Development of Quality Control Method for Glucofarmaka Antidiabetic Jamu by HPLC Fingerprint Analysis

Hanifullah Habibie[1], Rudi Heryanto[1,2], Mohamad Rafi[1,2,*], and Latifah Kosim Darusman[1,2]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University*
Jl. Tanjung Kampus IPB Dramaga, Bogor 16680, Indonesia

[2]*Tropical Biopharmaca Research Center, Bogor Agricultural University,*
Jl. Taman Kencana No. 3 Kampus IPB Taman Kencana, Bogor 16128, Indonesia

Received October 31, 2016; Accepted January 6, 2017

ABSTRACT

Herbal medicines become increasingly popular all over the world for preventive and therapeutic purposes. Quality control of herbal medicines is important to make sure their safety and efficacy. Chromatographic fingerprinting has been accepted by the World Health Organization as one reliable strategy for quality control method in herbal medicines. In this study, high-performance liquid chromatography fingerprint analysis was developed as a quality control method for glucofarmaka antidiabetic jamu. The optimum fingerprint chromatogram was obtained using C18 as the stationary phase and linear gradient elution using 10–95% acetonitrile:water as the mobile phase within 60 min of elution and detection at 210 nm. About 20 peaks were detected and could be used as a fingerprint of glucofarmaka jamu. To evaluate the analytical performance of the method, we determined the precision, reproducibility, and stability. The result of the analytical performance showed reliable results. The proposed method could be used as a quality control method for glucofarmaka antidiabetic jamu and also for its raw materials.

Keywords*: fingerprint analysis; glucofarmaka; antidiabetic jamu; HPLC; quality control*

ABSTRAK

Pengobatan herbal semakin populer di seluruh dunia baik untuk tujuan preventif maupun terapeutik. Upaya kendali mutu obat herbal menjadi hal yang penting agar terjamin keamanan dan khasiatnya. Sidik jari kromatografi telah diterima oleh Organisasi Kesehatan Dunia (WHO) sebagai strategi yang dapat diandalkan untuk metode kendali mutu obat herbal. Pada penelitian ini, analisis sidik jari kromatografi cair kinerja tinggi dikembangkan sebagai metode kendali mutu jamu antidiabetes glucofarmaka. Kromatogram sidik jari optimum diperoleh menggunakan C18 sebagai fase diam dengan elusi gradien linear 10–95% asetonitril:air sebagai fase gerak selama 60 menit dan deteksi pada 210 nm. Sebanyak 20 puncak mampu terdeteksi dengan metode ini dan dapat digunakan sebagai sidik jari jamu glucofarmaka. Untuk mengevaluasi kinerja analitik metode ini ditentukan nilai presisi, kedapatulangan, dan stabilitas. Hasil pengujian kinerja analitik menunjukkan hasil yang memuaskan. Metode yang diusulkan ini dapat digunakan sebagai metode kendali mutu jamu antidiabetes glucofarmaka dan bahan bakunya.

Kata Kunci: *analisis sidik jari; KCKT; jamu antidiabetes; glucofarmaka; kendali mutu*

INTRODUCTION

Nowadays, diabetes is one of the most worrisome health issues. Its prevalence has increased significantly in recent years. In 2015, a patient with diabetic reached about 415 million people all around the world. Diabetes contributes up to 14.5% to global death [1]. Treatment for this disease usually involves synthetic drugs to decrease the blood sugar level. However, the use of the synthetic drugs sometimes causes negative side effects [2]. This situation makes some people change their choice of drugs to herbal medicines which are considered as relatively safer. Biopharmaca Research Centre-Bogor Agricultural University has developed a new formula of antidiabetic *jamu* called glucofarmaka. This antidiabetic *jamu* consists of four plants (*Tinospora crispa, Zingiberoffcinale, Blumea balsamifera,* and *Momordica charantia*). *In vivo* assay for glucofarmaka using streptozotocin-induced mice showed a fairly good result in decreasing blood glucose level after 2 weeks of treatment [3].

The use of different sources of raw material in a large scale production will result in inconsistency of efficacy and quality of herbal medicine. Raw materials from different sources will give the different composition of chemical components that in the end, it

* Corresponding author.
Email address : mra@ipb.ac.id

DOI: 10.22146/ijc.23616

will affect the final product's efficacy. Thus, a quality control method is required to guarantee the quality of the raw materials used in the production of the herbal medicines in order to provide consistency in efficacy. In general, there are two approaches to the development of the quality control method for herbal medicines, namely compound-based and pattern-based approaches [4].

The compound-based approach uses one or some chemical compounds in the herbal medicines related to their quality. However, the use of marker compound sometimes is not relevant because generally, the efficacy of *jamu* comes from many compounds work in synergy [5]. The pattern-based approach sometimes is also called fingerprint analysis, will evaluate all detectable compounds in herbal medicines from an analytical instrument, such as TLC, HPLC, or FTIR without the necessity to characterize all compounds. Therefore, this approach will give a comprehensive representation of all compounds in the herb materials for evaluating the quality in order to guarantee the consistency of the raw materials and the herbal products. Accordingly, fingerprint analysis has the advantages to show all metabolites detected in a sample and their relative concentration proportion [6]. High-performance liquid chromatography (HPLC) is regarded as a popular analytical instrument applied to develop fingerprint of herbal medicines due to precision, sensitivity, and reproducibility. It is not limited by the volatility or stability of the sample compounds. Therefore, HPLC can be used to analyze almost all compounds that present in the herbal medicines [7].

In this study, we developed a fingerprint analysis method by using HPLC for quality control of glucofarmaka antidiabetic *jamu*. In addition, we also performed metabolite profiling using LC-MS (Liquid Chromatography-Mass Spectrometry) for identifying metabolites present in glucofarmaka *jamu* that can be used for further studies to find a marker compound of this *jamu*.

EXPERIMENTAL SECTION

Materials

Sembung leaves (*Blumeabalsamifera*), *brotowali* stems (*Tinosporacrispa*), ginger rhizomes (*Zingiberofficinale*), and *pare* leaves (*Momordicacharantia*) were used to make glucofarmaka antidiabetic *jamu*. All samples were obtained from Biopharmaca Research Centre, Bogor Agricultural University and identified in Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Indonesia. Voucher specimens of all samples were deposited at Biopharmaca Research Center, Bogor Agricultural University, Indonesia (BMK 0043052015–BMK 0046052015). Distilled water, methanol, and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Ekicrodisc 25R filter 0.45 µm was purchased from Gelman Science Inc. (Tokyo, Japan).

Instrumentation

HPLC LC-20A series (Shimadzu, Tokyo, Japan) with diode array UV detector, Shim-pack column VP-ODS C18 (150 mm x 4.6 mm i.d., particle size 4.6 µm) (Shimadzu, Tokyo, Japan) was used for HPLC fingerprint analysis. UPLC-QTOF-MS Waters Xevo G-2S using C18 column (50 mm x 2.1 mm i.d., particle size 1.7 µm) was used for identification of metabolites present in glucofarmaka antidiabetic *jamu*. MZmine 2,14,2 version software was used for data analysis from UPLC-QTOF-MS.

Procedure

Preparation of antidiabetic jamu extract

All samples were dried and pulverized prior to use. Powdered samples were weighed and mixed as the appropriate composition of the *jamu* formula to give 400 g total weight. Subsequently, the powder was poured into 5 L flask and added with 2800 mL of aquadest and boiled for 1 h. The extract was filtered by using a gray cloth to obtain a filtrate. The extraction was repeated twice. The resulting *jamu* filtrate was collected and concentrated using vacuum evaporator.

Optimization of HPLC fingerprinting of antidiabetic jamu

About 6 g of a powder mixture of all samples was diluted with 40 mL of methanol p.a and then sonicated for 2 x 15 min. After that, the extract was filtered through 0.45 µm membrane filter. This solution was injected into HPLC with an injection volume of 20 µL. The column temperature was set to 35 °C with a flow rate of the mobile phase is 1 mL/min. The parameters varied in the optimization of HPLC fingerprint analysis were the type and the composition of the mobile phase, and the length of analysis time. The wavelengths used were 210, 254, and 280 nm. A mixture of water and an organic modifier (acetonitrile or methanol) was used for mobile phase. First, elution was performed with a linear gradient of 5–95% organic modifier. Next, the initial and the final points of the organic modifier were determined by the elution profile obtained in the first step [8].

Analytical performance of HPLC fingerprint method

Analytical performance of HPLC fingerprint was determined by using chromatograms of glucofarmaka *jamu* extract. The parameters used for evaluation were

precision, reproducibility, and stability expressed as relative standard deviation value (RSD) of the relative retention time (RRT) and relative peak area (RPA) towards reference peak. The precision test was based on the RSD value of the RRT and the RPA of 5 injections of the same sample solution. Reproducibility test was performed by injecting 5 sample solutions prepared independently. The stability test was done by analyzing the sample solutions at 0, 3, 6, 24, and 48 h after prepared at room temperature.

Sample preparation and metabolite profiling using LC-MS

About 200 mg of glucofarmaka *jamu* extract powder was diluted with 10 mL of methanol p.a, sonicated for 5 min and filtered by using 0.45 µm membrane filter. 5 µL sample was injected into LC-MS. Mass Spectrometer was operated in negative ion mode with approximation at *m/z* 30 to 700. ESI parameters used include the capillary temperature at 120 °C, 50 L/h gas injection, + 2.9 kV potential source, temperature source at 41 °C. The eluent used was 10–90% ACN-water for 10 min in gradient elution system.

Data analysis

LC-MS output was converted into NetCDF format so that it could be easier to process. Then, LC-MS chromatogram was processed by MZmine software to change the chromatogram data into a mass array. MZmine data processing was carried out in some steps, i.e. filtering and baseline correction, peak detection, deisotoping, alignment, gap filling, and normalization [9]. The mass arrays are given contained information of the detected peak mass, retention time, intensity, and normalized peak area.

Interpretation and metabolite identification of glucofarmaka jamu extract

Identification of metabolite from the output data of LC-MS was carried out by comparing *m/z* of the mass array to the metabolite compound listed in *KNApSAcK* database and comparing the mass fragmentation patterns with the mass fragmentation pattern of the predicted compounds in literature.

RESULT AND DISCUSSION

Optimization of HPLC Fingerprint of Antidiabetic *Jamu* Extracts

Chromatographic fingerprint analysis can be used as a quality control method of plant extracts. In this study, HPLC fingerprint was used to develop a quality control method for glucofarmaka antidiabetic *jamu*. Optimization of the HPLC condition was conducted in order to obtain a good separation profile of chemicals present in the samples. The samples used in this work are powder sample of each plant, a mixture of 4 plants powder sample as the ingredients of the glucofarmaka *jamu* formula and water extract of the glucofarmaka antidiabetic *jamu*. A good fingerprint profile will show the greatest amount of well-separated peaks on the chromatogram with short analysis time [10].

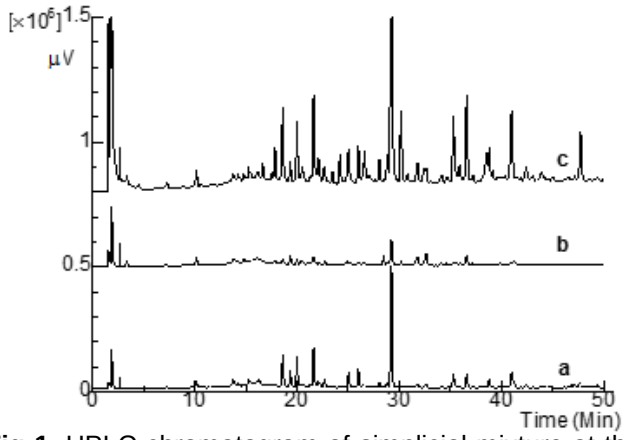

Fig 1. HPLC chromatogram of simplicial mixture at the wavelength of 280 nm (a); 254 nm (b); and 210 nm (c)

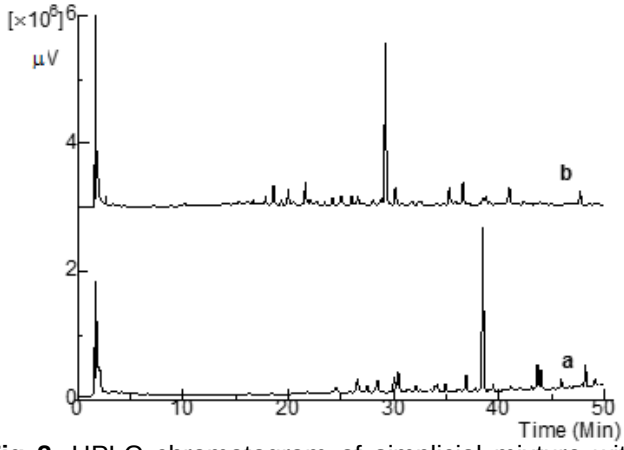

Fig 2. HPLC chromatogram of simplicial mixture with organic modifiers of MeOH (a) and ACN (b)

In the initial step of optimization, the sample solution was eluted in HPLC using an organic modifier, i.e. ACN or MeOH (5–95%), for 50 min. The detection wavelengths used were 210, 254, and 280 nm. The 210 nm wavelength was chosen based on its typical usage in HPLC analysis for 3 plants of *jamu* constituents, i.e. sembung [11], brotowali [12], and pare [13]. The 254 nm wavelength was used because most of the organic compounds would have aromatic rings that will absorb radiation at this wavelength. Meanwhile, 280 nm detection was conducted because ginger extract shows excellent chromatogram profile at

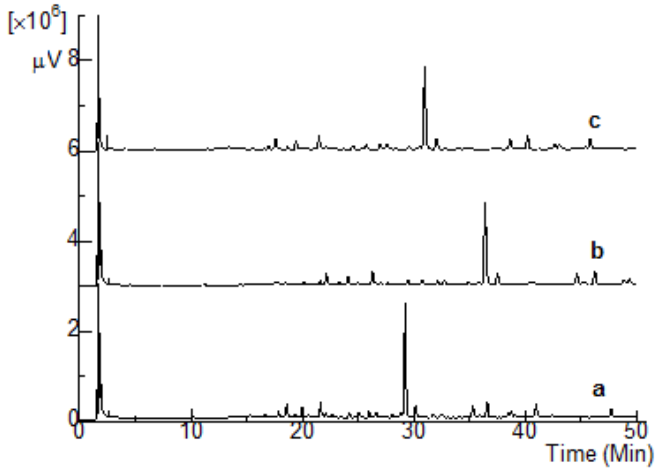

Fig 3. HPLC chromatogram of simplicial mixture with the organic modifier percentages of 5–95% (a); 5–70% (b); and 10–80% (c) of CAN

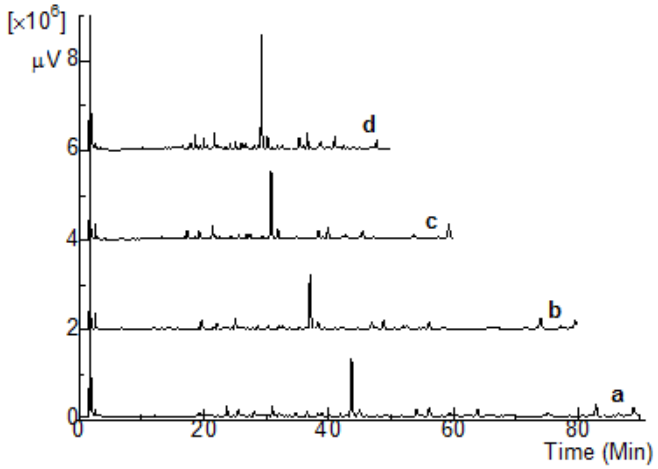

Fig 4. HPLC chromatogram of simplicial mixture with the analysis times of 90 min (a); 80 min (b); 60 min (c); and 50 min (d)

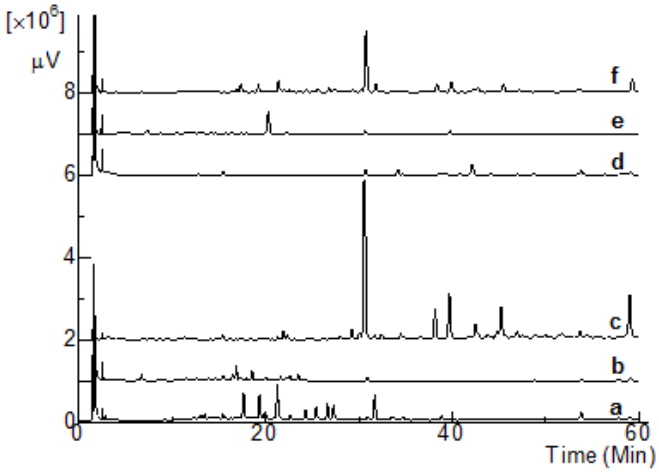

Fig 5. HPLC chromatogram profiling of sembung leaves (a); brotowali stem (b); ginger rizhome (c); pare leaves (d); *jamu* extract (e); and simplicial mixture (f)

this wavelength [14]. Among these detection wavelengths, the best result was obtained at 210 nm (Fig. 1). At this wavelength, the obtained number and the intensity of the peaks were higher than others and gave the best resolution. The use of organic modifiers, such as ACN, gave better chromatogram profile than MeOH. Fig. 2 showed that the chromatogram baseline of the MeOH-water mobile phase tended to rise as the MeOH percentage increased. It is believed that the MeOH will absorb more UV radiation at the wavelength of 205–235 nm as its concentration increases [15]. Thus, the 210 nm wavelength and ACN organic modifier were used in the optimization step.

Next, the upper and lower limits of ACN percentage in mobile phase were varied. As can be seen in Fig. 3, 5–70% ACN gave fairly good separation, but some peaks did not appear. This could be due to the compounds were retained in the column and could not be eluted with the ACN concentration below 70%. The 10–80% ACN percentage gave better chromatogram profile but some peaks did not appear. The 5–95% ACN percentage gave the best amount of peaks. Therefore, the upper limit of ACN percentage was determined at 95%. Some peaks which shown at the retention time below 2 min were not affected significantly by the modification of ACN lower limit, from 5 to 10%. It is expected that those peaks come from compound components which did not retain in the column. According to literature, the peaks with a retention time of 2 min or less is expected to come from unretained compounds [16]. Thus, the resolution was not affected by the percentage of the organic modifier. Consequently, the concentration of the composition of ACN organic modifier used was 10–95%.

The analysis time length parameter is one of the important factors which should be considered in the development of HPLC fingerprint method. The longer the analysis time, the better the separation and resolution that overlapped peaks will be separated well. On the other hand, it will not be efficient if this method applied to many samples. Thus, it is required to find the optimum time to compromise them. As shown in Fig. 4, the obtained higher resolution and the peak number increase as the length of analysis time increases. The best chromatogram profile was obtained at the analysis time of 90 min. However, it was too long and not efficient to be applied. The fairly excellent compromise result was obtained at the analysis time of 60 min which gave a fairly good peak resolution and acceptable analysis time period.

The optimized values of the HPLC fingerprint profile

Table 1. List of prediction secondary metabolite compounds in the glucofarmaka *jamu*

RT (min)	$[M-H]^-$	Alleged Compound	RT (min)	$[M-H]^-$	Alleged Compound
4.1707	135.0297	Tricyclene	6.6394	351.0871	Diacetoxy-[4]-gingerdiol
		3-Carene			[10]-Gingerdiol
		1R-α-Pinene	2.7226	371.1376	Unidentified
		β-Pinene	6.5888	377.1389	Unidentified
		α-Pinene	6.1709	379.1547	Unidentified
		Sabinene	6.5441	393.1333	Unidentified
		Camphene	6.5888	394.1414	Unidentified
		Terpinolene	2.9159	395.1380	Tinocordiside
2.5800	149.0609	Unidentified	6.5774	395.1492	Tinocordiside
5.5549	183.0119	Unidentified	6.5774	395.1492	Tinocordiside
5.2179	183.0119	Unidentified	6.1061	395.1496	Tinocordiside
0.4349	189.0764	Unidentified	7.1819	407.1492	[8]-Gingerdiol
4.5058	265.1480	[4]-Isogingerol	6.9735	409.1651	Shogasulfonic acid C
		[4]-Gingerol	6.5845	409.1659	Shogasulfonic acid C
6.5888	267.0297	Unidentified	4.9570	413.1605	β-Sitosterol
6.9040	277.2172	[6]-Paradol			Kuguacin C
7.3904	279.2335	Unidentified	7.5456	423.1818	Shogasulfonic acid B
6.1023	283.0245	Unidentified	5.1598	427.1761	Kuguacin D
5.2560	293.1883	[6]-Gingerol			Kuguacin P
6.5888	295.0245	[6]-Gingerdiol	2.8968	437.1814	Shogasulfonic acid A
6.5850	307.0247	[7]-Gingerol	6.7764	463.2123	Unidentified
6.5888	311.0921	Unidentified	2.2209	599.2344	Charantoside III
4.8809	311.1687	Unidentified			Charantoside IV
5.0066	311.1687	Unidentified	4.1916	633.3642	Momordicoside L
5.2179	325.1842	Unidentified			Karavilagenin B 3-alloside
3.1882	327.2177	Unidentified	3.8041	661.3596	Goyaglycoside-d
3.4405	329.2330	3,5,2'-Trihydroxy-7,5'-dimethoxyflavone			Goyaglycoside-c
		4-Gingesulfonic acid			Charantoside VIII
		[10]-Dehydroshogaol	4.1726	663.3745	Unidentified
6.5888	339.0872	Unidentified	4.1278	663.3747	Unidentified
5.6787	339.2000	Unidentified	4.2944	679.4058	Unidentified
5.6030	339.2008	Unidentified	4.7847	679.4060	Unidentified

parameter from the mixture of *jamu* raw material were applied to the antidiabetic *jamu* extract and each plant extract of the *jamu* constituents. There were 20 peaks that could be detected at the glucofarmaka *jamu* chromatogram and could be used as fingerprint markers. The peak number was counted by referring to Bliesner criteria (2006), i.e. S/N ratio ≥ 3 and the resolution ≥ 1.5 [17]. According to the obtained fingerprint profile (Fig. 5), the result was considered as excellent for HPLC fingerprint of *jamu* extract and every constituent plant showed a good chemical separation.

Evaluation of the Analytical Performance of the Developed HPLC Fingerprint Analysis Method

Evaluation of analytical performance is the verification process to make sure that the chosen condition for analysis is appropriate for the purpose. The results of the evaluation can be used to consider the quality, reliability, and consistency of the resulting analysis data [18]. Analytical performance of HPLC fingerprint analysis method was evaluated by RSD values of RRT and RPA towards a single reference peak. The chosen reference peak was the peak at the retention time of 20 min, which is in the intermediate position and area. The precisions of the RSD values of the RRT and the RPA were less than 0.3% and 3.9%, respectively. For the reliability parameter, the RSD values of the RRT and the RPA were less than 0.3% and 4.7%, respectively. Based on these results, it was indicated that the developed method was adequate, valid, and applicable.

Metabolite Profiling of Glucofarmaka Antidiabetic *Jamu* Extracts

Secondary metabolite identity information of the *jamu* extract can be obtained from LC-MS data which combines the chromatography separation principles and mass spectrometry measurement. This data contain retention time, the peak intensity of LC chromatogram, *m/z* values, and compound fragmentation pattern. However, the data size is too large and complex that it requires software with a certain

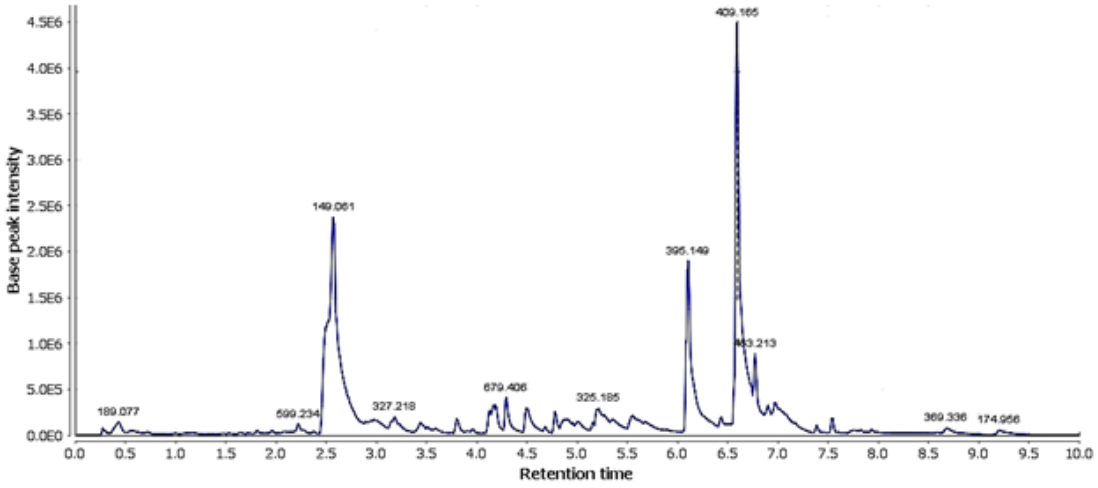

Fig 6. Base peak chromatogram of glucofarmaka *jamu* extract

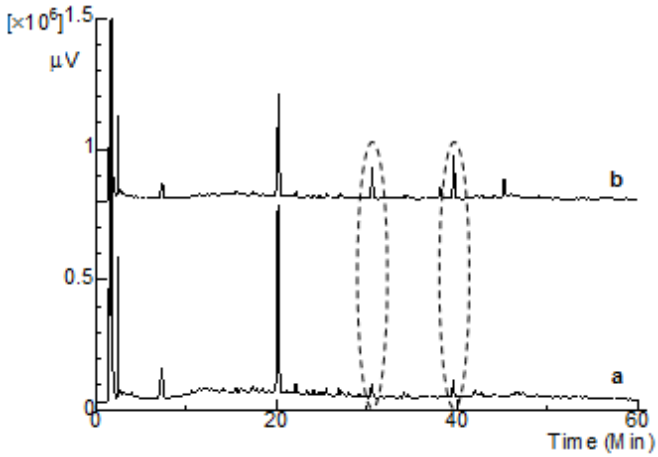

Fig 7. HPLC chromatogram of glucofarmaka *jamu* extract before standard addition (a); and after standard addition (b)

algorithm to simplify the raw data of LC-MS. There is some common software to process LC-MS data, such as MarkerLynx™, *MZmine*, and XCMS. In this study, the Java programming language-based and open licensed *MZmine* was used. One of the advantages of *MZmine* is the ability to handle raw data format so that it can process data from various types of LC-MS instrument from different producers [19].

Actually, a single peak on the LC-MS chromatogram can represent more than one compound. Thus, the comprehensive integration of *m/z* data from the mass spectrum is required to identify the compounds in the sample. Data processing results using *MZmine* are shown in mass array form. There were 47 features of LC-MS peaks obtained successfully after the data processing of the *jamu* extract (Table 1). Among those peaks, 22 of them could be identified as the predicted compounds. Some of the resulting peaks of the LC-MS analysis could not be identified because of the limited available information in the LC-MS database until this research was reported.

At the base peak chromatogram (Fig. 6), the highest intensity peak with retention time of 6.58 min (*m/z* 409.165) was expected as shogasulfonic acid C compound from the ginger. The second highest intensity peak (*m/z* 149.061) was not able to be identified. The peak with retention time of 6.11 min (*m/z* 395.149) was expected as tinocordiside from brotowali.

As an effort to identify the marker compound in the *jamu* extract, gingerol and shogaol internal standards (6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol compounds) were added to the jamu extract because they are commonly used as the marker for ginger extracts, which is one of this antidiabetic *jamu* constituent plants. After adding the internal standards, the peak area increased significantly at the retention times of 31 and 39 min (Fig. 7). The marker compound peaks which eluted at those retention times were 6-gingerol and 6-shogaol, respectively. In this case, the peak of the LC-MS in the metabolite profiling data analysis at the retention time of 31 min was expected as 6-gingerol. This expectation was supported by the MS^2 fragmentation data of LC-MS which showed the presence of this compound in the *jamu* extract. Fragments with *m/z* of 293.1798; 275.2017; 96.9599; and 79.9575 were suitable with the 6-gingerol fragmentation based on the *MassBank* database.

CONCLUSION

The optimum conditions of HPLC fingerprint profile of raw material mixture, single extract of the plant, and glucofarmaka antidiabetic *jamu* extract were obtained with 10–95% ACN-water as the mobile phase for 60 min at the wavelength of 210 nm. An analytical

performance test conducted to this method gave a good result. The peak in the jamu extract chromatogram with a retention time of 31 min is expected to be 6-gingerol potential to be a marker compound.

ACKNOWLEDGEMENT

We gratefully acknowledged Mrs. Erna Dwiyanti for her help in preparation of this manuscript. Hanifullah Habibie also gratefully acknowledged to PT Adaro Indonesia who has given the master scholarship at the Bogor Agricultural University, Indonesia.

REFERENCES

[1] International Diabetes Federation, 2015, *IDF Diabetes Atlas,* 7th Ed., ed. Cavan, D., Fernandes, J.R., Makaroff, L., Ogurtsova, K., Webber, S., Brussel, Belgium, 57.

[2] Nissen, S.E., and Wolski, K., 2007, Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes, *N. Engl. J. Med.,* 356 (24), 2457–2471.

[3] Habibie, H., 2014, Pengujian *In Vivo* Terhadap Efikasi Formula Jamu Antidiabetes dan Analisis Sidik Jari Kromatografinya, *Undergraduate Thesis*, Institut Pertanian Bogor, Bogor.

[4] Rafi, M., Wulansari, L., Heryanto, R., Darusman, L.K., Lim, L.W., and Takeuchi, T., 2015, Curcuminoid's content and fingerprint analysis for authentication and discrimination of *Curcuma xanthorrhiza* from *Curcuma longa* by high-performance liquid chromatography-diode array detector, *Food Anal. Methods*, 8 (9), 2185–2193.

[5] Xie, P.S., and Leung, A.Y., 2009, Understanding the traditional aspect of Chinese medicine in order to achieve meaningful quality control of Chinese materia medica, *J. Chromatogr. A*, 1216 (11), 1933–1940.

[6] Yang, L., Wu, D., Tang, X., Peng, W., Wang, X., Ma, Y., and Su, W., 2005, Fingerprint quality control of Tianjihuang by high-performance liquid chromatography-photodiode array detection, *J. Chromatogr. A*, 1070 (1-2), 35–42.

[7] Yang, X., Yang, S.P., Zhang, X., Yu, X.D., He, Q.Y., and Wang, B.C., 2014, Study on the multi-marker components quantitative HPLC fingerprint of the compound Chinese medicine Wuwei Changyanning granule, *Iran. J. Pharm. Res.*, 13 (4), 1191–1201.

[8] Alaerts, G., Matthijs, N., Smeyers-Verbeke, J., and vander Heyden, Y., 2007, Chromatographic fingerprint development for herbal extracts: A screening and optimization methodology on monolithic columns, *J. Chromatogr. A*, 1172 (1), 1–8.

[9] Castillo, S., Gopalacharyulu, P., Yetukuri, L., and Oresic, M., 2011, Algorithms and tools for the preprocessing of LC–MS metabolomics data, *Chemom. Intell. Lab. Syst.*, 108 (1), 23–32.

[10] Dejaegher, B., Alaerts, G., and Matthijs, N., 2010, Methodology to develop liquid chromatographic fingerprints for the quality control of herbal medicines, *Acta Chromatogr.*, 22 (2), 237–258.

[11] Xu, J., Jin, D., Liu, C., Xie, C., Guo, Y., and Fang, L., 2012, Isolation, characterization, and NO inhibitory activities of sesquiterpenes from *Blumea balsamifera*, *J. Agric. Food Chem.,* 60 (32), 8051–8058.

[12] Praman, S., Mulvany, M.J., Allenbach, Y., Marston, A., Hostettmann, K., Sirirugsa, P., and Jansakul, C., 2011, Effects of an *n*-butanol extract from the stem of Tinospora crispa on blood pressure and heart rate in anesthetized rats, *J. Ethnopharmacol.*, 133 (2), 675–686.

[13] Mekuria, D.B., Kashiwagi, T., Tebayashi, S., and Kim, C.S., 2006, Cucurbitane glucosides from *Momordica charantia* leaves as oviposition deterrents to the leafminer, *Liriomyza trifolii*, *Z. Naturforsch*, 61c, 81–86.

[14] Rafi, M., Lim, L.W., Takeuchi, T., and Darusman, L.K., 2013, Simultaneous determination of gingerols and shogaol using capillary liquid chromatography and its application in discrimination of three ginger varieties from Indonesia, *Talanta*, 103, 28–32.

[15] Sadek, P.C., 2002, *The HPLC solvent guide*, John Wiley and Sons Inc., New York.

[16] Kromidas, S., 2005, *More Practical in Problem Solving in HPLC*, Wiley-VCH Verlag GmbH & Co., Weinheim.

[17] Bliesner, D.M., 2006, *Validating Chromatographic Methods: A Practical Guide*, John Wiley & Sons, Inc., New Jersey.

[18] Kazusaki, M., Ueda, S., Takeuchi, N., and Ohgami, Y., 2012, Validation of analytical procedures by high-performance liquid chromatography for pharmaceutical analysis, *Chromatography*, 33 (2), 65–73.

[19] Theodoridis, G.A., Gika, H.G., and Wilson, I.D., 2008, LC-MS-based methodology for global metabolite profiling in metabonomics/metabolomics, *TrAC, Trends Anal. Chem.*, 27 (3), 251–260.