Comparison of bioactive compounds and their antioxidant potentials of three varieties of *Labisia pumila* Benth. extracts obtained from different extraction methods

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Abstract

Extraction methods are very important processes for separation of bioactive compounds of medicinal plants before analyses. Phenolic and flavonoid compounds include a broad variety of structures and functionalities that are required for the production of foods and food additives and there are different methods for the their extraction. In this study, total phenolic and flavonoid contents of leaf extracts from three varieties of *Labisia pumila* Benth (variety *alata*, *pumila*, and *lanceolata*) were investigated by microwave assisted extraction (MAE) and reflux extraction methods. Furthermore, DPPH radical-scavenging and ferric-reducing antioxidant power (FRAP) assays were applied to test the antioxidant activities. Results revealed that total phenolic (3.14, 3.03, 2.94 mg GAE g^{-1} DW) and flavonoid contents (2.08, 2.1, 1.85 mg Rutin g^{-1} DW) in the leaf extracts of *Labisia pumila* varieties *alata*, *pumila*, and *lanceolata* by MAE method were significantly higher than reflux extraction method, which was possibly responsible for the higher antioxidant activities in all three varieties of *L. pumila* Benth. These findings further illustrate that MAE has a bright prospect for extracting active ingredients from plant materials.

Keywords: microwave assisted extraction; reflux extraction; *Labisia pumila* Benth

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Introduction

Extraction is the first basic step in the recovery and isolation of bioactive phytochemicals of medicinal plants before analysis. It is affected by different parameters like chemical nature, sample particle size, and the presence of interfering substances (Romanik et al., 2007). Extraction and characterization of several bioactive compounds from medicinal herbs have given birth to some high activity profile drugs such as vincristine and vinblastine that have potential

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natural anticancer activity (Huie, 2002). Conventional extraction methods such as maceration, heating, boiling, refluxing, and soxhlet extraction are the most commonly used procedures prior to the analysis of the secondary metabolites from the plants, but they have shown low efficiency and potential environmental pollution due to large volumes of organic solvent used and long extraction time required in those methods (Li et al., 2005). In recent years, various novel extraction techniques have been developed for the extraction of bioactive compounds from the plants including microwave-assisted extraction (Wang and Weller, 2006). This extraction has shown tremendous research interest and potential due to considerable reduction in time and solvent as compared to conventional techniques (Eskilsson and Bjorklund, 2000).

Now a days, research on medicinal plants has drawn enormous global attention and exploitation of the herbal plant for commercial purposes is gradually increasing (Karimi et al., 2013). *Labisia pumila*, locally known as Kacip Fatimah, is the queen of the herbs in Malaysia. It is a genus of small woody and leafy plants of the Myrsinaceae family. There are three varieties of *L. pumila* namely, *L. pumila* var. *pumila*, *L. pumila* var. *alata*, and *L. pumila* var. *lanceolata* each having its own use. It is widely used as post-partum medication for centuries and lots of studies have been carried out on the phytochemical identification of its biological and toxicological potentials (Karimi et al., 2011). Phytochemicals found in the herbal extract showed high biological activities and pharmacological properties (Jaafar et al., 2012). The present study is aimed at the comparison of the bioactive constituent contents by two extraction methods, namely, Reflux and Microwave Assisted Extraction in relationship to their antioxidant activities in three varieties of *Labisia pumila* Benth.

Materials and Methods

Plant material

Seedlings of *Labisia pumila* varieties *alata, pumila*, and *lanceolata* were collected from five places of origin at Sungkai, Perak, Hulu Langat, Selangor and Kota Tinggi, Johore, respectively and raised under similar glasshouse condition (3° 0' 35.27" N latitude and 101° 42' 19.38" E longitude) for 18 months before they were used in the study. Healthy and uniform seedlings in term of leaf numbers were selected from the three varieties. The leaves of the three varieties of *Labisa pumila* Benth. were cleaned, separated, and freeze-dried (-15 ˚C for 20 h) for further analysis.

Hydrolysis extraction method (reflux extraction)

Samples were extracted using ethyl acetate as a solvent based on Crozier et al. (1997). Two grams of freeze-dried leaves were weighed and placed in 100 ml conical flask, and added with 40 ml of 80% (v/v) ethyl acetate. This was followed by an addition of 10 ml of 6 M HCl. The mixture was refluxed for 2 hours at 90 ˚C and filtered through Whatman No. 1 filter paper (Whatman, England) followed by evaporation of the filtrate using a vacuumed Rotary Evaporator (Buchii, Switzerland).

Microwave assisted extraction (MAE)

MAE was performed on microwave apparatus using closed vessel system with pressure (ETHOS® T Microwave digestion/ extraction system, Milestone Co., Italy) based on the method explained by Karimi et al. (2011). One gram of leaf part of the three varieties of *Labisia pumila* were weighed before they were transferred into the vessel of the Ethos E Microwave Extraction System for extraction by 30 ml of ethyl acetate as solvent for 2 min (p=750w). The extraction temperature was applied to 80 ˚C. After extraction, the vessels were allowed to cool at room temperature before opening. Then the extracts were filtered and stored in a refrigerator.

Total phenolics and flavonoids determination

Total phenolics and flavonoid contents were determined using a Folin–Ciocalteu reagent and aluminum chloride colorimetric assay, respectively (Karimi et al., 2014). Total phenolic

Table 1
Total phenolics and flavonoids content in the leaves of *Labisa pumila* Benth varieties with different extraction techniques

Sample	Phenolic Content[1]		Flavonoid Content[2]	
	Microwave Extraction	Reflux Extraction	Microwave Extraction	Reflux Extraction
Alata	3.14±0.15	2.60±0.05	2.08±0.02	1.16±0.13
Pumila	3.03±0.07	2.43±0.03	2.10±0.04	1.24±0.05
Lanceolata	2.94±0.11	2.42±0.11	1.85±0.05	1.15±0.04

[1] mg gallic acid equivalents g^{-1} DW, [2] mg rutin equivalents g^{-1} DW, Means ± standard deviation (n = 3).

Table 2
DPPH scavenging activities and total antioxidant (FRAP) activities of the leaf extract in all varieties of *L. pumila* Benth at concentration of 300 µg/mL with different extraction techniques; BHT and α-tocopherol were used as positive controls.

Sample	DPPH Assay (%)		FRAP Assay (%)	
	Microwave Extraction	Reflux Extraction	Microwave Extraction	Reflux Extraction
Alata	58.33±0.26	55.47±0.76	54.85±0.38	51.80±0.08
Pumila	56.71±2.61	53.12±0.99	52.50±1.44	50.78±0.06
Lanceolata	52.08±0.23	51.25±0.29	51.39±0.76	50.68±0.15
BHT	99.24±0.25	99.24±0.25	99.18±0.22	99.18±0.22
α-tocopherol	99.73±0.19	99.73±0.19	96.17±0.19	96.17±0.19

All analyses were mean of triplicate measurements ± standard deviation.

and flavonoid contents were expressed as mg Gallic acid equivalents g^{-1} and mg Rutin equivalent g^{-1} dry matter of the plant material, respectively.

Antioxidant activity

DPPH radical-scavenging activity

Free radical scavenging activities of the leaf extract were determined using the DPPH assay as described by Gulcin et al. (2004). One ml of each plant extracts at different concentrations were mixed with 3 ml 0.1 mM solution of 1,1-diphenyl-2-hydrazil (DPPH) in methanol and incubated for 30 min in the dark condition. The absorbance of the mixture was read using a visible spectrophotometer (Novaspec II visblespectro) at 517 nm. BHT and α-tocopherol were used as an antioxidant standard.

Ferric-reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) of the extracts was determined as described by Yen and Chen (1995). BHT and α-tocopherol were used as standard antioxidants.

Statistical Analysis

The experiment was carried out as completely randomized design and was analyzed using analysis of variance (ANOVA) with SAS Version 9 (SAS Institute, Cary, NC). Significant differences among means from triplicate analyses ($p < 0.05$) were determined by Duncan's Multiple Range Test.

Results

Total phenolics (TP) and total flavonoids (TF) content

Total phenolic and flavonoid contents in the leaves of *L. Pumila* Benth varieties with different extraction methods are summarized in Table 1. The results obtained from Microwave and Reflux Extraction methods demonstrated that the highest total phenolic content was found in *L. pumila* var. *alata* as 3.14 and 2.60 mg GAE g^{-1} DW. Also, the highest total flavonoids content from the

leaves of *L. pumila* Benth. was found in *L. pumila* var. *pumila* as 2.08 and 1.16 mg Rutin g^{-1} DW from Microwave and Reflux Extraction methods, respectively.

Antioxidant activity (DPPH and FRAP)

The 1,1-diphenylpicrylhydrazyl (DPPH) free radical scavenging activity and ferric-reducing antioxidant power (FRAP) were used to evaluate the antioxidant activity of the leaf extracts in all varieties of *L. pumila* Benth. at concentration of 300 µg/mL with different extraction methods. The results of the DPPH assay of the leaf parts of the three varieties of *L. pumila* are reported in Table 2. The obtained results indicated that *L. pumila* var. *alata*, had higher antioxidative activity (58.33%, 55.47%) compared to var. *pumila* (56.71%, 53.12%) and var. *lanceolate* (52.08%, 51.25%) using microwave and reflux extraction, respectively. However, these values were lower than the tested antioxidant standards, BHT (99.24%), and α-tocopherol (99.73%).

Similar to DPPH results, the reductive potential of *L. Pumila* Benth. in all three varieties increased in a dose-dependent manner. The reductive potential of *Labisia pumila* extracts in all three varieties and the standards at a concentration of 300 µg/mL (Table 2) were found to be in the descending order of BHT > α-tocopherol > *L. pumila var. alata* > *L. pumila var. pumila* > *L. pumila* var. *lanceolate* in both tested extraction methods.

Discussion

The results from the present study showed that Microwave Extraction method had higher total phenolic and flavonoid contents than Reflux Extraction. This is consistent with previous studies such as extraction of essential oil and bioactive compounds from the fresh stems and leaves of *Lippia alba* (Stashenko et al., 2004), extraction of embelin from dried berries of *Embelia ribes* (Latha, 2007), extraction of sanguinarine and chelerythrine from dry fruit of *Macleaya cordata* (Zhang et al., 2005), extraction of geniposidic and chlorogenic acid from dried bark of *Eucommia ulmodies* (Li et al., 2004), extraction of solanesol from tobacco leaves (Zhou and Liu, 2006), and extraction of polyphenols and caffeine from green tea leaves (Pan et al., 2003) where Microwave Extraction was more effective compared to traditional extraction methods like Reflux because of reduction of extraction time and solvents, selectivity, volumetric heating, and controllable heating process.

Antioxidants are phytochemicals, vitamins, and other nutrients that are extremely useful to humans. They alleviate and avoid various diseases related to lungs, kidneys, heart, cardiovascular system, muscle, and brain, and they help to retard the aging process (Karimi et al., 2014). These chemicals prevent or delay the formation of free radicals and lipid peroxidation in human bodies. Plants provide us with rich sources of natural antioxidants. Nutrients with antioxidant properties such as phenolics, flavonoids, carotenoids as well as vitamins C, E, and selenium, and possibly other nutrients and food components help to protect the proteins, lipids, and DNA in cells from damage by oxygen (Surai, 2005). Comparing antioxidant activity in relation to reference antioxidants such as vitamin C, vitamin E, and BHT provides useful information on the antioxidant potential of the plant materials.

Microwave assisted extraction showed obvious advantages in terms of high extraction efficiency and antioxidant activity of extract within shortest extraction time than conventional extraction. Previous studies revealed that microwaves can penetrate plant matrix directly, resulting highly localized temperature and pressure inside plant cells, which can cause selective migration of target compounds into the solvent rapidly (Upadhyay et al., 2012). THerefore, microwave has been widely employed to extract bioactive compounds as well as their antioxidant potentials from different plant materials (Sutivisedsak et al., 2010, Tsubaki et al., 2010, Song et al., 2011).

Conclusions

In this study two reliable extraction methods (Microwave and Reflux) were developed for the analysis of total phenolic and flavonoid contents and their antioxidant activities in the leaf extract in all varieties of *L. pumila* Benth. In general, the Microwave Extraction method gave

the greatest secondary metabolites content and also showed valuable antioxidant compared to Reflux method. Based on the results of the study, it can be conclude that Microwave Extraction may be widely applied to obtain such valuable natural compounds from medicinal plants as a more efficient method of extraction compared to conventional methods.

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