Research Article

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# Effect of Extraction Conditions on Color, pH, Flavor Profile and Ribonucleotide Contents of *Limnophila aromatica* (Lam.) Merr. Extracts

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**Abstract**
The extracts of *Limnophila aromatica* was conducted using 5 temperatures and 6 extraction times and the obtained color, pH, flavor profile and the contents of 5´ribonucleotides were compared. Extraction at higher temperature and longer time resulted in decrease of L* and a* values, but increase of b* value. The pH values of extracts had significantly decreased with longer time of extraction. Nine flavor terms and their intensities were identified. There was no ribonucleotides detected in the distilled water extracts. In contrast, when using methanol as the solvent for the extraction of dried samples, the 5´AMP and 5´UMP were detected.

**Keywords**: *Limnophila aromatica* (Lam.) Merr., pH, Color, Flavor profile, 5´-ribonucleotides

## 1 Introduction

*Limnophila aromatica* (Lam.) Merr. is an aromatic herb mostly found in the rice field. It grows well in flooding land or watery environment and temperate climate [1]. This plant belongs to the Scrophulariaceae family. The common name of *Limnophila aromatica* are rice paddy herb, finger grass or "Ka-yang" (in Thai). It is a native plant found in Australia and Asia countries including, Bangladesh, Bhutan, China, India, Indonesia, Japan, South Korea, North Korea, Laos, Malaysia, Myanmar, Philippines, Taiwan, Viet Nam and Thailand [2]. In the North-East of Thailand, *Limnophila aromatica* has been used as food ingredient to give or enhance umami taste of many types of soups and curries. This plant had been reported to have the bioavailability. Its essential oil showed antioxidant activity against free radical and exhibited anti-lipid peroxidation [3]. Effect of ratio and various types of solvents to extract bioactive compounds from *Limnophila aromatica* was studied. Extraction using 100% ethanol showed the best result with antioxidant $IC_{50}$ = 70.06 µg/mL, total phenol of 40.50 mg GAE/g and flavonoid content of 31.11 QCE/g [4].

Umami is originated from Japanese word referring to a delicious or savory taste. In 1908, terminology of umami was firstly described by Prof. Kikunae Ikeda who had extracted monosodium glutamate (MSG) from seaweed (*Laminaria Jaoanica*) which gave the umami taste to various kind of foods [5]. MSG has been used as food additive to give umami taste in food and food products for decades. However, many research attempted to find a natural ingredient to substitute or reduce the use of MSG.

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There were many food ingredients with umami enhancing property included yeast extract, mycoprotein, fermented soy bean paste, soy sauce, tomato sauce and tomato extract [6] The main compounds responsible for umami taste are glutamic acid (Glu), aspartic acid (Asp), L-glutamate and 5′-ribounacleotides including 5′-adenosine monophosphate (5′-AMP), 5′-guanosine monophosphate (5′-GMP), 5′-inosinate monophosphate (5′-IMP), 5′-uridine monophosphate (5′-UMP) and 5′-xanthosine monophosphate (5′-XMP) [7]. The presence in high level of Glu, Asp and 5′-GMP gave the most typical umami taste or palatable taste that is a characteristic of MSG taste [8].

The content of 5′-nucleotides in commercial mushrooms; *Flammulina velutipes*, *Lentinula edodes* (strain 271), *Pleurotus cystidiosus* and *Pleurotus ostreatus*, of 8.60, 11.6, 1.6, 5.52 and 6.09 mg/g (dry weight) were reported [9]. Effect of temperatures and times of extraction on the quantity of 5′-ribonucleotides in shitake mushroom (Lentinus edodes) were investigated which higher temperature and longer time of extraction resulted in greater quantity of each 5′-ribonucleotide [10].

This research aimed to study the physicochemical properties of the distilled water extract from *Limnophila aromatica* which could be a new product that have the same function of MSG and could be used as a food condiment. Different temperatures (room temperature, 50, 70, 90 and 100°C) and times (0, 15, 30, 60, 90 and 120 min) of extraction were performed. The physicochemical properties including L*, a*, b* color values, pH and the simple flavor profiles of *Limnophila aromatica* extracts were examined. The contents of 5′-ribonucleotides including 5′-AMP, 5′-GMP, 5′-IMP, 5′-UMP, and 5′-XMP were also determined.

## 2 Materials and Methods

### 2.1 *Materials*

Fresh *Limnophila aromatica* was bought from a local market in Sakon Nakhon province, Thailand. The roots were cut. After that, the stems and leaves were washed twice and kept in zip lock plastic bag at 4°C before extraction. The extraction were conducted within 24 h after washing.

### 2.2 *Chemicals and reagents*

All standard and reagents used in this study were HPLC grade. The 5′- ribonucleotides and phosphoric acid were from Sigma-Aldrich (Steinheim, Switzerland). Methanol was from Macron (California, USA).

### 2.3 *Methods*

#### *2.3.1 Extraction*

*Limnophila aromatica* was extracted using method adapted from Maria *et al*. [10]. Fresh leaves and stalk of *Limnophila aromatica* (30 g) was cut into small pieces and then extracted by adding 100 mL of distilled water using water bath shaker for extraction (SWB-35, HanYang Scientifc Equipment Ltd, Korea). The 5 different temperatures; room temperature (RT; 32°C), 50, 70, 90 and 100°C for 0, 15, 30, 60 and 120 min were performed. After extraction, the samples were cooled to reach room temperature and filtered through Whatman No.1 filter paper and PVDF Syringe filter (0.45 µm, Sigma aldrich, Switzerland), respectively. The extracts were kept at –18°C prior to the analysis of 5′ribonucleotides.

#### *2.3.2 Measurement of color*

The color of distilled water extracts from *Limnophila aromatica* were determined in CIE L*, a*, b* system using Hunter LabScan colorimeter (Miniscan XE plus, Hunter Associates Laborator, Virginia, USA). Ten milliliters of each of the extracts were analyzed in triplicate.

#### *2.3.3 Measurement of pH*

The pH values of distilled water extracts (10 mL) from *Limnophila aromatica* were determined using pH meter (Ultra Basic, Denver instrument, USA). The sample were analyzed in triplicate.

#### *2.3.4 Sensory consensus descriptive analysis*

Flavor profiles of the distilled water extracts from *Limnophila aromatica* were examined by 2 trained panels (female). The sensory analysis was conducted in a room with no noise interruption. All extracts were labelled with random 3-digit codes and presented at

once in white paper cups at 25°C. References were presented in clean glass (pre-heated at 100°C for 2 h) covered with aluminium foil. Panels were asked to rinse their mouth with drinking water before testing first sample and after testing each sample. Flavor terms, definitions and references were consensus-based identified in agreement of 2 panels. Panel was individually asked to range the intensities of each flavor in 5 points scale which 1 = very light, 2 = light, 3 = moderate, 4 = strong and 5 = very strong. After individual ranging, the intensities of each flavor were consensus determined in agreement of 2 panels and reported as a spider plot.

*2.3.5 Analysis of 5´-ribunucleotides*

Five ribonucleotide contents of distilled water extracts from *Limnophila aromatica* were determined using High Performance Liquid Chromatography (HPLC) with photodiode array detector (L-4500, Hitachi, Japan). An Inertsil ODS-2 column (4.6 cm × 250 mm; GL Sciences Inc., Tokyo, Japan) was used. Injection volume was 20 µL. The solvents were methanol (A) and 0.05% phosphoric acid (B) with the ratio of A : B (90 : 10). Isocratic mode of solvent elution was applied at 30°C, using the flow rate of 0.7 mL/min. Concentrations of 5´-ribonucleotides were reported as mg/100 g of sample (wet basis). Each 5´-ribonucleotide was quantified using a 5 point calibration curve of the pure 5´-ribonucleotide. $R^2$ of standard curve of 5´-ribonucleotide was calculated.

*2.3.6 Statistical analysis*

Two factors; temperature (5 levels) and time (6 levels) of extraction, were evaluated using a 2*2 factorial design in Completely Randomized Design (CRD) with three replicates of extraction. The pH value, L*, a* and b* values of water extract from each extraction were further measured in triplicate. All experimental results were reported as mean values. Statistical significance was performed using 2-way ANOVA, followed by Duncan´s multiple range tests. A value of $p \leq 0.05$ was considered statistically significant. The software SPSS statistic (SPSS 11, IBM SPSS statistics, New York, USA) was used.

## 3 Results and Discussions

*Limnophila aromatica* has been used as ingredient in many types of soups and curries in Thailand. Our previous sensory study (data not shown) revealed that 63% of untrained panel perceived umami taste in a soup containing *Limnophila aromatica*. This brought us to the interest of umami responsible compounds in this plant. There are many natural foods containing umami responsible compounds. One of them is soy sauce which is a common condiment in Thailand. To imitate this product, distilled water was used as a solvent to extract umami responsible compounds from *Limnophila aromatica*. Different temperatures and times of extraction were performed. Color, pH values, flavor profile and the contents of 5´ribonucleotide were analysed.

### 3.1 *Color of distilled water extracts from Limnophila aromatica*

The CIE L*, a*, b* values of distilled water extracts from fresh *Limnophila aromatica* at different temperatures and times of extraction were shown in Table 1, 2 and 3. The L* value represents a darkness (L* = 0)/lightness (L* = 100). The a* value represents a redness (+a*)/greenness (–a*). The b* value represents a yellowness (+b*)/blueness (–b*). Main effects and 2-way interaction between temperature and time of extraction were statistically significant with $p \leq 0.05$ for L*, a* and b* values. Over times and temperatures of extraction, L*, a* and b* values of extracts were in the range of 14.96 to 10.57, –1.81 to –0.38 and –2.46 to 3.69, respectively. While L*, a* and b* values of distilled water were 17.42, –0.69, and 0.40. These indicated the similarity in color of distilled water and the extracts. However, at higher temperatures and longer times of extraction, L* and a* values had decreased, but b* had increased. These indicated decreases of lightness and redness, but increase of yellowness of extracts.

**Table 1**: The L* values of distilled water extract from *Limnophila aromatica*

| Time (min) | L* values | | | | |
|---|---|---|---|---|---|
| | Temperature (°C) | | | | |
| | RT | 50 | 70 | 90 | 100 |
| 0 | $19.52^{a}$ | $17.89^{c}$ | $17.25^{de}$ | $16.79^{efg}$ | $15.81^{jk}$ |
| 15 | $19.04^{ab}$ | $17.19^{def}$ | $16.57^{fgh}$ | $16.15^{hij}$ | $15.92^{ijk}$ |
| 30 | $19.28^{ab}$ | $16.80^{efg}$ | $16.55^{fghi}$ | $15.75^{jkl}$ | $15.34^{klm}$ |
| 60 | $19.57^{a}$ | $17.45^{cd}$ | $16.88^{defg}$ | $15.79^{ijk}$ | $14.96^{m}$ |
| 90 | $18.75^{b}$ | $17.99^{c}$ | $16.31^{ghij}$ | $15.75^{jkl}$ | $15.13^{lm}$ |
| 120 | $19.14^{ab}$ | $16.12^{hij}$ | $16.12^{hij}$ | $15.44^{klm}$ | $15.39^{klm}$ |

Significant difference of values are indicated by different letters ($p \leq 0.05$) as the effect of interaction between extraction temperature and time. Room temperature indicated by RT.

**Table 2**: The a* values of distilled water extract from *Limnophila aromatica*

| Time (min) | a* values | | | | |
|---|---|---|---|---|---|
| | Temperature (°C) | | | | |
| | RT | 50 | 70 | 90 | 100 |
| 0 | $-0.52^{abcd}$ | $-0.39^{ab}$ | $-0.45^{abc}$ | $-0.94^{defg}$ | $-0.60^{abcd}$ |
| 15 | $-0.38^{ab}$ | $-0.42^{ab}$ | $-0.65^{abcd}$ | $-1.26^{fgh}$ | $-1.54^{hi}$ |
| 30 | $-0.45^{a}$ | $-0.35^{a}$ | $-0.81^{bcde}$ | $-0.97^{defg}$ | $-1.34^{gh}$ |
| 60 | $-0.46^{a}$ | $-0.81^{bcde}$ | $-0.80^{abcde}$ | $-1.37^{gh}$ | $-1.81^{i}$ |
| 90 | $-0.57^{abcd}$ | $-0.46^{abc}$ | $-0.88^{cdef}$ | $-1.14^{efgh}$ | $-1.37^{gh}$ |
| 120 | $-0.57^{abcd}$ | $-0.62^{abcd}$ | $-0.96^{defg}$ | $-1.36^{gh}$ | $-1.30^{fgh}$ |

Significant difference of values are indicated by different letters ($p \leq 0.05$) as the effect of interaction between extraction temperature and time. Room temperature indicated by RT.

**Table 3**: The b* values of distilled water extract from *Limnophila aromatica*

| Time (min) | b* values | | | | |
|---|---|---|---|---|---|
| | Temperature (°C) | | | | |
| | RT | 50 | 70 | 90 | 100 |
| 0 | $-0.24^{n}$ | $-2.46^{n}$ | $-1.96^{mn}$ | $-0.79^{k}$ | $-1.69^{m}$ |
| 15 | $-2.36^{n}$ | $-1.62^{m}$ | $-0.94^{kl}$ | $0.31^{fghi}$ | $1.35^{bcd}$ |
| 30 | $-1.45^{lm}$ | $-0.33^{ijk}$ | $0.11^{hij}$ | $0.20^{ghi}$ | $0.90^{cdef}$ |
| 60 | $-1.86^{mn}$ | $0.55^{efgh}$ | $0.24^{ghi}$ | $1.47^{bc}$ | $3.69^{a}$ |
| 90 | $-1.97^{mn}$ | $-0.47^{jk}$ | $0.04^{hij}$ | $1.03^{cde}$ | $1.65^{b}$ |
| 120 | $-1.85^{mn}$ | $-0.53^{jk}$ | $-0.44^{jk}$ | $0.76^{defg}$ | $0.99^{cde}$ |

Significant difference of values are indicated by different letters ($p \leq 0.05$) as the effect of interaction between extraction temperature and time. Room temperature indicated by RT.

Natural color of *Limnophila aromatica* is light green which chlorophyll is a pigment responsible for a color of this plant. The total chlorophyll and lipid contents of *Limnophila aromatica* was approximately 4 g/100 g [11]. According to L*, a* and b* values and by self-observation, the color of extracts from *Limnophila aromatica* had increased in brownness and yellowness when high temperature and long time of extraction were applied. The possible reasons could be as followed. First, even though chlorophyll is an oil soluble compound, high temperature incorporated with long time of extraction could enhance the solubility of chlorophyll into water. Upon extraction, degradation products of chlorophyll like pheophytins and pheophorbides could be formed, resulting brownish color of extracts [12]. Secondly, a yellowness of extract could contribute to carotenoid which is an chlorophyll accompanied pigment found in most plant [12]. Extraction conditions could also enhance carotenoid's solubilty as well as those to chlorophyll. Carotenoid is more heat stable than chlorophyll, when degradation of chlorophyll took place, caratenoid could contribute to the color of extracts [13].

Thirdly, there were 2 types of browning reactions, enzymatic browning and Maillard reactions that could be related to this phenomenon. At room temperature, 50 and 70°C, the browning related enzyme such as polyphenol oxidase and peroxidase were not inactivated. Processing temperature above 80°C were suggested to inactivate enzyme activities [14]. Their activities could play an important role on a browness of the extracts of room temperature, 50 and 70°C. However, when temperature above 80°C were applied, browning was remaining observed. In this case, chlorophyll degraded compounds, carotenoids and Maillard reaction could play an important role in an occurrence of brownness in these extracts. There was no reported on nutritive composition of *Limnophila aromatica*, but small amount of reducing sugar and amino-grouped compounds which normally found in plant, could lead to a formation colored polymeric Mailllard compounds. The rate, extent, and course of Maillard reactions are influenced by several factors including, but not limited to, type of reactants, temperature/time combinations, pH, and water activity [15]. In this study, using high temperature above 70°C of extraction could accelerate the Maillard reaction and resulted in brown color of the extracts.

### 3.2 *The pH values of distilled water extracts from Limnophila aromatica*

Table 4 showed the pH values of distilled water extracts from fresh *Limnophila aromatica*. Main effects and 2-way interaction between temperature and time of extraction had statistical influence on pH values of the extracts ($p \leq 0.05$). The pH values of extracts were in the range of 5.35 to 6.54, while the pH of distilled water was 7.15. The highest pH value was observed in the extract of 90°C, 15 min. The lowest pH value was observed in the extract of room temperature, 0 min.

**Table 4**: The pH values of distilled water extract from *Limnophila aromatica*

| Time (min) | pH value | | | | |
|---|---|---|---|---|---|
| | Temperature (°C) | | | | |
| | RT | 50 | 70 | 90 | 100 |
| 0 | 5.35/27 | 5.76/14 | 5.70/15 | 6.23/3 | 6.04/5 |
| 15 | 5.45/25 | 5.65/17 | 5.66/16 | 6.54/1 | 5.96/8 |
| 30 | 5.87/12 | 5.58/20 | 5.62/18 | 6.26/2 | 6.02/6 |
| 60 | 5.96/8 | 5.56/21 | 5.51/22 | 5.95/9 | 5.86/13 |
| 90 | 6.01/7 | 5.51/22 | 5.48/23 | 5.59/19 | 5.92/10 |
| 120 | 6.18/4 | 5.47/24 | 5.43/26 | 5.95/9 | 5.91/11 |

Significant difference of values are indicated by different superscript numbers ($p \leq 0.05$) as the effect of interaction between extraction temperature and time. Room temperature indicated by RT.

It was noticed that when higher temperature were applied, pH of extracts tended to increase. Temperatures of 50 and 70°C resulted in lower pH of extracts than those of room temperature, 90 and 100°C. In contrast, decreasing of pH was observed in the extracts of 50, 70, 90 and 100°C when longer extraction times were applied. The reason of pH changing according to extraction conditions cannot be clearly identified. In case of pH decrease for extracts of 50, 70, 90 and 100°C when longer extraction times applied, it was possible that longer time of extraction allowed transferring of H+ atoms from plant material into water to occur. H+ atoms could be originated from organic acids contained in plant cell. Result of pH values from this study could be used in consideration if the pH of future food product using distilled water extract from *Limnophila aromatica* as a prototype is crucial.

### 3.3 *Sensory descriptive profiles of distilled water extracts from Limnophila aromatica*

Two trained panels (female) with more than 1 year experience in flavor and sensory analysis in foods were selected to conduct a simple consensus descriptive analysis. Trained panels were additionally trained for 20 h before this study. Definitions, references and intensity scores of each flavor were consensus-based defined by 2 trained panels. Scale of flavor intensity score was range from 1 = very light to 5 = very strong. Flavor terms, definition and references of 5 odors, 2 tastes and 2 trigeminal characteristic were identified as green odor, mint odor, aloe vara like odor, bark odor, metallic odor, bitter taste, sour taste, astringency and cool feeling (Table 5). Flavor profiles of extracts at room temperature, 50, 70, 90 and 100°C were reported as spider plots (Figure 1). Similar trends of developments in brownness and flavor intensities of the extracts when extracting at high temperature and long time was observed. These formation of brown pigment and aroma of the extracts could relate to Maillard reaction. From Figure 1, when high temperature and long time of extraction applied to the extracts, it resulted in the increase of intensities of most flavor characteristics. However, temperature seemed to have more effect to the flavor profile rather than time of extraction. The astringency was the most dominant flavor characteristic perceived from distilled water extracts in most extraction conditions. Bitter taste was dominant in the extracts using the temperature above 50°C. At 70, 90, and 100°C, the extracts had increased in astringency, bitter taste, cool feeling, bark odor and aloe vera like odor comparing to the extracts using lower temperature. Sour taste was detected in 70, 90, and 100°C extracts, but time of extraction did not affect their intensity. Panels had detected unacceptable responses of the extracts using the extraction time of more than 60 min at every temperature of extraction. These extracts had unacceptable bitter taste and metallic odor (data not shown). Strong intensities of bitter taste and astringency could lead to rejection of using these extracts as food ingredient.

**Table 5**: Flavor terms, definitions and references used in consensus descriptive analysis of distilled water extracts from *Limnophila aromatica*

| Flavor Term | Definition | Reference |
|---|---|---|
| Green odor | Odor of freshly cut grass | 10 g of freshly cut grass |
| Mint odor | Odor of fresh pepper mint | 20 g of freshly crushed pepper mint leaves |
| Aloe vera like odor | Odor of aloe vara gel | 20 g of freshly cut aloe vera |
| Bark odor | Odor of fresh bark | 5 g of fresh bark |
| Metallic odor | Odor of metal and sulfur | No physical standard |
| Sour taste | Taste of citric acid | 0.5 g/L of citric acid solution |
| Bitter taste | Taste of caffein solution | 1 g/L of caffein solution |
| Astringency | Perception of alum solution | 3 g/L of alum solution |
| Cool feeling | Perception of sparking and cooling | 25 mL of soda |

### 3.4 *Contents of 5′-ribonucleotides*

Contents of 5 types of 5′-ribonucleotides including 5′-AMP, 5′-GMP, 5′-IMP, 5′-UMP and 5′-XMP could play an important role in enhancing umami taste in food using *Limnophila aromatica* as ingredient. In this study, distilled water was firstly used as a solvent. Since, a molecular structure to 5′-ribonucleotides were containing phosphate group and ribose sugar which are hydrophilic. Moreover, we would like to develop a food product which have similar use of soy sauce. The extraction procedure was applied from Dermiki *et al*. [1], which extract the 5′-ribonucleotides and free amino acids from dried shiitake mushroom.

Methanol (100 mL) and ultrasonic treatment were used to extract 5′-ribonucleotide in the second attempt. One gram of dried *Limnophila aromatica* (dried at 60°C and 90°C, for 4 h in tray dryer) was used as the

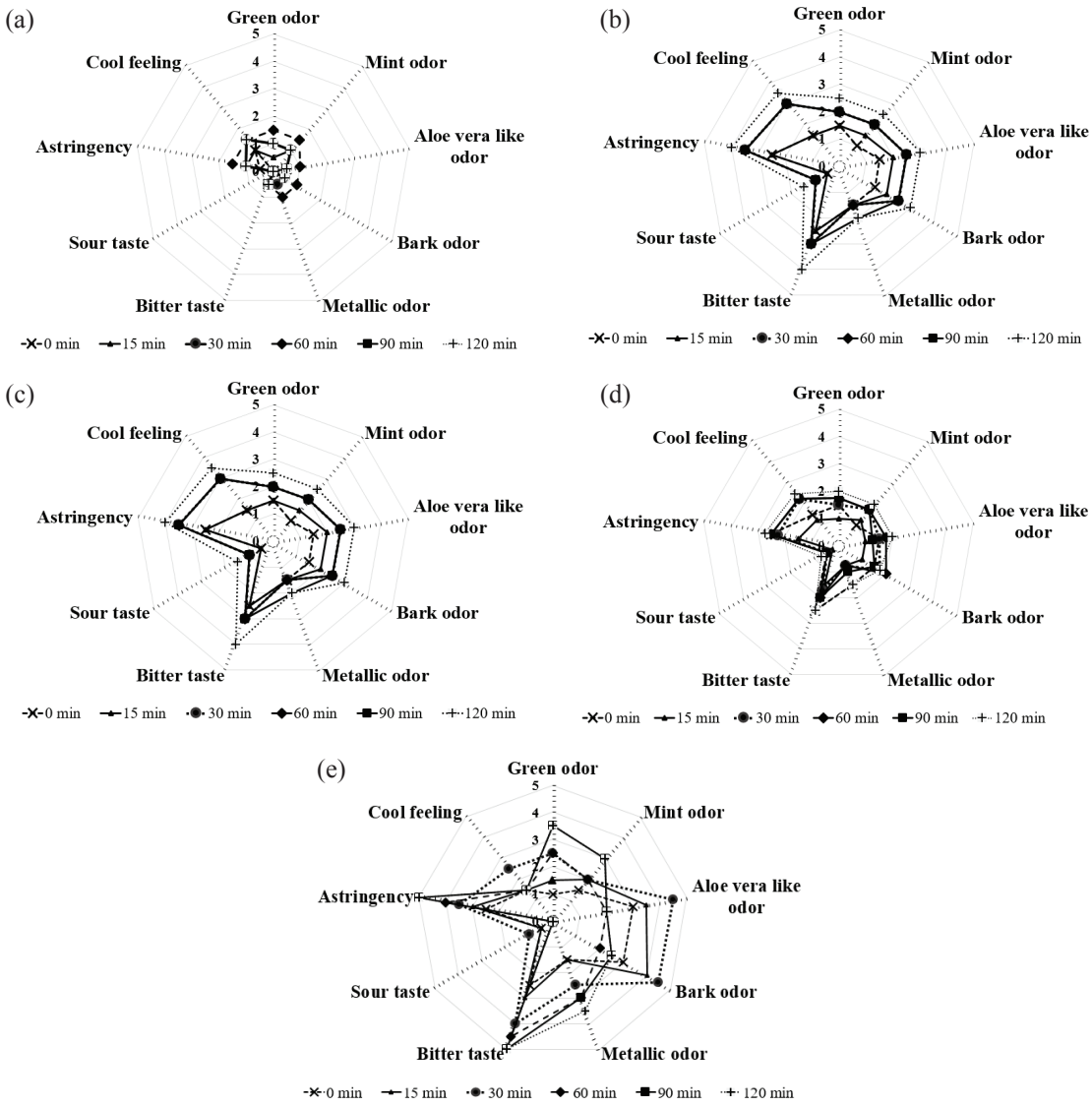


**Figure 1**: The flavor profile of distilled water extracts from *Limnophila aromatica* (a) room temperature extraction, (b) 50°C extraction, (c) 70°C extraction, (d) 90°C extraction, and (e) 100°C extraction.

sample. Prior to extraction using water bath shaker, methanol containing dried sample was ultra-sonicated at room temperature (32°C) for 30 min at a frequency of 200 Hz. After that, extraction in water bath shaker was performed at room temperature for 1 h. There were 5′-AMP and 5′-UMP founded in dried sample extracts (Table 5). Contents of 5′-AMP in 60 and 90°C dried extracts were 62.96 and 63.07 mg/100 g of sample (wet basis), respectively. Contents of 5′-UMP in 60 and 90°C dries extracts were 41.00 mg/100 g of sample (wet basic). The $R^2$ of standard curve of 5′-AMP and 5′-UMP were 0.9608 and 0.9513, respectively. These results suggested that methanol had more extracting power than distilled water to extract 5′-ribonucleotides from *Limnophila aromatica*. Difference in polarity of methanol and distilled water could have influence on extraction efficiency of 5′-ribonucleotides, and ultrasonic treatment helped facilitating the extraction.

**Table 5**: The contents of 5´-ribonucleotides in distilled water extracts from *Limnophila aromatic*

| Com-pound | 5´-Ribonucleotide (mg/100 g sample, wet basis) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Distilled Water | | | | | | Methanol | |
| | RT | | 70°C | | 100°C | | 60°C Dried Sample | 90°C Dried Sample |
| | 30 min | 60 min | 30 min | 60 min | 30 min | 60 min | | |
| 5´-AMP | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 62.96[a] | 63.07[a] |
| 5´-GMP | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 5´-IMP | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 5´-XMP | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 5´-UMP | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 41.00[a] | 41.00[a] |

Significant difference of values are indicated by different letters ($p \leq 0.05$) as the effect of interaction between extraction temperature and time. Room temperature indicated by RT. Not detected indicated by n.d.

Principle of Ultrasound-Assisted Extraction (UAE) is using high frequency sound to facilitate mass transfer between immiscible phases through super agitation. The benefit of UAE is reducing extraction time, extraction temperature, energy input and consumption of organic solvents [16]. These results could lead us to the future study to use various kind of solvents using ultrasonic assisted extraction.

## 4 Conclusions

This study was investigated the physicochemical properties of *Limnophila aromatica* extracts. Extraction temperatures had effect on the quality of pH and color of distilled water extracts. Darkness, greenness and yellowness of extracts had increased with longer times and higher temperatures of extraction. The pH change of water extracts had decreased when the temperature above 50°C with longer time extraction were applied. Flavor profiles of distilled water extracts showed a strong perception of bitterness, astringency and metallic odor when high temperature and long time of extraction were applied. There were no 5´-ribonucleotide detected when using distilled water as a solvent, while using methanol resulted in detection of 5´-AMP and 5´-UMP.

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