Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 10814 - 10920
Received 05 March 2021; Accepted 01 April 2021.

Evaluation of *Labisia pumila* Extract Using Two Dimensional Gel Electrophoresis (2-DE) Proteomics Approach

Noraliza A[1], Nor Datiakma MA[2], Farah Fazwa MA[1], Saeid Reza Doustjalali[3*], Negar Shafiei Sabet[3], Jamal Hussaini[4,5], Nazmul MHM[6], Marzalina M[1*].

[1]Forest Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor
[2]Natural Product Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor
[3]Faculty of Medicine, SEGi University, Kota Damansara, Selangor, Malaysia
[4]Institute for Pathology, Laboratory and Forensic Medicine (l-PPerForM). Universiti Teknologi MARA (UiTM), Sungai Buloh, Selangor, Malaysia
[5]Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Selangor, Malaysia
[6]Graduate School of Medicine, Perdana University, 9.2, Wisma Chase Perdana, Damansara Heights, 50490 Kuala Lumpur, Malaysia.

[*]Corresponding authors: Saeid Reza Doustjalali (Email:saeidrezad@gmail.com) and Marzalina Mansor (Email:mzalina@frim.gov.my)

Running title: Evaluation of *Labisia pumila* Extract, 2-DE

Keywords:*Labisia pumila* Extract, 2-DE, Kacip Fatimah Extract

ABSTRACT

There are three verieties of *L. pumila* (Kacip Fatimah) that have been reported, namely *L. pumila* var. *alata*, *L. pumila* var. *pumila* and *L. pumila* var. *lanceolata*. These three varieties can be characterised through the size of petiole. Preliminary studies have shown that the var. *alata* and var. *pumila* are more commonly used as medicinal plants than var. *lanceolata*. Malay women traditionally consume water-soluble extract of the *L. pumila var. alata* leaves as a herbal medicine to treat menstrual irregularities, painful menstruations and to help contracting the birth channel after delivery. Over time, our herbal industry will face insufficient supply of local raw materials to feed the growing industry.Due to high demand of this species in the market, farmers started to produce the leaves via different method such as cultivation and tissue culture. We are also aware that in proteomics, the most promising technique with sufficient resolving power is two-dimensional gel electrophoresis (2-DE) that provides a unique platform for the simultaneous separation of proteins in a complex mixture.This study was designed to evaluate the quality and standardization of the *L. pumila* var. *alata* leaves extract using 2-DE proteomics approach. Our results showed the typical 2-DE protein profile of water-soluble extract obtained from wild, cultivated and tissue-cultured sample of *L. pumila var. alata* leaves respectively. No protein spot was observed at pH 3-10 with 1 mg water-soluble extract loading. In conclusion, our study may be used as a tool for evaluating the quality and standardization of the *L. pumila* var. *alata* leaves extract from wild, cultivated and tissue-cultured samples using 2-DE proteomics approach.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 10814 - 10920
Received 05 March 2021; Accepted 01 April 2021.

Hence, we recommend that no protein spot should be observed at pH 3-10 with 1 mg water-soluble extract loading of *L. pumila var. alata*using 2-DE proteomics approach.

INTRODUCTION

Herbal plants are widely used as medicinal herbs and are important part of health care since the ancient times. *Labisia pumila* (Myrsinaceae) or locally known as Kacip Fatimah is one of the high-value herbal product in the herbal industry. It has been used for women health care. Kacip Fatimah contain phytoestrogen that has been used in the form of water decoction for confinement mother. The advancement of biotechnology in Malaysia has discovered the benefit of *L. pumila* in various fields of applications, particularly in pharmaceutical and cosmeceutical purpose. Nowadays, there are three varieties of *Labisia* in Malaysia. These three verieties of *L. pumila* have been reported, namely *L. pumila* var. *alata*, *L. pumila* var. *pumila* and *L. pumila* var. *lanceolata*[1]. These three varieties can be characterised through the size of petiole. Preliminary studies have shown that the var. *alata* and var. *pumila* are more commonly used as medicinal plants than var. *lanceolata*[2].

Malay women traditionally consume water-soluble extract of the *L. pumila var. alata* leaves as a herbal medicine to treat menstrual irregularities, painful menstruations and to help contracting the birth channel after delivery[3]. The water extract of this variety has been found to inhibit estradiol binding to antibodies raised against estradiol, suggesting the presence of estrogen-like compounds in the extract[4]. These varieties based products is in the increasing trend which there are tendency in violation of the product due to the insufficient supply of the sample.Over time, our herbal industry will face insufficient supply of local raw materials to feed the growing industry.Due to high demand of this species in the market, farmers started to produce the leaves via different method such as cultivation and tissue culture.We are also aware that in proteomics, the most promising technique with sufficient resolving power is two-dimensional gel electrophoresis (2-DE) that provides a unique platform for the simultaneous separation of proteins in a complex mixture[5]. Therefore, this study was designed toevaluate the quality and standardization of the *L. pumila* var. *alata* leaves extract from wild, cultivated and tissue-cultured samples using 2-DE proteomics approach.

MATERIALS AND METHODS

Sample collection and water-soluble extraction

Plant Improvement Programme, Forest Research Institute Malaysia (FRIM), Kepong has supplied the leaves sample of Kacip Fatimah (*L. pumila var. alata)* from wild collection. The leaves from cultivated samples were collected from Kacip Fatimah (*L. pumila var. alata)* clone gene bank field in FRIM, Kepong while samples from tissue-cultured was supplied by Center of Biotechnology Bioentreprenuer, FRIM. All selected samples were cleaned, washed, and air dried

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 10814 - 10920
Received 05 March 2021; Accepted 01 April 2021.

in an oven at 40 ^{o}C with fully open valve of air changes setting until the moisture content of the samples reach less than 10%. The moisture content was determined by using Halogen Moisture Analyzer HB43 (Mettler, USA). Dried samples were ground into powder and sieved using 6 mm mesh size. Fine powder of dried samples was prepared for aqueous extraction.Ultra-pure water was used for the extraction with ratio of 1: 10 (plant sample: water). The samples were refluxed in a Soxhlet apparatus for 2 hours. The samples were filtered using cotton wool and were concentrated onto a hotplate at 60 ^{o}C until the volume was reduced to 10% of the initial volume. Then these samples were freeze-dried and used for 2-DE analysis[6].

2-DE gel electrophoresis

The first-dimension Isoelectric Focusing (IEF) was performed by using PROTEAN IEF system (Bio-Rad Laboratories, USA). One (1) mg of water soluble extract was supplemented with 60 µl sample buffer solution (9 M urea, 0.5% v/v Triton X-100, 2% v/v IPG buffer pH 3-10 and 60 mM DDT) and left at room temperature (20ºC) for 30 minutes. The mixture was added with rehydration solution (8 M urea, 0.5% v/v Triton X-100, 0.5% v/v IPG buffer pH 3-10, 12 mM DDT and 0.002% of Orange G) to make final volume of 200 µl for 11 cm IPG Strip gel, pH 3-10 (Bio-Rad Laboratories, USA), respectively[7-13]. The IPG strips were then rehydrated with the sample mixture in the Immobiline Dry Strip Reswelling Tray. The reswelling tray and IPG strips were rehydrated at room temperature for 16 hours.

Isoelectric focusing (IEF) was performed under the following conditions: 300V for 30 minutes, 3500V for remaining hours till reached 12000 V/hr. Upon completion of IEF the strips were equilibrated in equilibration buffer (6 M urea, 1.5 M Tris-HCl, pH 8.8, 30% v/v Glycerol, 2% SDS, 0.002% bromophenol blue, 0.06 M DTT) for 15 minutes, followed by the same buffer containing 240 mM iodoacetamide instead of DTT for another 15 minutes. The second dimension separation was carried out at 16ºC on 12.5% SDS slab gels using 2-DE system (Bio-Rad Laboratories, USA), with the IPG strips sealed on the top of the gels with 0.5% agarose. SDS-PAGE was run for 40 mA/gel at 50V for the first 30 minutes. The voltage was subsequently increased to 600V until the bromophenol blue marker reached the bottom of the gel[7-13].

Silver staining

The 2-DE gels were developed by silver staining as previously described by Heukeshoven and Dernick[14].

Image analysis

Protein spots were analyzed and images of stained 2-DE gels were acquired with Platinum Image Master Scanner (Amersham Biosciences) and stored as TIF file. All samples were analyzed in triplicate.

RESULTS

Figure 1 shows the typical 2-DE protein profile of water-soluble extract obtained from wild sample of *L. pumila var. alata* leaves. No protein spot was observed at pH 3-10 with 1 mg water-soluble extract loading.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 10814 - 10920
Received 05 March 2021; Accepted 01 April 2021.

Figure 1: Showing typical 2-DE protein profile at pH3-10 with 1mg loading of water-soluble extract obtained from wild sample of *L. pumila var. alata* leaves.

Figure 2 shows the typical 2-DE protein profile of water-soluble extract obtained from cultivated sampleof *L. pumila var. alata* leaves. No protein spot was observed at pH 3-10 with 1 mg water-soluble extract loading.

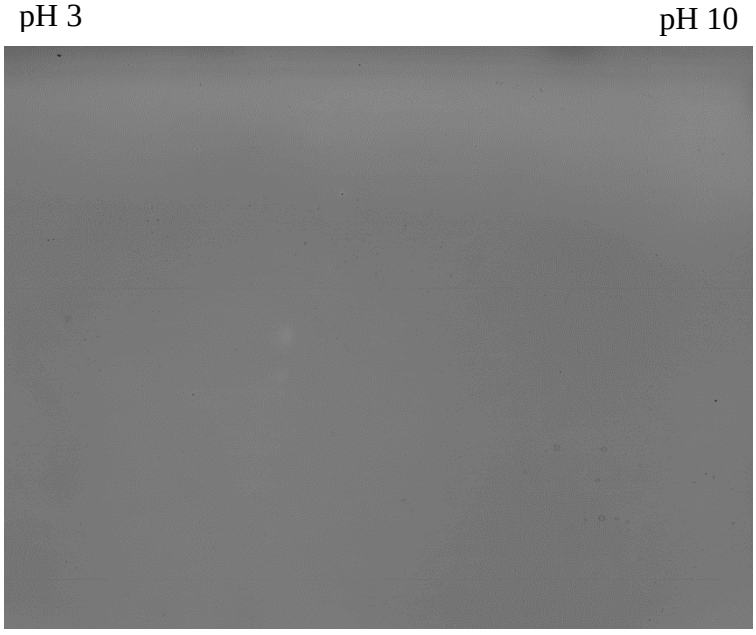

Figure 2: Showing typical 2-DE protein profile at pH3-10 with 1mg loading of water-soluble extract obtained from cultivated sample of *L. pumila var. alata* leaves.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 10814 - 10920
Received 05 March 2021; Accepted 01 April 2021.

Figure 3 shows the typical 2-DE protein profile of water-soluble extract obtained from tissue-cultured sample of *L. pumila var. alata* leaves. No protein spot was observed at pH 3-10 with 1 mg water-soluble extract loading.

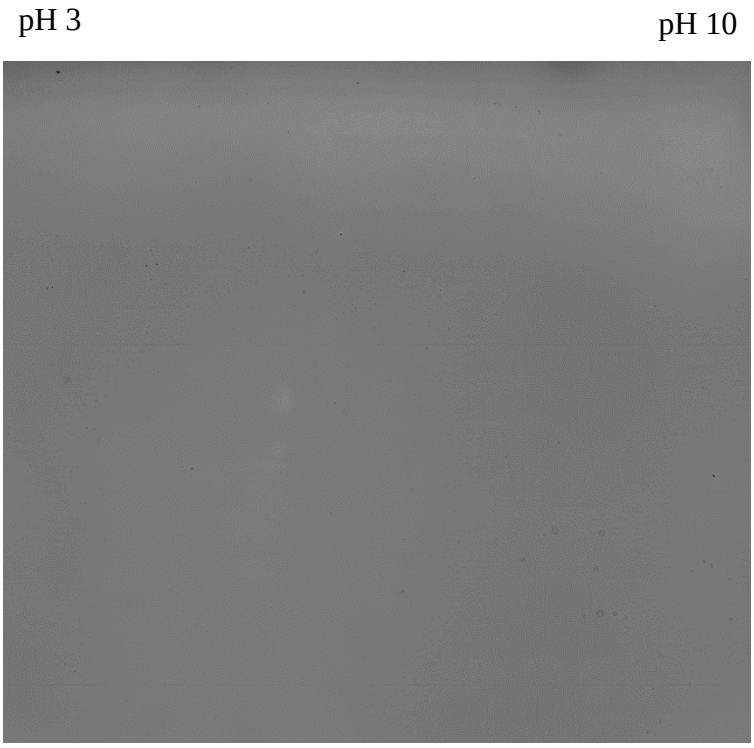

Figure 3: Showing typical 2-DE protein profile at pH3-10 with 1mg loading of water-soluble extract obtained from tissue-cultured sample of *L. pumila var. alata* leaves.

DISCUSSION

Over time, our herbal industry will face insufficient supply of local raw materials to feed the growing industry.Due to high demand of water-soluble extract of the *L. pumila var. alata* leaves in the market, farmers started to produce the leaves via different method such as cultivation and tissue culture. The applications of proteomics in herbal plant research is a valuable tool for quality control, toxicity studies and standardization of preparations and decoctions[15]. We are also aware that in proteomics, the most promising technique with sufficient resolving power is two-dimensional gel electrophoresis (2-DE) that provides a unique platform for the simultaneous separation of proteins in a complex mixture[5]. Therefore, this study was designed to evaluate the quality and standardization of the *L. pumila* var. *alata* leaves extract using 2-DE proteomics approach. Our results showed the typical 2-DE protein profile of water-soluble extract obtained from wild, cultivated and tissue-cultured sample of *L. pumila var. alata*

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 10814 - 10920
Received 05 March 2021; Accepted 01 April 2021.

leaves respectively (Figure1, Figure 2 and Figure 3). No protein spot was observed at pH 3-10 with 1 mg water-soluble extract loading. No similar study was performed and reported by other scientist. In conclusion, our study may be used as a tool for evaluating the quality and standardization of the *L. pumila* var. *alata* leaves extract from wild, cultivated and tissue-cultured samples using 2-DE proteomics approach. Hence, we recommend that no protein spot should be observed at pH 3-10 with 1 mg water-soluble extract loading of *L. pumila var. alata*using 2-DE proteomics approach.

COMPETING INTERESTS

The authors declared they have no conflict and competing interests.

ACKNOWLEDGMENTS

This study was supported by Forest Research Institute Malaysia (FRIM) and SEGi University, Faculty of Medicine matching grant.

AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: SRD NSS. Performed the experiments: NA NDMA SRD NSS. Analyzed the data: NA NDMA SRD NSS. Wrote the paper: SRD NSS NA NDMA. Revised the paper: SRD NSS NA NDMA FFMA JH NMHM MM. All authors read and approved the final manuscript to be published.

REFERENCES

1. Sunarno B: Revision of the Genus Labisia (Myrsinaceae). Blumea 2005, 50: 579 –597.
2. Jamia AJ: Malay traditional medicine. Tech Monitor (Special Feature: Traditional Medicine: S & T Advancement) 2006, 37-49.
3. Norhayati MN, Annie G, Nik-Hussain NH, Abdul-Kadir A, Hassan-Intan I, Kim SL, Ismail SB, Wan MWZ, Eckehard L, Abas A: Efficacy and Safety of *Labisia pumila var alata* Water Extract Among Pre- and Postmenopausal Women. Journal of Medicinal Food 2014, 17(8): 929-938.
4. Husniza H, Wan NWM, Khozirah S, Jamia AJ, Balaspramaniam A, Paridah J, Khalid BAK: Estrogenic properties of Kacip Fatimah (*Labisia pumila*): A preliminary study. Proceedings: 3rd Scientific Conference, National Institutes of Health, Malaysia. 2000, 77.
5. Doustjalali SR, Munira B, K Al-Jashamy, et al: Two dimensional gel electrophoresis: An overview of proteomic technique in cancer research. J Proteomics Bioinform 2014, 7: 077-081.
6. Abdullahi RA, Mainul H: Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci. 2020, 12(1): 1–10.
7. Doustjalali SR, Yip CH, Looi LM, Pillay B, Yusof R, Hashim OH: Aberrant expression of acute-phase reactant proteins in sera and breast lesions of patients with malignant and benign breast tumors. Electrophoresis 2004, 25:2392-2401.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 10814 - 10920
Received 05 March 2021; Accepted 01 April 2021.

8. Doustjalali SR, Yusof R, Govindasamy GK, Bustam AZ, Pillay B, Hashim OH: Patients with nasopharyngeal carcinoma demonstrate enhanced serum and tissue ceruloplasm in expression. The Journal of Medical Investigation 2006, 53:20-28.
9. Emida M, Abdul-Rahman PS, Doustjalali SR, Chen Y, Lim BK, Omar SZ, Bustam AZ, Singh VA, Mohd-Taib NA, Yip CH, Hashim OH: Lectin-based electrophoretic analysis of the expression of the 35 kDa inter-alpha-trypsin inhibitor heavy chain H4 fragment in sera of patients with five different malignancies. Electrophoresis 2008, 29:2645-2650.
10. Ng MH, Doustjalali SR, Pathak R, Sabet NS, Khor SF, Yogeswaran L, Ghani AN, Aminuddin S, Marzalina M, Ruszymah I: Two dimensional protein map standardization of human bone marrow stromal cells. J Proteomics Bioinform 2013, 6(4): 85-88.
11. Doustjalali SR, Munira B, K. Al-Jashamy, et al: Differential expression of serum ceruloplasmin and α2-HS glycoprotein among nasopharyngeal carcinoma patients. Journal of Molecular Biomarkers & Diagnosis 2015, http://dx. doi. org/10.4172/2155-9929. S2-010.
12. Doustjalali SR, Ali Y, K. Al-Jashamy, et al: Protein map standardization of human saliva using two dimensional gel electrophoresis (2-DE). Journal of Molecular Biomarkers & Diagnosis 2015, 6: 6:249.
13. Doustjalali SR, Shafiei NS, Aidanurashikin AR, et al: Protein map standardization of human urine using two dimensional gel electrophoresis (2DE). International Journal of Pharmaceutical Research 2019, 11(3): 101-104.
14. Heukeshoven J and Dernick R: Improved silver staining procedure for fast staining in PhastSystem development unit. I. Staining of sodium dodecyl sulphate gels. Electrophoresis 1988, 9: 28-32.
15. Ravindra P, Rajkumar T, Shiv-Shankar S: Omics: A newer technique in herbal drug standardization and quantification. J Young Pharm 2016, 8(2): 81-76.