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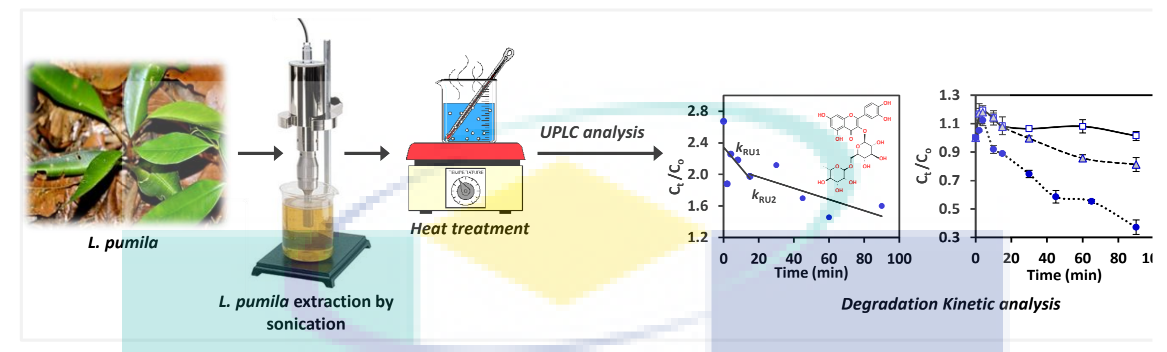

ELUCIDATION OF THERMAL DEGRADATION KINETICS OF LABISIA PUMILA'S POLYPHENOLS

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Leader: Assoc. Prof. Dr. Jolius Gimbun

Member:
Prof. Dr. Mashitah M. Yusoff
Dr. Sureena Abdullah
Nur Syuhada' Asmar
Nurul Azra Bakaruddin

Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), Universiti Malaysia Pahang, 26300 Gambang Pahang Malaysia
E-mail: jolius@ump.edu.my
FRGS Field: Chemical Technology

ABSTRACT

Labisia pumila (Kacip fatimah), contains many useful polyphenols leading to various activities such as pre and post-menopausal issues as well as effective for controlling obesity due to the presence of phytochemical profiles (e.g. phenolics, flavonoids and resorcinols) in the extracts. Processing stage of *L. pumila*, often involves high temperature causing bioactive compounds degradation. The kinetics of polyphenols degradation is not understood and this is the aim of this work. Polyphenols degradation at various temperature and time exposure was quantified via UPLC, and then degradation kinetics was evaluated using both the reaction and Arrhenius approach. Standards gallic acid, protocatechuic acid and epigallocatechin produced single, double and triple degradation rate constant (k) values, respectively with second-order rate law indicating the existence of a multistage degradation. Among all the target compounds studied, epigallocatechin was found to be the most sensitive to thermal degradation with the shortest half-life of 4.78 min. In the case of mixed polyphenols of *L. pumila* extracts, no distinctive degradation kinetics was found due to simultaneous synthesis and degradation of polyphenols as the heating experiment is performed.

1. INTRODUCTION

Labisia pumila (ver. name: Kacip fatimah) is a wild forest sub-herbaceous plant with creeping stems often found on the hill forest floor and lowland of peninsular Malaysia, Thailand, Indochina, Philippines, and New Guinea (Ibrahim et al., 2014). The plant is widely found at an altitude between 300 and 700 m (Melissa et al., 2012; Shahrim et al., 2006). There are three varieties of *L. pumila* in Malaysia, i.e., var. *alata*, var. *lanceolata* and var. *pumila* but the most common variety is var. *alata* (LPva) (Abdullah et al., 2013). *Labisia pumila* is often consumed by women in South-East Asia for general women wellness e.g. to alleviate post-menopausal syndrome and menstrual discomfort, to induce or ease childbirth as well as a post-partum medicine. Scientific study shows that LPva polyphenols alleviates post-menopausal issues (Al-Wahaibi et al., 2008) as well as effective for controlling obesity (Pandey et al., 2014). All the aforementioned medical benefit is due to the presence of phytochemical profiles (e.g. phenolics, flavonoids and resorcinols) in LPva extracts.

A supplement derived from LPva often undergoes conventional aqueous extraction by decoction followed by spray drying, both of which are thermal intensive process. However, phenolics and flavonoids are unstable thermo-labile constituents that may degrade due to thermal exposure during processing, which consequently affect the product nutritive value. For instance, Chen et al. (2001) studied the stability of tea catechins (flavonoid) under various processing conditions and found that 20% of catechins degraded within 7 hours at 98 °C. Earlier, Pang et al. (2014) showed up to 55% of polyphenols degradation during the spray drying of *Orthosiphon stamineus* extracts. Fruits vitamins and catechin are also shown to suffer from thermal degradation, with over 80% degradation of vitamin B5 observed after 20 min at 120 °C from Averrhoa bilimbi fruits extract (Muhamad et al., 2015). Generally, it is understood that the total phenolic content decreases as temperature increased (Muhamad et al., 2015; Solyom et al., 2014). The thermal degradation is undesirable because the degraded product is of low nutritional value and consequently, hampers the intention to produce a nutraceuticals product. Therefore, it is very important to understand the degradation kinetics of bioactive compound from *L. pumila*, which is vital for development of high quality product. Thermal degradation kinetics of flavonoid from *L. pumila* has never been studied before and hence this is the aim of this work.

Objectives

The main objectives of this program are:

- To determine the thermal degradation kinetics of *Labisia pumila's* polyphenols
- To discover the new molecules formed by the *Labisia pumila's* polyphenols degradation via LC-QTOF analysis
- To elucidate mechanism of *Labisia pumila's* polyphenols encapsulation using a transmission electron microscope-energy dispersive x-ray spectroscopy

Scopes

The following are the scope of this research:

i) Collection and preparation of sample
ii) Extraction of polyphenols via sonication technique
iii) Analysis of polyphenol content via liquid chromatography
iv) Thermal degradation kinetics study of polyphenols
v) Enhancement of polyphenol stability by microencapsulation

2. RESEARCH METHODOLOGY

Chemicals and plant material

L. pumila used in this work belonged to the variety alata (LPva) obtained from the state of Pahang in Peninsular Malaysia and was identified to be similar to one that has been deposited in the Herbarium of Forest Research Institute, Malaysia (voucher specimen no. FR154816). The plant was further dried at 37 °C in oven for consecutive 3 days before grounded into powder. The chromatography grade acetonitrile (ACN) was acquired from Merck (Darmstadt, Germany) and trifluoroacetic acid (TFA) was purchased from Fisher Scientific (Leicestershire, UK). Ultrapure water was produced by Milli-Q unit (Milipore, Bedford, MA, USA). The polyphenols were quantified using authentic standard of gallic acid obtained from Agros Organics (Geel, Belgium) whereas protocatechuic acid, epigallocatechin and rutin were purchased from Sigma Aldrich (St. Louis, MO). Standards were stored in -80 °C freezer prior to use.

Ultrasonic assisted extraction

L. pumila extracts were prepared using ultrasonic-assisted extraction at 50 °C for 90 min at 45 kHz. The extraction time of 90 min was chosen from the initial study on the effect time to the concentration of phenolic compounds, which indicate 90 min as optimum extraction time (Pang et al., 2013). Total of 8 g of powdered *L. pumila* was added to 100 ml of water. The supernatant was then separated from the residue by filtration using Whatman no. 1 filter paper. Extract was concentrated by evaporating out excessive solvent from the extract at 40 °C in *vacuo*. The total solid content for each sample was determined by evaporating the liquid from 5 ml solution completely in an oven.

Heating treatments

Kinetics of thermal degradation of gallic acid, protocatechuic acid, rutin and epigallocatechin in *L. pumila* extract were performed at three different temperatures, i.e., 90 °C, 100 °C and 120 °C. For comparison the degradation kinetics of pure compounds i.e., gallic acid, protocatechuic acid, rutin and epigallocatechin at similar concentration as the fruit extract were studied at 90 °C. Sample was placed into a clean and dry screw cap stainless steel tube to prevent evaporation. The samples were heated using an oil bath for times ranging from 1 to 40 min. Heated sample was cooled rapidly by plunging into iced water to stop further degradation. Unheated and filtered extract were used as a control sample.

Mathematical model for thermal degradation kinetics

The zero-order model was employed to describe the thermal degradation of rutin is given by the Eqn. (1).

$$[C]_0 - [C]_t = kt \qquad \text{Eqn. (1)}$$

where C_t is a rutin concentration at any time t, C_0 is initial concentration and k is the reaction rate constant.

The second-order model was employed to describe the thermal degradation of gallic acid, protocatechuic acid and epigallocatechin. The second order rate law is given by the Eqn. (3).

$$\frac{1}{[C]_t} = kt + \frac{1}{[C]_0} \qquad \text{Eqn. (2)}$$

k_2 in Eqn. (2) is the second-order degradation rate constants. The half-life ($t_{1/2}$) value which is the time needed to achieve 50% degradation based on the zero- and second-order model was calculated from Eqn. (3) and (4), respectively.

$$t_{1/2} = \frac{[C]_o}{2k} \qquad \text{Eqn. (3)}$$

$$t_{1/2} = \frac{1}{k_2[C]_o}$$ Eqn. (4)

Analysis of polyphenols content

Quantitative determinations of LPva extract target constituents (gallic acid, protocatechuic acid, epigallocatechin and rutin) before and after heat treatments were performed on a Waters Acquity UPLC H-Class (Milford, MA) fitted with Kinetex®1.7 µm XB-C18 LC column (100 x 2.1 mm) and Phenomenex Security Guard™ Ultra-cartridges 2 µm (2.1 mm). The UPLC system is equipped with photodiode array detector and connected to a computer running Waters Empower 3 software. The mobile phase consists of solvent A (0.1% trifluoroacetic acid (TFA)) and solvent B: (0.1% TFA in acetonitrile). The sample was filtered with 0.22 µm nylon membrane filter before injected into the UPLC system. The peaks for gallic acid (2.65 to 2.68 min), protocatechuic acid (4.43 to 4.5 min), epigallocatechin (5.62 to 5.75 min) and rutin (8.78 to 8.84 min) were detected at λ = 210, 260, 280 and 355 nm, respectively (Yeop et al., 2017a; Gimbun et al., 2018a; 2018b).

Microencapsulation and x-ray photoelectron spectroscopy analysis

The *L. pumila* extracts were encapsulated with either 10 wt.% whey protein isolate (WPI), 10 wt.% gum arabic (GA) and a combination between WPI and GA (1:9). The solution was spray dried using a lab scale spray dryer (Lab Plant SD06A, UK) fitted with 0.5 mm atomizer and air velocity of about 4.1 m/s which was set constant throughout the experiment. The inlet air temperature was set at 180 °C and sustained at ±1 °C by the proportional-integral-derivative controller. The method is similar to those used by Gimbun et al. (2019).

Statistical analysis

Analysis of variance (ANOVA) of the triplicate data was performed by using the data analysis tools in Microsoft Excel 2010, and a least significant difference (LSD) test was used to compare the means with a confidence interval of 95%.

3. LITERATURE REVIEW

Phenolics and flavonoids exposed to very high temperatures undergoes a series of thermal degradation reactions. The degradation pathway of gallic acid (Heleem et al., 1982; Boles et al., 1988) protocatechuic acid (Khuwijitjaru et al., 2014; Vallverdu-Queralt et al., 2014), epigallocatechin (Song et al., 2015) and rutin (Vallverdu-Queralt et al., 2014; Buchner et al., 2006; Ravber et al., 2016; Chaaban et al., 2016) had been studied by several researchers. In most of the previous work, the temperature was proposed to be the main factor that accelerates the degradation. Song et al. (2015) reported that epigallocatechin are vulnerable to several degradative reactions accelerated by heat, food ingredients, elevated pH (>5) conditions, and the presence of dissolved oxygen or other reactive oxygen species. The degradation of polyphenols may produce an unwanted by-product or reduce the efficacy of the functional food. Therefore, it is vital to understand the polyphenols degradation kinetics to enable an effective mitigation during the processing stages. To our knowledge, no data on the heat induced degradation of *L. pumila* polyphenols is available in the literature, hence this is the aim of the current work. In the present work the degradation kinetics of target LPva's polyphenols, i.e., gallic acid, protocatechuic acid, epigallocatechin and rutin are elucidated experimentally. In addition, the degradation of the polyphenols from LPva extract is also studied.

Microencapsulation is an effective way to reduce thermal degradation of polyphenols during spray drying (Pang et al., 2014). Such effect is affected by the choice of wall material (microencapsulation agent) employed to preserve the active compounds during spray drying. A common microencapsulation agent such as whey protein isolate (WPI) and gum arabic (GA) is often used for herbal or plant related product.

For instance, Rajabi et al. (2015) reported that gum arabic can securely maintained over 80% retention of bioactive components from saffron during spray drying. Another common microencapsulation agent often used for herbal or plant related product is the whey protein isolate (WPI). Pang et al. (2014) demonstrated an excellent retention of *O. stamineus* polyphenols by microencapsulation with WPI and maltodextrin. Earlier, Pang et al. (2013) reported that the use of a mixture made by polysaccharide and protein provides better encapsulation efficiency on polyphenols than the single wall material made of either polysaccharide or protein. However, there is a limited study about the microencapsulation of polyphenols from *L. pumila* available in the literature. Therefore, this work aims to reduce the degradation of bioactive compounds from *L. pumila* extract via microencapsulation technique using encapsulating agents such as whey protein isolate, gum arabic and its mixture.

4. FINDINGS

Thermal degradation of polyphenols derived from *L. pumila* has been elucidated. Among the main polyphenols from *L. pumila*, epigallocatechin is more susceptible to thermal degradation, followed by rutin, gallic acid and protocatechuic acid (Gimbun et al., 2018a; 2018b). The kinetics of degradation follows the Arrhenius approach. It is possible to minimize the thermal degradation of polyphenols during spray drying by microencapsulation process. Up to 92.06% retention of polyphenols can be achieved by microencapsulation by maltodextrin (Yeop et al., 2019). Gimbun et al. (2019) shows the encapsulation mechanism to be an irregular hollow multilayer system.

5. CONCLUSION

We elucidated the thermal degradation kinetics of polyphenols derived from *L. pumila* and has succesfully developed a method to advert such issue during the processing stage. Details of the findings is available from the published paper on this work as well as the students' thesis.

The research program was succesfully completed according to research plan. Despite the difficulties, our team managed to achieve the following:

a) 7 papers produced
b) 4 conference presentation
c) 5 students trained (2 PhD)
d) 1 awards nationally and internationally
e) 1 patent filed

ACHIEVEMENT

i) **Name of articles/ manuscripts/ books published**

[1] Yeop, A., Sandanasamy, J., Pang, S. F., Abdullah, Sureena., Yusoff, M. M., Gimbun, J. (2017). The effect of particle size and solvent type on the gallic acid yield obtained from Labisia pumila by ultrasonic extraction, MATEC web of conferences, 111, 02008 (2017). Scopus indexed.

[2] J. Sandanasamy, M. M. Yusoff, J. Gimbun, (2017), Optimization of Ultrasonic-Assisted Extraction of Polyphenolic and Flavonoids from Labisia pumila, Materials Science Forum, Vol. 890, pp. 167-170. Scopus indexed.

[3] Afiqah Yeop, Sook Fun Pang, Woon Phui Law, Mashitah M Yusoff, Jolius Gimbun, 2019. Assessment of size reduction and extraction methods on the yield of gallic acid from Labisia pumila leaf via microstructures analysis, Material Today Proceeding. Scopus indexed.

[4] Afiqah Yeop, Sook Fun Pang, Sureena Abdullah, Mashitah M. Yusoff and Jolius Gimbun, 2019. Effect of Ultrasonic and Microwave Assisted Extraction of Pyrogallic Acid from Labisia Pumila, Journal Engineering Applied Sciences. (accepted). Scopus indexed.

[5] J. Sandanasamy, Afiqah Yeop, S.F. Pang, M. M. Yusoff, J. Gimbun, 2019. Thermal stability of phenolic acids and flavonoids from Labisia pumila extract - a kinetic study, Talanta (in prep), Q1 ISI.
[6] Afiqah Yeop, S.F. Pang, J. Sandanasamy, Sureena Abdullah, M. M. Yusoff, J. Gimbun, 2019. Thermal stability and controlled release enhancement of Labisia pumila's polyphenols by surface protein encapsulated microcapsule, Journal of Controlled Release (in prep), Q1 ISI.
[7] Gimbun, J., Nguang, S.L., Pang, S.F., Yeong, Y.L., Kee, K.L. and Chin, S.C., 2019. Assessment of phenolic compounds stability and retention during spray drying of Phyllanthus niruri extracts. *Industrial & Engineering Chemistry Research*. 58 (2), 752–761. Q1 ISI.

ii) **Title of Paper presentations (international/ local)**
[1] Yeop, A., Sandanasamy, J., Pang, S. F., Abdullah, Sureena, Yusoff, M. M., Gimbun, J. (2017). The effect of particle size and solvent type on the gallic acid yield obtained from Labisia pumila by ultrasonic extraction. 2nd International Conference on Fluids & Chemical Engineering (FluidsChE) 2017, 4-6 March 2017, Sabah, Malaysia.
[2] Yeop, A., Sandanasamy, J., Pang, S. F., Yusoff, M. M., Gimbun, J. (2017). Essence D'Herbe KF for Woman Wellness. Creation, Innovation, Technology and Research Exposition (CITREX) 2017, 15-16 March 2017, Universiti Malaysia Pahang, Kuantan, Malaysia.
[3] Jessinta Sandanasamy, Mashitah M Yusoff and Jolius Gimbun, Optimization of Ultrasonic-Assisted Extraction of Polyphenolic and Flavonoids from Labisia pumila, ICMSET 2016, Tokyo, Japan, October 29-31, 2016.
[4] Jolius Gimbun, Jessinta Sandanasamy, Afiqah Yeop, Sook Fun Pang and M. M. Yusoff (2018) Thermal degradation kinetics of Labisia pumila's polyphenols. In: International Conference On Recent Trends In Analytical Chemistry (ICORTAC-2018), 15 - 17 March 2018, University of Madras. India.

iii) **Human Capital Development**
PhD student
[1] Jessinta Sandanasamy (PKB13005), pass viva-voce November 2018.
[2] Afiqah Yeop (MKC16024), Master converted to PhD. expected to graduate October 2019.

iv) **Awards**
[1] **Silver medal**, Yeop, A., Sandanasamy, J., Pang, S. F., Yusoff, M. M., Gimbun, J. (2017). Essence D'Herbe KF for Woman Wellness. Creation, Innovation, Technology and Research Exposition (CITREX) 2017, Kuantan.

v) **Patent**
[1] J. Gimbun, M.M. Yusoff, Pang Sook Fun, Jessinta Sandanasamy, Afiqah Yeop, Method for producing a high quality functional food ingredient from *Labisia pumila*, 2018 PI2018400005 (filed by East Coast IP). 7 March 2018.

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