Jurnal Teknologi

Full paper

Fractionation of *Labisia Pumila* using Solid-Phase Extraction for Extraction of Gallic Acid

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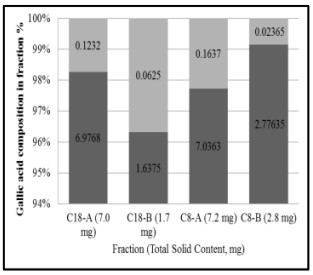
Article history

Received :4 October 2013
Received in revised form :
24 April 2014
Accepted :8 May 2014

Graphical abstract



Abstract

Phenolics are the most ample secondary metabolites of plants and have drawn increasing attention in their applications in health supplement and functional cosmetic. This is due to their strong antioxidant properties and their marked effects in the inhibition of several oxidative stress associated metabolic disorder such as cancer and aging. In *Labisia pumila*, gallic acid (3,4,5-Trihydroxybenzoic acid) was found to be one of the most abundant secondary metabolite. It has been proposed as a biomarker in cosmetic and food supplement applications. In this research the identification and isolation of gallic acid were carried out. Different sorbent of solid-phase extraction (SPE) column were used for the fractionation of *Labisia pumila var Alata* water extract and the amount of gallic acid present in the fractions were quantified. The aim is to identify the better sorbent-solvent combination in SPE that result in higher recovery of gallic acid from *Labisia pumila var Alata* water extract. A range of solvent mixtures of 20%, 40%, 60% and 80% methanol (Me Oh)-water was used. Gallic acid was successfully extracted and identified from *Labisia pumila*. From this discovery, C18 silica reversed phase solid phase extraction column could extracts highest gallic acid from *Labisia pumila var Alata* water extract. Retention of analyte from the sample from second elution using 80% methanol-water Solvent may be used as the best method to extract the compound from *Labisia Pumila var Alata* since it has been reported to have various bioactivities such as high antioxidant activity and cytotoxic against many diseases. Using C18 silica reversed phase in SPE as a fractionation and extraction of gallic acid from *Labisia pumila var Alata* can be achieved for application purpose or future studies.

Keywords: Solid phase extraction; gallic acid; fractionation

1.0 INTRODUCTION

Labisia pumila from Myrsinaceaeare family is also known as Kacip Fatimah among the natives from Asean region. Physically, *Labisia pumila* can be recognized as small herbaceous under a shrub that roots from the stem with a few leaves pointing upwards and pointed with a base that is tapered or rather broad rounded [1]. Local people in Asean especially in Malaysia and Indonesia generally used this small forest-floor herbaceous as a food supplement and medicine for a variety of illnesses. Most popular applications of this herb are to induce and facilitate childbirth as well as a post-partum medication to help contract the birth channel [2], to tone the abdominal muscles and to regain body strength. Recently researchers also found that *Labisia pumila* has high potential as aroma-active essential oil[1], natural antioxidant [3], anticancer and skin protector against photoaging [2].

In Malaysia, there are three types of *Labisia pumila* which are *Labisia pumila var Alata*, *Labisia pumila var Pumila* and *Labisia pumila var Lanceolata*. Although all three types have a similar general used in Malay traditional medicine, however, specifically there are differences in their bioactive compound, primary and secondary metabolite and potential in many areas such as anticancer, skin protector against photoaging and antioxidant activities. Hence, *Labisia pumila var Alata* and *Labisia pumila var pumila* are often used because their effect on several metabolite activities are more significant than *Labisia pumila var lanceolata*. Investigation of the plant leaves part are more popular than the roots maybe due to the differences in flavonoid, phenolic and others primary metabolite content in *Labisia pumila* parts.

1.1 Phytochemical Compound of *Labisia pumila var Alata*

Few phytochemical components have been identified in *Labisia pumila var Alata* [4] such as anthocyanins and antioxidant components which are also present in fruits and plants. The antioxidant activity of the aqueous extract has been reported as providing important protection to human dermal fibroblast, from

cell damage induced by UV irradiation [2], most likely due to the presence of flavonoids [4]. A study by Zhang and Ye (2008) [5] suggest that both phenolic acids and flavonoids are believed to be responsible for the full spectrum of pharmacological activities attributed to the herb. The antioxidant activity of the aqueous extract has been reported as providing important protection to human dermal fibroblast, from cell damage induced by UV irradiation [2] , most likely due to the presence of flavonoids [4].

Researches shown that gallic acid is one of the most abundant secondary metabolite in all three varieties in Malaysian Labisia pumila varieties [6][7]. Gallic acid is a part of phenolic compound and their role is significantly important because of their described protective roleagainst cancer and diseases [8]. This role perhaps attributed to their antioxidant activity which is reported to be greater than that of vitamins E and C [9].

An introductory screening of total phenolic content was done by Chua *et al*. (2011) [10] The study shows that methanol extract of this herb contained the highest amount of phenolic phytochemicals, followed by the 60% methanol extract. Flavonoids constituted a large portion of the phytochemicals in the 60% methanol leaf extract. The 40% methanol fraction was found to have the highest DPPH scavenging activity.

1.2 Solid Phase Extraction

Solid phase extraction (SPE) is based on the same principle of affinity-based separation as liquid chromatography. SPE enables retention and elution of analytes from complex mixtures, removal of interfering compounds, and sample concentration. SPE is available in normal phase, reverse-phase, and ion exchange modes, reversed phase being one of the most used formats [11].

Based on the wide range of physicochemical properties of the analytes, several commercial sorbents (e.g., C 18, C 8, C 2, phenyl, cyanopropyl, and ion exchange bonded materials, among others) are supplied to improve the versatility of SPE. Regarding this, in the peptidomic characterization of beer, Picariello *et al*., (2011) [12] applied the samples directly onto the C 18 pre-packed cartridges and eluted with acetonitrile/TFA to RP-LC. In other examples, Hernández-Ledesma *et al*. (2005) [13] treated the water-soluble extract of fermented milk with a Sep-Pak C 18 cartridge and acetonitrile elution, and a similar extraction step was used by Muguruma *et al*. (2009) [14] to desalt SEC-eluted fractions from porcine myosin B.

Based on similar principles of SPE techniques, innovative size-reduced devices have recently appeared for concentration, purification, and desalting of peptides prior to analysis by mass spectrometer. These devices support a membrane or micro column that can be of diverse nature (polar, nonpolar, and ion exchange) and feature an optimized procedure for sample preparation.

■2.0 EXPERIMENTAL

2.1 Sample Preparation Techniques

Fresh *Labisia pumila var Alata* leaves were procured from Forest Research Institute Malaysia (FRIM). The leaves were dried under the sun and ground to approximately 2.0 mm particle size.

2.2 Preparation of Water Extract

The dried ground leaves of *Labisia pumila var Alata* were extracted using conventional boiling methods using water with a ratio of 1:10 w/v grounded leaves in water and boiled for four hours in 100^0C using continuous stirred and this procedure was repeated twice using same leaves. After 4 hours the extract was filtered and concentrated to dryness using a rotary evaporator to obtain the crude extract. The crude extract then was kept in 50ºC oven until constant weight.

2.3 Fractionation of *Labisia pumila var Alata* using Solid Phase Extraction (SPE)

100 mg crude water extract of *Labisia pumila var Alata* was then suspended in 100 ml distilled water and fractioned using SPE with a range of solvent mixtures of 80%, 60%, 40% and 20% MeOh-water. 5 ml diluted crude water extract was first subjected to Carbon 18 (C18) sorbent SPE column. Fractionation starts with conditioning the sorbent with 100% methanol and equilibrates with 100% water. The sample was then added into the column and put under vacuum for not more than 20 psg. Retention from sample loaded was treated as fraction A. SPE column was eluted with a range of solvent mixtures of 80%, 60%, 40% and 20% methanol-water of increasing polarity to generate a total of six fractions (fraction B - fraction F) and last elution using 100% water wasreacted as fraction G. The experiment was repeated using Carbon 8 (C8) sorbent SPE column. Total 14 fractions were collected and proceed with quantification of gallic acid.

2.4 High Performance Liquid Chromatography (HPLC) Analysis

A HPLC system (Waters, USA) consisting of Waters 2690, photodiode array (detector Waters 996) was set up to monitor from 200 to 500 nm. Synergi 4u Fusion-RP 80A (4.6 mm i.d 150 mm) (Part no: 00F-4424-E0) (Phenomenex, USA) was used as acolumn. Data were integrated by Empower 2 software (Waters) at wavelength of 254 nm. Separation was achieved by flow rate of 0.6 ml/min with 3.0% Phosphoric acid (90%) / Acetonitrile(10%), in an isocratic programme. The injection volume was 20 µl.

■3.0 RESULTS AND DISCUSSION

3.1 Total Solid Content (TSC) in Fractions

Fractionation using SPE column C8 and C18 yield us to 14 fractions from initial 3 ml of 0.01 g/ml *Labisia pumila var Alata* water extract. Upon fractionation, the extract was analyzed using HPLC to determine the gallic acid and the concentration of total solid content (SC) was determined by the weight of crude samples collected after drying. Result from the HPLC was calculated using the following equations.

$$\text{GA (w/w)\%} = \frac{concentration\ (ppm) \times 0.01}{(solid\ content\ (g) \times 1000\ ) \times 100} \qquad (1)$$

Table 1 shows that the concentration of SC collected in the fraction varies from fraction A to G for both sorbent. The highest SC content collected by SPE C18 column was extracted into C18-A and C18-B fraction while in SPE C8 column, C8-A and C8-E shown the high solid content from all fourteen fractions. Fraction C18-A and C18-C shown highest content of solid content with 29% and 23% of total solid content in 3 ml 0.01g/ml of *Labisia pumila var Alata* water extract. The amounts of gallic acid detected were discussed further in the next section.

However, based on Table 2, the amount of solid collected in a fraction C18-A, contain 1.735219 GA µg/mg solid content meanwhile for fraction C18-B, 3.678788 GA mg/mg solid content was detected. Although fraction C18-C and C18-G shown higher

solid content compare to fraction C18-B, no gallic acid was detected in both fractions.

Meanwhile, for SPE Column C8, fraction C8-A and C8-E recorded higher solid content collected by 21% and 18% of total solid content in 3 ml 0.01 g/ml of*Labisia pumilavarAlata* water extract, butgallic acid only detected in fraction C8-A and C8-B with 2.2731and 0.8448 GA mg/mg solid content respectively.

Figure 1 shows the composition of gallic acid from the total solid content of fractions for both types of SPE sorbent. Fraction C8-B was fractioned using 80% methanol solvent and could fractions highest amount of solid content compare to others fractions. However, the amount of gallic acid detected was the smallest among the rest. Fraction C8-A shown that almost 50% of its content isgallic acid, although the amount of fraction obtained from SPE was lower than fraction C8-B. Same pattern occurred in C18 sorbent, however, to compare both sorbent, C8 shown more significant result, in order to extract more concentrate gallic acid.

This result was due to the Van der Walls interaction between sorbent, mobile phase and analyte from the sample. In this reversed phase extraction process using solid phase extraction method, we demonstrated the ability of solvent to dissolve polar organic compound in the sample. Methanol, which was known to be less polar than water are variances in the series of solvent (80-20% Me Oh) to create a different polarity in the solvent.

Generally polar compound spent more time in sorbent and non-polar compound spends more time in solvent. Gallic acid (polar compound) was adhering onto the surface of silica sorbent inside the column while the sample was introduced into the column. The retention of gallic acid was due primarily to the attractive forces between the carbon-hydrogen bonds in the gallic acid and the functional groups on the sorbent surface. These nonpolar–nonpolar attractive forces are commonly called Van der Waals forces or dispersion forces. Less polar solvent was used first to remove least polar compound first and increasing accordingly until all compounds completely remove from the system. The nonpolar solvent, which can disrupt the forces between the sorbent and compound, was used to elute an adsorbed compound from SPE column.

These results were supported by the finding from Chua *et. al* (2011) [10] which indicate that high concentration of methanol (100% methanol) could extract the highest amount of phenolic content from *Labisia pumila* which was indicated using gallic acid equivalent where it shown similarity with this experiment. However, in terms of the concentration of gallic acid extracted using two different SPE silica cartridges, C8 sorbent could extract more concentrate gallic acid using 80% methanol as solvent.This result was suggested that C8 could become better solid phase for fractionation of gallic acid from *Labisia pumila var Alata* water extract.

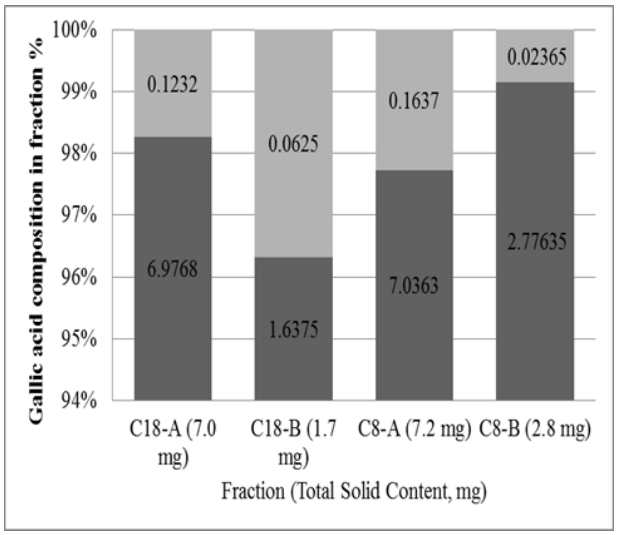


Figure 1 Composition gallic acid in fraction C18-A, C18-B, C8-A and C8-B. (A; Sample retention, B; elution 80% methanol)

4.0 CONCLUSION

Fractionation of *Labisia pumila* using solid phase extraction method using silica based sorbent shown that C8 silica SPE cartridge could extract more concentrate gallic acid when paired with 80% methanol as solvent compare to C18-80% methanol. This method can be used as a fractionationand extraction method forphenolics from *Labisia pumila var Alata*.

Acknowledgement

The authors are grateful to the Institute Bioproduct Development (IBD) of Universiti Teknologi Malaysia for laboratory facilities and the Ministry of Higher Education Malaysia for financing this work under the Research University Grant Scheme No. Q.J130000.7719.4H010.

Table 1 Total Solid Content (TSC) (w/w)% in all fractions in C8 and C18 SPE sorbent

Sorbent	Fraction (w/w)%						
	A	B	C	D	E	F	G
C8	0.441393	0.123916	0.202764	0.230555	0.223883	0.146895	0.155325
C18	0.546490	0.099282	0.458640	0.051740	0.021964	0.071573	0.105542

Table 2 Amount of solid content (SC) and gallic acid (GA) in each fraction for both SPE column C18 and C8

Fraction	SC in L.P fraction (w/w)%	*SC Percentage %	GA (w/w)%	GA (mg)	SC (mg/ml)	GA mg/mg Solid Content
C18 A	0.5465	29.3388	1.7352	0.1232	0.7100	1.7352
C18 B	0.0993	7.0248	3.6788	0.0625	0.1700	3.6788
C18 C	0.4586	23.1405	N/D	N/D	0.5600	N/D
C18 D	0.0517	4.9587	N/D	N/D	0.1200	N/D
C18 E	0.0220	2.4793	N/D	N/D	0.0600	N/D
C18 F	0.0716	14.4628	N/D	N/D	0.3500	N/D
C18 G	0.1055	18.5950	N/D	N/D	0.4500	N/D
C8 A	0.4414	21.8182	2.2731	0.1637	0.7200	2.2731
C8 B	0.1239	8.4849	0.8448	0.0237	0.2800	0.8448
C8 C	0.2028	14.8485	N/D	N/D	0.4900	N/D
C8 D	0.2306	17.2727	N/D	N/D	0.5700	N/D
C8 E	0.2239	18.7879	N/D	N/D	0.6200	N/D
C8 F	0.1469	12.4242	N/D	N/D	0.4100	N/D
C8 G	0.1553	6.3636	N/D	N/D	0.2100	N/D

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