

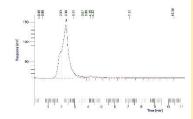
# Development of HPLC Fingerprint Analysis of Traditional Diabetes Herbal Jamu Diabetes Plant Materials

Gholamhossein Shams<sup>a</sup>, Hassan Fahmi Ismail<sup>b</sup>, Teh Liam Chee<sup>b</sup>, M Abbas Ali<sup>c</sup>, Fadzilah Adibah Abdul Majid<sup>b\*</sup>, Mohamad Roji Sarmidi<sup>d</sup>, Ramlan Abdul Aziz<sup>d</sup>

#### Article history

Received: 24 April 2013 Received in revised form: 30 January 2014 Accepted: 15 March 2014

#### Graphical abstract



#### Abstract

Selective and efficient analytical methods are required not only for quality assurance but also for authentication of herbal formulations. In the present study, high-performance liquid chromatography (HPLC) detection was developed for chromatographic fingerprint analysis of the extract from grounded plant materials of Jamu Diabetes (JD), a well known herbal formulation in Malaysia. The bioactive markers rosmarinic acid, curcumin, cinnamaldehyde and andrographolide were identified from the methanol/water (80:20) extract of JD materials. The data presented in this study showed that the developed method is simple, sensitive and specific for simultaneous determination of the indicated marker compounds either qualitatively or quantitatively, and may be used as a fingerprint profile for the standardization of extractives or herbal medicines from herbal formulation JD materials.

Keywords: Jamu diabetes; HPLC; fingerprint; bioactive markers

## Abstrak

Kaedah analisa yang selektif dan efisien bukan sahaja diperlukan untuk kesahihan kualiti malah juga sebagai pengesahan keaslian sesuatu formulasi herba. Dalam kajian ini, pengesanan menggunakan kaedah kromatografi cecair berprestatsi tinggi (HPLC) telah dibangunkan untuk analisa cap jari kromatografi ekstrak bahan-bahan tumbuhan dalam Jamu Diabetes (JD), iaitu satu formula herba yang terkenal di Malaysia. Bahan penanda bioaktif asid rosmarinik, kurkumin, cinemaldehida dan andrografolida telah dikenal pasti dari ekstrak metanol/air (80:20) bahan herba JD. Data yang dikemukakan di dalam kajian ini menunjukkan bahawa kaedah yang dibangunkan adalah mudah, sensitif dan spesifik bagi dapatan serentak kompaun-kompaun penanda bioaktif samada secara kualitatif atau kuantitatif, dan mungkin boleh menggunakan profil cap jari ini bagi tujuan pemiawaian pengekstrakan atau ubatan herba dari bahan formulasi herba JD.

Kata kunci: Jamu Diabetes; HPLC; cap jari; penanda bioaktif

© 2014 Penerbit UTM Press. All rights reserved.

# ■1.0 INTRODUCTION

Diabetes mellitus is a multifactorial disease. It is an endocrine and metabolic disorder characterized by chronic hyperglycemia [1]. Multiple biochemical impairments associate with micro- and macro-vascular complications, which is a major cause of morbidity and death in Diabetes mellitus [2]. With the numbers of people affected by diabetes multiplying worldwide, the disease is taking an ever-increasing proportion of national and international health care strategies [3]. It is projected to become one of the world's main

disablers and killers within the next 25 years [4, 5]. Traditional herbal medicines have been used throughout the world for a range of diabetes [6]. Herbal preparations used for the treatment of diseases and its knowledge are handed down from generation to generation and has been used in healthcare delivery in many parts of the world. The traditional herb medicines showed, since centuries, beneficial effects on health promotion, out of side effects, as compared with synthetic drugs [7, 8]. Herbal medicines have received great attention as alternative medicines in recent years in Malaysia and are sold as a dietary supplement [9]. Jamu is

<sup>&</sup>lt;sup>a</sup>Department of Chemical Engineering, Arak branch, Islamic Azad University, Arak, Iran

<sup>&</sup>lt;sup>b</sup>Tissue Culture Engineering Laboratory, Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia

<sup>&</sup>lt;sup>c</sup>Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia

<sup>&</sup>lt;sup>a</sup>Institute of Bioproduct Development, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia

<sup>\*</sup>Corresponding author: adibah@cheme.utm.my

a traditionally formulated herbs being used as traditional medicines for years to treat illnesses in the region of Indonesia and Malaysia. The plant materials mixture of Andrographis paniculata, Cinnamomum zeylanicum, Curcuma xanthorrhiza, Eugenia polyantha and Orthosiphon stamineus are used in the formulation of traditional JD. Andrographis paniculata is a plant that has been effectively used in traditional Asian medicines for centuries. It has been used for several purposes, primarily preventing diabetes mellitus [10]. Curcuma xanthorrhiza is a low growing plant with a root and reported to be useful for the treatment of diabetes. rheumatism, anticancer, hypertension and heart disorders [11]. Eugenia polyantha has been known since long time ago as a spice that can be used for therapy. Empirically, Eugenia polyantha can be used for hypertension, diabetic, diarrhea, gastritis and skin diseases [12]. Orthosiphon stamineus is the herb that traditionally used in Malaysia to treat kidney problem, gout, and diabetes

Recently, chromatographic fingerprint technique, as a more meaningful quality control method of herbal samples, has been attracting more and more people's attention, because the fingerprint technique emphasizes on the integral characterization of compositions of samples with a quantitative degree of reliability and focus on identifying and assessing the stability of the plants. Chromatographic fingerprint is a kind of method to show chemical information of medicines with chromatograms, spectrograms and other graphs by analytical techniques. To date, varieties of chromatographic techniques involving fingerprint include TLC, gas chromatography, HPLC, etc. [13]. Because of its advantages and popularization, HPLC fingerprint analysis has been regarded as the first choice. However, fingerprint analysis shows only the result of similarity calculated based on the relative value; retention time, with the selected marker compound as reference standard, and does not display the absolute quantity. Obviously, quantitative determination of some marker components is necessary.

Number of studies concerning chromatographic fingerprint analysis on different plant materials have been conducted [14, 15] and nothing has been reported on the HPLC fingerprint analysis on the herbal formulation JD plant materials. Consequently, the objective of this research was to develop the fingerprint method for ground plant materials of Jamu Diabetes (JD) by HPLC determination using rosmarinic acid, curcumin, cinnamaldehyde and andrographolide as standards, which are important constituents present in the formulation.

#### ■2.0 MATERIALS AND METHODS

#### 2.1 Plant Materials and Chemicals

The ground plant materials of JD was supplied by Naturemedic Supply, Johor, Malaysia. HPLC grade methanol, acetonitrile and formic acid were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents used in the present study were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

#### 2.2 Preparation of Plant Extracts

The ground plant materials of JD (100 g) was macerated with a 1 L solution of 80% methanol and 20% distillated water for 2 days in a tightly sealed vessel at room temperature, and stirred during the maceration. For water extraction, the plant material (500 g) was macerated with 1.5 L distillated water and the mixture was boiled for one hour. For both cases, the maceration was repeated until a colorless liquid was obtained. The resultant extracts were combined and combined extract was filtered, concentrated at reduced pressure

on rotary evaporator (Laborata 4000, Heidolph, Germany) or by freeze dryer (Heto PowerDry LL1500, Thermo Electron Corporation, UK). Afterword the sample was diluted with 2mL of methanol and the solution was centrifuged (Kubota KR- 20000T, Kubota Medical Appliance Supply, Japan) at 4000 rpm for 10 min at 25°C. After centrifugation the supernatant was filtered through a 0.45-µm PTFE membrane filter (Sartoriusstedium biotrch, Aubagne Cedex, France) prior to HPLC analysis.

## 2.3 Preparation of Standards

Stock solutions of each compound were prepared by dissolving weighed quantities of standard compounds in HPLC grade methanol and diluting to the desired concentration. By using the stock solutions, a series of mixed working standard solutions were prepared with different concentration ranges and all the solutions were stored under refrigeration.

#### 2.4 Development of HPLC Fingerprints

A Perkin Elmer Series 200 liquid chromatograph system furnished with automated gradient controller, SPD-M20A Photo Diode Array UV-Vis detector and a reversed-phase C18 analytical column (150 × 4.6 mm ID, ZORBAX Eclipse Plus) was used. The column was maintained at 37°C and 0.50 µL of sample was injected for development of the chromatogram. The flow-rate was maintained at 1 mL min<sup>-1</sup>. The mobile phase was optimized to give the best separation between the chromatographic peaks. Mobile phase consisted of acetonitrile (15 mL), de-ionized water (310 mL), methanol (170 mL) and acetic acid (5 mL) to set the pH around 3.5 was used for both water extract and water extract concentrated by vacuum rotary evaporator. On the other hand, the mobile phases consisted of acetonitrile (10 mL), de-ionized water (90 mL) and formic acid (5 mL) (A) and de-ionized water (10 mL), acetonitrile (90 mL) and formic acid (5 mL) (B) were used for methanol/water extract concentrated by vacuum rotary evaporator whereas the mobile phases consisted of de-ionized water (90 mL), methanol (10 mL) and formic acid (0.5 mL) (A) and de-ionized water (10 mL), methanol (90 mL) and formic acid (0.5 mL) (B) were used for freeze dried methanol/water extract. The reference compounds at different concentrations used were rosmarinic acid, curcumin, andrographolide and cinnamaldehyde for generating HPLC standard fingerprints. The fingerprint of extract was analyzed by interpolating data from fingerprint of extract and standard markers.

## ■3.0 RESULTS AND DISCUSSION

#### 3.1 Standardization of Fingerprint

The objective of the works was to establish the best way to have appropriate fingerprints for all the main phytochemicals present in the samples. The relationship between the fingerprints was notified. For traditional quality control system, rosmarinic acid, curcumin, andrographolide and cinnamaldehyde are used as the marker substances to evaluate the extract of JD plant materials. The contents of these compounds present in the sample were determined by linear regression analysis. To determine the linearity equations and linear scope for the analysts, a series of standard solutions at different concentrations were tested. All results indicated that the conditions for the fingerprint analysis were satisfactory.

#### 3.2 Sample Analysis by HPLC

HPLC analysis was performed using the different standard markers (Figures 1-4) dissolved in methanol at different concentrations. Each standard fingerprint in different concentrations was obtained and compared with the HPLC methanolic extract chromatogram data. Figure 5 shows two dominant peaks of water extract of JD plant materials which were not separated and others were not traceable. Then the concentrated water extract by rotary vacuum evaporator was prepared to raise the percentage of the components in the sample for better resolution. The curves still not separated and the components were not traceable with standard markers (Figure 6). As shown in Figure 7, freeze dried methanolic extract displayed 4 separated peaks; then it was decided to use the same mobile phase for HPLC test with standard markers to identify the

The rosmarinic acid standard curve was established by plotting concentrations (mg mL<sup>-1</sup>) versus area (UV\*sec). The equation 1 represents this data plotting by using microsoft excel.

different phytochemicals existed in the methanol extract.

$$y = 2 \times 10^{-7} x - 242.47 \tag{1}$$

Where y is area and x is concentration. The area 1,937,501 (UV\*sec) at 2.32 min from the fingerprint of methanolic extract of JD plant materials was considered and by puting this value in the above equation, the 0.0968 mg mL<sup>-1</sup> was computed for rosmarinic acid present in the sample. Similarly, by using standard curves of other markers, the concentrations of curcumin, andrographolide and cinnamaldehyde from the sample were calculated and tabulated accordingly in Table 1. As shown in Table 1, it can be generalized that the major constituents in JD extract are curcumin, rosmarinic acid, andrographolide and cinnamaldehyde. Therefore, HPLC fingerprint analysis was performed based on the relative retention time. The retention time was 2.10, 2.32, 2.67 and 3.22 min for curcumin, rosmarinic acid, andrographolide and cinnamaldehyde respectively. Methanol extract contained the higher amount of cinnamaldehyde and lower amount of curcumin.

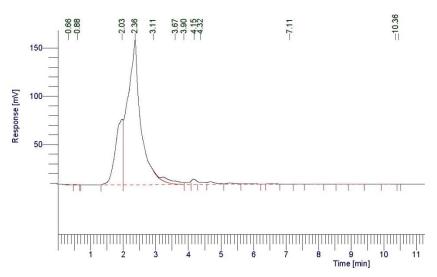


Figure 1 HPLC Chromatogram of Rosmarinic Acid standard marker

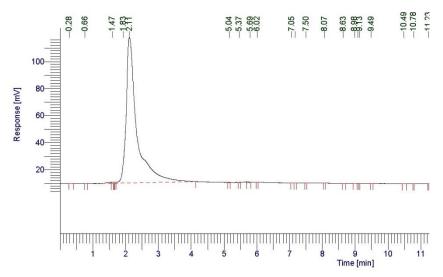


Figure 2 HPLC Chromatogram of Cucumin standard marker

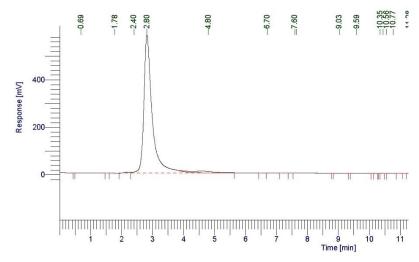


Figure 3 HPLC Chromatogram of Andrographolide standard marker

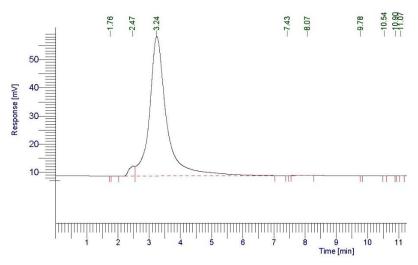


Figure 4 HPLC Chromatogram of Cinnamaldehyde standard marker

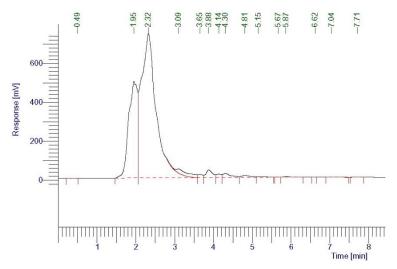


Figure 5 HPLC Chromatogram of water extract of JD plant materials

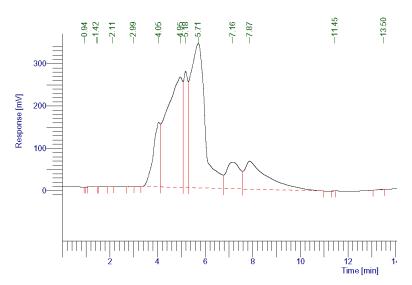


Figure 6 HPLC Chromatogram of concentrated water extract of JD plant materials

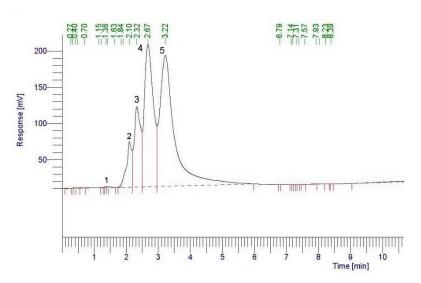


Figure 7 HPLC Chromatogram of the methanolic extract of JD plant materials. 1-unknown chemical, 2- curcumin, 3- rosmarinic acid, 4- Andrographolide, 5- cinnamaldehyde

Concentration Retention time Area Concentration Compounds Regression equation (mg per 500 mg (uV\*sec) (min)  $(mg\ mL^{\text{-}1})$ plant materials) 1.15 Unknown 2157 2.10 Curcumin  $y = 2 \times 10^{-7} x - 57448$ 0.0434 0.8672 924661 2.32 Rosmarinic Acid  $y = 2 \times 10^{-7} x - 242.47$ 1937501 0.0968 1.9360 2.67 Andrographolide  $y = 2 \times 10^{-7} x - 21377$ 3905579 0.1942 3.8840 3.22 Cinnamaldehyde  $y = 2 \times 10^{-7} x - 333271$ 5819776 0.2743 5.4860

Table 1 The amounts of curcumin, rosmarinic acid, andrographolide and cinnamaldehyde present in the sample

# ■4.0 CONCLUSION

A HPLC method was successfully developed for fingerprint analysis of JD plant materials. The HPLC fingerprint of rosmarinic acid, curcumin, andrographolide and cinnamaldehyde represent the characteristic markers of this herb's constituents for the first time. The fingerprint obtained by using our established

method provides a good repeatability in separation pattern which demonstrated that the fingerprint presented in this paper is a rapid, reliable and effective method suitable for either qualitatively or quantitatively determination of the constituents present in JD plant materials.

#### Reference

- Nickavar, B., and N. Yousefian. 2011. Evaluation of α-amylase Inhibitory Activities of Selected Antidiabetic Medicinal Plants. *Journal für Verbraucherschutz und Lebensmittelsicherheit*. 6(2): 191–195.
- [2] Berger, B., G. Stenstrom, and G. Sundkvist. 1999. Incidence, Prevalence, and Mortality of Diabetes in a Large Population. A Report from the Skaraborg Diabetes Registry. *Diabetes Care*. 22(5): 773–777.
- [3] WHO. 2012. Diabetes Programme, Retrieved 2012, from http://www.who.int/diabetes/en/.
- [4] King, H., R. E. Aubert, and W. H. Herman. 1998. Global Burden of Diabetes. 1995–2025: Prevalence, Numerical Estimates, and Projections. *Diabetes Care*. 21(9): 1414–1431.
- [5] Zhang, P, X. Zhang, J. Brown, D. Vistisen, R. Sicree, J. Shaw, and G. Nichols. 2010. Global Healthcare Expenditure on Diabetes for 2010 and 2030. Diabetes Research and Clinical Practices. 87(3): 293–301.
- [6] Odhav, B., T. Kandasamy, N. Khumalo, and H. Baijnath. 2010. Screening of African Traditional Vegetables for their Alpha-amylase Inhibitory Effect. *Journal of Medicinal Plants Research*. 4(14): 1502– 1507.
- [7] Yadav, N. P., and V. K. Dixit. 2008. Recent Approaches in Herbal Drug Standardization. *International journal of Integrative Biology*. 2(3): 195–203.
- [8] Hussain, K., Z. Ismail, A. Sadikun, and P. Ibrahim. 2009. Evaluation of Metabolic Changes in Fruit of *Piper Sarmentosum* in Various Seasons by Metabolomics Using Fourier Transform Infrared (FTIR)

- Spectroscopy. International Journal of Pharmaceutical and Clinical Research. 1(2): 68–71
- [9] Chin, J. H., H. H Abas, and I. Sabariah. 2009. Effect of Orthosiphon stamineus Leaf Extracts on Hepatic Cytochrome p450, UGT and GST Activity in Stz-Induced Diabetic Rats. Journal for the Advancement of Science & Arts. 1(1): 1–8.
- [10] Niranjan, A., S. Tewari, and A. Lehri. 2010. Biological Activities of Kalmegh (Andrograpis paniculata Nees) and Its Active Principles-A Review. Indian Journal of Natural Products and Resources. 1(2): 125– 135.
- [11] Sears, B. 2005. The Anti-Inflammation Zone-Reversing the Silent Epidemic that's Destroying Our Health. New York: Harper Collins Publishers. 75.
- [12] Utami, P., and L. Tim. 2005. Tanaman Obat Untuk Mengatasi Rematik Dan Asam Urat. Agromedia Pustaka, Jakarta. 57.
- [13] Vierling, W., N. Brand, F. Gaedcke, K.H. Sensch, E. Schneider, and M. Scholz. 2003. Investigation of the Pharmaceutical and Pharmacological Equivalence of Different Hawthorn Extracts. *Phytomedicine*. 10(1): 8–16.
- [14] Mohammad, J. A. S. and I. Zhari, 2011. Simultaneous Analysis of Bioactive Markers from *Orthosiphon stamineus* Benth Leaves Extracts by Reverse Phase High Performance Liquid Chromatography. *Tropical Journal of Pharmaceutical Research*. 10(1): 97–103.
- [15] Sirikatitham, A., T. Chuchom, and A. Itharat. 2007. Development of the Chromatographic Fingerprint Analysis of Dioscorealides and Dioscoreanone from *Dioscorea Membranacea* Pierre. Songklanakarin Journal of the Sciences and Technology. 29(Suppl. 1): 101–107.