TEST Engineering & Management

November-December 2019
ISSN: 0193-4120 Page No. 980- 986

Effects of Extraction Methods on Total Phenolic and Flavonoid Contents and Antioxidant Activity of *Barlerialupulina* Lindl

Ismail-Suhaimy N.W[1], Abd Gani S.S[*1,2], Zaidan U.H[3], Halmi M.I.E[4] and Bawon P[5]

[1]*Halal Products Research Institute (IPPH), Putra Infoport, noorwahidaismailsuhaimy@gmail.com.my*

[2]*Department of Agriculture Technology, Faculty of Agriculture*

**Corresponding author: ssalwaag@upm.edu.my*

[3]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences uswatun@upm.edu.my*

[4]*Department of Land Management, Faculty of Agriculture m_izuaneffendi@upm.edu.my*

[5]*Department of Forest Production, Faculty of Forestry paiman@upm.edu.my*

[1, 2, 3, 4, 5] *Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

Article Info
Volume 81
Page Number: 980- 986
Publication Issue:
November-December 2019

Article History
Article Received: *3 January 2019*
Revised: *25 March 2019*
Accepted: *28 July 2019*
Publication: *25 November 2019*

Abstract

BarlerialupulinaLindl. is a popular perennial herbal species commonly used by indigenous people in Malaysia as folk medicine due to its wide range of biological effects on human health. The present study reports on extraction and assessment of antioxidants from the species, with an aim of exploring potential antioxidants source for application in the production of pharmaceuticals, functional foods and other applications. Several methods of extraction were used in the study: microwave assisted extraction (MAE), soxhlet extraction (SE) and ultrasound assisted extraction (UAE) from leaves of B. lupulinaLindl. Each extraction method assessed phenolic content, total flavonoid content and antioxidant activity. The results have shown no variation between the three extraction methods ($P < 0.05$).The extracts of samples exhibited more than 80% inhibition scavenging activity against 2,2-diphenyi-1-picrylhydrazyl (DPPH) radical and almost 50% inhibition scavenging activity against Azino-bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) radical. The study identified that MAE was more efficient method of extraction for antioxidants from the species under study based on the short timeframe required. The present paper provides information to existing knowledge on available technologies for the good of mankind.

Keywords*: Barlerialupulina,* Total phenolic content(TPC), Total flavonoid content (TFC),Diphenyi-1-picrylhydrazyl (DPPH), Azino-bis-3-Ethylbenzothiazoline-6-sulfonic acid (ABTS).

November-December 2019
ISSN: 0193-4120 Page No. 980- 986

1 INTRODUCTION

*Barlerialupulina*Lindl., also known as *BisaUlar*or*PenawarSeribuBisa* in Malaysia, is a pantropical species from the genus Acanthaceae. It is a herbal shrub introduced from Mauritius and widely distributed throughout Africa and Asia [1,2]. The genus comprises of approximately 300 species and can grow up to 150cm in height [2]. Locally, it is cultivated mostly in the northern parts of PeninsularMalaysia (states of Perak, Kedahand Perlis) and generally used by the native people to treat several minor illnesses or infection caused by snake bites, dog bites, swelling, wounds andrheumatism [3]. A number of studies have reported that extract from the plant contains several bioactive compounds such as iridoid, phenylethanoid glycosides, alkaloids, saponins, tannins, flavonoids and proteins [3,4]. Leaf extracts have been documented to possess several health-related healing reactions including antibacterial, antioxidant, immunomodulatory, anti-inflammatory, anti-cancer, anti-HSV-2 activities, anti-diabetic, anti-arthritis and others [3, 5, 6, 7].

It is well-documented that plants have a large number of diverse bioactive compounds classified into three major classes: terpenoids, phenolic metabolites, and alkaloids [8]. Phenolic compounds including phenolic acids, polyphenols and flavonoids are essential for dietary purposes and have been widely studied by scientists compared to other classes of compounds [9]. These compounds act as protector of plants against oxidative damages and in human, they are used as antioxidants. Discoveries of new antioxidants from natural sources have become a major interest among researchers as the compounds can used as dietary supplements as well as innutraceuticals applications.

Methods of extraction are important in isolation of bioactive compounds as they can influence of quantity and type of phytoconstituents [10]. Traditionalmethods such as maceration, soxhlet, infusion, percolation and decoction require long extraction time and high-volume solvents. More recently, there has been widespread and effective use of methods like microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and supercritical fluid extraction (SFE).Known as Green Technology, it has become popular due to less use of solvents and achievable in shorter time although the techniques of extraction are continuously being modified for better results [11].

The current study was conducted to determine the effects of different techniques of extraction of antioxidants from *B. lupulina*using traditional and modern methods and their effects on (i) total phenolic content (TPC) (ii) total flavonoid content (TFC) (iii) Diphenyi-1-picrylhydrazyl (DPPH) scavenging activity and (iv) Azino-bis-3-Ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity.

2 MATERIALS AND METHOD

2.1 Plant materials

B. lupulina leaves were gathered from Serdang, Selangor, Malaysia. The plant specieswas authenticated at Institute Bioscience, Universiti Putra Malaysia (UPM), Malaysia with the voucher number MFI 0047/19. Leaves were washed, dried, ground to powder andstoredin airtight containers at 4^0C until further use.

Published by: The Mattingley Publishing Co., Inc.

2.2 Reagents

Sodium carbonate (Na_2CO_3), Folin–Ciocalteu's phenol reagent, Quecertin, DPPH, ABTS, potassium persulfate ($K_2S_2O_8$), Gallic acid has been bought from Biochem-Chemopharma (Loire, France), and ethanol was purchased from Friendemann Schmidt, Washington, USA.

2.3 Preparation of extract

Leaf powder was extracted using ethanol as solvent in a ratio of 1:10 (w/v) and three different extraction methods with minor modifications were performed: (i) Microwave-assisted extraction (MAE) was carried out using a domestic microwave oven system (Sharp Model R202ZS, Malaysia) at 800W for 60s, (ii) Soxhlet extraction (SE) was performed for 6h at high temperature, and (iii) ultrasound-assisted extraction (UAE) was conducted at room temperature using ELMA sonicator (135 W, 40 kHz) for 20min. All extracts used a rotary evaporator were concentrated and kept at -20^0C.

2.4 Determination of total phenolic content (TPC)

Total phenolic content (TPC) has been determined with minor changes using the Folin-Ciocalteau method[12]. In brief, 100µL of the extract was combined with 50µL Folin-Ciocalteau reagent and 4 minutes incubated.The mixture was added with a sodium carbonate (NaCO3) of 7.5 (w/v) % of 1.5 mL and incubated for 2 hours at room temperature. Using a UV-VIS microplate reader, the absorption of the reaction combination was read at 765 nm. Sample TPC was calculated on the basis of the standard calibration curve and was expressed as the mg gallic acid equivalent (mg/g GAE) of the sample extracted.

2.5 Determination of total flavonoid content

Each of the extracts was analyzed using the spectrophotometric technique with minor changes to the total flavonoid content (TFC) [13].An amount 100 uL (1 mg/mL) of each sample and 2% $AlCl_3$ were mixed and measured at λ= 415nm. The flavonoid concentration is conveyed as QE (mg quercetin/g extract) equivalent to quecertin.

2.6 DPPH Radical Scavenging Activity

The free radical scavenging operation of each extract was performed with some modifications in accordance with the method described by [14]. In brief, each extract was combined in a 96-well microliter plate with an ethanolic solution of 1-diphenyl-2-picrylhydrazyl (DPPH) radical. After 30 minutes of incubation, the samples were measured at 515 nm using a UV-VIS microplate reader. Radical scavenging activity was expressed as the percentage of inhibition and computed using the following formula (1):

$$\%\, Inhibition = \frac{(Acontrol\ -Asample\ )}{Acontrol}\ x\ 100 \quad (1)$$

2.7 ABTS Radical Scavenging Assay

The ABTS of each extract was determined based on the decolourization of the ABTS radical cation (ABTS•+) [15]. The mixture of ABTS radical cation and potassium persulfate ($K_2S_2O_8$) was allowed to stand in the dark at ambient temperature for 4 to 16 hours until the reaction was complete. Ethanol was added to generate ABTS•+ solution and mixed with 50µL of

each extract. The combination was vortexed and allowed to stand 15 minutes in the dark and read spectrophotometrically at 734 nm. The radical scavenging operation was expressed as the percentage of inhibition and computed using the following equation (2):

$$\%\ Inhibition = \frac{(Acontrol\ -Asample\ )}{Acontrol}\ x\ 100 \quad (2)$$

2.8 Statistical Analysis

One-way analysis of variance (ANOVA, SAS 9.4, SAS Institute Inc, Cary, NC, USA) was used to calculate the difference in means among different methods of extract (p<0.05).

3. RESULTS

3.1 Total Phenolic Content (TPC)

Figure 1 shows TPC results in the samples of various extraction methods. The highest phenolic content was observed in UAE (151.38 mg GAE/g) followed by MAE and SAE, respectively 149.5 mg GAE/g and 145.96 mg GAE/g. The TPC values were computed using the following linearequation based on the calibration curve of gallic acid:Y=0.0008X+0.0033, R^2= 0.9981.

3.2 Total Flavonoid Content (TFC)

Figure 2 shows the total flavonoid content for each extract. All extraction methods showed high flavonoid content with no significant difference among different methods: MAE (54.25mg QE/g), SE (52.34mg QE/g) and UAE (52.27mg QE/g). The TFC was computed using the following linear equation based on the quercetin calibration curve: Y=0.0216X+0.1075, R^2= 0.9986.

3.3 DPPH scavenging activity

Figure 3 shows the results of DPPH scavenging operation. DPPH's scavenging behavior was based on DPPH's free radical color solution shifts, measured by the use of a spectrophotometer. The DPPH solution's reduction color indicated the existence of antioxidant. DPPH scavenging activity showed high percentages of inhibition against DPPH radical, which were MAE (83.87%), SE (81.95%) and UAE (81.68%) respectively.

3.4 ABTS radical scavenging activity

Figure 4 shows the results of the radical scavenging operation of the leaf extracts. The stable bluish-green radical changed in colour intensity when reacted with antioxidant that can be measured by using spectrophotometer. MAE showed high percentages of inhibition with 46.71% followed by SE, 43.73% and UAE, 40.57%.

November-December 2019
ISSN: 0193-4120 Page No. 980- 986

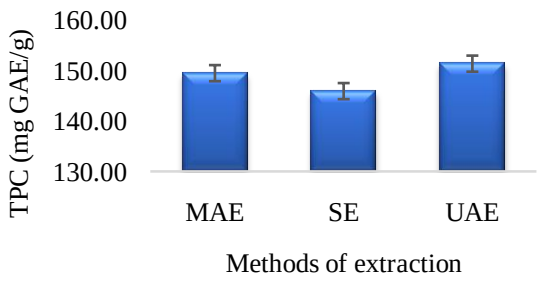

Figure 1. Total phenolic content from different extraction methods (*n*=3)LSD: least significant difference; level of significance: *p< 0.05.

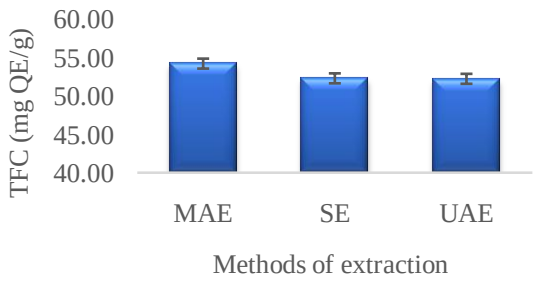

Figure 2. Total flavonoid content from different extraction methods (*n*=3) LSD: least significant difference; level of significance: * p< 0.05.

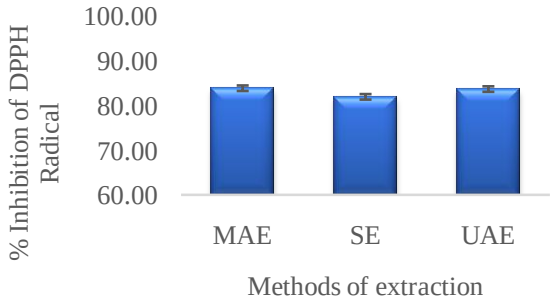

Figure 3. Percentage inhibition of DPPH radical from different extraction methods (*n*=3).LSD: least significant difference; level of significance: *p< 0.05.

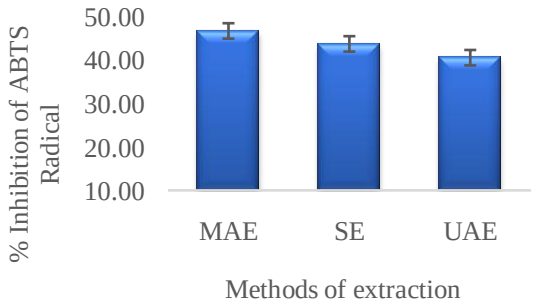

Figure 4. Percentage inhibition of ABTS radical from different extraction methods(*n*=3). LSD: least significant difference; level of significance: *p< 0.05.

Methods of extraction:
MAE : Microwave assisted extraction; SE : Soxhlet extraction; UAE : Ultrasound assisted extraction

4. DISCUSSION

Plants are one of natural antioxidants' rich sources. Epidemiological studies have shown that fruit and vegetable consumption is capable of protecting against many chronic diseases including cancer [16].The benefits of *B. lupulina*are widely known among native people of Malaysia, however no comprehensive studies have been reported on the antioxidant properties of the species.

The efficiency of extraction methods in separating soluble plant metabolites by using solvents depends on sample particle size, solvent type and chemical nature of phytochemicals. In the present study, ethanol was chosen as a solvent extraction because its suitability for polyphenol extraction, safe and less toxic [9]. Three methods of extraction were employed with different operation processes. Soxhlet extraction is a process using solvent heated in a flask, vaporizes into the sample then drips back and the cycle continues that usually takes several hours to complete. Microwave-assisted extraction is a process using microwave radiation, in which heat is transferred by conduction to the samples in a short distance and it usually takes less time to extract the compound. Ultrasound-assisted extraction is a process using mechanic

Published by: The Mattingley Publishing Co., Inc.

effects of acoustic cavitation to contact the solvent and sample of extract and this process usually takes several minutes to complete [10].

Based on the results, TPC decreased in the following order: UAE > MAE > SE. These results are contrary to previous studies, which showed high yield of TPC in MAE when compared other methods of extraction [17, 18]. These might be due to high microwave power as reported by[17]. Increase of microwave power to more than 600W, decreases or constant the yield of TPC. This was to SE due to prolonged exposure to heat which enable it to degrade of the compounds. However, the present study did not find significant difference between the three methods. The results are similar tostudy on *Piper betle* leaves [10] and propolis [19] when using traditional method of maceration.

The determination of flavonoid content in the present study show that all methods of extraction yielded more than 50mg QE/g of TFC, which made MAE the highest. These results are in agreement with several other findings showing the capability of MAE to extract TFC when compared with other method [17, 18]. In the various extraction methods, there was no significant difference in flavonoid content. Other studies reported high content of flavonoid in *B. lupulina*by using different solvent and different parts of the plant [3,4].

Phenolic content is responsible for bioactivities like antioxidant and antibacterial activities. The current study shows antioxidant activity of each ethanolic extract expressing better results with high percentage inhibition scavenging on DPPH and ABTS radicals. The high content of phenolic compounds and flavonoid content are likely to be related to these results.

5. CONCLUSION

The results indicate that there was no significant difference between the methods of extraction of *B. lupulina* leaves for total phenolic compound, flavonoid content and antioxidant activity. Optimization of procedures in the extraction processes is recommended in future studies.

ACKNOWLEDGEMENT

The study was supported by Halal Products Research Institute (IPPH), Putra Infoport, and Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia (UPM). The authors would like to thank for the support as well as for the use of all laboratory facilities in UPM.

REFERENCES

[1] M. J. Balkwill, and K. Balkwill. "A preliminary analysis of distribution patterns in a large, pantropical genus, *Barleria*L.(Acanthaceae)," *Journal of Biogeography*, vol. 25.1, pp. 95-110, Jan. 1998.

[2] T. Ghosh, S. K. Mukherjee, and H. S. Debnath. “Comparative taxonomic studies of four species of Barleria L. Tribe JusticieaeSeafft/Benth. & Hook f. Acanthaceae of N.E. India,” *Systematics of Flowering Plants*, pp.113-117, 2009.

[3] R. Kumari, S. Kumar, A. Kumar, K. K. Goel, and R. C. Dubey. “Antibacterial, antioxidant and Immuno-modulatory properties in extracts of *Barlerialupulina*Lindl.,” *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, pp. 484. 2017.

[4] S. R. Lee, J. Clardy, D. R. Senger, S. Cao, and K. H. Kim. “Iridoid and phenylethanoid glycosides from the aerial parts of

Barlerialupulina,"RevistaBrasileira de Farmacognosia, vol. 26, no. 3, pp. 281-284, 2016.

[5] P. M. Mazumder, A. Mondal, D.,Sasmal, S. Arulmozhi, and P. Rathinavelusamy. "Evaluation of antiarthritic and immunomodulatory activity of *Barlerialupulina*," *Asian Pacific Journal of Tropical Biomedicine* vol. 2, no. 3, pp. S1400-S1406, 2012.

[6] C. Yoosook, Y. Panpisutchai, S. Chaichana, T. Santisuk, and V. Reutrakul. "Evaluation of anti-HSV-2 activities of *Barlerialupulina* and *Clinacanthus nutans*," *Journal of ethnopharmacology*, vol. 67, no. 2, pp. 179-187, 1999.

[7] M. Wirotesangthong, and S. Rattanakiat. "Anti-herpes simplex virus type 2 activities of some Thai medicinal plants," *Thai J Pharm Sci*, vol. 30, no. 1-2, pp. 19-27, 2006.

[8] J. N. Walton, and D.E. Brown. "Chemicals from plants - Perspectives on plant secondary products," *Imperial College Press, London, UK*, pp. 1-25, 1999.

[9] Q. D. Do, A. E. Angkawijaya, P. L. Tran-Nguyen, L. H. Huynh, F. E. Soetaredjo, S. Ismadji, and Y. H. Ju. "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophilaaromatica*," *Journal of Food and Drug Analysis*, vol. 22, no. 3, pp. 296-302, 2014.

[10] S. Das, A. Ray, N. Nasim, S. Nayak, and S. Mohanty. "Effect of different extraction techniques on total phenolic and flavonoid contents, and antioxidant activity of betelvine and quantification of its phenolic constituents by validated HPTLC method," *3 Biotech* 9, no. 1, pp. 37, 2019.

[11] N. N. Azwanida. "A review on the extraction methods used in medicinal plants: Principle, Strength and Limitation," *Med Aromat Plants*, vol. 4, no. 196, pp. 2167-0412, 2015.

[12] V. L. Singleton, and J. A. Rossi. "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents," *American Journal of Enology and Viticulture*, vol. 16, no. 3, pp. 144-158, 1965.

[13] M. S. Stankovic. "Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubiumperegrinum* L. extracts," *Kragujevac J Sci*, vol. 33, no. 2011, pp. 63-72, 2011.

[14] M. S. Blois. "Antioxidant determinations by the use of a stable free radical," *Nature*, vol. 181, no. 4617, pp. 1199, 1958.

[15] E. A. Shalaby, and S. M. M. Shanab. "Antioxidant compounds, assays of determination and mode of action," *African Journal of Pharmacy and Pharmacology*, vol. 7, no. 10, pp. 528-539, 2013.

[16] G. Block, B. Patterson, and A. Subar, "Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence," *Nutrition and Cancer*, vol. 18, no. 1, pp. 1–29, 1992.

[17] F. Dahmoune, G. Spigno, K. Moussi, H. Remini, A. Cherbal, and K. Madani. "*Pistacialentiscus* leaves as a source of phenolic compounds: Microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction," *Industrial Crops and Products*, vol 61, pp. 31-40, 2014.

[18] F. Dahmoune, B. Nayak, K. Moussi, H. Remini, and K. Madani. "Optimization of microwave-assisted extraction of polyphenols from *Myrtuscommunis* L. leaves," *Food Chemistry*, vol. 166, pp. 585-595, 2015.

[19] B. Trusheva, D. Trunkova, and V. Bankova. "Different extraction methods of biologically active components from propolis: A preliminary study," *Chemistry Central Journal*, vol. 1, no. 1, pp. 13, 2007.