Journal of Applied Research on Medicinal and Aromatic Plants 3 (2016) 64–70

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Reflux extraction and cleanup process by column chromatography for high yield of andrographolide enriched extract

Lee Suan Chua*, Norliza Abd Latiff, Muna Mohamad

Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor Bahru, Johor, Malaysia

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 4 January 2016
Accepted 24 January 2016
Available online 16 February 2016

Keywords:
Andrographis paniculata
Reflux extraction
Column chromatography
Ethanolic extract
Andrographolide

ABSTRACT

Andrographis paniculata is widely used by local people from Southeast Asia countries for traditional medication. Recently, the demand for this herb and its extract is in the increasing trend, particularly for herbal related product development. Therefore, this study investigated the optimization of *A. paniculata* extraction in a reflux system, and the fractionation of the plant crude extract for andrographolide enriched extract using column chromatography. The extraction variables such as the ratio of plant leaves to solvent (1:9), extraction temperature (73 °C) and time (1.9 h) were optimized by the desirability, 0.85 based on the three levels and three factors of Box–Behnken design. The yield of extraction (17.2% w/w) and the concentration of andrographolide (140.5 ppm) were the responses for the optimization using a quadratic model in the response surface methodology. A pattern recognition tool of principle component analysis was used to determine the similarity of the andrographolide enriched extracts, in term of their chemical composition produced from the column chromatography at different ethanol concentrations (20–100%) as eluent. The first principle component explained for >40% of the total variance, which explained the high similarity of chemical composition in the extracts. The eluent system of 40% ethanol was chosen for andrographolide fractionation as it required the lowest volume of eluent (950 mL) for high concentration of andrographolide production (1.9% w/w). The combination of reflux extraction with column chromatography was successfully used for the preparation of andrographolide enriched extract.

1. Introduction

Andrographis paniculata is an important herbaceous plant known as "King of Bitters" in the family of Acanthaceae. It can grow well in tropical area up to 110 cm, especially in moist and shady places (Jain et al., 2015). It has widely been used in the formulation of traditional medicine for many ailments such as diabetes, worm infections, chronic bronchitis and skin diseases, particularly in the Traditional Chinese Medicine and Ayurveda since ancient time (Radhika and Lakshmi, 2010).

Recently, many entrepreneurs would like to expand their herbal business by the mean of processing herbal extract for the wide market demand. The number of small and pilot scale processing plant is remarkably growing. It is expected that the demand is not only on plant crude extract, but also for the bioactive compound enriched extract for value added product development, including nutraceutical, cosmeceutical and medical applications in the near future. Therefore, this study was focused on the extraction of plant crude extract and further fractionated into bioactive compound enriched extract from *A. paniculata*. The marker compound of *A. paniculata* is known as andrographolide which is also a labdane diterpenoid with the chemical formula of $C_{20}H_{30}O_5$. Andrographolide is the bioactive phytochemical with bitter taste, maximally found in the leaves (>2% w/w) (Wu et al., 2008; Pandey, 2011).

Reflux extraction is a solid–liquid extraction process at a constant temperature with repeatable solvent evaporation and condensation for a particular period of time without the loss of solvent. The system is widely used in herbal industries as it is efficient, easy to operate and cost effective (Wang et al., 2013). Column chromatography is another technique which is commonly used for cleanup process, especially for highly complex plant samples (Khoo et al., 2012). The affinity of solute is either to adsorb on the adsorbent or to suspend in the mobile phase depending upon the chemical properties of solute, stationary and mobile phases. The option of impurity retention and target compound collection could be the best method of choice because of faster elution process with lesser solvent consumption. Previously, Indonesian investigators reported the method of andrographolide enriched extract was prepared by the method of fractionation using *n*-hexane and ethyl acetate, and followed by reconstitution in 90–95% ethanol (Nugroho et al., 2013, 2014). Nevertheless, the method was different from this study because immiscible solvents (*n*-hexane and

* Corresponding author. Fax: +60 7 5569706.
E-mail address: chualeesuan@utm.my (L.S. Chua).

http://dx.doi.org/10.1016/j.jarmap.2016.01.004
2214-7861/

Table 1
Box–Behnken design for 3 levels and 3 factors of experiments.

Extraction variables	Level		
	−1	0	1
Ratio of plant leave to solvent	1:5	1:7	1:10
Extraction temperature (°C)	60	70	80
Extraction time (h)	1	2	3

Table 2
Performance of different scales of reflux extractors for andrographolide extraction.

Extraction variables	Reflux extractor		
	Small	Preparative	Large
Ratio of plant leaves to solvent	1/9	1/9	1/9
Temperature (°C)	73	73	73
Time (h)	1.9	1.9	1.9
Yield (%)	17.20	16.80	13.27
Andrographolide (ppm)	140.50	129.04	92.14

ethyl acetate) were used for phase separation in the sample cleanup process.

Response surface methodology (RSM) is widely used statistical approach to optimize the variables from a minimum set of experimental runs for cost reduction and time saving (Gu et al., 2012). The optimal variables could be determined from the response surface of a multiple factor plot. The similarity of the plant fractions, especially their chemical composition, can be analyzed by using a dimension reduction tool which is known as principle component analysis (PCA). Multivariate data can be reduced to a limited number of variables called principle factors without the compensation of data information. Therefore, this pattern recognition tool is useful for data mining.

In the present study, the leaves of *A. paniculata* were extracted in a reflux system and then the crude extract was further fractionated by different concentrations of ethanol using column chromatography. To our knowledge, extraction and followed by fractionation for sample cleanup in the preparation of andrographolide enriched extract is very limited in literature. Many studies were just focused on the preparation of crude extract and the application of the crude extract for the determination of pharmacological activities. Moreover, the integration of experimental works and multivariate statistical techniques could reliably prepare andrographolide enriched extract in cost effective manner. Nowadays, the application of multivariate statistical technique for data mining and optimization is increasingly accepted by herbal industry because of high reliability and cost effective, particularly for large dataset (Upadhyay and Mishra, 2016).

2. Methods and materials

2.1. Plant leaves and chemicals

The dried and finely ground leaves of *A. paniculata* was purchased from Fidea Resources, Selangor. Industrial grade of absolute ethanol (≥99.5% EMPLURA®, Merck, Darmstadt, Germany) was used for extraction process, reagent grade of ethanol (96% EMSURE®, Merck, Darmstadt, Germany) was applied for column chromatography during fractionation and acetonitrile (≥99.5% LiChrosolv®, Merck, Darmstadt, Germany) was bought for liquid chromatographic method. Distilled water and deionized water were used for extraction and analytical work, respectively.

2.2. Reflux extraction of plant crude extract

A reflux system of extraction process was used to prepare plant crude extract from dried and finely ground leaves of *A. paniculata*. The extraction process was carried out in a small scale (50 mL), preparative scale (1 L) and large scale (20 L) reactor using 50%v/v aqueous ethanol. The ratio of plant leaves to solvent was varied from 1:5, 1:7 and 1:10 at different temperatures ranging from 60 to 80 °C for 1–3 h. After extraction, the plant residue was filtered and supernatant was collected by centrifugation. The supernatant was dried by a rotary evaporator under vacuum.

2.3. Column chromatography for andrographolide enriched extract

The plant crude extract was further cleaned up by a column chromatography with the dimension of 4 cm diameter and 33 cm length packed with silica 60 (200 g) in order to prepare andrographolide enriched extract. Different concentrations of aqueous ethanol (20–100% ethanol) were used as the eluent to slowly elute andrographolide from the plant crude extract. The presence of andrographolide was screened by using high performance liquid chromatography at 254 nm based on the detection of peak at the same retention time with the standard chemical of andrographolide. The total volume of the eluent which was required for complete andrographolide elution was recorded. The andrographolide enriched extract was dried and kept for further investigation.

2.4. HPLC-PDA and LC–MS/MS

A high performance liquid chromatography (Waters Alliance e2695, Milford, MA) system combined with a photo diode array (Waters 2998, Milford, MA) was used to screen the presence of andrographolide during fractionation at 254 nm. A C18 reserved phase Xbridge column (5 μm, 4.6 × 250 mm) was used for the separation at 30 °C with a flow rate of 0.7 mL/min. The mobile phase was consisted of 60% aqueous methanol in an isocratic profile for 20 min. The injection volume is 20 μL. All samples were filtered with 0.45 μm nylon filters prior to injection.

A high sensitivity hybrid system of ultra performance liquid chromatography integrated with an electrospray ionization source of tandem mass spectrometer was used to determine the concentration of andrographolide in the fractions. A serial of standard andrographolide solutions ranging from 0.1 to 1.0 ppm was prepared for the construction of calibration curve. The scan mode of multiple reaction monitoring with two negative ion transitions, namely m/z 349 > 331 and m/z 349 > 287 were used for the quantitation. The capillary and voltage of the ESI source were maintained at 400 °C and −4.5 kV, respectively. All other parameters were as follows: nitrogen was used as ion source gas for nebulisation, 40 psi; for drying solvent, 40 psi; curtain gas, 10 psi; collision energy, −20 V; declustering potential, −70 V; collision exit energy, −15 V; dwell time, 300 ms and the scan rate was 1000 amu/s. The chemical profiles of both negative and positive ions were analyzed by the scan mode of enhanced mass spectra (m/z 100–1000) coupled to rolling collision energy of information dependent acquisition with enhanced product ion. The highly complex chemical profiles were compared for the similarity by reducing data dimensionality using a pattern recognition tool, namely unsupervised principle component analysis with Pareto scaling.

2.5. Statistical analysis

Response surface methodology with Box–Behnken design was used to optimize the operating conditions of the extraction