, reintroducing water as an extracting solvent should be considered. Although prepared in organic or water solvent, Jamu medicine must be simultaneously and scientifically confirmed regarding their pharmacological effect and chemical constituents. Finally, it is worth noting that the results of 44% those potent water extracts should also confirm the possibility of recorded Jamu components for antidiabetes and obesity through the inhibition of PTP1B activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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